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Stabilization of rat liver lysosomes by heparin *in vitro*

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WHEN a rat liver mitochondrial fraction is incubated in isoosmotic sucrose at pH 5 and 37°, the free activity of lysosomal enzymes gradually rises: there is an increase in permeability of the lysosomal membrane to exogenous substrates.[1] Certain steroids, retinol and tocopherol, are able to make lysosomes more sensitive to this treatment; on the contrary, cholesterol and, to a lesser extent, cortisone exert a protective effect on the granules.[2] In our search for other substances that might affect lysosome behavior during incubation at pH 5, we found that heparin is a potent lysosome protector under such conditions.

Mitochondrial fractions from the liver of Wistar rats, corresponding to the sum of fractions M and L of de Duve *et al.*,[3] were prepared in 0·25 M sucrose. Treatment of the granules at pH 5 and 37° was performed according to de Duve *et al.*[2] Acid phosphatase and acid ribonuclease were measured by the procedure of de Duve *et al.*,[3] and β-galactosidase was assayed according to Vaes.[4] Free activity was measured for 10 min in 0·25 M sucrose; total activity was measured in the presence of added 0·1% Triton X-100.[5] Experiments were carried out on mitochondrial preparations isolated from normal rats and in some cases, from rats injected with 170 mg Triton WR 1339 in saline 4 days before sacrifice, since it has been shown[6] that lysosomes from rats injected with this detergent are more stable at pH 5 and more sensitive to the stabilizing effect of cholesterol.

As illustrated in Fig. 1, free acid phosphatase activity increases during the incubation of mitochondrial fractions at pH 5 and 37°. When the granules are incubated with heparin, free activity does not increase as much. By way of comparison, the effect of cholesterol is also indicated. Heparin seems to be more effective than cholesterol with preparation from normal rat and slightly less effective with preparation from detergent-injected rat.

These results suggest that heparin is able to protect the lysosomes membrane to some extent during incubation of the granules at pH 5 and 37°. If this is true, one would expect that the free

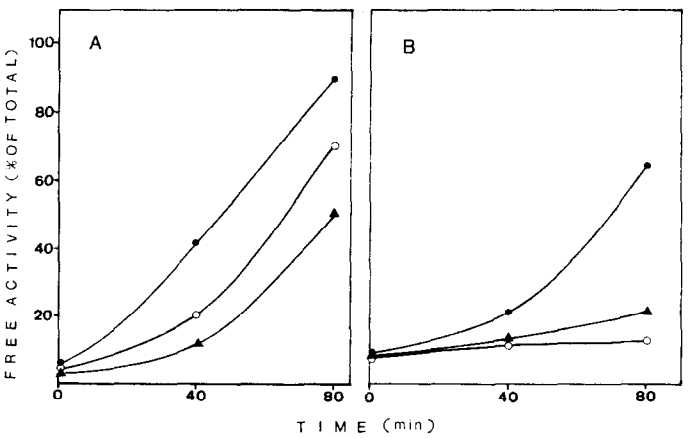

FIG. 1. Activation of acid phosphatase in a rat liver mitochondrial fraction incubated at 37° in 0·25 M sucrose, pH 5. Free activity expressed as percentage of activity in 0·1% Triton X-100. (A) Normal rat; (B) rat injected with 170 mg Triton WR-1339 in 1 ml isotonic saline, 4 days prior to sacrifice. ●——●: no addition; ○——○: in the presence of 0·125 mg/ml cholesterol; ▲——▲: in the presence of 0·125 mg/ml heparin.

activity of lysosomal enzymes other than acid phosphatase would likewise be affected by heparin. That such is the case is illustrated in Fig. 2. In one experiment (I) we recorded the changes in the free activities of acid phosphatase and of acid ribonuclease; in another (II) we recorded the changes in the free activities of acid phosphatase and β-galactosidase; all increase in a parallel manner. Heparin added to the granules affects the free activity of acid phosphatase and the other hydrolases in the same way.

As stated earlier, certain liposoluble compounds make lysosomes more sensitive to pH 5 treatment. Using two such substances, diethylstilbestrol and retinol, we attempted to determine whether their sensitizing effects would be inhibited by heparin. The results are recorded in Table 1. Both compounds

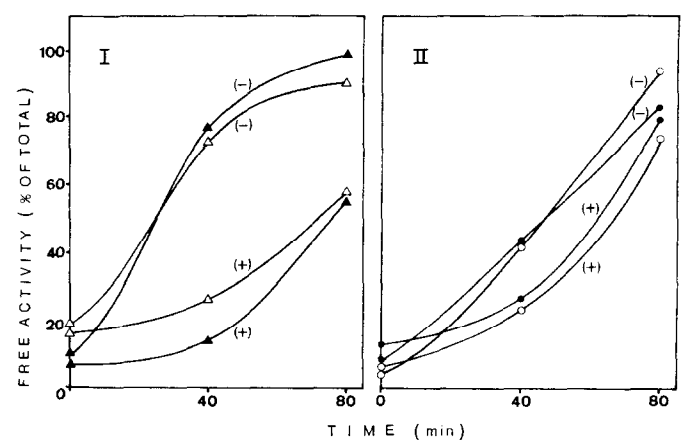

FIG. 2. Effects of heparin on activation of acid hydrolases. The results shown were obtained in two separate experiments. I. ▲——▲: acid phosphatase; △——△: β-galactosidase. II. ●——●: acid phosphatase; ○——○: acid ribonuclease. Incubation was performed without heparin (−) or in the presence of 0·125 mg/ml heparin (+).

greatly increase free acid phosphatase activity in granules incubated for 40 min at pH 5 and 37°. As Table 1 makes it clear, the addition of heparin to the suspension of granules counteracts the effect of the liposoluble substance.

TABLE 1. EFFECT OF DIETHYLSTILBESTROL, RETINOL AND HEPARIN ON THE ACTIVATION OF ACID PHOSPHATASE

	Free activity (in % of total activity)		
	Initial	Final	Increase
No addition	9	43	34
Diethylstilbestrol	10	95	85
Heparin	9	22	13
Diethylstilbestrol + heparin	7	36	29
No addition	10	58	48
Retinol	8	81	73
Heparin	9	25	16
Retinol + heparin	7	36	29

The compounds were tested at the concentration of 0·125 g/l. during a 40-min incubation at 37° and pH 5.

An explanation of the effect of heparin on rat-liver lysosomes can only be tentative, since the mechanism of lysosome activation at pH 5 is still unclear. It has been suggested that activation might originate with an attack on the lysosomal membrane by an acid hydrolase located inside the granules.[2] If this is the case, the protective effect of a compound such as heparin would result from an inhibition of this reaction at the enzyme level or at the substrate (i.e. the membrane) level.

It is not possible to know to what extent the effect of heparin on lysosomes has a physiological significance. However, it may be pointed out that heparin affects endocytosis,[7,8] a cellular function in which lysosomes are deeply involved.

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