A NOTE CONCERNING THE PRESENCE OF AN ACID NUCLEOSIDE TRIPHOSPHATASE IN RAT LIVER MITOCHONDRIA.

M. Huybrechts and R.Wattiaux

Laboratory of Physiological Chemistry, Facultés Universitaires Notre-Dame de la Paix, 5000 Namur, Belgium.

Received March 23, 1971

In a recent paper, Kalf and Gréce (I970) report that an acid nucleoside triphosphatase is present in rat liver mitochondria. However, the mitocondrial preparation they employ must contain large number of lysosomes; it can be estimated that their preparation corresponds approximately to the heavy mitochondrial fraction M of de Duve et al. (I955), in which 25-30% of rat liver lysosomes are recovered. Thus, one cannot rule out the possibility that the acid ATPase which Kalf and Gréce situate in the mitochondria may in fact be associated with the lysosomes. It should be pointed out that Brightwell and Tappel (I968) have shown that an acid pyrophosphatase is present in rat liver lysosomes. Results reported here indicate that acid ATPase activity recovered in the mitochondrial fraction of rat liver and measured according to Kalf and Gréce is located in lysosomes and not in mitochondria.

Methods.

Mitochondrial fractions were prepared from the livers of Buffalo rats by the procedure of de Duve et al.(I955). Density gradient centrifugations were performed according to Beaufay et al.(I964). Cytochrome oxidase was assayed as described by Appelmans et al. (I955), sulfite cytochrome c reductase by the procedure of Wattiaux De Coninck and Wattiaux (I97I), and acid phosphatase according to de Duve et al. (I955). Acid nucleoside triphosphatase was measured as described by Kalf and Gréce (I970) using ATP as substrate.

Results and discussion.

We compared the distributions of reference enzymes of mitochondria and lysosomes with those of acid ATPase after isopycnic centrifugation in a sucrose gradient. Mitochondrial fractions were isolated from normal rats and from rats injected with Triton WR 1339. This non-ionic detergent when injected into rats accumulates in liver lysosomes and lowers their density considerably (Wattiaux et al., I963). As a result, the distribution curves of lysosomal enzymes in a sucrose gradient are shifted toward low-density

regions while the distribution of mitochondrial enzymes remains unchanged. This phenomenon is illustrated in Fig.1. Two mitochondrial enzymes were measured: cytochrome oxidase, which is firmly bound to the inner membrane, and sulfite cytochrome c reductase, a soluble enzyme from the inter mem-

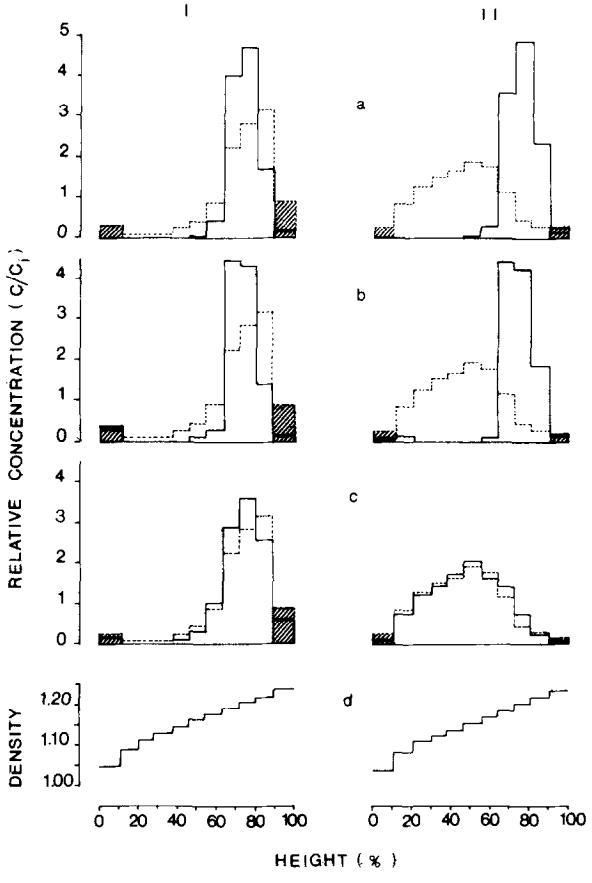

Fig. 1.

Distribution of particle bound enzymes after centrifugation (for 2.5h at 39,000 rev./min in.SW 39 head of the Spinco model L-HV preparative ultracentrifuge) of a mitochondrial fraction of rat liver through a 0.77 - 2.97 molal sucrose gradient in water. Abscissa: percentage of the height of the liquid column in tube (H). Ordinate: relative concentration, i.e, ratio of the observed activity (c) to that which would have been found if the enzyme had been homogeneously distributed throughout the whole gradient (Ci). Filled blocks (▨) are used for the bottom and top subfractions to indicate that they include material falling beyond the limits of the gradient. Solid line: (a) cytochrome oxidase; (b) sulfite cytochrome c reductase; (c) acid phosphatase, (d) Average density of the subfractions. Dotted line: acid ATPase. (I) Normal Rat; (II) rat injected intraperitoneally with 200 mg of Triton WR 1339 dissolved in saline and killed four days after injection. Recoveries were 86.0% and 86.7% for cytochrome oxidase, 108.8% and 106.5% for sulfite cytochrome c reductase, 100.2% and 98.0% for acid phosphatase and 98.9% and 106.1% for acid ATPase.

brane space (Wattiaux-De Coninck and Wattiaux, I97I); acid phosphatase was used as a reference enzyme for lysosomes. It can be seen that similar distributions of mitochondrial enzymes were obtained for the two mitochondrial preparations; in contrast, the distribution of acid phosphatase is shifted toward the top of the gradient when the preparation originates from the liver of a Triton WR I339 injected rat. Acid ATPase distribution is significantly different from that of the two mitochondrial enzymes and resembles the distribution of acid phosphatase in the normal rat. The distribution of this enzyme after injection of the detergent leaves little doubt that it is associated with subcellular structures similar to those containing acid phosphatase, since the distributions of the two hydrolases are affected to the same extent by Triton WR 1339 administration. We therefore conclude that the acid nucleoside triphosphatase recovered in the rat liver mitochondrial fraction is located in the lysosomes and not in the mitochondria.

Aknowledgments

This work was supported by a grant from the Fonds National de la Recherche Scientifique.

References.

Appelmans, F., Wattiaux, R. and de Duve, C. (I955) Biochem. J. 59, 438.
Beaufay, H., Jacques, P., Baudhuin, P., Sellinger, O.Z., Berthet,J. and de Duve, C. (I964). Biochem.J. 92, 184.
Brightwell,R., and Tappel, A.L. (I968). Arch.Biochem. Biophys. 124,333.
de Duve,C., Pressman, B.C., Gianetto,R., Wattiaux,R. and Appelmans,F.(I955) Biochem.J. 60, 604.
Kalf, G.F. and Gréce, M.A. (I970) Biochemistry 9, 4049.
Wattiaux-De Coninck,S. and Wattiaux,R. (I97I) European J. Biochem. (in press)
Wattiaux,R., Wibo,C. and Baudhuin,P. (I963). Ciba Found. Symp. Lysosomes. 176 (J. and A. Churchill, Ltd., London, 1963).