STRUCTURAL HOMEOSTASIS AND ITS POSSIBLE MECHANISMS DURING FORMATION OF THE PARENCHYMAL RESPONSE TO LIVER DAMAGE

V. A. Shkurupii

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Ideas on the ability of organisms to maintain the constancy of their own internal milieu have been formulated on the basis of investigations chiefly of the mechanisms ensuring constancy of their various physiological constants [2]. Meanwhile, the mechanisms of structural homeostasis of the organism have received far less study [2, 5].

The aim of this investigation was to study morphological manifestations of the probable mechanisms of structural homeostasis of the liver parenchyma during formation of the response to injury.

EXPERIMENTAL METHOD

The liver of C57BL/6 mice aged 2 months and weighing 19-21 g was investigated. The animals inhaled CCl_4 in a dose of 0.025 ml/liter of air during an exposure of 10 min in a chamber [8]. The mice were decapitated 6, 24, 48, 72, and 96 h after the end of inhalation of the poison, between 9 and 10 am. The liver from five mice was studied in each of these groups and in the control. Samples of liver for light and electron microscopy were fixed in 1% OsO_4 solution in phosphate buffer for 2 h, dehydrated in alcohols, and embedded in Epon. Epon sections 1 μm thick were stained with toluidine blue and used for karyometry, measurement of the nucleo-cytoplasmic ratios, and calculation of the volumes of the hepatocytes, their nuclei, and cytoplasm. Ultrathin sections were studied in the JEM 100S/SEGZ/ACID electron microscope. Morphometry was carried out in accordance with the recommendations in [11]. Hepatocytes outside zones of destructive changes were investigated stereometrically. Differences between mean values compared were considered significant at the $P < 0.05$ level (Student's t test).

EXPERIMENTAL RESULTS

Six hours after injection of CCl_4 hepatocytes with the most marked changes of fatty degeneration type were observed in the central and intermediate zones of the hepatic lobules [7]. At this stage the volume of the hepatocytes and of their nuclei was reduced (Table 1, Fig. 1), possibly as a result of division of the cells before they developed dystrophic changes. After 24 h, the hepatocytes in the center of the lobules showed necrotic changes.

TABLE 1. Results of Morphometry of Liver Parenchyma of Male C57BL/6 Mice (M ± m)

Parameter	Control	Time after injection of CCl_4, h				
		6	24	48	72	96
Volume of parenchyma in liver, %	89,5±0,93	86,9±1,60	87,5±1,30	86,3±1,20	87,0±0,86	86,1±1,10
Volume of hepatocytes, μm^3	3883,2±351	2487,0±358*	5231,0±463*	5203,0±266*	5634,2±468*	5737,8±518*
Number of mitochondria in hepatocyte	570±57	226±38*	535±68	572±68	715±77	837±98
Total volume of nucleoli	3,1±0,20	2,9±0,22	4,8±0,31*	3,6±0,34	3,7±0,20	3,7±0,24

Legend. Volume of nucleoli expressed as percentage of volume of nucleus. Here and in Table 2, asterisk indicates significant difference from control.

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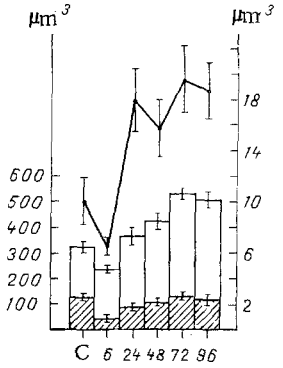

Fig. 1

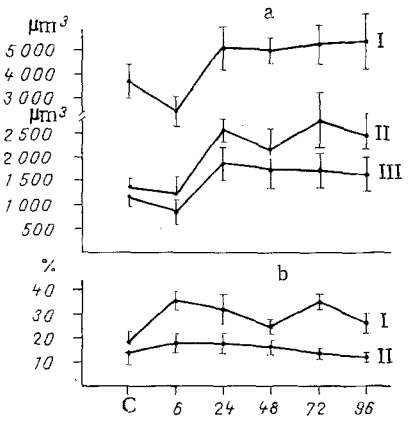

Fig. 2

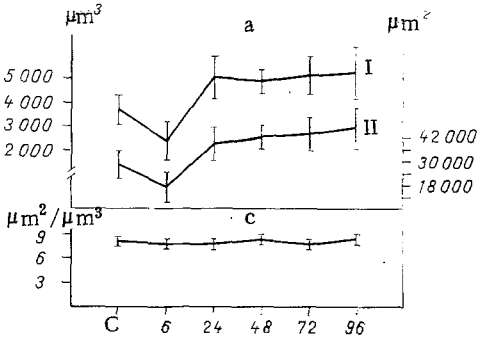

Fig. 3

Fig. 1. Results of morphometry of hepatocyte nuclei. Unshaded part of column — volume of nuclei, shaded part — volume of condensed chromatin of hepatocyte nuclei (both parameters plotted along ordinate on left, in μm^3). Top curve shows total volume of nucleoli (plotted on ordinate on right, in μm^3). Here and in the other figures: abscissa, time after end of CCl_4 inhalation (in h). C) Control. Results of morphometry of 100 nuclei (20 from each animal).

Fig. 2. Results of investigation of volumes (in μm^3) and bulk densities (in %) of cytoplasm and of cytoplasmic structures of hepatocytes. a: I) Volume of cytoplasm of hepatocytes; II) volume of cytoplasmic structures (including glycogen and lipids) in one hepatocyte; III) total volume of cytoplasmic organoids. b: I) Bulk density of cytoplasmic structures (including glycogen and lipids) of hepatocytes; II) bulk density of cytoplasmic organoids.

Fig. 3. Results of investigation of volumes (in μm^3) of hepatocyte cytoplasm, of surface areas of cytoplasmic organoids (in μm^2), and of surface density of membranes of cytoplasmic organoids of hepatocytes (in $\mu m^2/\mu m^3$ of hepatocyte cytoplasm). a: I) Mean volume of cytoplasm of hepatocytes; II) total surface area of membranes of cytoplasmic organoids. b: Surface density of membranes of cytoplasmic organoids.

TABLE 2. Results of Study of Surface Densities (in $\mu m^2/\mu m^3$ volume of cytoplasm) of Membranes of Intracellular Organoids (M ± m)

Test object	Control	Time after injection of CCl_4, h				
		6	24	48	72	96
Mitochondria						
outer membrane	1,42±0,11	0,87±0,04*	1,16±0,05*	0,98±0,04*	1,10±0,04*	0,91±0,04*
inner membrane	3,67±0,29	3,25±0,24	3,42±0,23	3,32±0,23	3,31±0,24	3,55±0,25
Rough endoplasmic reticulum	2,57±0,19	2,50±0,16	2,68±0,18	3,49±0,18*	2,88±0,17	3,30±0,18*
Peroxisomes	0,31±0,03	0,27±0,03	0,22±0,02*	0,26±0,03	0,26±0,03	0,30±0,03
Lysosomes	0,24±0,02	0,30±0,04	0,27±0,03	0,33±0,04*	0,19±0,02	0,19±0,02

The volume of the undamaged cells and of their nuclei and cytoplasm was increased compared with the control. Signs of activation of nucleolar function were observed. The nucleoli became honeycombed in structure and their total volume was increased. The volume of condensed chromatin in the nuclei was reduced, evidence of activation of genome transcription. Evidently as a result of this the number of free and attached ribosomes, counted per cell, increased. These changes in the nuclei, and the volume of the hepatocytes and number of ribosomes increased until 48 h (the peak of necroses in the center of the lobules), and were maximal 72 and 96 h after inhalation of CCl_4 — the period of marked reparative regeneration (Table 1, Fig. 1).

The weight of the liver was restored after injury by proliferation and hypertrophy of the cells. Polyploidy, one cause of hypertrophy of cells [4], is a variant of proliferation in which the mitotic cycle does not proceed to its end [1]. Depending on the type of liver damage, DNA synthesis in hepatocyte nuclei reaches a peak between 22 and 34 h after poison-

ing [10]. Liver damage in mice led to entry of nearly all remaining hepatocytes into the mitotic cycle [6]. In accordance with these findings and the results shown in Table 1 and Fig. 1, it can be postulated that processes of hypertrophy of hepatocytes as a result of their polyploidization predominated in the mouse liver 24-96 h after inhalation of the poison.

A decrease or increase in functional activity of cells has a material basis [5]. The decrease or increase in volume of the cytoplasm of the hepatocytes at different periods of formation of the response to inhalation of CCl_4 was in fact accompanied by corresponding changes in the total volume of the cytoplasmic organoids (Fig. 2, I, III).

Since the endoplasmic reticulum of smooth type is masked by glycogen, and 6 h after inhalation of CCl_4 it was "uncovered" and its membranes and those of the Golgi complex disintegrated, making over- or underestimation of their content in the cell possible, they were not subjected to morphometry.

The relative total volume of the organoids remained constant and did not depend on changes in the volume of cytoplasm of the hepatocytes (Fig. 3a, I; b, II). Changes in the relative total volume of all subcellular structures, including glycogen and lipids, did not agree in "sign" with changes in volume of the cytoplasm (Fig. 3b, I; a, I). The increase in the relative total volume of the structures 6 h after inhalation of CCl_4 (Fig. 3b, I) was due to an eightfold increase in the lipid content (compared with the control), whereas 72 h after inhalation it was due to a ninefold increase in the volume of glycogen (Fig. 3b, I). Consequently, even the considerable changes in volume of the cytoplasmic inclusions could not affect the volume of cell cytoplasm, as was observed previously [9] under different experimental conditions. Changes in permeability of the cytoplasmic membranes in different functional states of the organoids essentially determine the volumes of the compartments formed by them. For that reason, to judge the true character of changes in concentration of structures in the hepatocytes associated with fluctuations in the volume of their cytoplasm, it is more appropriate to study the concentration of cell membranes. The total concentration (in $\mu m^2/\mu m^3$ cytoplasm) of membranes of the organoids was constant in hepatocytes of animals of all groups, but the total area (in μm^2), calculated per cell, corresponded to fluctuations in volume of the cytoplasm (Fig. 3), indicating proportionality of the change in content of cell membranes in hepatocytes and changes in the volume of their cytoplasm, i.e., it indicates constancy of their concentration in the hepatic parenchyma. The character of the change in the number of ribosomes at different periods of observation was similar in its trend to changes in the volume of the cytoplasm (Figs. 2 and 3a, I). It has been shown that changes in dry weight of hepatocytes, enzyme activity, protein synthesis [12-15], and total area of nucleoli [1] vary in accordance with the dose of the genes with a change in ploidy of hepatocytes. For instance, in the present investigation the relative total volume of the nucleoli, including the period of 24 h after inhalation of CCl_4, remained constant (Table 1). At the same time, the concentration of membranes of individual organoids in some cases changed significantly from one stage of the investigation to another (Table 2) whereas the total concentration of membranes of the organoids studied remained within constant limits. The concentration of the inner membranes of the mitochondria was constant at all times of the investigation, despite changes in the number and size of these organoids (Tables 1 and 2). However, in the case of a considerable increase in volume of the mitochondria, forms of them with very few cristae appeared. Despite changes in volumes of hepatocytes during formation of the response to CCl_4, the relative volume of the parenchyma in the liver, like the concentration of subcellular membranes in it, remained constant (Table 1). Consequently, changes taking place in the liver parenchyma were "aimed" at restoring the lost number of genomes and preserving its structural constancy. The realization of the various discrete functions of the hepatocytes under these circumstances was accompanied by structural transformations at cellular and subcellular levels under conditions of limited reserves of plastic materials. Differences which exist in the organization of membranes of different cell structures prevent exchange of membranes between organoids, either directly or through realization of the reconstructive function of the lysosomes [3], for activation of autophagy was not observed in this study. It can be tentatively suggested that, with a decrease in the initial number of genomes and weight of the structures in the case of loss of part of the cell population of the tissue, in cells which still remained there was not just a simple multiple change in the weight of the intracellular structures, but a distinctive kind of redistribution of the limited reserves of plastic materials for synthesis of the various structures depending on the functional demands made on the organ.

This may perhaps explain the fluctuation in concentration of membranes of individual subcellular structures while the total concentration of membranes of all the organoids studied remained constant.

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ACTION OF ANGIOTENSIN ON ULTRASTRUCTURE OF THE RAT THYROID GLAND

L. V. Gerbil'skii

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Angiotensin is a peptide hormone which regulates the functional state of cells of various organs [4, 9]. We know, in particular, that angiotensin controls the function of several endocrine organs, namely the hypothalamus, adenohypophysis, and adrenals [5, 14, 15, 16]. After injection of angiotensin into rats the blood flow in the thyroid gland has been shown to be significantly reduced [13]. On incubation of fragments of rat thyroid gland in the presence of angiotensin, accumulation of radioactive iodine by the cells of this gland is considerably inhibited [5]. However, the action of angiotensin on the structure of the thyroid gland has not previously been studied. The aim of the present investigation was accordingly to study the ultrastructure of exchange microvessels and follicles of the thyroid gland in rats receiving angiotensin.

EXPERIMENTAL METHOD

Experiments were carried out on 32 male rats weighing 200-300 g. Sixteen rats were used for the radiometric tests. Angiotensin was injected intraperitoneally into six experimental animals in a dose of 1 mg in 1 ml physiological saline; ten control animals received 1 ml of physiological saline alone. All animals were given an intraperitoneal injection of ^{131}I in a dose of 3 μCi at the same time.

Department of Histology, Dnepropetrovsk Medical Institute.

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