EFFECT OF LYSOSOMAL LOADING WITH TRITON WR 1339 ON THE DISTRIBUTION OF ALBUMIN-^{14}C IN THE LIVER OF RATS WITH CHRONIC HEPATITIS

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The mechanism of the protective effect of the lysosomotropic detergent Triton WR 1339 in chronic hepatitis was examined. Assuming that the improvement in the condition is connected with potentiation of the heterophagous function of the lysosomes, the intensity of uptake of albumin-^{14}C by the liver and its subcellular distribution in the liver of rats were studied during administration of the detergent to animals with chronic carbon tetrachloride hepatitis. Preliminary injection of the detergent did not affect the intensity of uptake of albumin-^{14}C, but subsequent injection of Triton WR 1339 into rats with toxic hepatitis reduced the protein uptake to values obtained in intact rats. In chronic hepatitis albumin-^{14}C is concentrated in the lysosomal fraction. After injection of Triton WR 1339 into the poisoned animals the peaks of labeled protein and lysosomal enzymes did not coincide. The selective role of lysosomes of the Kupffer cells of the liver in producing the more rapid recovery of the liver from chronic hepatitis is examined.

KEY WORDS: heterophagous function of lysosomes; Triton WR 1339; toxic hepatitis.

The role of lysosomes in the ingestion and digestion of macromolecules and particles entering the cell by endocytosis has been studied in detail in recent years [4-7]. The protective, heterophagous function of the lysosomes assumes great importance in the development of several pathological processes accompanied by overloading of the lysosomal apparatus of the cell. Injection of lysosomotropic substances, increasing the uptake of foreign substances by the lysosomes and their elimination from the cell may contribute toward restoration of the normal structure and function of the cell. The writers have shown that Triton WR 1339 has a beneficial action on the structure and function of the liver in toxic hepatitis [1].

Considering that Triton WR 1339 accumulates selectively in the lysosomes the effect of the detergent on the ability of the liver to take up protein was investigated, on the grounds that the protective effect of Triton WR 1339 must be connected with a change in this function.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200 g were used. Chronic hepatitis was produced by inhalation of CCl_4 for 21 days [1]. The animals were decapitated 1, 3, and 7 days after the last inhalation. The animals of the second group received an intraperitoneal injection of Triton WR 1339 in a dose of 85 mg/100 g body weight 4 h before the first inhalation of CCl_4. The rats of group 3 received the detergent in the same dose but 24 h after the last inhalation of CCl_4 and they were decapitated 3 or 7 days later. Intact animals and rats receiving the detergent formed the appropriate control groups.

The preparative and analytical procedures were taken from De Duve et al. [3, 4]. The uptake of albumin-^{14}C by the liver and the intracellular distribution of labeled protein were determined 30 min after intravenous injection of albumin-^{14}C in a dose of 0.25 mg/100 g body weight. The results were expressed as percentages

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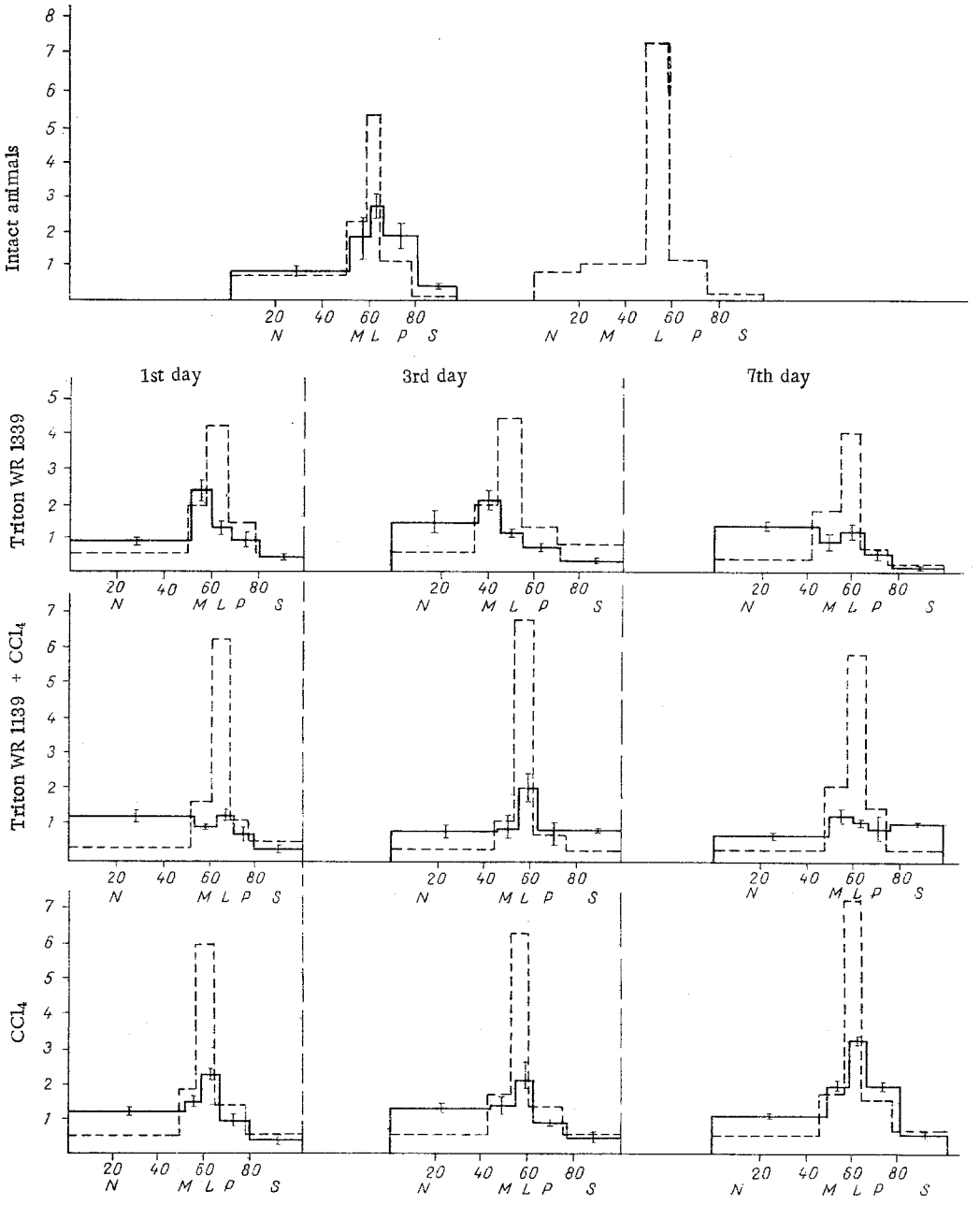

Fig. 1. Subcellular distribution of acid RNase and albumin-^{14}C in rat liver. Distribution of acid RNase shown for intact animals, RSA of acid RNase and relative specific radioactivity shown for animals receiving albumin-^{14}C. Abscissa, percentage protein content of subcellular fraction; ordinate, RSA values. Broken line – RNase; continuous line – albumin-^{14}C. N) nuclear fraction, M) heavy mitochondria, L) light mitochondria, P) microsomes, S) supernatant.

of the injected dose of protein calculated per gram of liver tissue [7]. The intracellular distribution of labeled protein and of the marker enzyme of the lysosomes (acid RNase) was represented as the value of relative specific activity (RSA) [3].

Just as in the experiments of Mego [6], who used albumin-^{125}I, we modified the same groups (amino groups) of the protein; in this way stable preparations of albumin-^{14}C were obtained. Acetic anhydride-^{14}C in 10% dimethylformamide (specific activity 0.1 mCi/mmole) was added gradually, with continuous titration, to a solution of bovine serum albumin containing 5 mg/ml at pH 11.0. The protein was precipitated and washed with ethanol, dissolved in 7 M urea, and dialyzed against water. The specific activity of the modified albumin was 0.14 μCi/mg protein. A fraction containing from 0.05 to 0.3 ml (protein content 0.5-2.0 mg) was precipitated

TABLE 1. Effect of Injection of Triton WR 1339 on Uptake of Albumin-^{14}C by the Liver of Intact Rats and Rats with Toxic Hepatitis (M ± m)

Experimental conditions	Uptake of albumin-^{14}C (in % of injected dose per gram liver)	Group compared	P
CCl_4 (21 days)			
A. 1 day after period of poisoning	4.60 ± 0.07	A–K	< 0.001
B. the same, 3 days later	5.70 ± 1.25	B–K	< 0.01
C. the same, 7 days later	1.40 ± 0.24		
CCl_4 preceded by Triton WR 1339			
D. 1 day after period of poisoning	4.10 ± 0.25		–
CCl_4 followed by Triton WR 1339			
E. 3 days after poisoning	1.40 ± 0.30	E–B	< 0.01
F. the same, 7 days later	1.55 ± 0.03	E–G	< 0.001
Triton WR 1339			
G. 3 days	5.80 ± 0.40	G–K	< 0.001
H. 7 days	3.70 ± 0.20	H–K	< 0.001
I. 21 days	3.8 ± 0.20	I –K	< 0.001
K. Intact animals	1.80 ± 0.13	–	–

with 5% TCA and filtered through an AUFS (Czechoslovakia) ultrafilter. Radioactivity was measured in toluene scintillator on a Mark I (USA) counter. The results were subjected to statistical analysis, using Student's criterion.

EXPERIMENTAL RESULTS

The uptake of modified protein by the liver of intact rats was 1.8%/g tissue (Table 1). Injection of the detergent into intact rats and the development of chronic hepatitis (1 and 3 days after the period of poisoning) were accompanied by an increase in the albumin-^{14}C uptake by the liver. This index was back to normal 7 days after the last inhalation of CCl_4. Preliminary injection of the detergent into the poisoned animals did not affect the intensity of albumin-^{14}C uptake; injection of Triton WR 1339 after poisoning into rats with toxic hepatitis, however, reduced the uptake of the protein (after 3 days) to the values obtained in intact rats. Normalization coincided in time with a considerable improvement in the morphological picture of the liver.

In the intact rats and injected protein was concentrated in the lysosomal fraction, as shown by coincidence of the peaks of the RSA values for the uptake of albumin and for acid RNase (Fig. 1). A redistribution of protein of the subcellular fractions and an increase in RSA of acid RNase were observed in the fraction of heavy mitochondria. The latter is evidence of the formation of heterolysosomes, which take up protein and are sedimented at lower accelerations [6], in the liver cells. Similar changes were found in all the groups of experimental rats receiving albumin-^{14}C. The development of chronic hepatitis was not accompanied by any change in the subcellular distribution of labeled protein; an increase in the RSA value was found only in the nuclear fraction.

After injection of Triton WR 1339 into intact rats the peaks of the RSA values for albumin uptake and acid RNase for the lysosomal fraction did not coincide at any time of investigation. Meanwhile RSA for the uptake of the nuclear fraction was increased, indicating retention of protein in heterophagosomes [5, 6]. Injection of the detergent into the poisoned animals was accompanied by a marked decrease in relative specific radioactivity of the lysosomal fraction with an increase in radioactivity in the supernatant.

In chronic hepatitis the lysosomes of liver cells take up an increased amount of protein and concentrate it in the lysosomal fraction. Meanwhile, after injection of Triton WR 1339 into intact and poisoned animals, the distribution of labeled protein and lysosomal enzymes did not coincide completely. These results can be inter-

preted as follows. As Davies [2] found, if the detergent and albumin-^{125}I were injected simultaneously, the latter was located in the heterophagosomes, which did not merge with heterolysosomes containing Triton WR 1339. Consequently, the albumin-^{14}C may be present in heterophagosomes, which are larger in size and more sensitive to the action of damaging procedures. During homogenization the particles are ruptured and solubilization of the radioactive protein takes place, especially during the combined action of Triton WR 1339 and CCl_4. The fact that the distribution of the injection of protein (horseradish peroxidase) did not coincide exactly with the distribution of lysosomal enzymes after loading of the lysosomes with Triton WR 1339 also was observed by Wattiaux [8]. However, when comparing the results of biochemical tests with those of electron-microscopic examination of the liver, Wattiaux selects the second alternative as the most probable explanation: Lysosomes of the Kupffer cells of the liver participate in the segregation of foreign proteins.

Modified proteins are known to be largely ingested by the Kupffer cells of the liver, especially is administered in small doses as was the case in the present investigation. It is therefore possible to regard the selective role of the lysosomes of the Kupffer cells of the liver as a response ensuring the more rapid regeneration of the liver in chronic hepatitis.

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LITERATURE CITED

1. T. A. Korolenko, V. G. Titova, S. G. Dobrovol'skaya, et al., Byull. Éksp. Biol. Med., No. 1, 21 (1975).
2. M. Davies, Biochem. J., 136, 57 (1973).
3. C. De Duve, B. C. Pressman, R. Gianetto, et al., Biochem. J., 60, 604 (1955).
4. C. De Duve, T. De Barsy, B. Poole, et al., Biochem. Pharmacol., 23, 2495 (1974).
5. A. H. Gordon, in: Lysosomes in Biology and Pathology (ed. by J. T. Dingle and H. B. Fell), Vol. 3, Elsevier, Amsterdam (1973), p. 89.
6. J. L. Mego, in: Lysosomes in Biology and Pathology (ed. by J. T. Dingle and H. B. Fell), Vol. 3, Elsevier, Amsterdam (1973), p. 527.
7. J. L. Mego and J. A. Cain, Biochim. Biophys. Acta, 297, 343 (1973).
8. R. Wattiaux, Étude Expérimentale de la Surcharge des Lysosomes, Louvain (1966), p. 60.