EFFECT OF SOME EXTREMAL FACTORS ON THE SUBMICROSCOPIC ORGANIZATION OF THE LIVER IN RATS POISONED WITH CARBON TETRACHLORIDE

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UDC 615.917'412.133.07:616.36-091-092.9

The submicroscopic study of the parenchymatous cells of the liver of rats receiving carbon tetrachlorde (CCl_4) in a dose of 0.12 ml/g body weight repeatedly over a period of 25 days was studied. After the end of poisoning the animals were exposed to various extremal stimuli, namely immobilization, sunburn, or a combination of the two. Administration of CCl_4 caused the appearance of many lipid inclusions in the cytoplasm of the hepatocytes, the basis for fatty infiltration of the liver. Under the influence of extremal factors, accumulation of lipid material not found and not previously described in any other pathological condition, and evidently reflecting profound disturbances of intracellular metabolism, was discovered in the spaces of the rough cytoplasmic reticulum.

KEY WORDS: electron microscopy; liver; carbon tetrachloride; stress syndrome.

Various states of stress caused by certain extremal factors (nervous and emotional stress, exposure to various temperatures, physical exertion, prolonged immobilization) in man and experimental animals lead to definite disturbances both of metabolism and of the structure of organs concerned somehow or other in these processes [1, 2, 6, 8].

The character of the reparative reactions during experimental toxic hepatitis has been studied previously [11, 12] in investigations which established the regulatory effect of the pituitary–adrenal system on intracellular regeneration. This system is also known to play a role in the mechanism of stressor effects [9].

The object of the present investigation was to study the effect of various extremal exogenous factors on the submicroscopic structure of the pathologically changed liver.

EXPERIMENTAL METHOD

We investigated the liver tissue from male rats poisoned by subcutaneous injection of carbon tetrachloride (CCl_4) in a dose of 0.12 ml/100 g body weight on alternate days for 25 days. The animals were exposed to the action of the extremal factor as soon as administration of CCl_4 ended. Immobilization was carried out in the animal house by tying the experimental rats in the prone position for 10 min on alternate days for 20 and 35 days after the last dose of CCl_4. Immobilization by this method was carried out in the open air directly under the sun's rays (combination of immobilization and solar radiation). To study the isolated effect of solar radiation, the cage containing the animals was kept in the sun for 10 min once a day on alternate days for 20 and 35 days.

Pieces of liver for electron-microscopic study were fixed in a 6% solution of glutaraldehyde and postfixed in 1% OsO_4 solution. The material was embedded in Epon-812. Ultrathin sections were studied in the ÉM-100V electron microscope.

EXPERIMENTAL RESULTS

On the 25th day of CCl_4 administration the characteristic feature of hepatocyte ultrastructure in the animals in this series of experiments was that their cytoplasm contained numerous lipid granules of different sizes. No special selectivity could be observed in their localization, although in some cells migration of the lipid granules toward the sinu-

Research Institute of Local Medicine, Ministry of Health of the Uzbek SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 498-501, October, 1978. Original article submitted January 20, 1978.

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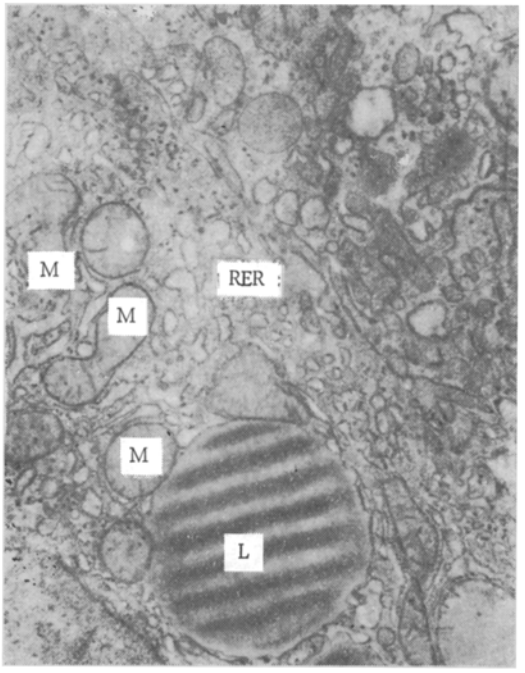

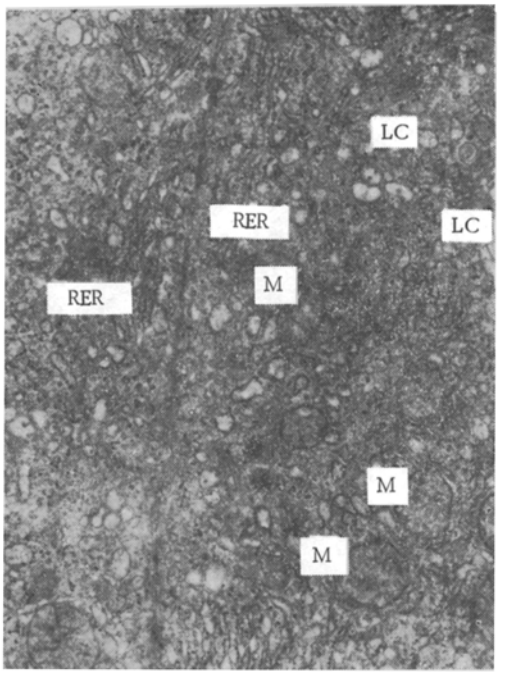

Fig. 1 Fig. 2

Fig. 1. CCl_4 poisoning (25th day). Lipid granules in cytoplasm of hepatocytes. Concentration of mitochondria in zone of lipid drops. Here and in Figs. 2 and 3: L) lipids; M) mitochondira; RER) rough endoplasmic reticulum; 22,500×.

Fig. 2. CCl_4 poisoning (60th day). Proliferation of rough endoplasmic reticulum. Mitochondria swollen, with fragmented cristae. LC) Lamellar complex; 22,000×.

soidal pole of the liver cells could be observed. Mitochondria were grouped in the zone where these granules were located; osmiophilic inclusions were present in the matrix of the organelles. The rough endoplasmic reticulum was composed of irregularly shaped cisterns with electron-transparent contents (Fig. 1).

By the 60th day "pale" cells were found much less frequently than at the time of the last injection of CCl_4, and they evidently consisted of hepatocytes in a state of fatty degeneration, detectable under the light microscope. Many lipid inclusions, varying in size and location, were observed in the cytoplasm of these cells. The mitochondria was swollen and the rough endoplasmic reticulum was in a state of vesicular fragmentation, wich numerous RNP-granules fixed on its membranes.

Cells of the second type had a darker matrix ("dark" cells) and their rough endoplasmic reticulum was relatively well developed. Many free ribosomes and polysomes were found in their cytoplasm. The lamellar complex, represented mainly by a system of flattened cisterns, occupied a large area (Fig. 2).

The literature devoted to changes in the ultrastructure of the liver cells in CCl_4 poisoning now numbers many titles [13]. In some of them [14] an attempt is made to explain the picture of fatty degeneration of the rat liver in response to CCl_4 administration as a result of disturbance of oxidative processes in the affected swollen mitochondria, the changes in which are evidently primary. However, this view is not supported by other investigators [4].

According to some workers [15] CCl_4 inhibits oxidative processes in the cell, and consequently the mitochondria are damaged, which causes a change in the energy balance of the cell. Although not postulating that the injuries to the mitochondria in the liver are primary, Yakobson [11] has suggested on the basis of his own observations and data in the literature a scheme of damage to the liver and adrenals by CCl_4; he suggests that this hepatotrophic poison interacts with the lipid membranes of organelles of the hepatocytes and also of cells of certain other organs and tissues, and induces the formation of peroxides, which impair the function of the mitochondria and of certain enzyme systems of the liver.

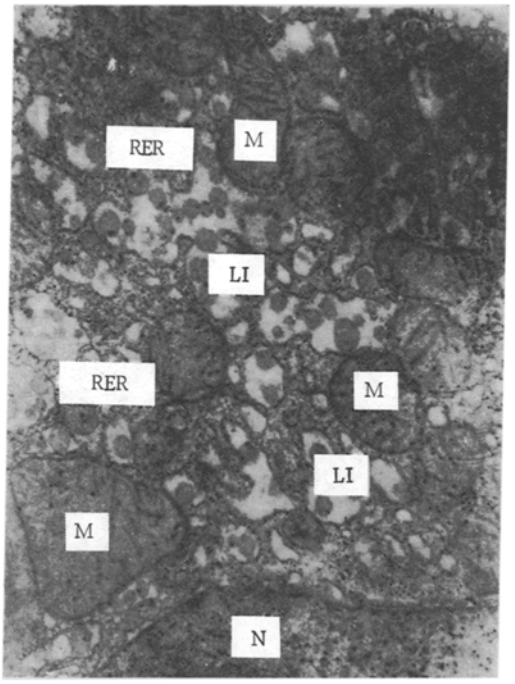

Fig. 3. CCl_4 poisoning (25th day), solar radiation (20 days). Dilatation of cisterns of rough endoplasmic reticulum and concentration of electron-dense contents — lipid inclusions (LI) — in them.

The results of a study of the submicroscopic structure of the hepatocytes during exposure to extremal factors showed that under the influence of exposure to sunlight for 20 days after the end of CCl_4 administration, besides numerous lipid granules, a considerable number of free ribosomes and clusters of polysomes also appeared in the cytoplasm of the liver cells. One of the most characteristic features was the presence of osmiophilic lipid-like inclusions in the spaces of rough endoplasmic reticulum (Fig. 3).

In animals immobilized for 35 days after the last injection of CCl_4 the number of lipid granules fell a little, although they were still found in the cytoplasm in a certain proportion of cases. Lipid inclusions disappeared from the cisterns of the rough endoplasmic reticulum. The number of mitochondria in the zones where the lipid granules were located was small.

A combination of solar radiation and immobilization led to the appearance of the two types of cells described above, evidence of different forms of their intracellular organization and differences in their functional state. Lipid-like inclusions were observed in cisterns of the rough endoplasmic reticulum.

The presence of a substance evidently of lipid nature in the cisterns of the endoplasmic reticulum, in the writers' view, is of considerable interest. Similar changes were found by Bondar' and co-workers [3] in the liver of patients with fatty hepatosis, but as these workers showed, the lipid inclusions were located chiefly in the agranular endoplasmic reticulum. Descriptions of the inclusions found by the present writers in the rough endoplasmic reticulum could not be found in descriptions given by other workers. The effect of extremal factors was evidently so considerable that profound disturbances of intracellular metabolism took place in the liver cells, and these were expressed in the submicroscopic organization of the hepatocytes.

Administration of CCl_4 rapidly leads to biochemical and ultrastructural changes in the parenchymatous cells of the liver. Frunder [10], 1 h after injection of small doses of CCl_4 into rats, observed reduction and fragmentation of the rough endoplasmic reticulum and swelling of the mitochondria, with consequent disturbance of the synthetic function of the liver. Later multiple infiltration of the liver cells with lipid drops appeared.

Injury to the rough endoplasmic reticulum, leading to depression of synthesis of lipoproteins, which are responsible for the evacuation of lipids from hepatocytes, is considered to be responsible for the accumulation of lipids in the liver.

The ultrastructural organization of the liver following exposure to extremal factors is nonspecific, although it has been suggested that the noxious agent causes nonspecific changes in the cell against the background of which the characteristic features of the reaction appear.

The effect of stress on the submicroscopic organization of the cells is also determined by the fact that, as a "syndrome of functional overstrain," stress behaves as a general pathological phenomenon which can take place at all levels of organization of living matter, including the ultrastructural level [5]. In every case the effect of exposure to extremal factors consists of functional overstrain of the corresponding structure. This standard syndrome has been defined by Kryzhanovskii [5] as the "standard form of intracellular dystrophic process."

Termination of exposure to stress alone led to intensified regeneration of the ultrastructures, expressed as the appearance of many "dark" cells, which reflect intracellular reparative processes aimed at restoring the functional and morphological capabilities of the cells [7].

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