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Study on the stability of lysosome membranes—V
Changes in the stability of liver lysosomes of rats kept at different temperatures after treatment with chlorpromazine

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CHLORPROMAZINE (CPZ) in high concentrations labilizes lysosomes *in vitro*.[1] The same effect is obtained after treating experimental animals with high doses of the drug.[2–6] It was presumed that *in vivo*, CPZ damages lysosome membranes indirectly.[3–6] Probably this action is due to primary disturbances in a bioenergetic pathway, leading to low energy production. Experimental data from previous works partly support this conception.[4–6] If *in vivo* CPZ labilizes lysosomes indirectly, then the animals treated with the drug and kept at lower temperatures might show more severe damage of these particles in comparison to the animals treated with the same doses but kept at temperature 35–37°, the temperature at which the loss of energy for production of heat is minimum. The data

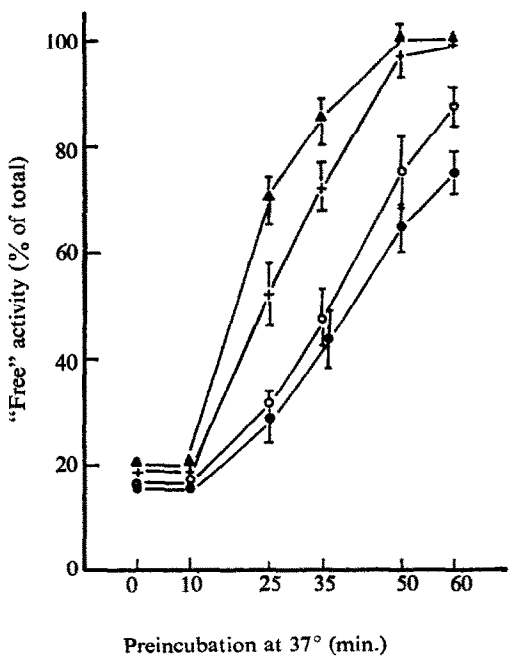

FIG. 1. Release of acid phosphatase from ML fractions isolated from liver homogenates of control and treated rats.

Amounts of the fractions used for determining the enzyme activity were preincubated for 0, 10, 25, 35, 50 and 60 min at 37° in a media containing 0, 25 M sucrose and 0·1 M acetate buffer with pH 5. Substrate (Sodium β-glycerophosphate) ensuring a concentration of 0·05 M was added at the end of the period of time indicated for preincubation, after which the incubation continued for 10 min.

The "free" activity is expressed in percentages to the total one determined in the presence of Triton X-100 (final concentration 0·1% v/v).

●—● controls; ○—○ treated with CPZ and kept at 35–37°; +—+ treated with CPZ and kept at 19–20°; ▲—▲ treated with CPZ and kept at 14–16°.

The vertical bars depict S.E.M.

obtained in the present study show an interdependence between lysosome labilizing effect of CPZ and the temperature at which the rats were kept after treatment with this drug.

Male and female albino rats of the Wistar breed with body weight of 140–200 g were used in the experiments. After 12 hr of starvation the animals were injected subcutaneously with CPZ (from EGYT—Hungary) in doses of 0·01 g/kg body wt. Some of the treated rats were kept at a temperature of 14–16°, some at 19–20° and the remaining ones at 35–37°. Some non treated rats were included at all the three temperatures as controls. Five hr after injection of CPZ, the rats were killed by decapitation. Livers were rapidly removed and were put into an ice-cold 0·25 M solution of sucrose. Preparation of homogenates and their centrifugal fractionation was done as described earlier.[5] An assessment of lysosome membrane stability was made according to the rate of release of acid phosphatase, during preincubation of large-granule fractions (mitochondrial–lysosomal fractions sedimented at 20,000 *g* for 20 min and washed twice, which will be referred to further on as ML fractions) as indicated in the text for figure. The activity of the acid phosphatase was determined by the method of Gianetto and De Duve.[7]

The results from the experiments are summarised in the figure. The data from rats of all three control groups are given as one because when compared, there were no differences above their individual variations. There were large differences in the rate of release of the acid phosphatase from the ML fractions isolated from rats of three experimental groups: ML fractions isolated from rats kept at 14–16° after treatment with CPZ, the lysosome-marker enzyme is released very rapidly, from rats kept at 35–37°; the rate is approximately similar to that of controls and the ones from rats kept at 19–20°; the rate takes a middle course (See Fig. 1).

It is commonly accepted that rapid release of granule-bound hydrolases is an indication for labilization of lysosomes. Taking this into consideration, it can be concluded that with rats treated with CPZ and kept at 35–37°, the damage to liver lysosomes is very small. When the environmental temperature is decreased, the labilization effect increases though the animals were treated with the same doses of the drug.

Rats treated with CPZ and kept at 35–37° do not undergo a hypothermic phase induced by the drug. It seems that this fact has no connection with the damaging action of CPZ on lysosomes, because, promethazine at doses which causes hypothermia does not provoke damage of lysosomes. When rats treated with CPZ are kept at 5–7° or 19–20° they undergo practically the same degree of hypothermia, but as can be seen from the figure, lysosomes of rats kept at various temperatures after treatment with CPZ have different stability.

Department of Chemistry and Biochemistry,
Higher Institute of Veterinary Medicine,
Sofia 13, *Bulgaria*

CH. S. POPOV

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