CHANGES IN LIVER LYSOSOMES OF RATS WITH CHRONIC TOXIC HEPATITIS

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Permeability of the lysosomal membranes and the subcellular distribution of acid hydrolases were investigated in rats with chronic hepatitis induced by inhalation of CCl_4 and during repair of the liver after injury. Until 14 days after the last inhalation of CCl_4 no sign of restoration of the parameters studied to normal was observed: changes in the stability of the lysosomal membranes persisted and a redistribution of acid hydrolases was observed. This last effect is associated with the processes of both injury and repair of the liver.

KEY WORDS: *inhalation of CCl_4; liver lysosomes; acid phosphatase; acid ribonuclease.*

Sufficient evidence has now been obtained to demonstrate the participation of lysosomes in certain physiological and pathological states, including necrosis, degeneration, and regeneration [3]. Changes in the lysosomes in these situations can be regarded as an indication of the universal cellular response to the development of injury and a manifestation of the mechanism of cell defense. In acute toxic hepatitis caused by CCl_4, for instance, changes in the lysosomes coinciding with the times of development of morphological evidence of liver damage include labilization of the membranes of the particles, changes in their population composition, and an increase in their susceptibility to injury during standard treatment in vitro [1, 2, 10]. Even more complex changes can be expected during the development of chronic toxic hepatitis, a model that resembles most closely in its manifestations the clinical picture of hepatitis.

This paper gives the results of a study of the permeability of the lysosomal membranes and the subcellular distribution of acid hydrolases in chronic toxic CCl_4-induced hepatitis and in the course of repair of the liver after injury.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. Chronic toxic hepatitis was induced by inhalation of CCl_4 for 3 weeks [8]. The properties of the liver lysosomes were studied on the 1st, 3rd, 7th, and 14th days after the last inhalation of CCl_4. Fractionation of the liver tissue was carried out by the scheme described by De Duve et al. [4]. The protein content and the total activity of the lysosomal marker enzymes, acid phosphatase and acid ribonuclease (RNase) were determined in the five fractions obtained. The results of the study of the protein distribution were expressed as percentages of the total protein content in each fraction. The distribu-

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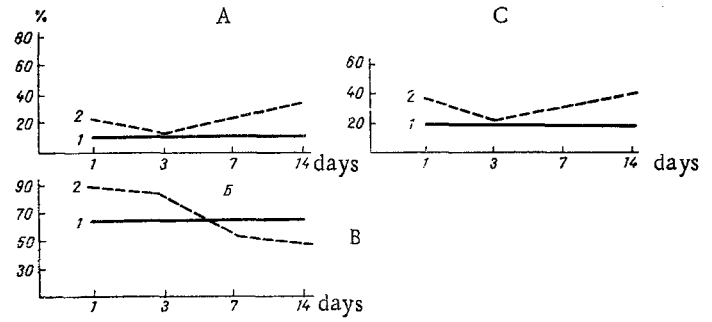

Fig. 1. Characteristics of lysosomal membrane of rat liver during period of spontaneous recovery after chronic toxic hepatitis: A) free acid phosphatase activity in light mitochondrial fraction; B) free acid phosphatase activity determined after preliminary incubation of fraction at pH 5.0 and 37°C for 30 min; C) free acid phosphatase activity determined after hypotonic treatment (0.125 M sucrose, 0°C, 15 min); 1) intact lysosomes; 2) lysosomes after exposure to CCl_4. Abscissa, time after exposure to CCl_4 (in days); ordinate, free acid phosphatase activity (in % of total).

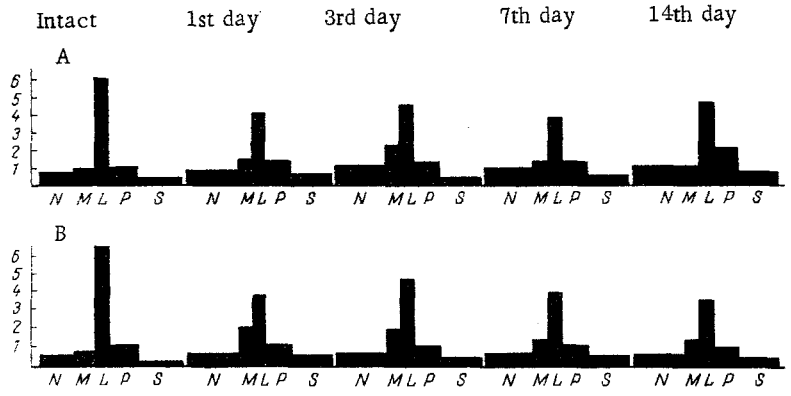

Fig. 2. Intracellular distribution of acid phosphatase (A) and acid ribonuclease (B) during reparative regeneration of the liver. Abscissa, protein content of fractions (in %); ordinate, RSA.

tion of enzymes was presented as values of relative specific activity (RSA) as determined by de Duve et al. [4]. The susceptibility of the lysosomal membranes to damage was estimated as described previously [2].

EXPERIMENTAL RESULTS

Labilization of the membranes and greater sensitivity of the lysosomes to treatment in vitro at pH 5.0 and 37°C were observed 24 h after the last inhalation, i.e., at the time of maximal damage to the liver (Fig. 1). Changes were found only in respect of acid phosphatase. During this period a redistribution of the protein of the subcellular fractions was observed: an increase in its content in the nuclear fractions and a decrease in the fraction of heavy mitochondria. The distribution of the mitochondrial and microsomal marker enzymes — succinate dehydrogenase and glucose-6-

phosphatase — was indistinguishable from their distribution in intact animals. RSA of acid phosphatase and acid RNase was reduced in the light mitochondrial fraction but at the same time it was increased in the heavy mitochondrial fraction and in the supernatant (solubilization of the enzyme) (Fig. 2).

In the later stages of recovery the response of the lysosomal membranes to standard treatments in vitro varied. A decrease in free acid phosphatase activity, regarded as evidence of stabilization of the membranes, was accompanied on the 3rd day after the last inhalation by an increase in their sensitivity to treatment at pH 5.0 and 37°C (Fig. 1). On the 7th and 14th days, parallel with labilization of the membranes, an increase in their resistance to the same procedure at pH 5.0 and 37°C was observed. During the study of the effect of another harmful factor — treatment of the particles in 0.125 M sucrose at 0°C — no increase in the vulnerability of the particles during hypotonic treatment could be found at any stage of recovery of the liver.

On the 3rd, 7th, and 14th days after the last inhalation the study of the intracellular distribution of acid hydrolases revealed the same tendencies as on the 1st. day: an increase in RSA of acid phosphatase and acid RNase in the heavy mitochondrial fraction, to reach its maximum on the 3rd day after the last inhalation of CCl_4. On the 7th and 14th days RSA of acid phosphatase of the heavy mitochondrial fraction was indistinguishable from this parameter in intact animals, whereas RSA of the acid RNase of this fraction remained high. At all times of investigation solubilization of acid RNase into the supernatant was well marked. On the 14th day an increase in RSA of acid phosphatase was observed in the microsomal fraction, where usually the primary lysosomes are located [7].

During the recovery period investigated, none of the parameters studied returned to normal, i.e., the changes in stability of the lysosomal membranes persisted and abnormalities in the intracellular distribution of acid hydrolases were observed. The results may be associated with processes of both injury and repair of the liver. A similarity between changes in the lysosomes during regeneration and injury was observed by Kerr [5]. Moreover, the opinion is held that the constancy of changes in the lysosomes during hepatitis may reflect a process of regeneration which develops simultaneously with damage to the liver [5, 10]. The redistribution of marker enzymes found in the earlier stages, when processes of injury predominate, may be regarded as a sign of an increase in the number of heterophagosomes, which are sedimented at lower accelerations. The work of Mego et al. [6] showed that lysosomes loaded with protein possess similar properties. Consequently, an increase in the intensity of heterophagosome formation can be postulated at this period. Differences in the subcellular distribution of acid phosphatase and acid RNase can evidently be attributed to the fact that the enzymes belong to different subclasses of lysosomes [9]. Differences in the cellular origin of the lysosomes containing these enzymes cannot be ruled out. Meanwhile the constant increase in acid RNase activity in the supernatant may point to the cytoplasmic origin of this enzyme. Support for this view is given by the absence of labilization of the lysosomes during investigation of acid RNase.

Distinctive changes were found in the lysosomal membranes. Labilization of the lysosomal membrane (an increase in free acid phosphatase activity) one day after the last inhalation of CCl_4 was accompanied by increased sensitivity of the particles to treatment at pH 5.0 and 37°C. This similarity of direction of the changes was not subsequently observed. For instance, on the 3rd day restoration of the "normal" stability of the lysosomes was accompanied by greater susceptibility to injury during treatment in vitro, whereas on the 7th and 14th days labilization of the lysosomes was not accompanied by any increase in the fragility of the particles under the same conditions. These results indicate continuous involvement of the lysosomes in lytic processes during repair after liver damage. Probably the population composition of the lysosomes (the ratio between the numbers of primary and secondary forms) and also infiltration of the liver by lymphocytes and macrophages, probably has the greatest influence on the parameters studied.

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