Sugar transport in rat liver lysosomes

Direct demonstration by using labelled sugars

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1. Purified rat liver lysosomes ('tritosomes') were prepared from rats injected with Triton WR-1339. 2. The water space of tritosomes, measured by using [^3H]water and [^{14}C]sucrose, was $2.15 \pm 0.72 \mu\text{l}/\text{mg}$ of protein (mean $\pm$ S.E.M., $n = 12$). 3. Tritosomes, when compared with a crude preparation of normal lysosomes by an indirect method of study, showed sugar specificity but decreased stereospecificity of sugar uptake. 4. At 125 mM the relative rates of net uptake of D-[^{14}C]ribose, D-[^{14}C]glucose and 2-deoxy-D-[^3H]glucose were the same as that inferred from the indirect study. 5. The entry of D-[^3H]glucose into tritosomes showed concentration-dependence suggestive of saturation, with a K_m of $48 \pm 18 \text{ mM}$ (4). 6. D- and L-glucose, D-ribose, 2-deoxy-D-glucose and D-mannose competed with D-[^{14}C]glucose or D-[^{14}C]ribose for uptake. 7. Cytochalasin B inhibited D-[^3H]glucose uptake. 8. Uptake of 1 mM-L-[^{14}C]glucose was slower than for 1 mM-D-[^{14}C]glucose. 9. It is concluded that a facilitated-diffusion transport system is present in purified rat liver lysosomes.

The products of intralysosomal digestion must cross the lysosomal membrane to reach the cytoplasm of a cell. Many workers have concluded that this occurs by a process of simple diffusion (for review, see Weissmann, 1979), whereas Hales (1978) proposed that specific transport processes were involved. Docherty *et al.* (1979), using an indirect technique (the osmotic-protection method), demonstrated that sugars entered rat liver lysosomes by a process that was sugar specific, stereospecific, inhibitable by phlorrhizin and cytochalasin B, exhibited competition between sugars and had a characteristic Q_{10} (rate difference for a 10°C temperature rise) for transport.

The osmotic-protection technique suffers from the disadvantage that it does not measure sugar uptake directly and necessitates the use of iso-osmotic (250 mM) sugar solutions, which make kinetic studies impossible. Therefore a direct study using purified lysosomes was undertaken. Lysosomes may be purified by a variety of techniques involving alteration of the lysosomal density (Dean, 1977). In the present paper the use of lysosomes purified from the livers of rats injected with Triton WR-1339 ('tritosomes') for sugar-uptake studies is described. Their sugar-transport characteristics were compared with those of a crude preparation of normal lysosomes by

using the osmotic-protection technique. Radioactively labelled sugars were used to study directly sugar uptake into tritosomes. The results confirm the presence of a facilitated-diffusion sugar-transport system in rat liver lysosomes.

Experimental

Chemicals

D-[1- ^3H]Glucose, D-[U- ^{14}C]glucose, L-[1- ^{14}C]glucose, [6,6(n)- ^3H]sucrose, [U- ^{14}C]sucrose, 2-deoxy-D[2,6- ^3H]glucose and D-[1- ^{14}C]ribose were purchased from Amersham International, Amersham, Bucks., U.K. Tyloxapol (Triton WR-1339) was purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. Dow Corning silicone fluids DC550 and DC556 were obtained from BDH Chemicals, Dagenham, Essex, U.K. All other chemicals were of the purest grade available.

Preparation of lysosomes

Impure lysosomal suspensions were prepared as described by Docherty *et al.* (1979). Tritosomes were prepared as described by Trouet (1974) and a similar degree of purification from other subcellular structures was achieved.

Sugar-uptake measurement: indirect method

The osmotic-protection method as described by Docherty *et al.* (1979) was used. A crude lysosomal

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or tritosomal suspension in 250 mM-sucrose/10 mM-Tes (2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino]ethanesulphonic acid) adjusted to pH 7.4 with Tris was prepared. Each suspension contained about 160 units ($\mu\text{mol}/\text{min}$) of β -N-acetylglucosaminidase (EC 3.2.1.30)/ml. Samples (25 μl) of suspension were added to each of several sugar solutions at 25°C, such that the final concentrations were 250 mM-sugar, 10 mM-Tes adjusted to pH 7.4 with Tris, and the final volume was 500 μl . At the stated times free β -N-acetylglucosaminidase was determined as follows. Triplicate 25 μl samples of each sugar solution were added to 100 μl of assay cocktail, containing 0.5 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside, 270 mM-sucrose and 100 mM-citrate, pH 5.0, and, for total activity determinations, 0.1% Triton X-100. The assay was terminated after 3 min by addition of 1.6 ml of 0.5 M- Na_2CO_3 and the released 4-methylumbelliferone was determined by fluorimetry. At each time point the free activity was expressed as a percentage of the total activity.

Sugar-uptake measurement: direct method

All solutions were kept at 25°C in a water bath.

Samples (50 μl) of tritosomes containing 4 mg of protein/ml in 0.25 M-sucrose/10 mM-Tes/Tris, pH 7.4, were added, to start the incubation, to quadruplicate 25 μl sugar solutions, containing 0.5 μCi of ^3H and 0.1 μCi of ^{14}C such that the final concentrations were 250 mM- ^{14}C - or ^3H -sucrose, 10 mM-Tes adjusted to pH 7.4 with Tris, and ^{14}C - or ^3H -sugar of interest (and inhibitor when present) at the stated concentration. No significant difference was found in the behaviour of a ^3H -labelled sugar when compared with the same sugar labelled with ^{14}C . The tubes were placed in a water bath at 25°C and removed 5 s before the desired incubation time (for incubations of 5 s, the tubes were placed immediately in the centrifuge). Then 300 μl of a mixture of silicone fluids of density 1.055 g/ml at 25°C was added and the incubation was stopped at the desired time by centrifugation for 45 s in an Eppendorf 5412 centrifuge followed by freezing in solid CO_2 . The time taken to sediment the tritosomes was equivalent to an additional incubation of about 10 s (results not shown). For short incubations (<1 min) each of four tubes at every time point was processed individually. For longer incubations they were processed simultaneously, which led to a difference in the incubation times between the quadruplicate observations of less than 5 s. When all the tubes had been processed, the tips containing the pellets were sawn off. Each pellet was dispersed into 500 μl of water by vortex mixing. The resulting suspension was centrifuged on an Eppendorf 5412 instrument to separate the oil and water phases. Then 400 μl of the supernatant was removed

to a scintillation vial and 5 ml of FisoFluor 2 (Fisons) scintillation cocktail added. For total radioactivity 25 μl of sugar solution was diluted to 500 μl with water and 400 μl of this was taken. Scintillation counting was performed in a Packard Tri-Carb liquid-scintillation counter adjusted to give 10% cross-over of ^{14}C in the ^3H channel.

Sucrose was used as a marker of the extra-tritosomal water space associated with the tritosome pellet. Thus, for each observation, the fraction of the labelled sucrose in the pellet was subtracted from that of the labelled sugar, and the remainder was assumed to represent sugar uptake. The water space was determined by using ^3H -water and ^{14}C -sucrose and the separation procedure described above.

Protein

This was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Statistics

The results are expressed as means $\pm$ S.E.M., with the numbers of experiments (or observations in the case of a single experiment) in parentheses. Significance testing was performed with Student's paired *t* test.

Results

Analysis of marker enzymes in the tritosome preparation indicated a 57 ± 3 -fold (6) purification (Table 1). The amount of plasma membrane in the preparation may be calculated from the formula of Leighton *et al.* (1968). The plasma membrane contains 2.6% of the total cell protein (Wattiaux *et al.*, 1978). The relative specific activity of the plasma-membrane marker 5'-nucleotidase is 1.8. Therefore 4.7% of the protein in the tritosome preparation is of plasma-membrane origin. The water space of the tritosomes corrected for the sucrose space [which was $47 \pm 1\%$ (12) of the total water space] was $2.15 \pm 0.21 \mu\text{l}/\text{mg}$ of protein (12). The isolated tritosomes were broken to the extent of 40% as judged by the free activity of β -N-acetylglucosaminidase.

Indirect study by the osmotic-protection method

Initial experiments were undertaken to determine whether tritosomes exhibited similar sugar uptake to that of normal liver lysosomes. It was found that tritosomes exhibited similar sugar specificity, but lower stereospecificity (Table 2). It is unlikely that the rupture of 40% of the tritosomes accounted for the lower degree of stereospecificity, since normal lysosomes deliberately damaged to a similar extent by suspension in 50 mM-sucrose retained stereospecific sugar uptake (results not shown).

Table 1. *Distribution of marker enzymes in the fractions obtained during tritosome preparation*

Tritosomes were prepared as described by Trouet (1974) and the fractions obtained are named similarly. Protein was assayed by the method of Lowry *et al.* (1951). β -N-Acetylglucosaminidase was assayed as described in the Experimental section. Cytochrome oxidase (EC 1.9.3.1) was assayed by the method of Cooperstein & Lazarow (1951). Catalase (EC 1.11.1.6) was assayed by the method of Beers & Sizer (1952). 5'-Nucleotidase (EC 3.1.3.5) was assayed by the method of Avruch & Wallach (1971) as described by Luzio *et al.* (1976). Glucose 6-phosphatase (EC 3.1.3.9) was assayed by the method of Kitcher *et al.* (1978) and galactosyltransferase (EC 2.4.1.38) was assayed by the method of Bretz *et al.* (1980). The numbers of experiments are shown in parentheses.

Marker	Percentage in fraction					Recovery
	E + N	N	PS	R	T	
Protein (6)	100	43.0 $\pm$ 2.7	53.0 $\pm$ 6.1	7.7 $\pm$ 1.1	0.17 $\pm$ 0.04	104.7 $\pm$ 6.4
β -N-Acetylglucosaminidase (6)	100	31.2 $\pm$ 1.6	44.0 $\pm$ 4.1	12.9 $\pm$ 0.9	9.7 $\pm$ 1.4	97.8 $\pm$ 5.0
Cytochrome oxidase (5)	100	46.0 $\pm$ 7.5	13.5 $\pm$ 2.0	26.8 $\pm$ 4.5	0.08 $\pm$ 0.02	86.3 $\pm$ 9.0
Catalase (5)	100	38.4 $\pm$ 1.7	39.6 $\pm$ 4.7	17.6 $\pm$ 5.3	0.04 $\pm$ 0.01	95.4 $\pm$ 6.4
5'-Nucleotidase (5)	100	62.2 $\pm$ 7.2	32.8 $\pm$ 3.1	1.3 $\pm$ 0.3	0.31 $\pm$ 0.03	96.6 $\pm$ 6.3
Glucose 6-phosphatase (4)	100	46.5 $\pm$ 3.9	23.2 $\pm$ 4.3	8.1 $\pm$ 2.9	0.09 $\pm$ 0.01	78.0 $\pm$ 3.3
Galactosyltransferase (1)	100	52	41	nil	0.02	93

Table 2. *Loss of structural latency of a lysosomal enzyme (β -N-acetylglucosaminidase) after incubation of purified lysosomes (tritosomes) or a crude lysosomal fraction in 250 mM-sugars*

A crude lysosomal fraction or tritosomes were resuspended in 250 mM-sugar/10 mM-Tes, adjusted to pH 7.4 with Tris and incubated at 25°C. The percentage of free β -N-acetylglucosaminidase activity was determined at the indicated times. All values are the means $\pm$ S.E.M. of four experiments.

		Activity (%)			
Tritosomes	Incubation time (min) ...	0	5	10	15
D-Fucose		42.6 ± 3.3	52.8 ± 3.3	63.8 ± 4.8	71.3 ± 4.3
L-Fucose		44.0 ± 2.9	56.0 ± 2.6	69.0 ± 3.3	76.3 ± 3.5
D-Ribose		41.0 ± 3.3	65.0 ± 4.9	81.3 ± 4.0	87.8 ± 2.7
L-Ribose		43.8 ± 3.1	63.0 ± 5.3	75.8 ± 4.5	84.8 ± 3.9
		Activity (%)			
Crude lysosomal fraction	Incubation time (min) ...	0	5	10	15
D-Fucose		3.6 ± 0.4	7.1 ± 0.8	13.2 ± 0.9	20.5 ± 1.6
L-Fucose		3.5 ± 0.4	11.6 ± 1.4	24.2 ± 2.6	36.9 ± 3.4
D-Ribose		3.9 ± 0.5	16.6 ± 2.5	37.8 ± 4.1	50.8 ± 4.0
L-Ribose		3.7 ± 0.5	11.4 ± 1.6	23.8 ± 3.0	35.7 ± 3.6

Direct study with labelled sugars

The rate of uptake of 20 mM-D-[3 H]glucose varied between preparations. However, in each preparation tested the uptake was linear with time for greater than 5 s. Fig. 1 illustrates a single representative experiment. The curve in Fig. 1 intersects the abscissa at -10 s. This interval is due to the delay involved in separating the tritosomes by the procedure used as described above. It was not possible to measure the initial rate of uptake at higher concentrations, because at the first practicable time point (5 s) the rate of uptake was not linear. Therefore, the kinetic studies were performed by using a 5 s incubation and concentrations of glucose up to 20 mM. The pooled results from four

experiments were used to construct a Hanes plot (Wilkinson, 1961), which is shown in Fig. 2. The results from the individual experiments gave a K_m of 48 ± 18 mM.

The rapid uptake of 20 mM-D-[3 H]glucose into tritosomes appeared to differ markedly from that inferred from the osmotic-protection method. However, the latter method necessitated the use of 250 mM-D-glucose. Both approaches measure the net rate of sugar entry, which is the difference between influx and efflux. Fig. 3 illustrates a comparison between the rates of equilibration of 125 mM- and 20 mM-D-[3 H]glucose in a single experiment at each concentration. In a larger series the ratios of the D-[3 H]glucose to [3 H]water spaces after 1 min were

Fig. 1. Time course of 20 mM-D-[^3H]glucose uptake into tritosomes

Tritosomes were incubated at 25°C in 20 mM-D-[^3H]glucose, 250 mM-[^{14}C]sucrose and 10 mM-Tes adjusted to pH 7.4 with Tris. At the indicated times the incubation was stopped by centrifugation through oil and the D-[^3H]glucose uptake was determined as described in the Experimental section. The values shown are the means $\pm$ S.E.M. of the quadruplicate observations from a single representative experiment.

0.15 ± 0.02 (5) and 0.34 ± 0.05 (4) at 125 mM and 20 mM respectively. The slower rate of equilibration at the higher concentration was consistent with the time course of breakage in the osmotic-protection studies and with the theoretical considerations discussed by Stein (1967), concerning the movement of sugars when they are studied at concentrations in excess of their affinities for a transport system.

If the theoretical explanation of Stein (1967) is applicable to the lysosomal transport system, the relative rates of uptake of different sugars at concentrations high compared with their K_m will be the reverse of their affinities for the system. At high external concentrations in relation to their K_m values for a transport system, the influx of sugars will be at their $V_{\max}$ values. However the efflux of high-affinity sugars will be greater at early times, as the internal concentrations will be low. Hence the net rate of sugar entry will be lower for sugars of high affinity,

Fig. 2. Hanes plot of the effect of concentration on D-[^3H]glucose entry into tritosomes

Tritosomes were incubated for 5 s at 25°C in 250 mM-[^{14}C]sucrose, 10 mM-Tes, adjusted to pH 7.4 with Tris, and D-[^3H]glucose at the indicated concentrations. The sugar uptake was determined as described in the Experimental section. s/v = concentration (mM) divided by uptake (nmol/5 s per μl of water space). The values are the means $\pm$ S.E.M. for four experiments.

whereas at low concentrations the relative rates of uptake will be in the order of their affinities. The uptakes of D-[^{14}C]glucose and D-[^{14}C]ribose were compared at both 1 and 125 mM. At the lower concentration D-[^{14}C]glucose uptake was faster, whereas at the higher concentration D-[^{14}C]ribose uptake was faster (Fig. 4). 2-Deoxy-D-[^3H]glucose uptake at the higher concentration was slower than that of D-[^3H]glucose. In a series of experiments, comparing D-[^3H]- or D-[^{14}C]-glucose with either D-[^{14}C]ribose or 2-deoxy-D-[^3H]glucose uptake at 125 mM over 1–10 min, the differences were $+25.0 \pm 2.0$ nmol/ μl of tritosomal water space (mean

Fig. 3. Comparison of the uptake into tritosomes of D-[^3H]glucose at 125 mM and 20 mM

Tritosomes were incubated for the indicated times at 25°C in 20 mM- or 125 mM-D-[^3H]glucose, 250 mM-[^{14}C]sucrose and 10 mM-Tes, adjusted to pH 7.4 with Tris. The sugar uptake was determined as described in the Experimental section. The results are expressed as the ratios of the D-[^3H]glucose to [^3H]water spaces and are the means (the S.E.M. values are smaller than the symbols) of the quadruplicate observations from a single representative experiment at each concentration. The first time point at each concentration was 5 s.

and range, $n = 2$) and $-4.6 \pm 0.8 \text{ nmol}/\mu\text{l}$ of tritosomal water space (mean $\pm$ S.E.M., $n = 3$) respectively. The relative rates of uptake of these sugars at 125 mM are the same as those deduced from the osmotic-protection study performed at 250 mM (Docherty *et al.*, 1979).

If the sugars share a common transport system, they should compete for uptake in the same order as their relative affinities deduced above. Table 3 gives the results of a single representative experiment in which various sugars were tested for their ability to inhibit D-[^{14}C]glucose or D-[^{14}C]ribose uptake. The order of competition was similar in both cases and in the order of their affinities for the erythrocyte transport system.

Cytochalasin B is a potent competitive inhibitor of glucose uptake (Estensen & Plagemann, 1972). Fig. 5 illustrates the effects of cytochalasin B on the uptake of 1 mM-D-[^3H]glucose. At 2 μM -cytochalasin B, there is a $15.2 \pm 2.6\%$ ($6; P < 0.01$) inhibition of D-[^3H]glucose uptake. The inhibition was competitive, since the inhibitory effects of cytochalasin B could be overcome by using higher D-[^3H]glucose concentrations. At 20 μM , cytochalasin B had a small and statistically insignificant effect on 125 mM-D-[^3H]glucose uptake.

In view of the relatively high concentrations of cytochalasin B used here, the possibility of non-specific effects was investigated. Cytochalasin B did

Fig. 4. Comparison of the uptake into tritosomes of D-[^{14}C]glucose and D-[^{14}C]ribose at (a) 1 mM and (b) 125 mM. Tritosomes were incubated for the indicated times in 250 mM-[^3H]sucrose, 10 mM-Tes adjusted to pH 7.4 with Tris and D-[^{14}C]glucose or D-[^{14}C]ribose at (a) 1 mM and (b) 125 mM. The sugar uptake was determined as described in the Experimental section. The values are the means $\pm$ S.E.M. (where this is greater than the symbol) for (a) three experiments and (b) quadruplicate observations from a single experiment.

Fig. 5. Effect of cytochalasin B on D-[³H]glucose uptake into tritosomes

Tritosomes were incubated at 25°C for 30 s in 1 mM-D-[³H]glucose, 250 mM-[¹⁴C]sucrose, 10 mM-Tes adjusted to pH 7.4 with Tris and the indicated concentration of cytochalasin B. The sugar uptake was determined as described in the Experimental section. The results are expressed as a percentage of the D-[³H]glucose uptake occurring in the absence of cytochalasin B and are the means $\pm$ S.E.M. for three experiments.

not affect the uptake of D-[³H]glucose if it was added after D-[³H]glucose was nearly at its equilibrium concentration. Furthermore, cytochalasin E, which has similar cytoskeletal effects as cytochalasin B, but lacks the effects on glucose transport, had only 20% of the inhibitory action on D-[³H]glucose uptake as cytochalasin B (results not shown).

The results from the osmotic-protection studies suggested that the lysosomal sugar-transport system did not discriminate well between stereoisomers. However, the uptake into tritosomes of D- and L-[¹⁴C]glucose exhibited several differences: 1 mM-L-[¹⁴C]glucose entered tritosomes more slowly than the D isomer {after 30 s the intralysosomal concentration of L-[¹⁴C]glucose was only $73 \pm 3\%$ (4; $P < 0.05$) that of D-[¹⁴C]glucose}, and was also less sensitive to cytochalasin-B inhibition (2 μM had no effect). However, at 125 mM, they entered at a similar rate (results not shown). At 50 mM, L-glucose

Table 3. Effect of sugars on the uptake into tritosomes of D-[¹⁴C]glucose or D-[¹⁴C]ribose

Tritosomes were incubated for 30 s at 25°C in 10 mM-D-[¹⁴C]glucose or D-[¹⁴C]ribose, 250 mM-[³H]sucrose, 10 mM-Tes adjusted to pH 7.4 with Tris and the sugars at the indicated concentrations. The apparent spaces were calculated as described in the Experimental section. The values are the means $\pm$ S.E.M. of the quadruplicate observations from a single representative experiment.

Additions to 10 mM-D-[¹⁴ C]glucose	Apparent D-[¹⁴ C]glucose space (μl/mg)	Inhibition (%)
Nil	0.86 ± 0.01	—
50 mM-D-ribose	0.61 ± 0.03	29
50 mM-L-glucose	0.58 ± 0.01	33
50 mM-D-mannose	0.51 ± 0.01	41
50 mM-D-glucose	0.42 ± 0.01	51
50 mM-2-deoxy-D-glucose	0.37 ± 0.02	57

Additions to 10 mM-D-[¹⁴ C]ribose	Apparent D-[¹⁴ C]ribose space (μl/mg)	Inhibition (%)
Nil	0.38 ± 0.01	—
50 mM-L-glucose	0.34 ± 0.01	11
50 mM-D-ribose	0.33 ± 0.02	13
50 mM-D-mannose	0.30 ± 0.01	21
50 mM-2-deoxy-D-glucose	0.25 ± 0.01	34
50 mM-D-glucose	0.24 ± 0.01	37

competed for uptake with 10 mM-D-[¹⁴C]glucose or 10 mM-D-[¹⁴C]ribose less effectively than did 50 mM-D-glucose (Table 3).

Discussion

Stein (1967) has listed the properties which characterize a transport system that operates by means of facilitated diffusion. Our previous results, obtained by an indirect method, provided suggestive evidence that such a system exists in the membrane of rat liver lysosomes (Docherty *et al.*, 1979). The present results with purified lysosomes complement and extend the earlier work and lead to the same conclusion. They show that the rate of D-[³H]-glucose entry into lysosomes is potentially saturable, although it was not practicable to measure the initial rates of uptake at high concentrations. Another technical limitation of these studies was that, to ensure a linear rate of uptake, the sugar space was only allowed to reach a small percentage of the (small) internal water space. This meant that the determinations were inherently subject to error. Nevertheless, the K_m observed was close to that quoted for the liver cell (Baur & Heldt, 1977; Elliott & Craik, 1982), and somewhat higher than that for the fat-cell (Gliemann, 1982) or the erythrocyte (LeFevre, 1961).

Tritosomes are secondary lysosomes (Henning & Plattner, 1975) and would be expected to contain membrane derived from both the plasma membrane and the primary lysosome. Schneider *et al.* (1981) demonstrated in rat embryo fibroblasts not only that plasma-membrane elements were found in lysosomes but that they could return to the cell surface. In the present studies it was calculated that 4.6% of the tritosomal protein was of plasma-membrane origin. This is similar to the value of 3% quoted by Trouet (1974). The relative amount of 5'-nucleotidase compared with β -N-acetylglucosaminidase found in tritosomes is about half that found by Pletsch & Coffey (1972). This difference may reflect the lower purification (34-fold) of their preparation. Cytochemical studies have shown that 5'-nucleotidase is genuinely located within the lysosome (Wattiaux *et al.*, 1978; Widnell & Little, 1977). Therefore it is likely that the plasma membrane associated with the tritosomes is an integral part of their structure and does not represent plasma-membrane contamination.

It is noteworthy that the relative rates of osmotic rupture of tritosomes in various sugars resembled that of impure lysosomes in which the populations of primary and secondary lysosomes had not been separated. It is not certain whether the lower degree of stereospecificity that tritosomes exhibited in comparison with the mixed population was a feature of the behaviour of secondary lysosomes or was an artefact of their preparation by this means. It is likely that the lipid-soluble detergent is present in the lysosomal membrane and may therefore affect membrane function.

The isolated tritosomes were broken to the extent of 40%. This is greater than that reported by Ruth & Weglecki (1978), who found that tritosomes were less than 10% broken, or Schneider (1981), who found that tritosomes were less than 20% broken. The reason for the difference is not known. However, it is unlikely that the glucose-transport system is possessed only by the more stable tritosomes that survived the preparation procedure. Several techniques for purifying normal lysosomes have now been published (Wattiaux *et al.*, 1978; Dobrota & Hinton, 1980). We have attempted to investigate the sugar-transport properties of normal liver lysosomes purified on a Percoll gradient. However, their sucrose space was >90% of their water space, which made accurate assessment of sugar uptake impossible.

By using the osmotic-protection method it has not proved possible to show consistently a difference between the behaviour of D- and L-glucose (K. Docherty, G. A. Maguire & C. N. Hales, unpublished work). However, the present direct studies have shown that at low concentrations L-[¹⁴C]-glucose enters tritosomes more slowly than does D-[¹⁴C]-glucose, although the difference observed is

not as great as might have been expected. The observed uptake of L-[¹⁴C]-glucose could represent free diffusion into a space which is not accessible to sucrose, or a genuine rate of transport. Consistent with the former explanation is the observation that the uptake at a low concentration of L-[¹⁴C]-glucose is not inhibited by low concentrations of cytochalasin B, whereas that of D-[³H]- or D-[¹⁴C]-glucose is. However, 50 mM-L-glucose inhibited D-[¹⁴C]-glucose uptake. It is often assumed that L-glucose uptake is simply by free diffusion (see, e.g., Ludvigsen & Jarrett, 1979). There is some evidence, however, that L-glucose can be transported by the D-glucose transporter; in both fat-cells and erythrocytes, L-glucose entry, which was very slow, was nevertheless inhibitable by cytochalasin B (Czech, 1976; Lin *et al.*, 1978). A combination of free diffusion and a low affinity for the transport system could account for the behaviour of L-[¹⁴C]-glucose reported here.

D-[³H]-Glucose entry into tritosomes was about tenfold less sensitive to cytochalasin B inhibition than that into liver cells (Bauer & Heldt, 1977) or fat-cells (Czech, 1976). One explanation for the difference in sensitivities could be that, in the present experiments, cytochalasin B is present at the cytoplasmic side of the membrane. Alternatively, if a proportion of the uptake equivalent to the L-[¹⁴C]-glucose uptake was by free diffusion, the true inhibition of transport would be much greater.

The permeability of human leucocyte lysosomes to cystine has now been investigated (Gahl *et al.*, 1982). These authors demonstrate the presence of a lysosomal ATP-dependent cystine-transport system in normal leucocytes. They also demonstrate its absence from the leucocytes of patients suffering from the rare inborn error of metabolism cystinosis. The present paper provides further evidence for the existence of a facilitated-diffusion sugar-transport system in rat liver lysosomes. These two independent demonstrations of transport systems in lysosomes indicate, as predicted, that transport mechanisms are a general property of lysosomal membranes.

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