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Lysosomal localization of sphingomyelinase in rat liver

Several recent studies have shown that rat-liver lysosomes contain an acid hydrolase that splits sphingomyelin to ceramide and phosphorylcholine[1–4]. However, it is not clear from the data given whether the enzyme is restricted solely to lysosomes or is present also in other hepatic cell organelles. The results of HELLER AND SHAPIRO[1] suggest a predominant lysosomal localization of sphingomyelinase, but these authors have found no detectable activity in the post-mitochondrial supernatant fraction, even though this fraction contained 30% of the total acid phosphatase activity. The data of WEINREB *et al.*[3] are even more puzzling. The distribution pattern reported by these authors for the enzyme in normal rat liver allows for an association with

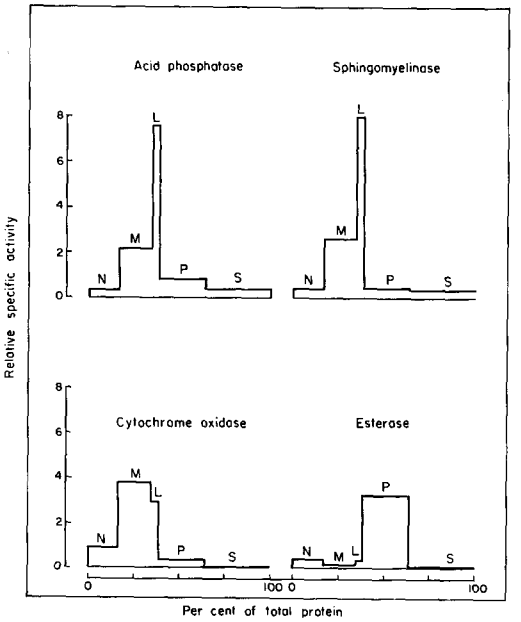

Fig. 1. Intracellular distribution of enzymes in livers of normal rats. Fractions are represented separately in the ordinate scale by their relative specific activity (percentage of total recovered activity/percentage of total recovered protein). In the abscissa scale, each fraction is represented (cumulatively from left to right) by its protein content, expressed as percentage of total recovered protein.

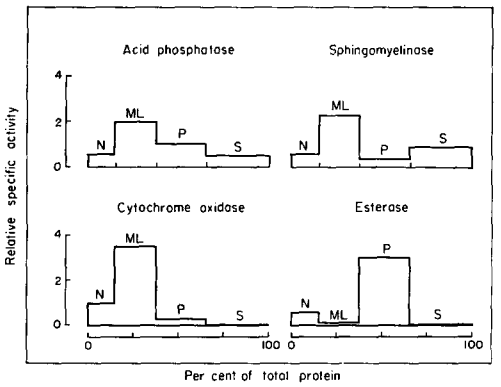

Fig. 2. Intracellular distribution of enzymes in livers of rats treated with Triton WR-1339. Results are represented as in Fig. 1.

lysosomes of only a minor fraction of the total sphingomyelinase activity of the liver. Nevertheless, they obtained a 97-fold purification of sphingomyelinase in lysosomes purified by floatation from the livers of rats treated with Triton WR-1339, as opposed to a 31-fold purification of the lysosomal marker acid phosphatase. These discrepancies suggest that some unknown factors may have complicated the assay of sphingomyelinase in these experiments.

The subcellular distribution of sphingomyelinase in rat liver has been reinvestigated in the present work. The following experimental conditions were used for assay of the enzyme. The reaction mixture, in a total volume of 0.25 ml, contained 7.5 mM sphingomyelin (dispersed by sonication in the presence of 2% Triton X-100), 0.2 M acetate buffer (pH 5.0), 0.8% Triton X-100, and enzyme. After incubation at 37° for 1 h, the reaction was stopped, and the remaining substrate removed by extraction into chloroform as described in a previous paper[4]; the aqueous phase was then assayed for total phosphate. The excess of water-soluble phosphorus found in the test system over that recovered from the enzyme and from the substrate incubated separately was taken as a measure of enzyme activity. Under these conditions, good proportionality with amount of enzyme was obtained if less than 15% of the added

TABLE I

DISTRIBUTION OF ENZYMES BETWEEN SUBFRACTIONS FROM LIVERS OF RATS TREATED WITH TRITON WR-1339

The ML fraction of Fig. 2 was further subfractionated by flotation through a discontinuous sucrose gradient[5]. Results are given as percentage of the content of the ML fraction.

Fraction	*Density of sucrose*	*Protein (%)*	*Acid phosphatase (%)*	*Sphingomyelinase (%)*	*Esterase (%)*	*Cytochrome oxidase (%)*
I	1.06	2.5	46.3	60.9	4.9	0.0
II	1.15	1.1	13.9	11.3	3.8	0.7
III	1.21	86.6	40.5	38.6	85.0	88.0

TABLE II

COMPLEMENTARY DATA ON EXPERIMENTS OF FIGS. 1 AND 2

Enzyme	*Untreated rats*		*Rats treated with Triton WR-1339*	
	*Activity (units/g liver)**	*Recovery* (%)***	*Activity (units/g liver)**	*Recovery (%)***
Acid phosphatase	9.77	104.3	9.00	96.6
Sphingomyelinase	0.037	101.2	0.036	102.9
Esterase	121.0	99.9	124.4	94.0
Cytochrome oxidase	14.8	90.9	17.5	89.2
Protein	217.5	98.7	200.0	98.2

* One unit of cytochrome oxidase activity is the amount of enzyme oxidizing 90% of the reduced cytochrome *c* present in 100 ml of incubation mixture per min; one unit of hydrolase activity is the amount of enzyme splitting 1 μmole of substrate per min (ref. 6). Proteins are given in mg/g of liver .

** Sum of activities of fractions expressed as percentage of activity of homogenate (nuclear fraction *plus* cytoplasmic extract).

substrate was consumed during the reaction. Assay methods for reference enzymes were those described previously[5] except for the determination of esterase, which was done by an automated method developed in our laboratory by Dr. Marco Baggiolini, using α-naphthyl acetate as substrate.

In Fig. 1 are shown the results obtained in a fractionation experiment performed according to DE DUVE *et al.*[6] on normal rat liver. An abbreviated version of the same fractionation scheme, in which the heavy (M) and light (L) mitochondrial fractions were separated as a single fraction, was followed in another experiment performed on the livers of rats killed 3.5 days after an intraperitoneal injection of Triton WR-1339 (0.85 g/kg body weight). As shown by WATTIAUX *et al.*[7], this treatment causes the lysosomes to be filled with the low-density detergent, allowing their further purification by floatation in a density gradient[5]. Fig. 2 and Table I show the results obtained on the animals treated with Triton WR-1339. Enzyme recoveries in the fractionation experiments are listed in Table II. Esterase was chosen as a microsomal marker and cytochrome oxidase as a mitochondrial marker.

There is a striking similarity in the distribution patterns of sphingomyelinase and acid phosphatase. The only difference is a somewhat higher level of acid phosphatase in the microsomal fraction and a correspondingly lower level of this enzyme in the final supernatant. This difference is more pronounced after treatment of the animals with Triton WR-1339, when a greater breakage of lysosomes upon homogenization is likely to occur. The known ability of acid phosphatase to adsorb onto microsomes[8] is probably responsible for it. It should be noted further that all the enzyme recoveries were quite satisfactory in these experiments, thus providing no indication of unknown interferences with the enzyme assays.

This conclusion has some bearing on the pathogeny of Niemann-Pick disease, which is characterized by the accumulation of sphingolipids in a number of tissues. It has recently been found that sphingomyelinase activity is very low or absent in the tissues of patients afflicted with this disease[9–11]. Morphological observations

indicate that the abnormal lipid deposits occur within membrane-bounded cytoplasmic vacuoles[12-14]. These results suggest that Niemann-Pick disease is a typical inborn lysosomal disease, as defined by HERS[15].

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