BIOCHEMICAL LETTERS

Lysosomal sugar transport: facilitated or free diffusion?

Bird *et al.* (1987) in a recent communication showed the results of experiments which they claim were a test of Hales's hypothesis that sugars enter rat liver lysosomes by a facilitated diffusion system (Hales, 1978; Docherty *et al.*, 1979; Docherty & Hales, 1979; Hales *et al.*, 1982; Maguire *et al.*, 1983). In particular, they focussed upon the problem of why D-ribose apparently enters these lysosomes at a higher net rate than D-glucose. They explain this observation on the basis of uptake mediated by simple diffusion with a rate determined by molecular size of the molecule such that smaller molecules like D-ribose enter faster than larger ones such as D-glucose. In contrast, our explanation (Docherty *et al.*, 1979) is based upon the properties of a carrier-mediated process. These are such that, at high concentrations, the net rates of uptake of a series of sugars may, paradoxically, be in the reverse order of their affinities for the system (Stein, 1967).

Bird *et al.* (1987) attempted to test the hypothesis advanced by Hales in experiments using the osmotic protection method. This method only very indirectly measures sugar uptake. It involves incubating intact lysosomes in sugar solutions of iso-osmotic (250 mmol/l) concentration. The consequent gradual increase in extralysosomal enzyme activity is attributed to rupture of the lysosomes, caused by the entry of sugar and accompanying water. The time course of the appearance of lysosomal enzyme activity is, therefore, governed as much by the distribution of the abilities of the lysosomes to withstand osmotic swelling as by the rate of sugar uptake. Although for a given preparation of lysosomes any differences observed between their behaviour in different sugars at the same concentration must be the result of differences in the rates of sugar uptake, it is not possible to estimate any rates of uptake nor to calculate any kinetic parameters.

It was observed by Bird *et al.* (1987) that the rate of release of lysosomal enzyme (and therefore the overall rate of sugar uptake) in a mixture of 125 mmol/l glucose and 125 mmol/l ribose was intermediate between that in the presence of either sugar alone at 250 mmol/l. They conclude that this observation is inconsistent with Hales's hypothesis. They argue that for a carrier-mediated process glucose would effectively inhibit ribose influx and that glucose influx might itself be slower at 125 mmol/l than at 250 mmol/l. Thus, they argue, the overall uptake would be slower than in 250 mmol/l glucose alone. However they neglect any effect of either sugar on the other's efflux and they argue that for a carrier-mediated process glucose would 'powerfully inhibit' ribose influx such that it would behave as an 'impermeant sugar'. This is not the case. The effect of a competitive inhibitor

Fig. 1. Effect of K_m on the simulated time course of sugar uptake into lysosomes

The changes with respect to time of the combined intralysosomal concentrations of glucose and ribose were calculated allowing for the interaction of each sugar with the other. The external sugar concentrations were 250 mmol/l ribose (—), 250 mmol/l glucose (····) or 125 mmol/l ribose plus 125 mmol/l glucose (— · —). A model of the process was constructed whereby glucose and ribose each acted as a competitive inhibitor of transport via a single bidirectional carrier. Each of the four individual sugar fluxes (glucose influx and efflux and ribose influx and efflux) was calculated as (Widdas, 1954; Stein, 1967):

$$j = \frac{V_{\max} [S]}{[S] + (1 + [I]/K_i) K_m}$$

where j is flux, $V_{\max}$ is the maximum flux, $[S]$ is the sugar concn., $[I]$ is the inhibitory sugar concn., K_m is the Michaelis constant and K_i is the inhibitor constant (equal to the K_m of the inhibitory sugar). The $V_{\max}$ was set to 35 mmol/min per l (calculated from Maguire *et al.*, 1983). The process was simulated using the STELLA software package (High Performance Systems, Inc., 13 Dartmouth College Highway, Lyme, NH 03768, U.S.A.) run on an Apple Macintosh microcomputer. Details of the model are available upon request to J. D. S. K.

Table 1. Predicted lysosomal sugar concentration 10 and 60 min after suspension in iso-osmotic sugars of various hypothetical K_m values

Details as legend to Fig. 1.

External concn. (mmol/l)		Glucose... Ribose...	Hypothetical K_m				
Glucose	Ribose		20 50	25 100	50 100	50 500	50 1000
<hr/>							
			Internal concn. at 10 min (mmol/l)				
250	0		83	89	105	105	105
125	125		89	92	108	93	87
0	250		105	115	115	87	59
			Internal concn. at 60 min (mmol/l)				
250	0		177	187	212	212	212
125	125		190	197	217	190	166
0	250		212	226	226	221	194

is to increase the K_m by $(1 + [I]/K_i)$, where $[I]$ is the inhibitor concentration and K_i its inhibition constant (equal to its K_m ; Widdas, 1954). Given a K_m for glucose of 50 mmol/l (Maguire *et al.*, 1983), in the presence of 125 mmol/l glucose, the affinity of ribose would therefore be only 3.5-fold lower than in its absence.

For a carrier-mediated process it is difficult to predict what the overall rate of sugar uptake in the equimolar mixture of glucose and ribose would be. Consideration has to be given to the competition of each sugar on the other's influx and efflux; the latter is particularly difficult to determine since the internal concentration of each sugar would be changing. There will be other differences in the internal environment such as pH. Where there are difficulties, such as here, with intuitive reasoning, objective predictions are better made using quantitative modelling. We have simulated the time course of sugar uptake from the mixture of glucose and ribose and from either sugar alone, at various combinations of hypothetical K_m values. The predictions were (Fig. 1, Table 1) that the behaviour of the mixture may or may not be intermediate between that of either sugar alone, depending on their K_m values. The intermediate behaviour found in the experiments of Bird *et al.* (1987) therefore cannot be used to argue for the absence of a lysosomal transport system for ribose and glucose.

The osmotic protection method was used initially to test Hales's hypothesis that there would be nutrient transport processes in the membrane of lysosomes and provided data consistent with the hypothesis (Docherty *et al.*, 1979). The later direct study of unidirectional sugar uptake showed that D-glucose and D-ribose indeed compete for uptake (Maguire *et al.*, 1983). Bird *et al.* (1987) conclude that the simplest explanation of their data is that glucose and ribose enter lysosomes by simple diffusion. This is very difficult to accept without an explanation of our findings in the direct study, which, in addition to showing competition, showed the process to be sugar-specific and stereospecific, saturable and inhibitable by cytochalasin B with very little effect of cytochalasin E (Maguire *et al.*, 1983).

Lysosomes maintain a pH gradient. They also have transport systems for a variety of other small molecules such as L-cystine (Gahl *et al.*, 1982), sialic acid (Renlund *et al.*, 1986) tyrosine and other neutral amino acids (Bernar *et al.*, 1986) and vitamin B12 (Rosenblatt *et al.*, 1985), all of which have been shown to be the basis of genetically determined disorders. Thus in the 8 years since the original proposal that lysosomes would be found to have nutrient transport processes (Hales, 1978) the hypothesis has been amply confirmed. Indeed a recent review of lysosomal storage diseases (Anon, 1986) concluded 'the lysosomal transport defects may well prove to be as important and varied as the diseases caused by deficiencies of lysosomal hydrolases'.

It would be a pity if further studies in this area were inhibited by the reintroduction of the concept of the lysosomal membrane acting as a molecular-mass sieve for sugars. If the very strong evidence for sugar and other transport processes is to be rigorously tested and investigated it is essential that adequate methods are employed.

Gerald A. MAGUIRE,* Jonathan D. S. KAY* and C. Nicholas HALES†

†Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QR, and *Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

Anon (1986) Editorial, *Lancet* ii, 898–899

Bernar, J., Tietz, F., Kohn, L. D., Bernardini, I., Harper, G. S., Grollman, E. F. & Gahl, W. A. (1986) *J. Biol. Chem.* **261**, 17107–17112

Bird, S. J., Forster, S. & Lloyd, J. B. (1987) *Biochem. J.* **245**, 929–931

Docherty, K. & Hales, C. N. (1979) *FEBS Lett.* **106**, 145–148

Docherty, K., Brenchley, G. V. & Hales, C. N. (1979) *Biochem. J.* **178**, 361–366

Gahl, W. A., Bashan, N., Tietz, F., Bernardini, I. & Schulman, J. D. (1982) *Science* **217**, 1263–1265

Hales, C. N. (1978) *FEBS Lett.* **94**, 10–16

- Hales, C. N., Docherty, K. & Maguire, G. A. (1982) *Biochem. Soc. Trans.* **10**, 14–15
- Maguire, G. A., Docherty, K. & Hales, C. N. (1983) *Biochem. J.* **212**, 211–218
- Renlund, M., Kovanen, P. T., Raivio, K. O., Aula, P., Gahmberg, C. G. & Ehnholm, C. (1986) *J. Clin. Invest.* **77**, 568–574
- Rosenblatt, D. S., Hosack, A., Matiaszuk, N. V., Cooper, B. A. & Laframboise, R. (1985) *Science* **228**, 1319–1321
- Stein, W. D. (1967) in *The Movement of Molecules Across Cell Membranes*, pp. 126–176, Academic Press, New York and London
- Widdas, W. F. (1954) *J. Physiol. (London)* **125**, 163–180

Received 13 November 1987

Lysosomal sugar transport: facilitated or free diffusion?: a reply

The main point at issue is not whether a mono-saccharide transporter exists in the rat liver lysosome membrane, but the mechanism(s) by which sugars cross this membrane. In our recent paper (Bird *et al.*, 1987) we quoted both the evidence for a glucose carrier (evidence repeated in the Letter by Maguire *et al.*, 1988) and also the evidence that some sugars and their derivatives cross by passive diffusion. In their most recent experimental paper on sugar translocation across the lysosome membrane, Maguire *et al.* (1983) concluded (we believe wisely) that glucose crosses partly by facilitated and partly by passive diffusion. In their Letter they now consider the ‘reintroduction of the concept of the lysosomal membrane acting as a molecular-mass sieve for sugars’ as inhibitory to further studies. We cannot agree, and believe it would be more truly inhibiting if investigators ignored the possibility that passive diffusion may play an important role: if it does, molecular mass

and hydrogen-bonding capacity will indeed be the relevant molecular features.

We look forward to an opportunity to examine the detail of the program used and the assumptions made in the calculations presented by Maguire *et al.* (1988) in their Letter. But at face value their data constitute a valid criticism of our experiment, and we are grateful for their comments. Their Table and Figure suggest that there are some circumstances in which the net rate of carrier-mediated solute entry from an equimolar mixture of sugars would be greater, and not always less as we thought, than the rate from a simple solution of the sugar with the lower K_m . Such circumstances would seem to occur where the K_m values of the two sugars are not too dissimilar. The numerical values computed by Maguire *et al.* (1988) suggest, however, that under these conditions the net rates of entry of the two sugars (separately) are unlikely to be sufficiently different to explain the experimental data on osmotic protection by glucose and ribose.

We therefore hold to our opinion that the more rapid net entry of ribose into lysosomes in osmotic-protection experiments reflects a greater passive-diffusion component and not entry on the glucose carrier. This matter will only be settled by a rigorous analysis of the limits of passive diffusion as a translocation mechanism in the lysosome membrane. The osmotic-protection method, particularly with the added sophistication now introduced by Maguire *et al.*, is particularly valuable here, as we hope to show in forthcoming publications.

Susan J. BIRD, Susan FORSTER and John B. LLOYD
Biochemistry Research Laboratory, Department of Biological Sciences, University of Keele, Keele, Staffs. ST5 5BG, U.K.

- Bird, S. J., Foster, S. & Lloyd, J. B. (1987) *Biochem. J.* **245**, 929–931
- Maguire, G. A., Docherty, K. & Hales, C. N. (1983) *Biochem. J.* **212**, 211–218
- Maguire, G. A., Kay, J. D. S. & Hales, C. N. (1988) *Biochem. J.* **252**, 621–623

Received 11 January 1988