Morphological and functional characteristics of vesicles of vascular SMC thus enable them to be regarded as structures facilitating the elimination of calcium ions from the cell into the extracellular space against a high concentration gradient and damage to this apparatus in pathological disturbances of calcium metabolism.

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HEPATOCYTE ULTRASTRUCTURE IN MICE WITH CHRONIC T2 MYCOTOXICOSIS

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UDC 615.918:582.282].015.44:616.36-018.1-076.4

KEY WORDS: trichothecene mycotoxins; T2 toxin; hepatocyte ultrastructure.

T2 toxin is one of the commonest natural contaminants of food products belonging to the trichothecene mycotoxin group [7, 11]. Unlike most mycotoxins, the toxins of this group possess a wide range of toxic action, affecting the central nervous system, immunocompetent and hematopoietic organs, cardiovascular system, and gastrointestinal tract [7, 9, 10]. The principal organ in which most xenobiotics, including T2 toxin, undergo biotransformation and detoxication, is the liver [7]. The writers showed previously that administration of sublethal doses of T2 toxin to rats causes marked destruction of all intracellular organelles of the hepatocytes within 1-3 h, accompanied by disorganization of the liver enzymes [2].

The aim of this investigation was to study the effect of long-term administration of very small doses of T2 toxin on hepatocyte ultrastructure in mice.

EXPERIMENTAL METHOD

Experiments were carried out on male hybrid CBA × C57B1/6 mice receiving the normal balanced animal house diet and water ad lib. Animals of the experimental group received T2 toxin by gastric tube [8] in a dose of 0.33 mg/kg body weight (equivalent to 0.05 LD_{50}) for 1 month and in a dose of 0.45 mg/kg (0.067 LD_{50}) for the next 5 months. Mice of the control group received an equal volume of the solvent, namely 1% aqueous solution of ethanol. The animals were decapitated 24 h after the last injection. The liver tissue for electron microscopy was fixed in 4% paraformaldehyde solution in Hanks' buffer (pH 7.3) for 3 h. After washing to remove the fixative with buffer solution, the preparations were postfixed for 12 h with 1% OsO_4 solution in the same Hanks' buffer for 3 h at 4°C. The fixed prepara-

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 482-485, October, 1986. Original article submitted February 14, 1986.

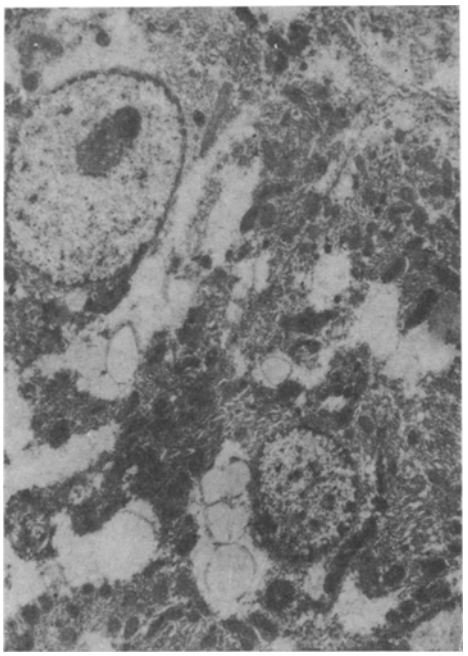

Fig. 1. General picture of liver lesion during chronic administration of T2 toxin. Heterogeneity of contents of hepatocytes. Foci of translucency of cytoplasm. 5000×.

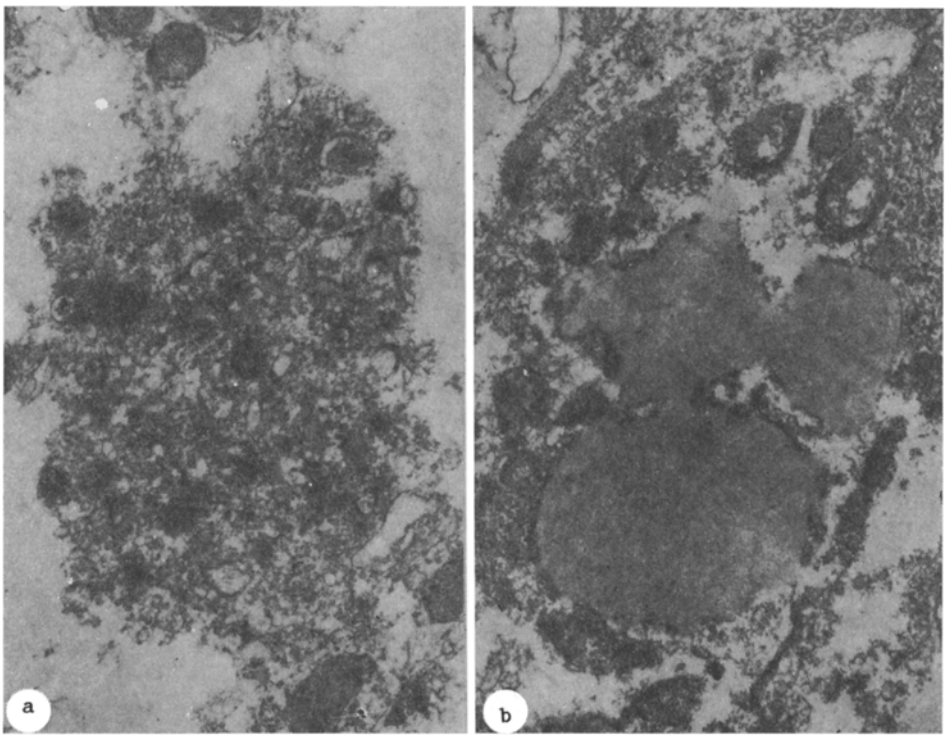

Fig. 2. Destructive processes visible in hepatocytes: a) zone of destruction of intracellular organelles (22,000×); b) accumulation of lipid droplets in hepatocyte cytoplasm (15,400×).

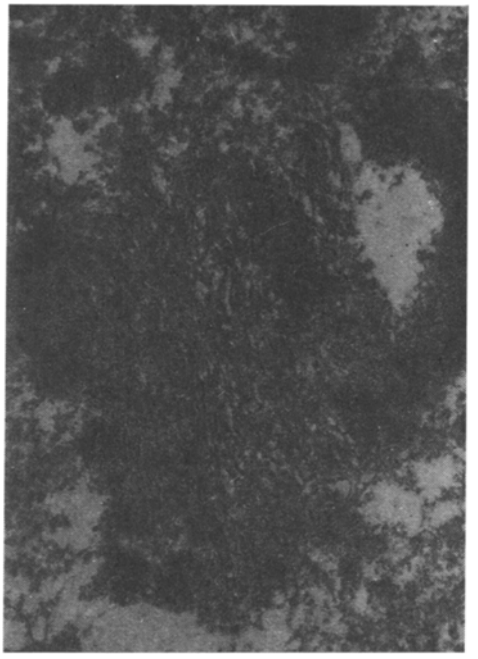

Fig. 3. Zone of intracellular regeneration of hepatocyte. System of endoplasmic reticulum. 17,300×.

tions were washed with buffer solution, dehydrated in acetone and alcohols, and embedded in a mixture of Epon and Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in JEM-7A and H-300 electron microscopes (Japan).

EXPERIMENTAL RESULTS

Throughout the experiments no toxic symptoms were observed in the mice of the experimental group, although toward the end of the 6th month their body weight was a little less than that of the control group. Macroscopically no changes likewise could be found in the internal organs of the experimental animals. However, examination of semithin sections revealed disturbances of the structure of the parenchymatous cells of the liver: they appeared paler and stained unevenly with toluidine blue.

Electron-microscopic study of the hepatocytes of the experimental animals revealed marked disturbance of the structure of all cells without exception. This was reflected mainly in the presence of large and small foci of translucency of the cytoplasm, distributed irregularly throughout the area of the hepatocytes (Fig. 1). They were located close to the nucleus and also near the cell membrane. Zones with homogeneous granular contents, most probably the disintegration product of intracellular organelles, in which the organelles were at different stages of destruction, also were seen in the hepatocytes (Fig. 2a). In the areas of translucency of the cytoplasm fragments of destroyed cisterns of the smooth and rough endoplasmic reticulum (ER) were observed. Frequently regions of cisterns of ER were fragmented, and on swelling, they came to look like vacuoles. Ribosomes in some cases lost their connection with the membrane of ER. Numerous lipid droplets, some of them of a considerable size, were located in the cytoplasm of the hepatocytes. These droplets were frequently grouped or fused together to form structures resembling bridges (Fig. 2b). The separate droplets had an uneven, eroded surface, evidence of active lipolytic processes taking place in the cells together with the formation of new lipid droplets. This conclusion was confirmed by the increase in the number of both primary and secondary (of cytosegresome type) lysosomes in the hepatocytes.

Besides destructive processes, foci of regeneration of different sizes also were noted in the overwhelming majority of liver cells. The contained an organized system of cisterns of the rough ER and many mitochondria (Fig. 3). The main mass of mitochondria in these foci showed no visible changes, but sometimes ring-shaped, branching, or simply elongated organelles could be seen. The presence of such atypical forms of mitochondria was more

characteristic of liver damage due to sublethal doses of T2 toxin [2]. Regions with evidence of regeneration most frequently occupied less than half of the total area of the hepatocyte cytoplasm, and only in occasional cells did they cover the whole cytoplasm. Zones of regeneration were located both near the nucleus and near the outer membrane of the hepatocytes. Incidentally, the ultrastructural changes found in the liver cells during chronic administratin of small doses of T2 toxin correlate well with changes in activity of organelle-specific liver enzymes [1]. Activity of the mitochondrial marker enzyme, succinate dehydrogenase, for example, and activity of enzymes located in ER (glucose-6-phosphate, aniline hydroxylase, carboxyl esterase, epoxy hydrolase) was significntly depressed. Meanwhile activity of the lysosomal hydrolases (β-galactosidase, β-N-acetylglucosaminidase, α-mannosidase, and aryl sulfatases A and B) was increased, evidently reflecting an increase in the number of lysosomes in the hepatocytes.

The marked destructive changes found in the smooth and rough ER and the fall in activity of enzymes bound with them may determine to a significant degree the toxic effects of T2 toxin, including its inhibitory action on protein biosynthesis [11]. The increase in functional activity of the lysosomal apparatus under conditions of marked destruction of the hepatocytes in chronic T2 toxicosis is connected with participation of lysosomes in the utilization of damaged cell components and it reflects their role in intracellular regenerative processes [4, 5]. Meanwhile activation of lysosomes can be regarded as a response to the development of a state of endogeneous protein insufficiency as a result of chronic exposure to T2 toxin, an inhibitor of protein insufficiency as a result of chronic exposure to T2 toxin, an inhibitor of protein synthesis, the end results of which are mobilization and redistribution of intracellular protein reserves [3, 5, 6].

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