# MORPHOLOGY AND PATHOMORPHOLOGY

# Tissue and Intracellular Reorganization of Rat Liver during Total Body Hypothermia

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We studied tissue and intracellular reorganization of the liver during total body hypothermia and evaluated regeneration strategies at different levels of structural organization. Hypothermia results in morphofunctional changes in the liver (degeneration, lysis, necrobiosis, and focal necrosis of hepatocytes developing against the background of disorders in blood and lymph circulation). Decreased sinusoid/hepatocyte volume ratio is the key factor in tissue reorganization of the liver. Intracellular reorganization of hepatocytes is characterized by dysproportional changes in the volume and surface densities of the main cytoplasmic organelles involved in biosynthesis and energy production.

**Key Words:** *liver; hepatocytes; total hypothermia; ultrastructure; stereology*

Morphogenetic processes in the body induced by exposure to extreme factors largely depend on reliability of the detoxification and synthetic systems of the liver, which are realized in the same subcellular structures of hepatocytes [2,7,9,11,13]. Pronounced changes in functional activity of tissue and cell systems are associated with their spatial reorganization, which, along with other processes (proliferation, differentiation, maturing, and hypertrophy) determines specific features of regeneration and compensatory-adaptive reactions of tissues and cells [1,3,4]. From this viewpoint, evaluation of regularities of spatial reorganization of tissue and cell systems is important for clearing out the regeneratory and adaptive strategies and direction of the morphogenetic processes. This circumstance necessitated the study of structural and functional rearrangement of the liver and regeneratory potential of hepatocytes during exposure to extreme ecological factors.

We studied tissue and intracellular reorganization of the liver in moderate total body hypothermia.

## MATERIALS AND METHODS

Complex morphological analysis of the liver was carried out in 5-month-old male Wistar rats ($n$=66). Control group consisted of 23 rats. Experimental animals were kept at 3-4°C in individual cages with free access to food and water for 6 and 8 weeks, after which they were sacrificed.

The liver was separated from adjacent tissues and rapidly weighed. Specimens of the liver were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin, by Van-Gieson's method with post-staining of elastic fibers by Weigert's resorcin-fuchsin, and PAS reaction was carried out. Liver specimens for electron microscopy were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and processed routinely. Semithin sections made on

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an LKB-III ultratome were stained with 1% Azur II and used for morphological study and stereological analysis. Ultrathin sections after contrasting with uranyl acetate and lead citrate were examined under a JEM 1010 electron microscope at accelerating voltage 40 kV.

Stereological analysis was carried out as described previously [4]. At the tissue level the volume density (relative volume) of hepatocytes, hepatocyte nuclei, sinusoids, summary connective tissue components, surface density (relative surface area) of hepatocytes, hepatocyte nuclei, and sinusoids were evaluated. Based on the values of the first stereological parameters, secondary parameters were estimated describing the quantitative relationships between different components of the stroma and parenchyma: surface/volume ratio of structures, stroma/parenchyma volume ratio, sinusoid/hepatocyte volume ratio, and sinusoid/hepatocyte surface volume ratio.

Ultrastructural stereological analysis of hepatocytes was carried out on photonegatives at a final magnification of 20,000 (primary magnification ×8000). Volume density of mitochondria, agranular endoplasmic reticulum (AER), granular endoplasmic reticulum (GER) was evaluated. Surface density was evaluated for mitochondria, AER, and GER. Based on the primary stereological parameters, secondary parameters were estimated: volume and surface/volume ratios of the main structures.

Statistical processing of the results included estimation of means for parameters, dispersion, and errors in the means. The significance of differences was evaluated using Student's *t* test.

## RESULTS

Total body hypothermia increased liver weight by 24% ($p<0.01$) and its relative weight by 22% ($p<0.05$) after 6 weeks of the experiment (Table 1). Changes in the liver tissue after hypothermia were stereotypical: hemodynamic disorders were paralleled by hepatocyte damage, causing mosaic staining of the liver parenchyma with acid stains. Along with destructively changed hepatocytes, there were hepatocytes with signs of intracellular regeneration and cells with different level of functional activity. These changes were expressed to a different degree for each term of the experiment. Loosening of the perinuclear zone, nuclear pyknosis were seen in some cells. Pronounced nuclear hypertrophy (including hypertrophy at the expense of huge lipid incorporation) was noted in hepatocytes with normal tinctorial characteristics, especially after week 6 of hypothermia.

Hypothermia for 6 weeks increased lipid production, which manifested in abundant lipid droplets in hepatocyte cytoplasm with their predominant accumulation at the sinusoidal poles (Fig. 1, *a*). Lipid incorporations, though less pronounced, were seen in

**TABLE 1.** Morphometry of Rat Liver after Total Body Hypothermia (*M*±*m*)

| Parameter | | Control | Duration of hypothermia | |
|---|---|---|---|---|
| | | | 6 weeks | 8 weeks |
| Body weight, g | | 275.0±7.3 | 284.0±11.5 | 410.7±29.7*** |
| Liver weight, mg | | 9800.0±600.0 | 12 200.0±500.0** | 13 600.0±1200.0* |
| Relative liver weight, mg/g body weight | | 35.7±2.0 | 43.7±2.5* | 33.1±1.1 |
| Volume density, $mm^3/cm^3$ | hepatocytes | 810.3±5.2 | 856.5±14.1* | 845.8±11.6* |
| | hepatocyte nuclei | 44.9±1.2 | 61.6±3.2** | 53.2±2.7 |
| | sinusoids | 141.2±8.6 | 96.8±6.5* | 108.3±7.6* |
| Surface density, $m^2/cm^3$ | hepatocytes | 0.2234±0.0058 | 0.2102±0.0030 | 0.2240±0.0018 |
| | hepatocyte nuclei | 0.0307±0.0009 | 0.0367±0.0019* | 0.0315±0.0028 |
| | sinusoids | 0.0887±0.0022 | 0.0677±0.0056* | 0.0802±0.0044 |
| Surface/volume ratio, $m^2/cm^3$ | hepatocytes | 0.276±0.006 | 0.246±0.008* | 0.265±0.004 |
| | hepatocyte nuclei | 0.683±0.009 | 0.596±0.020* | 0,588 ± 0,031* |
| | sinusoids | 0.643±0.040 | 0.705±0.042 | 0.779±0.026* |
| | sinusoids/hepatocytes | 0.109±0.002 | 0.079±0.008* | 0.095±0.012 |
| Volume ratio: | stroma/parenchyma | 0.234±0.008 | 0.168±0.019* | 0.183±0.016* |
| | nucleus/cytoplasm | 0.059±0.001 | 0.078±0.005* | 0.067±0.002* |
| | sinusoids/hepatocytes | 0.174±0.012 | 0.113±0.009* | 0.128±0.011* |

**Note.** *$p<0.05$, **$p<0.01$ compared to the control.

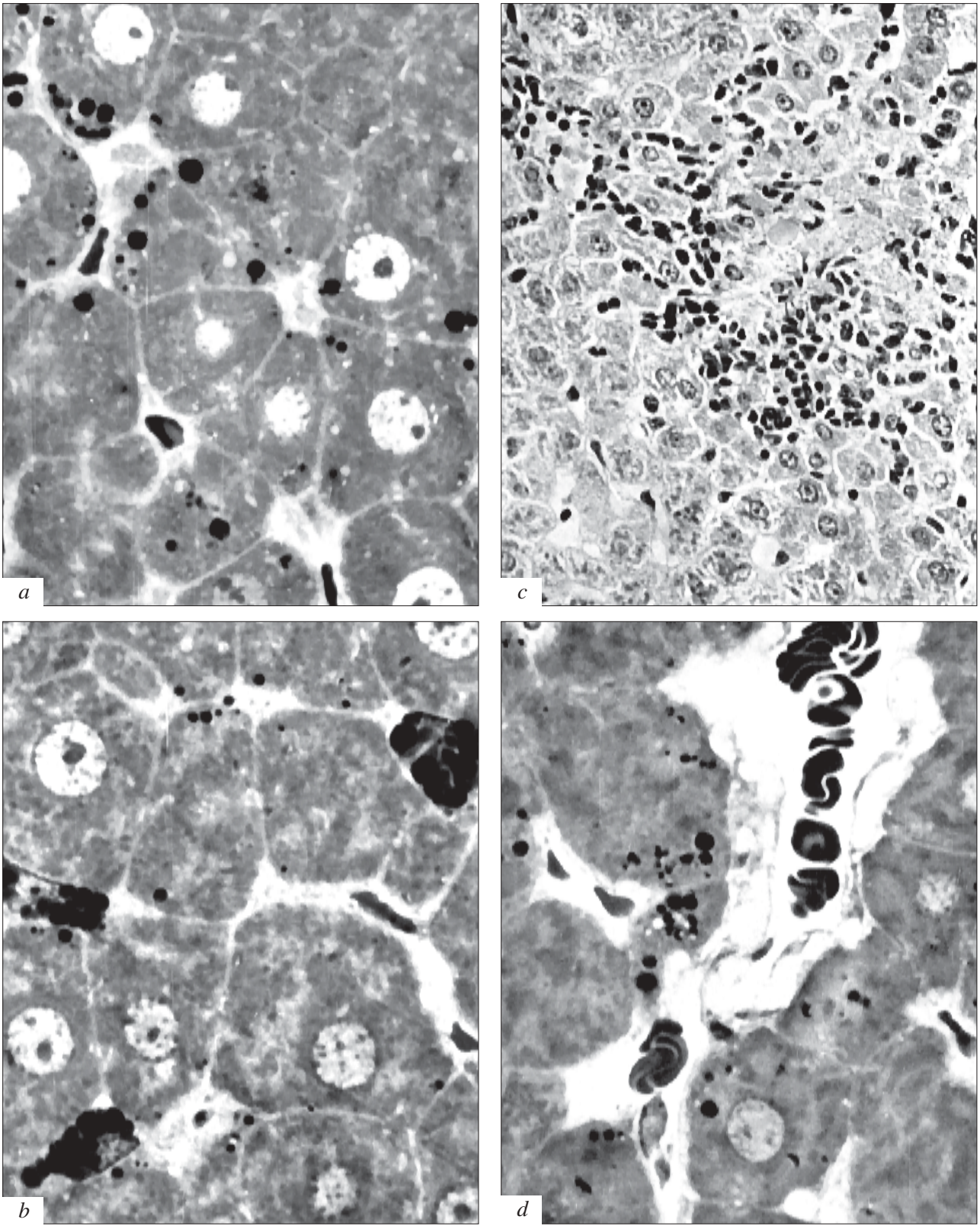


**Fig. 1.** Morphological changes in Wistar rat liver after hypothermia. *a*) accumulation of lipid droplets on hepatocyte sinusoidal poles after 6 weeks of hypothermia, Azur II staining, ×700; *b*) lipid accumulation in Ito cells after 8 weeks of hypothermia, Azur II staining, ×1000; *c*) mononuclear infiltration in foci of hepatocyte necrosis after 8 weeks, hematoxylin and eosin staining, ×250; *d*) perivascular edema after 8 weeks of hypothermia, Azur II staining, ×800.

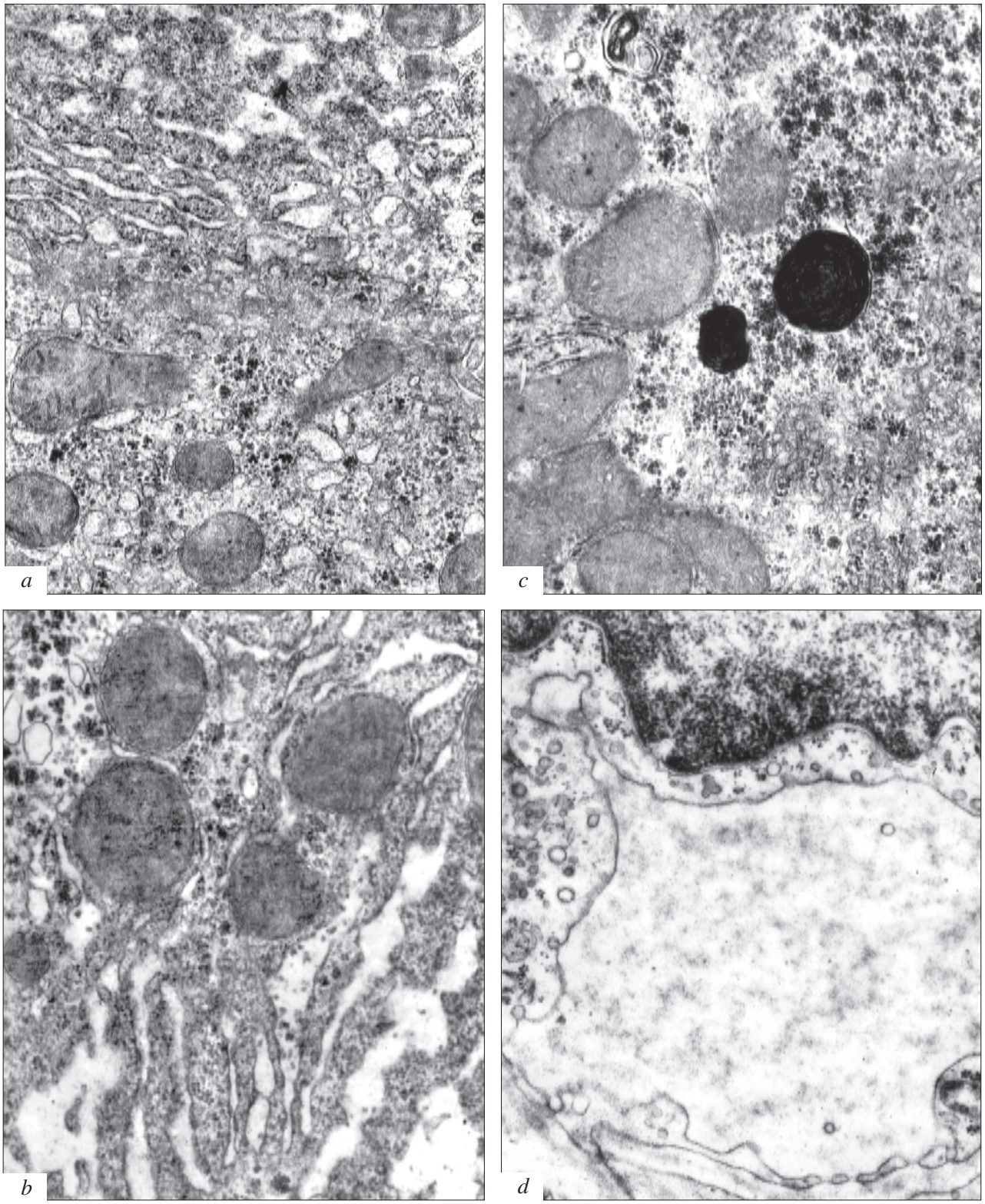


**Fig. 2.** Ultrastructural changes in Wistar rat hepatocytes after total hypothermia. *a*) vacuolation of smooth endoplasmic reticulum and dilatation of cisterns in granular endoplasmic reticulum after 6-week hypothermia, ×8000; *b*) lacuna-like foci of cytoplasm degradation after 6-week hypothermia, ×12,000; *c*) myelin-like and osmiophilic structures between glycogen rosettes after 8 weeks of hypothermia, ×10,000; *d*) lythic changes in the cytoplasm and reduction of organelles in the sinusoidal endotheliocyte after 8 weeks of hypothermia, ×12,000.

Ito's cells. By week 8 of the experiment redistribution of lipids was observed: their content in decreased appreciably in hepatocyte cytoplasm, but increased in Ito's cells leading to marked hypertrophy of these cells (Fig. 1, *b*).

During week 8 of moderate hypothermia lythic injuries to hepatocytes progressed in comparison with the previous term; we observed enlarged foci of parenchymatous necrosis infiltrated with mononuclears (Fig. 1, *c*). Mononuclear infiltration of the stroma and portal tracts also increased, perivascular edema of connective tissue developed (Fig. 1, *d*). Pronounced polymorphism of hepatocyte nuclei and their hypertrophy persisted. The number of binucleated cells decreased (from 8.9±0.8% in the control to 6.3±0.9 and 6.9±0.9%, respectively, after 6 and 8 weeks of total hypothermia), which attests to exhaustion of the main reserve for restoration of total hepatocyte population [1,8].

Spatial reorganization of the hepatic tissue microregion during total hypothermia manifested by increase of the volume density of hepatocytes (by 6 and 4% after 6 and 8 weeks of hypothermia, respectively, $p<0.05$), their surface density remaining unchanged. This caused a decrease of the surface/volume ratio of hepatocytes and indicated their hypertrophic remodeling (Table 1).

Hepatocyte remodeling was also characterized by increase in the volume and surface densities of their nuclei (by 37%, $p<0.01$, and 19%, $p<0.05$, respectively, after 6 weeks of hypothermia). These changes led to a decrease in the surface/volume ratio of the nuclei (by 14%) and indicated their hypertrophy and intensification of their functional activity. The nuclear/cytoplasmic ratio of hepatocytes increased by 32 and 14%, respectively, after 6 and 8 weeks of experiment ($p<0.05$).

Reorganization of the vascular network of the liver microregion consisted in decrease of the volume (by 31 and 23% after 6 and 8 weeks of experiment, respectively) and surface density of sinusoids (by 24 and 10%). This led to decrease in the sinusoid/hepatocyte volume ratio (by 35 and 26% after 6 and 8 weeks of hypothermia, respectively, $p<0.05$) and to a decrease of the sinusoid/hepatocyte surface-volume ratio (by 28 and 13%, respectively). An important aspect in the spatial reorganization of the liver resulting from total hypothermia was decrease of the stroma/parenchyma volume ratio by 28 and 22% during weeks 6 and 8 of experiment, respectively ($p<0.05$).

Total hypothermia caused essential disorders in the hepatocyte intracellular organization, which resulted inhibition of regeneratory plastic processes. Ultrastructural changes in hepatocytes after 6 weeks of total hypothermia manifested primarily in vacuolation of AER and GER (with reduction of the ribosomal system), lacuna-like foci of degradation, as a result of which the cytoplasm of these hepatocytes looked like a honeycomb (Fig. 2, *a*, *b*). By contrast, GER was well develo-

**TABLE 2.** Ultrastructural Stereological Analysis of Rat Hepatocytes after Total Hypothermia (*M*±*m*)

| Parameter | Control | Duration of hypothermia | |
|---|---|---|---|
| | | 6 weeks | 8 weeks |
| Volume density, $mm^3/cm^3$ | | | |
| mitochondria | 217.9±9.8 | 246.4±13.0 | 277.0±8.7* |
| agranular endoplasmic reticulum | 89.5±11.9 | 138.8±13.2* | 106.5±7.0 |
| granular endoplasmic reticulum | 166.7±14.8 | 164.3±9.5 | 111.1±11.9* |
| cytoplasm | 525.9±28.9 | 450.5±0.7* | 505.4±26.5 |
| Surface density, $m^2/cm^3$ | | | |
| mitochondria | 1.64±0.03 | 1.75±0.07 | 1.98±0.04* |
| agranular endoplasmic reticulum | 4.48±0.33 | 2.22±0.27* | 4.03±0.14 |
| granular endoplasmic reticulum | 4.67±0.12 | 5.47±0.17* | 3.86±0.16* |
| Surface/volume ratio, $m^2/cm^3$ | | | |
| mitochondria | 7.6±0.4 | 7.1±0.3 | 7.1±0.5 |
| agranular endoplasmic reticulum | 52.1±2.8 | 17.4±4.3** | 40.3±2.3* |
| granular endoplasmic reticulum | 30.5±1.5 | 33.1±2.7 | 38.4±1.9* |
| Volume ratio of organelles | | | |
| granular/agranular endoplasmic reticulum | 1.95±0.08 | 1.26±0.09* | 1.08±0.09** |
| granular endoplasmic reticulum/mitochondria | 0.82±0.07 | 0.67±0.01 | 0.39±0.05* |

**Note.** *$p<0.05$, **$p<0.01$ compared to the control.

ped in some hepatocytes, with dilated cisterns, indicating intensification of protein-synthesizing function.

A trend to normalization of fine structure of hepatocytes was observed on week 8 of experiment in comparison with week 6: abundant GER cisterns were observed (in some cells they remained reduced), the ribosomal system was well developed, vacuolation of AER tubules was less pronounced. Glycogen metabolism changed significantly: vast sites of glycogen lysis and sequestration were seen. Transformation of lipid droplets with the formation of myelin-like figures comparable to mitochondria by their size was noted (Fig. 2, *c*). Intensification of catabolic reactions led to activation of autophagic processes, this manifesting by formation of residual corpuscles (myelin-like structures) on the hepatocyte sinusoidal poles; these corpuscles were released into Disse space. Secondary lysosomes containing lipofuchsin were seen on the biliary pole. Lythic changes in the cytoplasm and reduction of organelles were detected in the sinusoidal capillary endotheliocytes (Fig. 2, *d*).

Ultrastructural stereological analysis of hepatocytes helped to determine the type of their intracellular reorganization resulting from total hypothermia (Table 2). Let us first of all note the increase of AER volume density by 55 and 19% after 6 and 8 weeks of moderate hypothermia, respectively, and decrease of GER volume density by 33% after 8 weeks of hypothermia. GER/AER volume ratio was decreased at all terms of the experiment (Table 2), indicating intensification of detoxication processes and increase of non-protein synthesis in comparison with protein production. Volume and surface densities of mitochondria increased (by 27 and 21%, respectively) after 8 weeks of experiment. Dysproportional changes in the volume density of the mitochondrial compartment and GER decreased ($p<0.05$) in GER/mitochondria volume ratio by 52%. Analysis of secondary stereological parameters showed a decrease in the surface/volume ratio of AER (by 67 and 23% after 6 and 8 weeks of hypothermia, respectively) as a result of its significant vacuolation.

The detected changes in the stereological parameters (volume and surface densities) of parenchymatous cells and capillaries in the rat liver reflect the fundamental regularities of spatial reorganization of these tissue compartments and specific features of their regeneration under the effect of untoward exposure. Decreased volume and surface densities of the sinusoids in parallel with increase of these parameters for hepatocytes and the general decrease of stroma/parenchyma volume ratio result from liver remodeling during the development of common adaptation syndrome under the effect of chronic hypothermia [10, 14]. Changes in the parenchymatous-stromal relationships in the liver result in disorders in the common correlations between the parenchymal and stromal structural elements at the tissue microregion level, which can be a "prelude" to development of pathological changes.

Ultrastructural changes in hepatocytes can be regarded as their nonspecific reorganization under conditions of redistribution of the plastic and energy resources for attaining the optimal level of thermal production [5-7,12]. This status of hepatocytes, if lasts for a rather long time, can eventuate in atrophy of some cells and their resorption by macrophages.

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