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CHANGES IN HEPATOCYTE STRUCTURE IN OLD RATS WITH POSTHEMORRHAGIC ANEMIA

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UDC 616.155.194-02:615-005.1]-07:616.36-091.8-053.9-092.9

KEY WORDS: aging; hepatocyte; blood loss; ultrastructure; morphometry.

Blood loss is accompanied by the development of processes aimed at replacing the lost plasma proteins. During aging the altered mechanisms of intracellular protein synthesis and its inadequate neurohumoral control [11] do not permit the initial plasma protein level to be fully restored [7]. Discovery of the factors constraining this process is therefore essential in the search for ways of correcting anemia and their particular features in old age.

The aim of this investigation was to discover age differences in ultrastructural changes effecting hepatocytes under conditions of posthemorrhagic anemia in order to determine the morphological substrate of the deficient protein-synthesizing function of the liver in old animals.

EXPERIMENTAL METHOD

Male laboratory albino rats of two age groups were used: 35 adult (aged 8 months) and 35 old (25 months) rats. The rats did not eat but had free access to water for 12 h before the experiment. Intact rats (group 1) served as the control. The animals were bled to the extent of 2% of body weight from the caudal artery once, and the liver was investigated after 15 min, 2.5, 5, 10, and 24 h, and 7 days later (animals of groups 2, 3, 4, 5, 6, and 7, respectively). Each group contained five adult and five old animals. Pieces of liver for light microscopy were fixed in acetic-alcohol-formalin [4]. Paraffin sections 7 μ thick were stained with hematoxylin and eosin; the number of binuclear hepatocytes per 1000 mononuclear cells was counted in them; the dimensions of the cytoplasm and nuclei were determined on a Leitz ASM instrument. Fixation with 3% glutaraldehyde in phosphate buffer (pH 7.4) and postfixation with 1% osmic acid solution were used for electron microscopy; the material was dehydrated and embedded in Epon-812. Sections cut on the LKB-111 ultrotome were stained by Reynolds' method [15] and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

A distinguishing feature of the liver in old animals on light-optical microscopy (LOM) was the accentuation of the structure of the lobules due to the abundance of connective tissue, the larger number of binuclear cells (Fig. 1), and the increased average area of the hepatocytes (Fig. 2). On electron microscopy (EM) age differences were manifested as a decrease in the number of glycogen rosettes in the cytoplasm, clarification of its matrix, polymorphism of the mitochondria, an increase in the number of secondary lysosomes with lipofuchsin granules, and the appearance of postlysosomes, including myelin-like structures, in the old animals.

Under experimental conditions during LOM of the liver at different times after blood loss, changes such as anemia, disturbance of the complex structure of the hepatic trabeculae, and dystrophic changes in the hepatocytes, described previously [2 5], were discovered. Mean-

Laboratory of Morphology and Cytology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 2, pp. 229-232, February, 1987. Original article submitted May 5, 1986.

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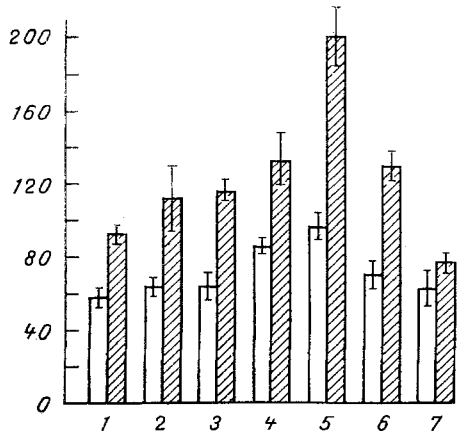

Fig. 1

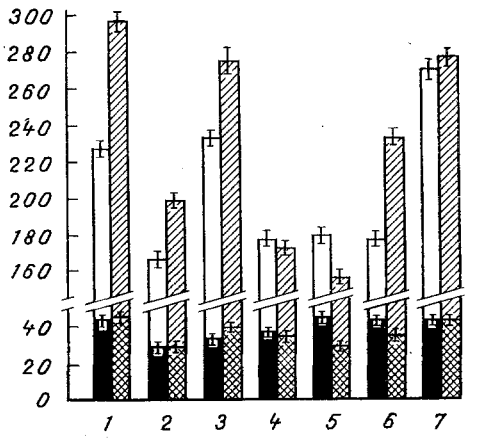

Fig. 2

Fig. 1. Changes in number of binuclear hepatocytes after blood loss in adult (unshaded columns) and old (shaded columns) rats. Abscissa (here and in Fig. 2), time after blood loss and groups of animals): 1) control (intact rats), 2) 15 min, 3) 2.5 h, 4) 5 h, 5) 10 h, 6) 24 h, 7) 7 days after blood loss; ordinate, number of binuclear hepatocytes per 1000 mononuclear cells.

Fig. 2. Changes in area of hepatocytes and their nuclei in adult and old rats. Unshaded columns — area of hepatocytes in adult animals; black columns — area of nuclei in adult animals; obliquely shaded columns — area of hepatocytes in old animals; cross-hatched columns — area of nuclei in old animals. Ordinate: mean area of hepatocytes and nuclei (in μ^2).

while clear age differences in the response of the hepatocytes in the early stages after anemia were discovered only with the aid of morphometric analysis and EM.

The area of the hepatocytes and their nuclei 15 min after blood loss was less than in the control (Fig. 2), possibly due to dehydration. The decrease in area of the cytoplasm, moreover, was more marked (34%) in the old animals than in the adults (27%), although the packing density of the cytoplasmic organelles was equal, as shown by EM at this stage of the experiment, possibly due to the larger quantity of hyaloplasm in cells of old intact animals. An increase in the area of condensed chromatin, located mainly near the karyolemma and in the perinucleolar region, took place in the hepatocyte nuclei. Very dense packing of the organelles, deaggregation of the mitochondrial cristae, and the formation of myelin-like structures were found in the cytoplasm; primary lysosomes and lipids were identified. Distinguishing features of the old animals included a larger number of injured mitochondria and lipid drops and the presence of glycogen rosettes in the cytoplasm of the hepatocytes (Fig. 3a).

After anemia for 2.5 h enlargement of the cells and nuclei was observed (Fig. 2). This process may be linked with rehydration and acidotic hyperhydration [1, 6, 13]. Under these circumstances, only the area of the hepatocytes in adult animals regained its initial value, due to the greater integrity of their subcellular structures. Processes of disintegration and death of the intracellular organelles, consisting of aggregation and disappearance of free ribosomes and polysomes, fragmentation and degranulation of the rough endoplasmic reticulum (RER), and destruction of mitochondria (Fig. 3b), took place more demonstratively in the cytoplasm of the hepatocytes of old animals (Fig. 3b).

The dimensions of the hepatocytes 5 h after blood loss in the adults and, to a greater degree, in the old animals were reduced, the result of death of the cytoplasmic organelles; the mean area of the nuclei changed variously: in the adult animals a tendency was observed for it to increase compared with the previous time (Fig. 2). This may be connected with the appearance of single nuclei rich in euchromatin.

The mean dimensions of the nuclei in adult animals continued to increase 10 h after the beginning of the experiment, and came close to the normal values, whereas in the old animals they were reduced (Fig. 2), possibly due to an increase in the number of binuclear (Fig. 1) and shrunken hepatocytes in that age group. At the same time activation of the protein-synthesizing apparatus was observed. In the adult animals this was manifested in the majority of

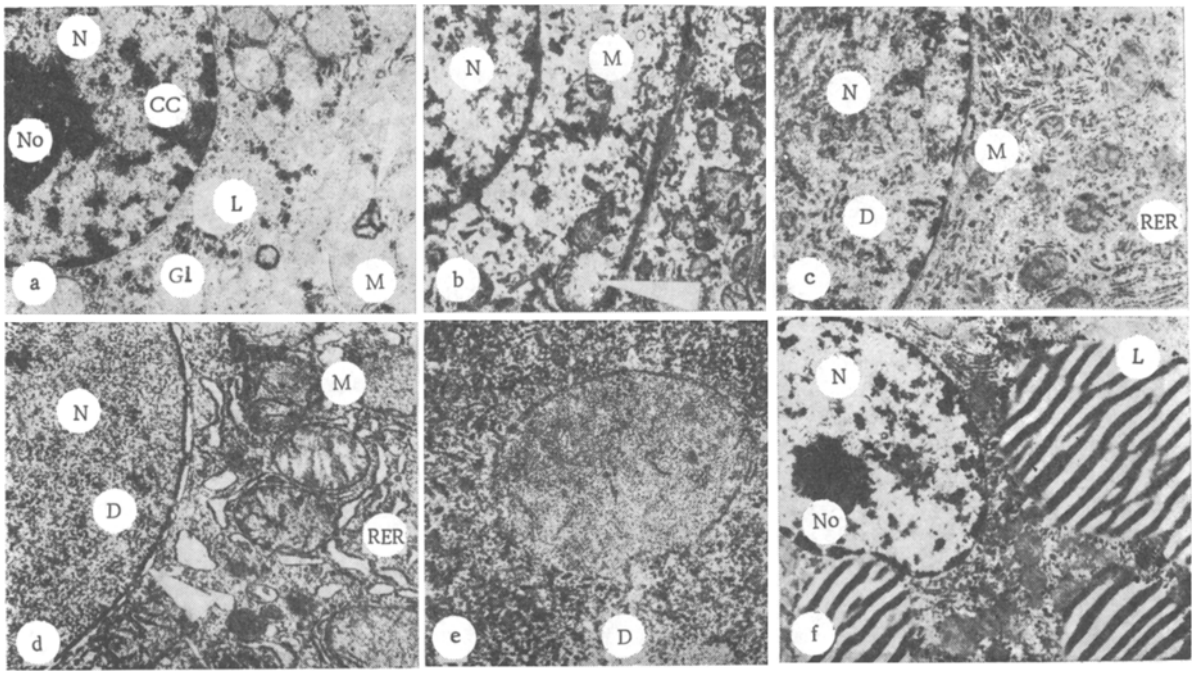

Fig. 3. Changes in number of injured mitochondria, lipid drops, and presence of glycogen rosettes in hepatocyte cytoplasm. a) 15 Min after blood loss, old rat (25 months): dense packing of cytoplasmic organelles, presence of glycogen rosettes and myelin-like structures in mitochondria (arrows). Here and in Fig. 3, b-f: N) nucleus, No) nucleolus, CC) condensed chromatin, L) lipids, Gl) glycogen. 9000×; b) 2.5 h after blood loss, old rats (25 months): edema of cytoplasm, fragmentation and degranulation of rough endoplasmic reticulum (RER), destruction of mitochondria (arrow). 6000×; c) 10 h after blood loss, adult rat (8 months): euchromatization, restoration of RER taking place in cytoplasm, with free ribosomes, polysomes, and mitochondria. DC) Decondensed chromatin. 10,000×; d) 10 h after blood loss, old rat (25 months): complete decondensation in nucleus with formation of osmiophilic type of chromatin; widened perinuclear space (arrow) and dilated tubules of RER, empty and degranulated, visible in cytoplasm. Mitochondria with translucent matrix and wide spaces between cristae. 17,000×; e) 10 h after blood loss, old rats (25 months); against the background of dense osmiophilic decondensation of chromatin in the nucleus round foci of delicate chromatin fibrils with bead-like structures can be seen in the nucleus. 30,000×; f) 7 days after blood loss, old rats (25 months): large-droplet fatty degeneration. 300×.

nuclei by euchromatization and by the nucleolonemal structure of the nucleoli, by an increase in the number of ribosomes and polysomes in the cytoplasm, and by restoration of the RER and mitochondria (Fig. 3c). The ultrastructural changes in the old animals were unequal. In cells with a few ribosomes and polysomes in their cytoplasm, with dilated and empty tubules of the RER and perinuclear space, and with a reduced number and size of the pores in the nuclear membrane, only osmiophilic, decondensed chromatin could be observed (Fig. 3d). In some of these cells, against the background of dense, decondensed chromatin, round foci of delicate electron-translucent chromatin fibrils with bead-like structures formed; these were perhaps active regions of the genome with a nucleosomal organization of the chromatin (Fig. 3e). Despite the fact that condensed chromatin was virtually completely absent in nuclei of this kind, they were functionally imperfect because of their reduced transcription activity, on account of the more compact packing of the chromatin fibrils [3, 14], as shown by the osmiophilic character of this chromatin. The deficient synthetic function of cells with such nuclei can be judged from the reduced and disturbed protein-synthesizing apparatus in the cytoplasm compared with the hepatocytes of adult animals, in which the nuclei contained euchromatin of an electron-translucent type. Other cells of the old animals at this time were shrunken, with densely packed organelles, and sometimes with partial necrosis of the cytoplasmic structures and with pycnotic nuclei.

Age differences could still be identified at the light-optical level of investigation 24 h after blood loss. Whereas in adult animals no signs of damage to the hepatocytes could be observed, in the old animals cloudy-swelling and fatty degeneration, and necrobiosis and necrosis of individual hepatocytes and of groups of cells located mainly interlobularly, were distinctly seen in the old animals. Under these circumstances karyopycnosis, karyorrhexis, and karyolysis were visible in the nuclei. The area of the hepat cytes and of their nuclei were increased in the old animals (Fig. 2), possibly due to the appearance of widespread fatty degeneration which, as could be seen on EM, was of the large-droplet type, and was accompanied by a decrease in the number of binuclear cells (Fig. 1).

On the 7th day the dimensions of the hepatocytes in the adult animals were increased, and exceeded their initial values; according to the EM data, this takes place mainly through an increase in the number of cytoplasmic organelles and glycogen accumulation and it can be regarded as a manifestation of intracellular regenerative hypertrophy [8]. In old animals this process is limited, as shown by the dimensions of the hepatocytes, which hardly reach their initial level (Fig. 2), on account of fatty degeneration (Fig. 3f). This last fact may indicate a decrease in the synthesis of transport proteins and (or) diminution of β-oxidation of fatty acids in damaged mitochondria [9] which, as shown by EM, are much more numerous in old animals at all times after blood loss. This state of affairs can be explained by increased activity of lysosomal enzymes and of phospholipase A_2, a "mitochondrial killer" under hypoxic conditions [12].

Almost throughout the duration of the experiment the formation of intranuclear lipid and membrane-like inclusions was observed, as is found in other organs and tissues also during aging [10]. Their presence may indicate profound changes in the nuclear apparatus of the cells, which are exhibited when their load is increased.

These results thus indicate that blood loss leads to considerable changes in the hepatocytes, which may either cause injury to them or stimulate their synthetic processes. Qualitative and quantitative analysis of age differences in their parenchymatous cells of the liver after blood loss indicate that injuries are manifested to a greater degree in the hepatocytes of old animals, whereas repair processes are not so pronounced and take place at later stages than in adult animals. Changes in the genetic apparatus under these circumstances may be blocking stages for the adequate functioning of hepatocytes in old animals.

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