MORPHOLOGICAL BASIS FOR HEPATIC FAILURE IN THE EARLY PERIOD OF THE CRUSH SYNDROME

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KEY WORDS: crush syndrome; liver; reticuloendothelial system for the liver; sinusoidal cells of the liver; stellate reticuloendotheliocyte (Kupffer cell).

Disturbances of liver function are of great importance in the clinical management of the crush syndrome (CS). The results of including the allogenic and xenogenic liver in the perfusion circuit of the crushed limb under clinical and experimental conditions indicate that the state of the liver can largely determine both the victim's recovery from shock and the development of acute renal failure [5]. Depression of many functions of the liver during crushing of the soft tissues has been observed during the first few hours of decompression, and may continue for a long time after trauma [3, 7, 12, 13]. For instance, disturbance of antitoxic function was described even 3 months after trauma [10] and depression of the protein forming function 5 years after trauma [11]. The mechanism of the disturbances of liver function in CS and its morphological basis have been inadequately studied. Nonspecific changes in the liver have been described, in the form of congestion and stasis of blood in the sinusoids, disappearance of glycogen from the hepatocytes, and microfocal necrosis [3, 4]. Insufficient attention has been paid in the pathogenesis of liver damage to the effect of the decompression factor, and no data are available on the state of the reticuloendothelial system (RES) of the liver in CS.

In the investigation described below the morphological basis and possible mechanisms of liver damage in the early period of CS were studied.

EXPERIMENTAL METHOD

The liver of rats and dogs with a severe form of CS, reproduced in animals with different typological features of their nervous system was studied; this was done in order to achieve the closest possible similarity between the model and CS in man, which is characterized by diversity of its clinical manifestations. Both hind limbs of 45 rats, under open ether anesthesia, were crushed with a force of 30 kg for 6 h. The soft tissues of one thigh of 20 dogs were crushed under thiopental anesthesia by metal forceps for 9 h. Morphological changes in the liver were studied at the end of the period of decompression and 2-7 h after decompression. Paraffin sections stained with hematoxylin and eosin and after the PAS reaction were examined. Pieces of liver for electron microscopy were fixed in 1% osmic acid and sections were stained with uranyl acetate and lead and studied in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

During the period of compression marked changes in structures of the microcirculatory system and cells of the RES and parenchyma, such as are observed in various types of shock, were found in the animals' liver. Arterioles of the portal tracts as a rule were in spasm, the sinusoids were dilated and congested in some places, and sometimes stasis was observed (Fig. 1a). Considerable changes were found in RES of the liver. Besides actively functioning (i.e., large, stellate) reticuloendotheliocytes (Kupffer cells, KC) with numerous processes, and with an increased content of endocytotic structures, lysosomes, and profiles of the rough endoplasmic reticulum, other KC whose morphology reflected a state of depressed function,

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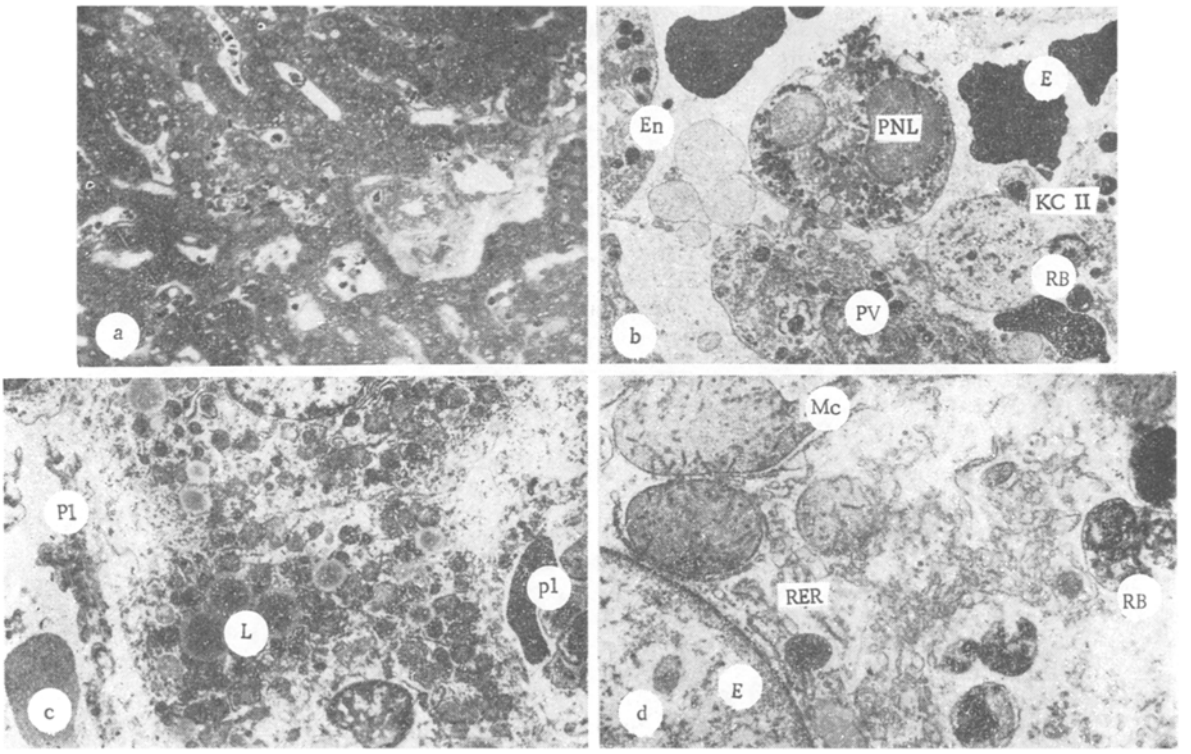

Fig. 1. Changes in the liver during compression of soft tissues: a) focal congestion of sinusoids, stasis. Semithin section. Methylene blue. 200×; b) KC (KC I) with many primary and secondary lysosomes (Ls) and KC (KC II) with signs of depressed function, containing residual bodies (RB), in a dilated sinusoid; endotheliocyte (En) contains many pinocytotic vesicles (PV) and lysosomes (Ls), concentrations of erythrocytes (E) and polymorphonuclear leukocytes (PNL). 6500×; c) lipid inclusions (L) can be seen in cytoplasm of hepatocytes, concentrations of platelets (Pl) in sinusoids. 5500×; d) chromatolysis in nucleus (N), translucency of cytoplasm, degranulated rough endoplasmic reticulum (RER), concentration of residual bodies (RB), swelling of mitochondria (Mc) with fragmented cristae. 25,500×.

and was characterized by single endocytotic structures and primary lysosomes, but with many large residual bodies (Fig. 1b), were constantly found. Changes in the endothelial cells of the sinusoids under these circumstances reflected an increase in their phagocytic activity: They contained many pinocytotic vesicles and primary and secondary lysosomes.

Hydropic, cloudy-swelling, and fatty degeneration were found in the parenchymatous cells of the liver (Fig. 1c), accompanied by enlargement of the nuclei, focal lysis of chromatin, degranulation of membranes of the rough endoplasmic reticulum, and concentration of residual bodies (Fig. 1d). Signs of a deficient energy supply for the liver cells were constantly found: A drastic decrease in the number of glycogen granules was accompanied by considerable swelling of the mitochondria and by a reduction in the frequency and length of the cristae. At the biliary poles of the hepatocytes large secondary lysosomes and lipofuscin granules were seen, in agreement with data of Lopatin [8], who found a tenfold increase in acid phosphatase activity in the liver during CS, with depression of the excretory function of the liver. As Lopatin showed, the half-elimination time of Ueviridin in dogs with a severe form of CS was lengthened from 3.08 min before crushing to 10.86 min ($P < 0.01$) after 4 h of crushing.

A leading role in the pathogenesis of the changes in the liver revealed during crushing evidently is played by the action of stressors such as catecholamines, whose excretion in traumatic shock may be increased by as much as 20-50-fold [6]. Such features of changes in the liver during CS as the development of hydropic and fatty degeneration of the cells, disturbance of blood clotting, and the paucity of changes of a compensatory character are also connected, evidently, with the harmful action of an excess of catecholamines on cell membranes, due to activation of lipid peroxidation (LPO) and of lipases and phospholipases in

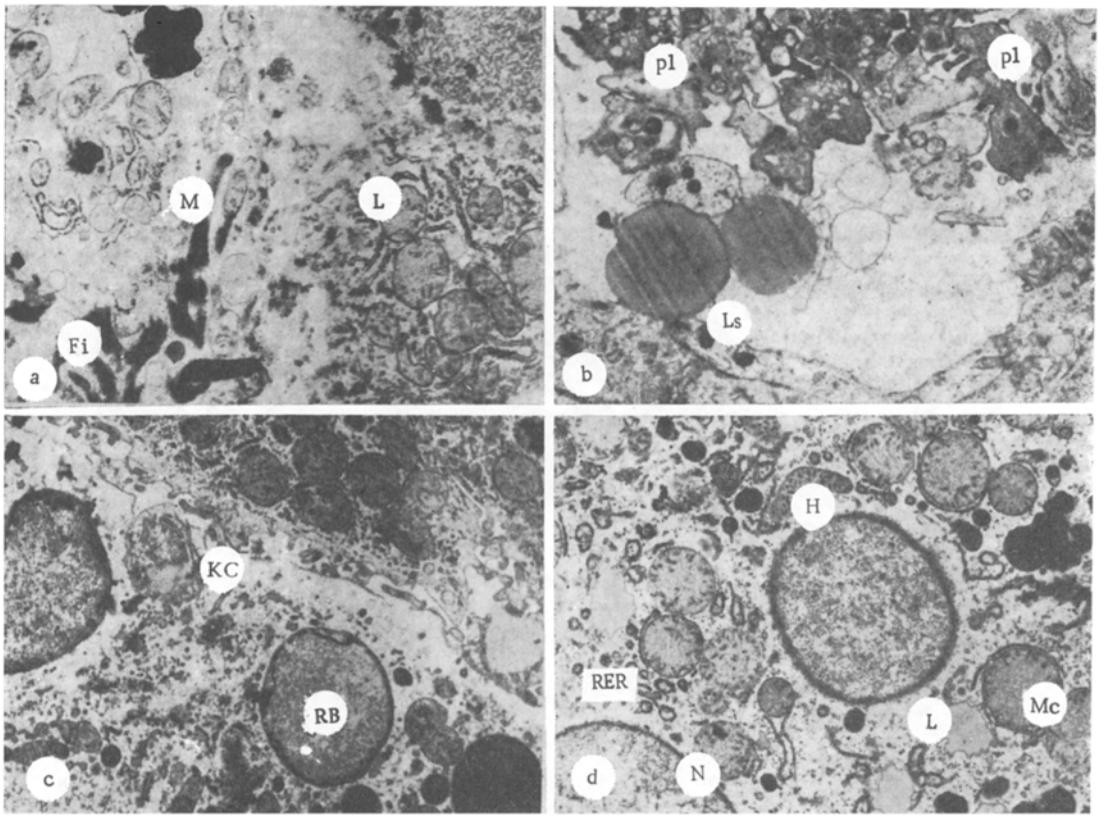

Fig. 2. Changes in liver during crushing: a) accumulation of degranulated platelets (Pl) in dilated sinusoid, solitary lysosomes (Ls) present in cytoplasm of swollen endotheliocytes. 10,000×; b) fibrin threads (Fi) and fragments of destroyed macrophages (M) in a sinusoid, destruction of sinusoidal pole of hepatocytes (H). 11,500×; c) swollen KC with residual bodies (RB) and fragments of "undigested" erythrocytes in a sinusoid, sinusoidal lining damaged. 11,500×; d) translucent cytoplasm of hepatocyte (H); nuclei (N) and mitochondria (Mc) swollen, rough endoplasmic reticulum (RER) is degranulated and forms circular structures. L) lipids. 11,500×.

them, and also on the microcirculatory system, with activation of contact activity of platelets and erythrocytes [2, 15, 16]. Changes in KC and endotheliocytes of the sinusoids reflect disturbance of the barrier function of the hepatic RES and the reduced ability of its cells to catabolize breakdown products, and they have a definite role in the mechanism of injury to the organ in the period of crushing.

Decompression of the limb led to progression of the destructive processes in the microcirculatory bed, RES of the liver, and hepatocytes, most marked in dogs. The dilated sinusoids, 2-4 h after removal of the press, contained not only erythrocytes but also fragments of destroyed hepatocytes, polymorphonuclear leukocytes, KC, and degranulated platelets (Fig. 2a), and 7 h after removal, they contained bundles of fibrin threads (Fig. 2b). The Disse's spaces were widened and often contained erythrocytes. Gross changes in RES of the liver were found, and were evidence of the development of failure and exhaustion of its function: The number of KC with indistinct outlines and with large residual bodies were observed more frequently in the sinusoids (Fig. 2c). The number of residual bodies also was increased in the endotheliocytes, which were "loaded" with secondary lysosomes, myelin figures, and "undigested" fragments of erythrocytes. Precursors of KC were not found, and lymphocytes also were absent. The morphological changes discovered in the sinusoidal cells of the liver are evidence of the development of failure and exhaustion of the hepatic RES. Insufficiency of this system evidently leads to progressive destruction of the parenchyma of the organ and to irreversibility of shock [14], for the performance of all the main functions of this organ, its powers of regeneration, and maintenance of immunologic homeostasis of the body is nowadays associated with the state of the hepatic RES [1, 8].

Changes in the hepatocytes correlated with the state of cells of the sinusoidal wall. With their more intensive destruction with an increase in the duration of decompression the

intensity of hydropic and fatty degeneration and signs of disturbances of protein synthesis increased in most hepatocytes: degranulation of membranes of the rough endoplasmic reticulum, fragmentation of its elements with the formation of circular structures, and a decrease in the number of ribosomes and polysomes (Fig. 2d). Changes in structures concerned with protein synthesis lead to the reduction in the total protein content of the liver discovered by Lopatin [8] during the first hours of decompression of the dog's limb. In this period signs of insufficiency of the antitoxic function of the hepatocytes became more marked: fragmentation and vacuolation of the smooth endoplasmic reticulum of the centrilobular hepatocytes. Meanwhile hyperplasia of the microperoxisomes, evidently aimed at compensating for this insufficiency, was observed in these cells. Evidence of a sharp decline in the detoxicating function of the liver during CS was given by the results of Lopatin's investigation [8], which showed shortening of the survival of paramecium in dogs' blood serum for 14 min in the control to 12 min toward the end of compression of the limb for 4 h, and to 5.2 min 1 h after removal of the crushing force.

After decompression, injuries in structures responsible for performance of the ingestive and excretory functions of the hepatocytes increased in severity: The microvilli of the sinusoidal pole were less prominent, subcytolemmal edema appeared, the number of pinocytotic vesicles was reduced, large lipofuscin granules were seen throughout the cytoplasm, the lamellar complexes became small, and the biliary tubules narrow. In central zones of the lobules of the liver areas of colliquative necrosis often were observed. Predominance of destructive over synthetic processes in the hepatocytes after removal of the crushing device [9] is evidently the result of the lytic effect of hydrolases, increased intensity of LPO, and disturbance of energy metabolism. The addition of such factors as plasmarrhea, hemoconcentration, and toxemia after decompression, while in the period of compression damaged cell membranes were present and catabolism activated, leads to progressive failure of the hepatic microcirculation, exhaustion of functions of the RES, and the development of necrobiotic and necrotic changes in the parenchyma.

Disturbances of liver function in CS under conditions of microculatory failure are thus due to destructive changes in the hepatocytes and cells of the hepatic RES, amounting in some cases to their necrosis. Morphological changes in the hepatic macrophages are evidence of disturbance of their barrier function, leading to toxemia. They play a role in the disturbance of regeneration of the liver and of the immunologic homeostasis of the organism.

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CHANGES IN STAINING PROPERTIES OF PURKINJE CELLS IN MICE WITH PROTEIN AND CALORIC DEFICIENCY AND DURING REHABILITATION

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Individual details revealed by the study of the cytologic structure of Purkinje cells of the cerebellum after exposure to various unfavorable conditions are largely associated with changes in their staining properties. In the ganglion-cell layer of the cerebellar cortex, besides pale neurons, dark, pyriform neurons are constantly found [12, 15]. An increase in the number of dark cells has been observed by many workers under a very wide range of experimental conditions, such as hypoxia [3], increased physical exertion [14], and protein and caloric deficiency [7]. There is also evidence that the number of dark neurons increases with an animal's age [11]. However, the question of the origin and functional role of the dark cells has not yet been finally explained. This is largely due to the fact that they are comparatively few in number if the brain is fixed by the perfusion method, but the number rises sharply if the brain is fixed by the immersion method [12].

Experimental results obtained by the writers previously [7] show no correlation between an increase in the number of dark cells and the specific character of the conditions to which the animal is exposed. An increase in the number of dark cells can most probably be regarded as a change in the type of their metabolism under different extremal conditions.

The aim of this investigation was to study changes in the staining properties of cerebellar Purkinje cells during modification of intracellular free fatty acid metabolism by the drug carnitine, in the course of dietary rehabilitation after prolonged underfeeding in early postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on 40 CBA mice. Protein and caloric deficiency was produced by reducing the concentration of nutrients in the experimental diet by 50% compared with the control. The casein content of the diet in the control group was 10%, in the experimental group 5% [5]. In the experiments of series I the cerebellum of young mice aged 40 days (n = 6) was studied immediately after a month of underfeeding. In the experiments of series II, underfed mice (n = 6) were transferred to a balanced diet from the 41st through the 70th day of life. In series III underfed mice (n = 6) received a balanced diet from the 41st through the 70th day of life, to which carnitine had been added. In all series of experiments mice (n = 12) receiving a synthetic balanced diet served as the control.

The animals were perfused with Karnovsky's fixative through the ascending aorta under pentobarbital anesthesia. The time from laparotomy until the beginning of fixation of the brain did not exceed 40 sec. Sagittal sections through the cerebellar vermis were postfixed in 2% OsO_4 solution, dehydrated, and embedded in Araldite. Under the light microscope, in semithin sections stained with methylene blue, the pale and dark pyriform neurons in the ganglion-cell layer of the cerebellar cortex were studied. The numerical results were subjected to statistical analysis by the method described in [4].

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