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CHANGES IN RAT LIVER LYSOSOMES DURING STIMULATION OF REGENERATION IN THE INJURED ORGAN BY TRITON WR-1339

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The effect of a single dose of the lysosomotropic agent Triton WR-1339 on the properties of the liver lysosomes of rats with chronic toxic hepatitis and on the course of the pathological process was investigated. The compound was shown to promote the more rapid restoration of liver structure and function. The possible mechanism of the beneficial effect of Triton WR-1339 is discussed. KEY WORDS: lysosomes; toxic hepatitis; Triton WR-1339.

Changes in the lysosomes under pathological conditions are connected both with the development of injury and with the formation of intracellular mechanisms leading to compensation of the disturbances and restoration of the normal structure and function of the organ [3, 5, 7, 11]. The writers showed previously that chronic toxic hepatitis, developing during loading of the vacuolar system of the liver cells by Triton WR-1339 (Triton), is distinguished by the milder development of zones of necrosis and collagenization of the tissue [1]. This effect may be due either to a decrease in the severity of injury to the organ or the more intensive development of repair processes.

To study this problem the effect of Triton on the development of repair processes in the liver of rats with chronic toxic hepatitis was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Chronic toxic hepatitis was induced by inhalation of CCl_4 for three weeks [1]. Triton in a dose of 85 mg/100 g body weight was injected intraperitoneally 24 h after the last inhalation of the poison. The animals were decapitated 3, 7, and 14 days after the end of the course of

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TABLE 1. Effect of Triton WR-1339 on Rate of Elimination of Bromsulphthalein (BSP) in Rats with Toxic Hepatitis during Regeneration of the Organ (M ± m).

Index	Intact rats	Time after poisoning, days					
		control (CCl_4)			experiment (CCl_4 + triton)		
		3	7	14	3	7	14
BSP concentration (retention coefficient) at different times after inject.							
5 min	19,9±1,8	58,7±2,6	44,7±2,0	36,6±1,4	61,1±0,7	21,4±2,3	28,9±2,9
10 min	6,8±0,9	38,4±1,9	15,6±0,9	13,4±1,4	28,2±1,8	10,4±2,0	7,6±0,7

TABLE 2. Effect of Triton on Total Acid Ribonuclease Activity and Total Radioactivity of Liver Homogenate from Intact Rats

Index	Intact rats	Triton		
		2 days	6 days	13 days
Total acid ribonuclease activity, $E_{260} \times 100$/min/mg protein	7,2±0,6	6,3±0,6	14,3±1,0 $P<0,001$	15,3±0,7 $P<0,001$
Total radioactivity, % of injected dose, calculated per gram wet weight of tissue	1,8±0,13	5,8±0,4 $P<0,01$	3,7±0,2 $P<0,01$	—

inhalations, and 2, 6, and 13 days respectively in the case of control rats receiving detergent. In all cases the liver function was assessed by determining the rate of excretion of bromsulphthalein [4]. The preparative and analytical procedures were carried out by methods described previously [1, 2]. The degree of damage to the lysosomal membranes was assessed from the degree of increase in free acid phosphatase activity in the fraction of light mitochondria after incubation of the particles in 0.125 M sucrose at 0°C for 15 min [8]. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Injection of Triton into rats with chronic toxic hepatitis led to a more favorable course of the pathological process. For example, histological investigation* of liver sections of rats poisoned with CCl_4 and of rats receiving Triton after inhalation of CCl_4 gave the following results. In the animals of the second group the number of foci of necrosis was reduced or they were absent altogether, the number of damaged hepatocytes (hydropic degeneration, fatty degeneration) was reduced, the intensity of collagenization of the liver was lower, and the amount of localization of glycogen were restored to normal. Additionally, one week after the end of the course of inhalation, in the animals receiving both CCl_4 and Triton the mitotic index was increased to 1.3% (compared with 0.4% in rats with "pure" hepatitis). Toward the end of the second week there was a shift in the nucleo-cytoplasmic and nucleo-nucleolar ratios in favor of nuclei and nucleoli (Fig. 1). Administration of Triton to rats with chronic toxic hepatitis led to the more rapid normalization of the excretory and assimilative function of the liver (Table 1). Meanwhile, investigation of the state of the liver lysosomes in the rats of this group showed greater (compared with "pure" hepatitis) solubilization of enzymes and lower resistance of the particles to osmotic shock (Figs. 2 and 3).

The results thus indicate that during accumulation of Triton in the vacuolar system of the cells of the damaged liver processes resulting in the more rapid normalization of the structure and function of the organ are stimulated. Administration of Triton shifted the dynamic equilibrium to some degree between the processes of injury and regeneration in favor of the latter. For instance, in rats with toxic hepatitis receiving Triton the mitotic index was increased by almost threefold, the necrotic changes were less severe, and degeneration of the hepatocytes was less marked. The excretory and assimilative function of the damaged liver was restored sooner.

Administration of a single dose of Triton WR-1339 leads to the formation of large Triton-lysosomes in the Kupffer cells and hepatocytes of the animals. Particles loaded with detergent were less resistant to osmotic shock but, at the same time, were more resistant than particles isolated from the intact liver to incubation in medium with pH 5 at 37°C [13]. Consequently, the high level of free and nonsedimented activity of lysosomal

* These investigations were carried out by T. N. Tsytsorina and S. V. Michurina, on the staff of the Pathomorphology and Histochemistry Group of the Central Research Laboratory.

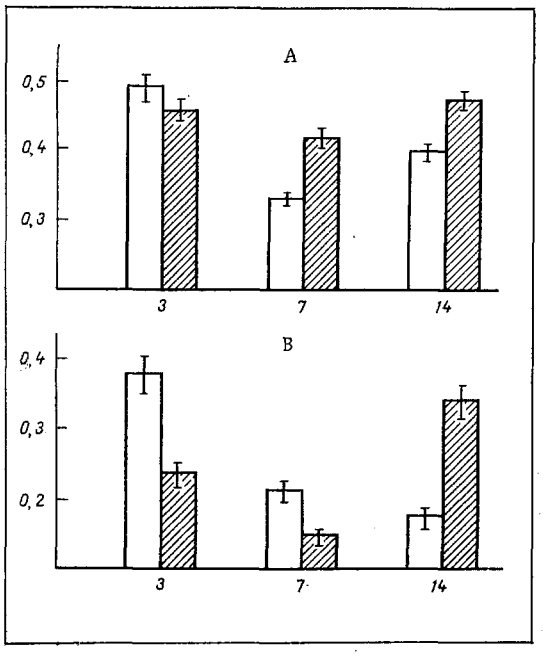

Fig. 1

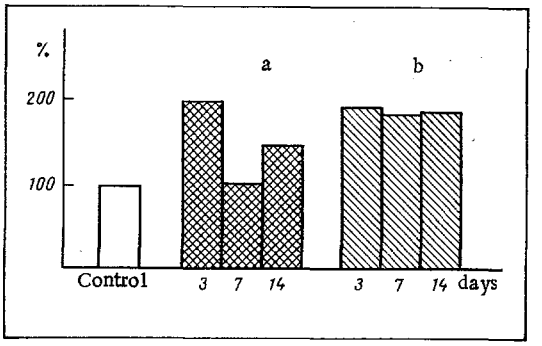

Fig. 2

Fig. 1. Nucleo-cytoplasmic (A) and nucleo-nucleolar (B) ratios in liver sections from rats with toxic hepatitis. Unshaded columns – CCl_4; shaded columns – CCl_4 + Triton. Abscissa, time after poisoning (days); ordinate, ratio.

Fig. 2. Effect of Triton WR-1339 on level of nonsedimented activity of marker enzyme lysosomes of liver homogenate from rats with toxic hepatitis. a) Acid phosphatase; b) acid ribonuclease; control – activity of enzymes obtained in rats treated with CCl_4 only, at the same period of recovery. Abscissa, time of recovery (days); ordinate, activity (in % of control).

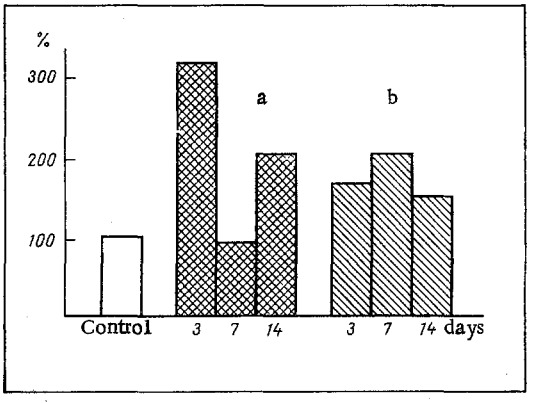

Fig. 3. Effect of Triton WR-1339 on osmotic resistance of liver lysosomes of rats with toxic hepatitis. Ordinate, osmotic resistance (in % of control). Remainder of legend as in Fig. 2.

enzymes and the lowered osmotic resistance of the particles observed in the experiments on rats with toxic hepatitis after injection of Triton indicate that the liver cells of these animals contain secondary Triton-lysosomes, less resistant to the isolation procedures [8]. The entry of Triton into the liver cells is also known to activate processes of endocytosis and auto- and heterophagy in them [6, 10, 13]. This is supported by the increased uptake of labeled modified protein by the liver of the rats and also by the increase in total acid ribonuclease activity observed in the present experiments (Table 2). The latter evidently reflects the induction of

lysomal enzymes of Kupffer cells, which contain from 2 to 2.5 times more lysosomal enzymes than hepatocytes [12]. Activation of auto- and heterophagy is accompanied by increased segregation of damaged structural elements of the cells, on the one hand, and by increased catabolic activity on the lysosomes. This latter effect facilitates the utilization of breakdown products for the construction of new macromolecules and promotes restoration of the structure and function of the organ.

The beneficial effect of Triton on the course of toxic hepatatis is thus connected with stimulation of repair processes in the liver. It can tentatively be suggested that this effect is mediated through the vacuolar system of the cells and, in particular, through the lysosomes, and that the Triton itself performs the role of inducer of the mechanisms of nonspecific defense in this case [9].

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