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TRAPPING OF MANNITOL IN RAT-LIVER MITOCHONDRIA AND LYSOSOMES

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When injected into rats, a certain amount of mannitol is taken up by the liver and is associated with sedimentable structures. Isopycnic centrifugation in a sucrose gradient shows that a large part of mannitol is present in mitochondria, what remains is located in the lysosomes. The hypotonic release of mannitol present in organelles shows that the polyol is shared between mitochondria and lysosomes. The trapping of mannitol in lysosomes could result from the heterophagic or autophagic function of the lysosomes; the mechanism of its accumulation in mitochondria is still unexplained.

To study the cytoplasm sequestration process during autophagy, Gordon and Slegen (1) introduced sucrose, as an inert marker of the cytosol fluid, into isolated hepatocytes after the electropermeabilization of the cell. Recently and unexpectedly, it was discovered that an important part of the sucrose was trapped in the mitochondria (2). Many years ago, we found that mannitol, a non-metabolizable sugar derivative considered as an osmotic protector of lysosomes and mitochondria, when injected into rats, entered in significant amounts into the liver cells and affected the lysosomes in the same way as sucrose (3) but less drastically. These results were interpreted by supposing that mannitol accumulated in these organelles after endocytosis but to a lesser extent than sucrose. The observations of Gordon, Slegen (1) and Tolleshaug et al. (2) prompted us to reinvestigate the subcellular localization of mannitol picked up by the liver. As described in this paper, we found that effectively a certain proportion of the polyol was associated with lysosomes but that a high amount was also localized in mitochondria.

METHODS

Experiments were performed on male Wistar rats weighing 250-300 g.injected intraperitoneally with 10 ml of a 20% mannitol solution containing (^{14}C) mannitol (Amersham). After perfusion with cold 0.15 M NaCl,the livers were homogenized in 0.25 M

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sucrose; then a nuclear fraction N and a cytoplasmic extract E were prepared by differential centrifugation according to de Duve et al (4). The cytoplasmic extract was fractionnated using the centrifugation schema described by these authors: a nuclear fraction N, a heavy mitochondrial fraction M, a light mitochondrial fraction L, a microsomal fraction P and a soluble fraction S were isolated.

In density gradients and hypotonic activation experiments a total mitochondrial fraction corresponding to the sum of the heavy and the light mitochondrial fractions was used. Density gradient centrifugation was performed according to Beaufay et al.(5) Cytochrome oxidase, acid phosphatase and glucose-6-phosphatase were measured according to de Duve et al.(4), catalase according to Baudhuin et al. (6) and proteins by the method of Lowry et al. (7). Radioactivity was determined with a liquid scintillation counter.

RESULTS

Differential centrifugation.

Table 1 illustrates the distribution of radioactivity and some reference enzymes after fractionation of a rat liver homogenate by differential centrifugation, 2 h. after a mannitol injection. 60% of the radioactivity is sedimentable, the major part being associated with the mitochondrial fraction. This is also the case for cytochrome oxidase (mitochondria) and acid phosphatase (lysosomes); glucose-6-phosphatase (endoplasmic reticulum) is mainly recovered in the microsomal fraction P.

Isopycnic centrifugation

In order to locate precisely the presence of labelled mannitol in the mitochondrial fraction, isopycnic centrifugation

TABLE 1
Intracellular distribution of enzymes and radioactivity.

	E + N	N	M	L	P	S
Cytochrome Oxidase	20.6	15.4	53.5	7.0	5.3	
Acid Phosphatase	5.4	16.0	48.9	12.8	12.4	13.2
Glucose-6-phosphatase	17.9	19.8	10.0	10.6	57.4	1.3
Radioactivity	76.550	15.7	34.3	4.4	6.7	41.9
Proteins	220	20.0	22.3	5.3	19.7	27.9

For E + N, absolute values are given in mg/g for proteins, in cpm/g for radioactivity and in units/g fresh weight for enzymes according to de Duve et al. (4). For N, M, L, P and S, percentage values are presented, 100 % corresponding to the activity recovered in E + N.

was performed in a sucrose gradient. To clearly separate lysosomes, mitochondria and peroxisomes, the rat was injected with Triton WR 1339, 4 days before being sacrificed (8). In this experiment, protein distribution was used as a reference for mitochondria (5). A slight amount of radioactivity is present in the lowest density zones of the gradient where the lysosomes are located as ascertained by the distribution of acid phosphatase. Most of the radioactivity exhibits a distribution similar to that of the proteins (mitochondria). Such a distribution is clearly distinct from that of catalase (peroxisomes) which is recovered at the bottom of the gradient. (Fig. 1)

Release of radioactivity and reference enzymes by hypotonic treatment.

Lysosomes are markedly more susceptible to hypotonicity than mitochondria. An additional procedure, that permits the distinction between a mitochondrial and a lysosomal localisation, is to subject the granule preparation to hypotonic treatment and to follow the release of the substance and of the reference enzymes. A mitochondrial fraction, isolated 2 h. after a (^{14}C)

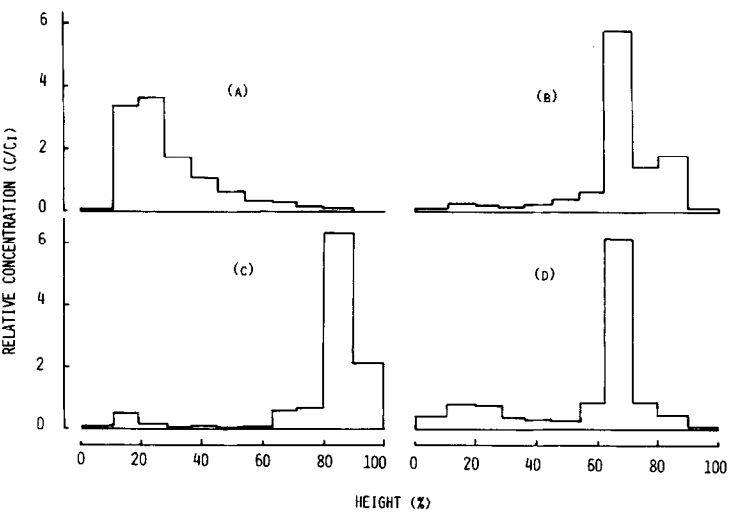

Fig.1 Distribution of acid phosphatase (A), proteins (B), catalase (C) and radioactivity (D) after centrifugation (for 2.5 h. at 39,000 rev/min in SW 39 head of Spinco rotor model L-HV preparative ultracentrifuge) of a mitochondrial fraction of a rat liver through a sucrose gradient extending from 1.09 to 1.26 g/ml density. The rat was injected intravenously with Triton WR 1339 (170 mg in 1 ml saline) 4 days before being sacrificed. Mannitol was injected intraperitoneally 2 h before killing. Abscissa: percentage of the height of the liquid column in tube. Ordinate: relative concentration i.e. the ratio of the observed activity (c) to what would have been found if the compound had been homogeneously distributed throughout the whole gradient (ci).

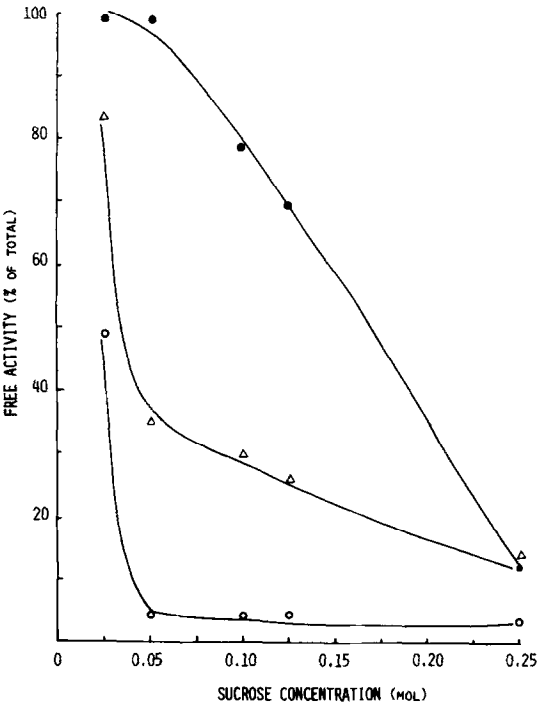

Fig.2 Osmotic release of acid phosphatase (●) glutamate dehydrogenase (○) and radioactivity (△). Mitochondrial fraction ML was exposed to the indicated sucrose concentrations. Then the enzyme free activity and the amount of released radioactivity were measured. They are expressed as a percentage of activity found in presence of 0,1% Triton X 100 (total activity). The rat was killed 2 h after the mannitol injection.

mannitol injection, was kept in media of different sucrose concentrations. The free activities of acid phosphatase (lysosomes) and glutamate dehydrogenase (mitochondria) were then measured together with the radioactivity that becomes unsedimentable. Results are presented in Fig.2. The way in which the latency changes strongly differ for acid phosphatase and glutamate dehydrogenase. The release of radioactivity agrees with a double localization of mannitol in lysosomes and mitochondria.

DISCUSSION

Our results show that after an intraperitoneal injection, mannitol is taken up by the liver and recovered, to a large extent, in the sedimentable structures of the homogenate. Centrifugation and activation experiments clearly indicate that the sedimentable polyol is shared between lysosomes and mitochondria,the largest part being associated with the latter.

How does mannitol actually reach these two cellular compartments ? In order to localise mannitol in mitochondria, the

plasma membrane must exhibit some permeability to this compound to allow it to penetrate into the cytosol. When present in the cytosol, mannitol could easily diffuse in the intermembrane space of mitochondria owing to the high permeability of the outer mitochondrial membrane. However if the polyol was localized in this space, it would rapidly diffuse in the external medium during the centrifugation procedures that last for many hours. Therefore the mannitol we find in mitochondria is probably located in the organelle matrix where it remains trapped after having crossed the inner membranes. To this day, we are not able to explain the procedure of this mechanism. The association of mannitol with lysosomes could be explained in a similar way to that of sucrose (9) by supposing that part of the polyol is taken up by fluid endocytosis and ends up in lysosomes like many endocytosed substances. However if mannitol can cross the plasma membrane, the presence of this compound within lysosomes could also result, at least partially, from its sequestration in these organelles by the autophagic process.

Our observations are parallel to the results that Tolleshaug et al (2) have obtained with sucrose, but in the case of mannitol, artificial permeabilization of the plasma membrane is not required and the entry of this substance into subcellular structures can be followed "in vivo". Therefore, the use of mannitol could be more appropriate to study the mechanism by which a cytosol soluble component is trapped in mitochondria and lysosomes.

REFERENCES

1. Gordon, P.B. and Seglen, P.O. (1982) Exp. Cell Res., 142, 1-14.
2. Tolleshaug,H., Gordon,P.B., Solheim, A.E. and Seglen,P.O. (1984) Biochem. Biophys. Res. Comm. 119, 955-961.
3. Wattiaux, R., Wattiaux-De Coninck,S., Tulkens,P. and Koninckx, Ph. (1965) 2d FEBS Meeting, Abst. of papers, 126.
4. de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux,R. and Appelmans, F. (1955). Biochem.J. 60, 604-616.
5. Beaufay,H., Jacques, P., Baudhuin,P., Sellinger, O.Z., Berthet J., and de Duve, C. (1964) Biochem.J., 92, 184-205.
6. Baudhuin,P., Beaufay,H., Rahman-Li, Y., Sellinger, O.Z., Wattiaux,R., Jacques,P., and de Duve,C. (1964) Biochem.J., 92, 172-184.
7. Lowry, O.H., Rosebrough,N.J., Farr,A.L., and Randall, R.J. (1951) 193, 265-275.
8. Wattiaux,R., Wibo,M. and Baudhuin,P. (1963) Ciba Found.Symp. Lysosomes, (J. and A. Churchill, Ltd, London) pp. 176-200.
9. Wattiaux, R., Wattiaux-De Coninck,S., Rutgeerts, M.J. and Tulkens, P. (1964) Nature, 203, 757-758.