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SUGAR UPTAKE AS A FUNCTION OF CELL VOLUME IN HUMAN ERYTHROCYTES

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Two main theories have been proposed to explain the specificity of uptake of sugars by human erythrocytes. The membrane theory (see for example, Bowyer & Widdas, 1956; LeFevre, 1954) suggests that the main barrier to diffusion is the cell membrane and that transfer across this barrier can occur only as a result of binding of the penetrant by a specific agent or carrier located in the membrane. Once inside the cell, however, the sugar is free to diffuse throughout the aqueous phase of the protoplasm. The 'sorption' theory (Troshin, 1961) on the other hand suggests that all the water within the protoplasm is bound, so that the solubility of the sugar in the aqueous phase of the cell is less than in free water. Specific binding of various compounds by the cell colloids is supposed to occur, however, and to account for at least part of the sugar appearing in the cell.

The present work seeks to determine the amount of binding of sugars occurring in human erythrocytes. To this end measurements were made of the amount of sugar entering cells whose volume was adjusted by varying the tonicity of the medium in which they were suspended. It would be expected that if all the sugar in the cells were bound the amount taken up by a given number of cells would be independent of their volume, since the amount of cell protein and hence the number of binding sites should be constant. On the other hand if the sugar was in solution within the cell the amount taken up would be proportional to the volume of free (or solvent) water present in the cell.

It was also the purpose of the present work to measure the kinetics of sugar uptake by cells of different volumes in an attempt to decide whether the greater resistance to diffusion occurs in the membrane or within the protoplasm. Since the surface area of erythrocytes remains constant as the cell volume is varied, the rate of sugar uptake should be independent of the volume if the membrane provides the greater resistance.

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METHODS

Static measurements. Fresh to one-day-old heperinized human blood was centrifuged and the cells washed with normal NaCl solution, 0·9 g/100 ml. A quantity of cells whose volume in normal saline had been determined previously were suspended in a known total volume at a hematocrit of 3–4 % in a medium having the following composition (mM): Trishydroxymethylaminomethane—HCl buffer, pH 7·35, 30; KCl 5; calcium acetate, 2·6; magnesium acetate 1·7; NaCl to bring the medium to the appropriate tonicity (usually between 0·5 and 2 × isotonic) and sugar at the required concentration. This suspension was incubated at 37·5° C for about half an hour, centrifuged down and resuspended in the same medium. Samples of a known volume were then removed after further 1, 2 and 3 hr periods, and centrifuged in hematocrit tubes whose lower portions consisted of graduated and calibrated capillaries. These allowed the volume of cells to be measured and the supernatant to be washed out of the tube above the surface of the column of packed cells. The cells themselves were then washed out and hemolysed with distilled water, the protein precipitated by Nelson's (1944) method and the sugar content determined. Glucose was analysed by the glucose-oxidase method (Marks, 1959) and fructose by Pogell's (1954) method.

Kinetic measurements. The entry of fructose at 30° C was chosen for this study since it was slow enough to allow the above sampling methods to be used with little error in timing. Further, by using a fructose concentration of 20 mM the volume changes with penetration were slight. Cells were incubated in this medium, less the sugar, for 1–2 hr, the sugar was added and a sample taken immediately. Further samples were taken at various intervals and centrifuged and analysed for fructose as specified in the previous section. In calculating the results the amount of sugar present in the first sample was subtracted from all others, thus correcting for any solution trapped in the cell column.

RESULTS

Static measurements. Table 1 column four lists the number of millimoles sugar found by analysis to be present in a quantity of cells whose volume in normal saline (0·9 %) was 1 l. Column three indicates the actual volume of the cells relative to that in normal saline, which they occupied following equilibration with the sugar. These figures are an average of at

TABLE 1

Sugar	External sugar concentration (mM)	Relative cell volume	Sugar content (m-mole/l. normal cell volume)	Non-solvent volume and standard deviation (% of normal volume)
Glucose	100	0·74	44	30 ± 3
		0·91	61	30 ± 3
		1·19	90	29 ± 4
		1·64	136	28 ± 4
Glucose	10	0·76	4·6	30 ± 2
		0·89	5·9	30 ± 2
		1·11	8·0	31 ± 2
		1·45	11·4	31 ± 2
Fructose	10	0·84	5·5	29 ± 2
		1·75	14·5	30 ± 2

least ten determinations made on blood provided by three different donors in each case.

Kinetic measurements. The sugar uptake was found to obey a simple first-order rate equation as predicted (see Discussion). At each relative cell volume listed in column one of Table 2, measurements of the first-order rate constant were made and are listed (together with their standard deviations) in column two (headed B/V). These represent the averaged values of determinations made at five or six different times during at least two experiments each done on blood obtained from different donors.

TABLE 2

	Relative total cell volume	B/V with standard deviations (min^{-1})	Relative solvent volume	B with standard deviations (ml./min)
a	0·72	0·085 ± 0·010	0·60	0·051 ± 0·006
	0·84	0·057 ± 0·003	0·77	0·044 ± 0·002
	1·00	0·0365 ± 0·0015	1·00	0·037 ± 0·001
	1·16	0·029 ± 0·003	1·23	0·036 ± 0·004
	1·37	0·024 ± 0·001	1·53	0·037 ± 0·001
b	0·76	0·049 ± 0·009	0·66	0·037 ± 0·006

DISCUSSION

Static measurements

It is immediately apparent from Table 1 that the amount of sugar taken up by a quantity of cells is far from independent of their volume, thus ruling out the possibility that all the sugar is bound by a fixed number of sites. The results appear to follow the predictions of the membrane theory, however, and we may test this theory quantitatively in the following way.

As the erythrocyte contains about 71 % water by weight (Hill, 1930) it may be assumed to consist of two phases, an aqueous or solvent phase throughout which the sugar freely diffuses and an non-aqueous phase from which it is excluded. As water enters or leaves the cell following readjustment of the external osmotic pressure, the resultant volume changes should be confined only to the solvent phase, the non-solvent volume remaining constant. If it is assumed that the sugar concentration in the solvent phase at equilibrium is the same as that in the external solution, the volume of this phase may be calculated directly from its sugar content. Such an assumption is probably valid, since it has been found that erythrocytes equilibrated at 37·5° C with media containing 100 mM glucose or fructose assume the same size as those in the media alone, indicating that no net osmotic pressure is exerted by sugars on either side of the membrane.

The non-solvent volume of a quantity of cells whose normal volume was 1 l. may be calculated from the figures in Table 1 by subtracting the solution volume (obtained by dividing sugar content of column four by the external sugar concentration, column two) from the total cell volume (column three). This expressed as a percentage of the initial volume (1 l.) is given in column five of Table 1 and can be seen to be a constant within the limits of error over a wide range of conditions.

From these results it may be concluded that if either of these sugars is adsorbed by cell colloids the amount thus held is negligible under ordinary conditions. Furthermore, the volume of the solvent phase of the erythrocytes in normal solutions is $0{\cdot}70 \pm 0{\cdot}02$ times the total isotonic cell volume, and varies directly as changes in water content of the cell. This indicates that almost all the water in the cell is 'un-bound' or free to act as solvent, a result in agreement with the findings of Hill (1930), MacLeod & Ponder (1936) and Dick & Lowenstein (1958).

Kinetic measurements

The time course of sugar entry into human erythrocytes is given by the equation (Widdas, 1954)

$$\frac{dS}{dt} = K\left(\frac{C}{C+\phi} - \frac{S/v}{(S/v)+\phi}\right), \tag{1}$$

where C is the outside sugar concentration, S the amount of sugar in the cell, ϕ the half-saturation concentration, K a constant and V the solvent volume of the cell. LeFevre (1954) has shown, however, that ϕ (called K_s by him) is very large relative to the fructose concentration employed here, so that since V is approximately constant eqn 1 reduces to

$$\frac{dS}{dt} = V\frac{dC'}{dt} = B(C-C'), \tag{2}$$

in which $B = K/\phi$ and C' is the internal concentration. Finally, since $C' = 0$ when $t = 0$, eqn. 2 may be integrated to the kinetically first-order equation

$$\ln\left(\frac{C}{C-C'}\right) = \ln\left(\frac{S_\infty}{S_\infty - S}\right) = \frac{B}{V}t, \tag{3}$$

where S_∞ is the equilibrium sugar content of the erythrocyte cell (at large t) and B/V is the first-order rate constant. According to the membrane theory B should be a constant independent of the volume of the cell. To obtain B from the figures in Table 2*a*, column 2, we must multiply by the respective relative solvent volumes. These are given by the expression

$$\frac{\text{Relative total volume} - 0{\cdot}30}{0{\cdot}70}$$

(since the inert volume is constant at 0·30 for all relative volumes) and are listed in column three. The values of B obtained by this procedure can be seen (column four) to show the predicted constancy only for relative volumes of 1·00 or greater. This suggests that either the theory is incorrect or the permeability of the membrane increases with salt concentration. If the latter is true, again there are two possibilities, the increased B may result from either a high osmotic pressure or an increased electrolyte concentration (salt effect). To test for a salt effect the osmotic pressure of normal media was increased by the addition of lactose, a non-penetrating disaccharide which does not contain fructose and hence does not seriously interfere with the analysis. At a relative total volume of 0·76 (Table 2*b*) B was found to be $0{\cdot}037 \pm 0{\cdot}006$ ml./min, showing that the membrane theory holds provided excessive electrolyte concentrations are avoided. It may be noted that Mawe (1956) compared glucose penetrations from normal and twice isotonic saline media; in the latter solution the half-times are shortened approximately by the ratio holding between the cell volumes. He concluded that the glucose permeability was unchanged by shrinkage.

The diffusion theory cannot be tested at normal and higher tonicities because of the complex shapes assumed by the erythrocytes. At its largest volume, however, the cell may be assumed to approach a spherical shape, allowing the application of standard diffusion equations to the calculation of sugar entry. This has been done for the last experiment listed in Table 2*a* for which the relative volume is 1·37. The equation used is

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp(-Dn^2\pi^2 t/r^2), \tag{4}$$

where r is the radius of the cell, t the time, D the diffusion coefficient and M_t/M_∞ the ratio of sugar which has entered the cell at time t to that at infinite time. The radius is calculated from the volume of the cell, which was found by multiplying the normal volume of 87 μ^3 (Ponder, 1955) by the relative volume 1·37. The diffusion coefficient was obtained from eqn 4 after substitution of the values $M_t/M_\infty = 0{\cdot}5$ and t = half-saturation time as estimated graphically from the results and came to $1{\cdot}0 \times 10^{-10}$ cm^2/min. With this value substituted in eqn 4, the remainder of the curve of sugar uptake against time as predicted by the diffusion theory was calculated and is plotted in Fig. 1. A first-order plot, a rate constant of 0·024 min^{-1} (Table 2) being used, is also presented in Fig. 1 along with the experimentally determined values. It is obvious that the latter follow much more closely the first-order curve than that of the diffusion theory, once again supporting the membrane theory.

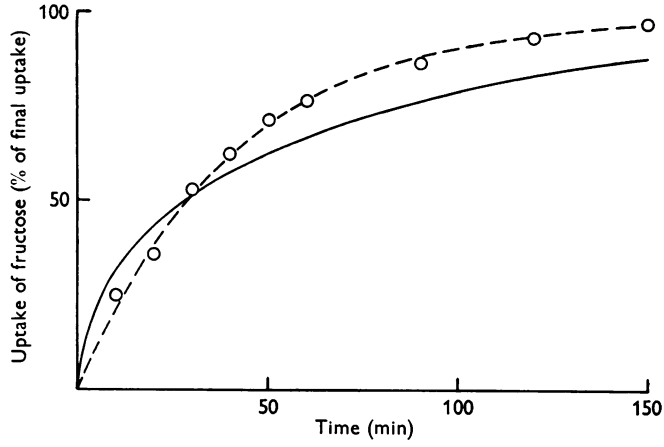

Fig.1. Theoretical rate curves and experimental points for uptake of fructose by spherical human erythrocytes at 30° C; fructose = 20 mM. —— uptake limited by internal diffusion; - - - uptake limited by membrane; ○ experimental values.

SUMMARY

1. The amount of sugar taken up by human erythrocytes was measured as a function of the cell volume and found to be proportional to the cell water content. This proportionality appeared to be independent of the type of sugar and its concentration. It is concluded that all the sugar within the cell is freely dissolved in the aqueous phase and virtually none is bound to cell colloids.

2. The solvent water within the cell was found to constitute $70 \pm 2\%$ of the normal cell volume. As this represents practically all the water present in the cell, the amount bound, and hence incapable of acting as a solvent must be negligible.

3. The kinetics of uptake of fructose by human erythrocytes was measured at 30° C also as a function of cell volume, and found to follow a first-order rate expression at all volumes as predicted by the membrane theory. These results were also tested against an equation predicting uptake as limited by internal (cytoplasmic) resistance to diffusion, and found to diverge widely from it.

4. The addition of large quantities of NaCl (up to twice isotonic) was found to increase the permeability of the membrane to fructose.

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