ACTION OF HIGH-MOLECULAR-WEIGHT TOXIN FROM BURNED SKIN ON THE LYSOSOMAL APPARATUS OF HEPATOCYTES

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In the last decade some convincing proof has been obtained of the formation of toxic products in burned skin [7]. Interest accordingly has risen in the analysis of functional changes in the body during simulation of burn toxemia following injection of purified toxic substances. The cardiotropic nature of "Rosenthal's glycoprotein" has been established [10, 11]. The toxic "Schoenenberger's lipoprotein" damages the liver mitochondria and leads to loss of glycogen by the cells [12, 13]. The activity of low-molecular-weight peptides revealed by R. I. Livshits [2] is linked with the state of the molecular-cellular-enzymic barrier.

The work of Fedorov et al., concentrated on histogenic factors of toxemia and, in particular, on a high-molecular-weight toxin of burned skin [4, 7]. The high pathogenicity of the toxin [5] and its diverse biological activity [3, 9] were demonstrated, and methods of detoxicating therapy were outlined [6, 7].

In its structure the toxin is a glycoprotein and, in this respect, it is similar to "Rosenthal's glycoprotein." The action of all the toxins known at the present time has been studied extremely inadequately. In particular, the action of toxins on permeability and other properties of biological membranes has received little study, although most of the vitally important processes, under both normal and pathological conditions, are connected with activity of membranes. A major contribution to the understanding of the mechanism of action of the toxin must be made by the study of its effect on permeability of lysosomal membranes and activity of lysosomal enzymes. It is these factors which determine the development of destructive changes in the organs and tissues in burns, and for that reason their study on a model of burn toxemia must help to elucidate the mechanism of the pathogenetic action of the toxin at cellular and intracellular levels.

EXPERIMENTAL METHOD

Experiments were carried out on 46 Wistar rats weighing 170-200 g, receiving the ordinary animal house diet. Skin from burned Wistar rats was used as the source of toxic material. Extracts of burned skin (EBS) were prepared and the high-molecular-weight toxin isolated as described previously [8]. Characteristics of the preparations were described in [5, 9].

The animals were given intraperitoneal injections of physiological saline in a volume of 1 ml (group 1), native EBS in a dose of 20 mg protein (group 2), EBS exhausted on immunosorbents in the same dose (group 3), incompletely purified toxin (IPT) in a dose of 2-3 mg (group 4), and highly purified unstable toxin (HPT) in a dose of 0.5-0.9 mg (group 5). The rats were killed 2-2.5 h after injection of the preparations. Liver tissue was subjected to morphological examination.

A lysosome-enriched fraction was isolated from liver homogenate in 0.25 M sucrose by differential centrifugation. Permeability of the lysosomal membranes of the hepatocytes was judged from the ratio between unsedimented and total cathepsin D activities. Cathepsin D

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TABLE 1. Unsedimented Cathepsin D Activity and Autolysis in Rat Liver in Simulated Burn Toxemia (M ± m)

Group of animals	Unsedimented cathepsin D activity		Autolysis, μg tyrosine/mg protein
	μg tyrosine/mg protein	% of total activity	
1) Control	5,5±0,7 (n=14)	18,3±2,3 (n=14)	5,3±1,3 (n=6)
2) Injection of native EBS	11,2±2,9 (n=6)	39,4±6,6 (n=6)	6,6±0,9 (n=6)
Change %	+104	+115	—
3) Injection of exhausted ERS	8,9±1,3 (n=5)	32,0±4,7 (n=5)	6,3±1,3 (n=3)
Change, %	+62	+70	—
4) Injection of IPT	9,4±1,6 (n=8)	36,0±7,1 (n=8)	7,4±0,3 (n=6)
Change, %	+71	+97	+40
5) Injection of HPT	9,1±1,0 (n=12)	32,3+4,4 (n=11)	—
Change, %	+65	+80	—

Legend. Changes relative to control group shown as percentages when differences statistically significant.

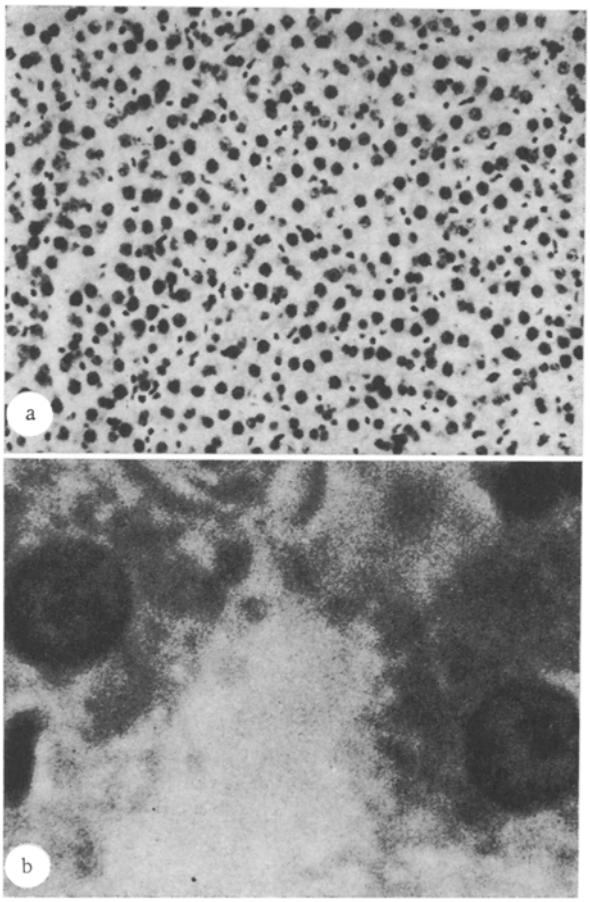

Fig. 1. Marked degenerative changes in liver tissue: Considerable dilatation of Disse's spaces, granular degeneration of liver cells — binuclear cells. Hematoxylin—eosin. Magnification: a) 80, b) 900 ×.

activity was expressed in micrograms tyrosine/mg protein/2 h of incubation with hemoglobin [1].

The increase in the content of protein breakdown products (aromatic amino acids), determined by the color reaction with Folin—Ciocalteu reagent, in liver homogenate incubated for 2 h at 37°C served as a quantitative characteristic of spontaneous autolysis.

EXPERIMENTAL RESULTS

The results of determination of unsedimented cathepsin D activity and spontaneous autolysis in the different groups of animals are given in Table 1.

The results showed that in all experimental rats the absolute values of unsedimented cathepsin D activity were considerably increased under the influence of preparations of high-molecular-weight toxins after precipitation of the lysosomal fraction of the liver. The increase was particularly marked after injection of native EBS (group 1:+104% compared with the control; $P < 0.05$). However, the remaining groups were characterized by a higher level of unsedimented proteolytic activity compared with the control. Differences between the experimental and control groups were significant in all cases.

An increase in the absolute value of unsedimented proteolytic cathepsin D activity is unambiguous evidence of the outflow of enzymes through the lysosomal membrane. An increase in the unsedimented fraction of total activity indicates that it was membrane permeability that increased. In all the experimental animals permeability of the lysosomal membranes of the hepatocytes was thus increased by the action of high-molecular-weight toxins from burned skin.

In the course of purification of the toxin from the original EBS, its injurious action on the lysosomal membrane was observed to diminish a little. It can be tentatively suggested that the membrane-toxic effect was due to the combined action not only of the toxin, but also of other products present in the native extract. This is confirmed indirectly by the fact that proteolytic activity was released from lysosomes by the "exhausted EBS" also — a preparation not containing toxin and not possessing toxic properties against animals or cell cultures.

That would explain why native EBS caused maximal migration of proteolytic lysosomal enzymes into the cytosol. The outflow of proteolytic and other lysosomal enzymes into the cytosol as a rule leads to intensification of autolysis and the development of destructive changes. No such changes as a rule were observed 1-1.5 h after burning, only edema and congestion of the liver tissue. However, 2-2.5 h after injection of IPT from burned skin into healthy rats, intensification of autolysis was observed by up to +40% compared with normal, and there were distinct changes in the morphological structure of the liver. Marked degenerative changes can be seen in Fig. 1, which shows the liver of a rat receiving an injection of IPT: dilatation of Disse's spaces and necrosis of single hepatocytes. Many binuclear cells could be seen in the substance of the liver tissue.

It can thus be concluded that burned skin contains substances with a marked membrane-toxic action, which may participate in the intensification of proteolysis in the course of burns. Whatever the case, a few hours after thermal burns of the IIIb degree, affecting 15% of the body surface, approximately the same degree of liberation of lysosomal enzymes took place [1], with the same action on the hemodynamics and the excretory and assimilative function of the liver, and on the permeability of the cell membranes of the hepatocytes.

Nevertheless, the question of the specificity of the injurious action of burn toxin on lysosomal membranes and the role of cooperative interactions between the toxin and other products from burned skin in the realization of its membrane-toxic action remains unsolved. Research in this direction will continue.

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