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## PRELIMINARY COMMUNICATIONS

# REVERSIBLE AND IRREVERSIBLE ALTERATIONS OF LYSOSOMES IN ISCHEMIC RAT-LIVER. EFFECTS OF CHLORPROMAZINE.

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The total ischemia of a rat-liver lobe causes a progressive decrease of acid hydrolase latency indicating that the lysosomes are altered (1). It is not known to what extent these alterations are reversible after re-establishment of the blood flow. In the present work we are particularly concerned with the fate of two lysosomal hydrolases: acid phosphatase and β-galactosidase in rat-liver lobes rendered ischemic during one or two hours, the animals being killed immediately after the ischemic period or 20 hours after re-establishment of the circulation. Some of the rats were pretreated with chlorpromazine. This experimental schema was adopted because according to some morphological and biochemical observations (2,3) cell injury induced by ischemia seems reversible when the reflow in the liver occurs one hour after the blood supply has been cut off, but is irreversible when the reflow takes place two hours after the interruption of circulation. Moreover, it has been shown that the reversible period can be markedly extended when the rats have been pretreated with chlorpromazine (4).

Methods:

In our experiments we used male Wistar rats weighing 200 to 300 g.which had been starved overnight. They were laparotomized under ether anaesthesia and the vascular pedicle of the left liver lobe was clamped with a bull-dog forceps. To re-establish flow, the animals were again anaesthetized, the abdomen was re-opened, the forceps removed and the wound closed. After killing the animals the left lobe and in some cases the right one (used as control) were removed, weighed in ice cold 0.25 M sucrose and then homogenized in the same medium by three strokes of a motor driven Teflon pestle in a Potter homogenizer (A.H. Thomas Co. Philadelphia). The homogenized suspension was brought to a volume corresponding to 10 times the weight of the tissue. Part of the homogenates was spun for 45 min at 40.000 rev/min in the n° 40 rotor of the Spinco preparative ultracentrifuge.

The degree of latency of the lysosomal hydrolases was appreciated by measuring free and total acid phosphatase activity of the homogenates according to de Duve et al. (5) and total β-galactosidase activity on the homogenates and on the high speed supernatants according to Vaes (6). Proteins were determined according to Lowry et al.(7). Units of enzymic activity are defined as the amount of enzyme causing the decomposition of 1 μmole of substrate per min.

Chlorpromazine was administred 30 min. before surgery by an intraperitoneal injection of 4 mg/ml solution in 1 mM imidazole pH 7.4, 0.15 M NaCl with a dose of 2 mg/100 g body weight.

Results and discussion:

As shown in Fig.1, the free activity of acid phosphatase is increased two fold in the homogenates of livers deprived of blood for one hour. Twenty hours after the return of blood flow, a quasi normal free activity is recovered.

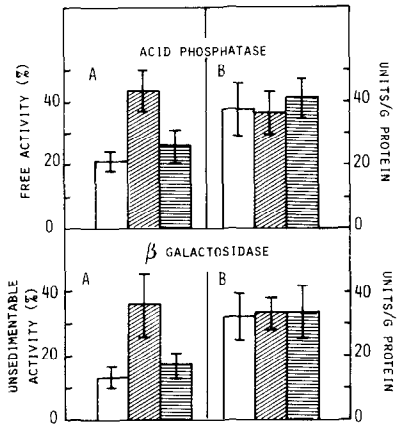


Fig.1. Influence of 1 hour ischemia on the release of liver acid phosphatase and galactosidase. Free and unsedimentable activities (A) are given as percentages of the total activity; total activities (B) are given in units/g. of protein. Means of at least 7 animals with S.D. are presented. ☐ control values: as significant differences were not observed for the unligated lobes whatever their origin, in one or another group of operated rats, all the determinations were pooled to calculate the mean values. ▨ :without re-establishment of blood flow; ▤ : after re-establishment of blood flow during 20 hours.

The change of unsedimentable β-galactosidase parallels that of free acid phosphatase. Total activities of both hydrolases are unaffected by ischemia. Thus one hour ischemia causes a lysosomal lesion which is reversible to a large extent.

When the blood supply has been cut off for two hours, the increase of the free and soluble activities of acid hydrolases is more impressive (Fig.2). Acid phosphatase free activity is about 60% and β-galactosidase unsedimentable activity about 50% of the total activities. Re-establishment of the circulation does not lead to recovery; on the contrary, 20 hours after the reflow took place, the unmasking of acid hydrolases is still more marked. The total activity of β-galactosidase is not modified but that of acid phosphatase is significantly reduced. Therefore, the lysosomal lesion induced by a two hour ischemia is irreversible.

Also illustrated in Fig.2 are the results obtained when the rats have been injected with chlorpromazine. Obviously, chlorpromazine treatment opposes the irreversible effect of ischemia on the lysosomal hydrolase latency, 20 hours after the reflow of blood, acid phosphatase free activity and β-galactosidase unsedimentable activity are nearer their normal values. Moreover, the

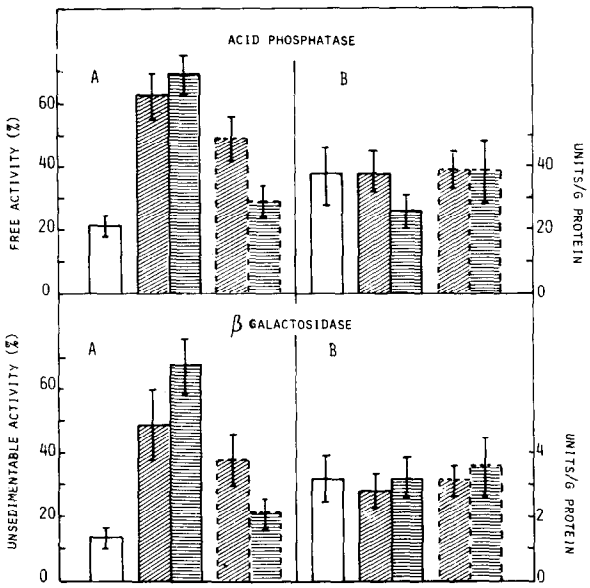


Fig.2. Influence of a 2 hour ischemia on the release of liver acid phosphatase and βgalactosidase. Free and unsedimentable activities (A) are given as percentages of total activity; total activities (B) are given in units/g of protein. Means of at least 7 animals with S.D. are presented. ☐ : control values, see legend of Fig.1; ▨ : without re-establishment of blood flow; ▨ :id, after chlorpromazine treatment; ▤ : after re-establishment of blood flow during 20 hours; ▤ : id.after chlorpromazine treatment.

acid phosphatase total activity is not affected like in the untreated animals.

The immediate relevance of these findings is to show that a lysosomal lesion may be reversible and that it is possible, with the help of a drug, to influence favorably this reversibility. As for the nature of this lesion, it should be noted that the loss of acid hydrolase latency observed in a homogenate may have two origins. This results from a true release of these enzymes inside the cells owing to an abnormal permeability of the lysosomal membrane, or from an increase in the fragility of the lysosomes causing them to be more easily disrupted during homogenization. We are not sure as to which of these interpretations is responsible for the latency change induced by ischemia, however we think that a detailed study of the reversibility of the process will help to understand the nature of the lysosome lesion that causes the unmasking of hydrolases. In this respect, the treatment with chlorpromazine is particularly interesting since it allows the change of an irreversible lysosome lesion into a reversible one.

## REFERENCES

1. C. de Duve and H. Beaufay. Biochem.J. 73, 610 (1959).
2. M.Bassi and A.Bernelli-Zazzera. Exp.Mol.Pathol. 3, 332 (1964)

3. K.R. Chien and J.L. Farber. Arch.Biochem.Biophys. 180, 191 (1977)
4. K.R. Chien, J.Abrams, R.G. Pfau and J.L. Farber. Am.J.Pathol. 88, 539 (1977)
5. R.Wattiaux and C. de Duve. Biochem.J. 63, 606 (1956).
6. G.Vaes. Methods Enzymol. 8, 509 (1966).
7. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall. J.Biol.Chem. 193, 265 (1951).