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Ultrastructural Stereological Analysis of the Liver of Muridae from Regions with Various Levels of Anthropogenic Pollution

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Ultrastructural stereological analysis revealed the fundamental reorganization of hepatocytes in Muridae from natural ecosystems exposed to anthropogenic factors. An appreciable decrease of the volume density of mitochondria and granular endoplasmic reticulum was associated with a many-fold increase of the volume density of glycogen. The detected hepatocyte glycogenosis was characterized by a redistribution of organelles and the formation of large depots of glycogen with reduced electron density.

Key Words: *anthropogenic pollution; Muridae; hepatocytes; ultrastructure; stereology*

Analysis of the fine structural changes and identification of the patterns of intracellular reorganization under novel ecological conditions play an important role in the comprehensive assessment of the biological effects determined by exposure to technogenic factors. This is especially true for the biological effects caused by prolonged exposure to low-level industrial factors, which have recently become dominant in many natural regions [8]. However, these effects have been little studied from the viewpoint of both their phenomenology and the mechanisms of their development.

This study was aimed at assessing the intracellular reorganization of hepatocytes of Muridae captured in two regions with different levels of anthropogenic pollution.

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MATERIALS AND METHODS

Liver samples from 20 adult male common field mice (*Apodemus sylvaticus*) and 18 common voles (*Microtus arvalis*), captured in three regions of the Altai with different levels of pollution, were examined under an electron microscope. Two regions (Uglovskoe and Lokot') have been exposed to radionuclides for a long time as a result of nuclear tests at the Semipalatinsk testing grounds [6,11]. In addition, the soil in the Lokot' district is contaminated with heavy metal salts. The third district (Tyumentsevo) served as a control.

For electron microscopy, liver specimens from the large lobe were fixed in 4% paraformaldehyde in Millonig's buffer (pH 7.4), postfixed in 1% osmium tetroxide, and after dehydration embedded in Epon-Araldite mixture. Ultrathin slices were made with an LKB III ultratome and after contrast staining with uranyl acetate and lead citrate examined under a JEM 100C electron microscope at an accelerating voltage of 80 kV.

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Ultrastructural stereological analysis of hepatocytes was carried out on negatives at a final magnification of 30,000 (initial magnification 10,000). The volume density of mitochondria, granular endoplas-

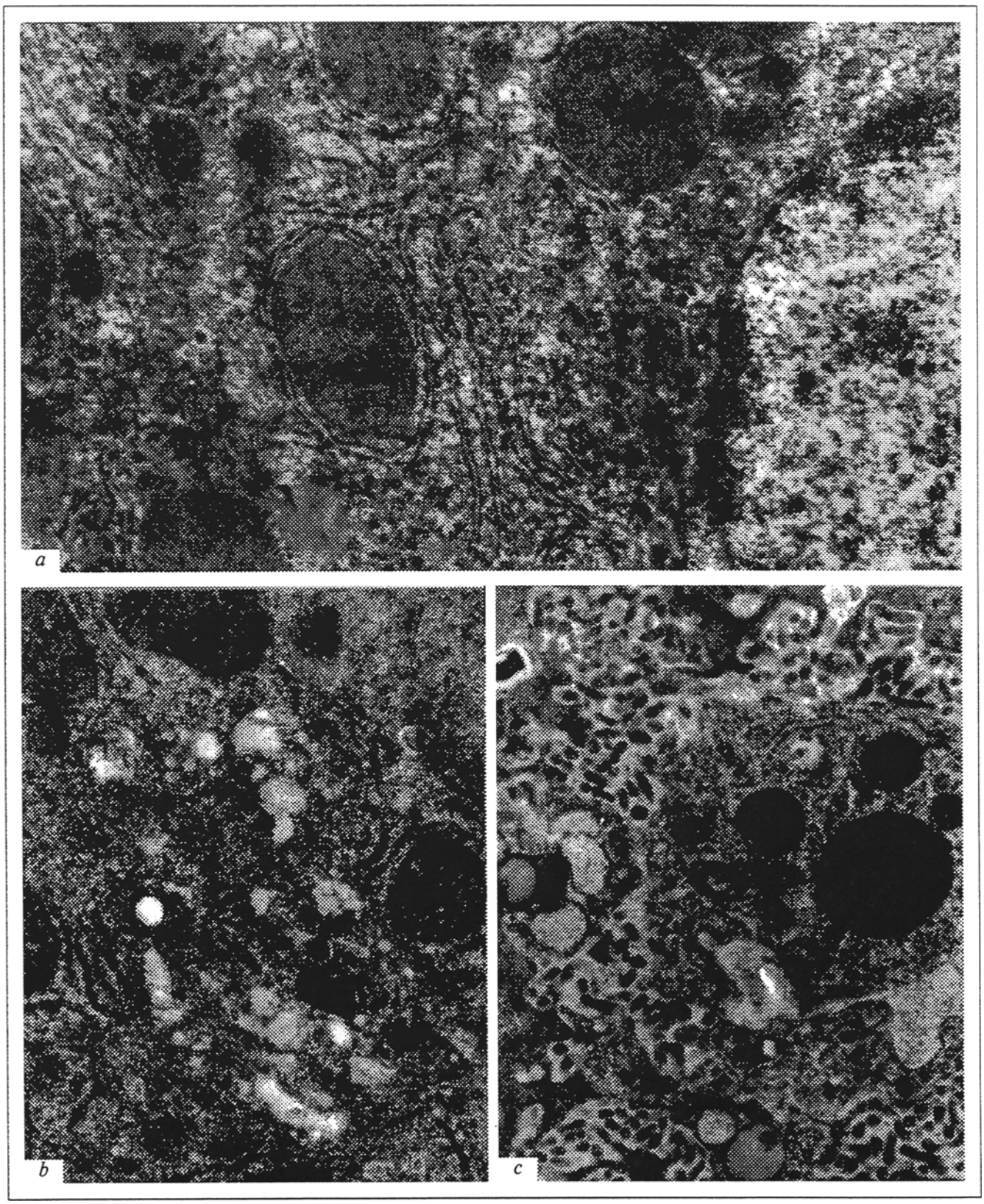

Fig. 1. Ultrastructure of hepatocytes of mice and voles from the control region. *a*) fragment of a hepatocyte of a field mouse, × 10,000; *b*) Golgi complex in a hepatocyte of a vole, × 10,000; *c*) fragment of a Kupffer cell with heterogeneous phagosomes, × 8300.

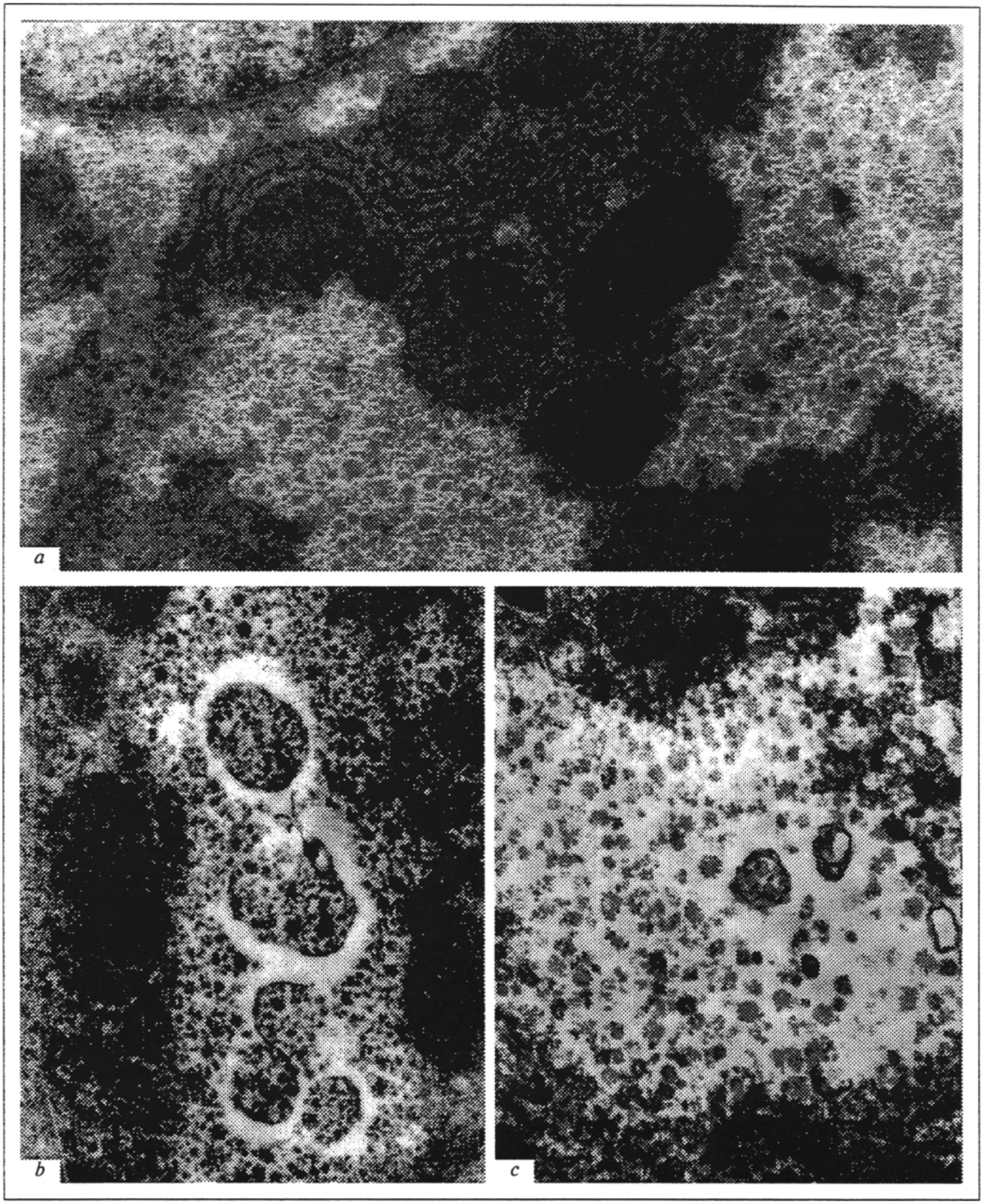

Fig. 2. Ultrastructure of hepatocytes of mice and voles from regions with an appreciable level of pollution. *a*) massive deposition of glycogen and redistribution of organelles in a hepatocyte of a field mouse, × 10,000; *b*) sequestration of glycogen, fragment of a hepatocyte of a vole, × 10,000; *c*) diffuse lysis of glycogen in a hepatocyte of a field mouse, × 13,000.

mic reticulum (ER), smooth ER, and the cytoplasm (including glycogen, lipid droplets, lysosomes, and the cytoplasmic matrix proper), as well as the surface density of mitochondria and membranes of granular ER were assessed using a multi-purpose test system [9,14]. Secondary stereological parameters — volume and surface-volume ratios of the basic ultrastructures — were calculated from the primary parameters. The data were statistically processed and the mean values compared using Student's *t* test.

RESULTS

The ultrastructure of the majority of hepatocytes of both species of rodents captured in the Tyumentsevo district was in general the same as in other small rodents [2,5]. Cisternae of granular ER, polymorphous mitochondria, and vesicles of smooth ER were clearly seen in moderately dense cytoplasm (Fig. 1, *a*); a well-developed Golgi complex was seen in virtually all cells (Fig. 1, *b*). The mitochondria were located in the median zones of the cells and were most numerous at the sinusoidal pole. Glycogen was represented by the α-form and as a rule was evenly distributed in the cell. Lipid droplets of various size were more often seen in hepatocytes of voles than of mice; sometimes their partial depletion and membranous transformation were observed. Sinusoidal endotheliocytes were mainly electron-dense and fenestrated; Kupffer's cells were frequently seen in the sinusoidal lumens and perisinusoidally (Fig. 1, *c*).

In the rodents captured in the Lokot' and Uglovskoe districts the ultrastructural changes of hepatocytes manifested themselves primarily in a peculiar hyperglycogenosis: abundant depositions of the α-form of glycogen, occupying the greater part of the cells (Fig. 2, *a*). The electron density of glycogen was reduced, this often being associated with its sequestration (Fig. 2, *b*) and focal or diffuse lysis (Fig. 2, *c*). The rest of the organelles in such hepatocytes were as if compressed and condensed, which resulted in an increase of their electron density. A redistribution of organelles in the cells was observed: the mitochondria with the surrounding cisternae of granular ER were most frequently located near the nucleus or marginally. It is noteworthy that such chang-

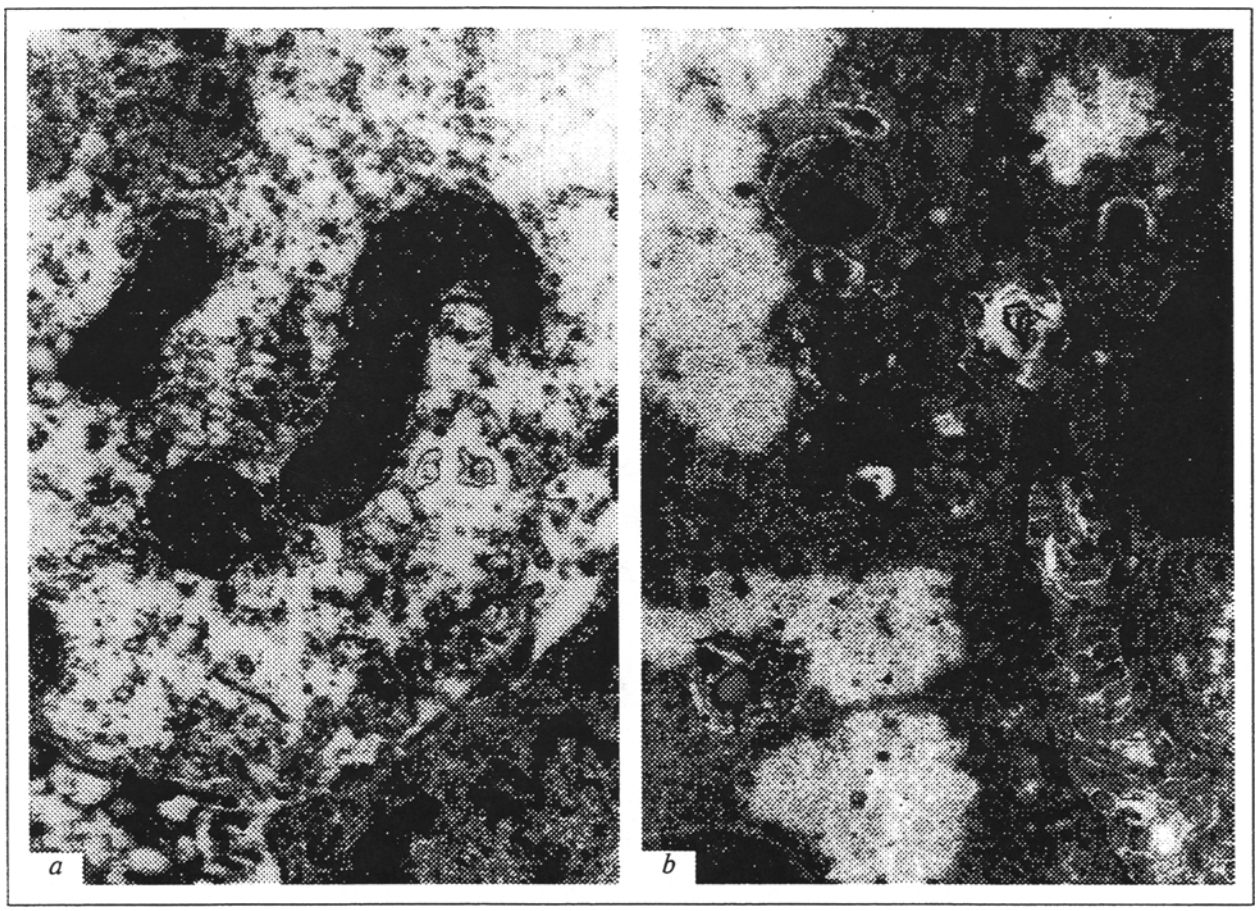

Fig. 3. Ultrastructure of hepatocytes of mice and voles from ecologically impacted regions. × 10,000. *a*) lytic changes of the cytoplasmic matrix and numerous vesicles of smooth ER in a hepatocyte of a vole; *b*) focal accumulations of glycogen with lowered electron density and secondary lysosomes at the biliary pole of a hepatocyte of a field mouse.

TABLE 1. Stereological Analysis of Hepatocytes of Field Mice from Regions with Different Levels of Anthropogenic Pollution (*M±m*)

Parameter	District		
	Tyumentsevo	Lokot'	Uglovskoe
Volume density of, mm^3/cm^3:			
mitochondria	335.1±34.5	225.8±10.4*	236.8±11.9
granular ER	46.2±4.7	31.0±5.7	31.2±0.8*
smooth ER	3.0±0.6	10.1±1.2*	0.9±0.3
cytoplasm	615.7±39.3	733.1±16.1	731.1±12.3
Surface density of, m^2/cm^3:			
mitochondria	2.024±0.173	1.497±0.044*	1.533±0.155
granular ER	2.933±0.102	1.933±0.260*	2.016±0.206*
Surface-volume ratio of, m^2/cm^3:			
mitochondria	6.2±0.7	6.7±0.5	6.6±1.0
granular ER	64.7±6.7	63.9±11.7	64.8±6.1
Volume ratio, number of:			
mitochondria and granular ER	7.6±1.7	7.6±1.0	7.6±0.2
glycogen and mitochondria	0.06±0.007	1.7±0.3	1.4±0.1

Note. Here and in Table 2: *$p<0.05$.

TABLE 2. Stereological Analysis of Hepatocytes of Voles from Regions with Different Levels of Anthropogenic Pollution (*M±m*)

Parameter	District		
	Tyumentsevo	Lokot'	Uglovskoe
Volume density of, mm^3/cm^3:			
mitochondria	396.4±42.1	214.9±23.6*	248.2±28.1*
granular ER	40.9±17.7	29.1±4.8	39.5±13.0
smooth ER	8.1±6.3	31.1±7.2*	2.1±0.6
cytoplasm	554.6±56.3	724.9±19.9*	710.2±37.9
Surface density of, m^2/cm^3:			
mitochondria	2.098±0.261	1.413±0.170	1.511±0.105
granular ER	1.852±0.233	2.350±0.287	3.006±0.409
Surface-volume ratio of, m^2/cm^3:			
mitochondria	5.4±0.4	6.6±0.2	6.0±0.2
granular ER	56.9±13.3	82.2±5.4	85.5±14.2
Volume ratio, number of:			
mitochondria and granular ER	14.1±5.3	8.1±2.2	8.2±3.0
glycogen and mitochondria	0.03±0.001	1.1±0.2	1.1±0.5

es were more pronounced in the rodents captured in the Lokot' district: in some cases virtually total lysis of glycogen was observed with the formation of "blank" spaces, in which just solitary ramified particles of β-glycogen were seen. In both mice and voles we found hepatocytes with cytoplasm containing mainly vesicles of smooth ER and scanty mitochondria, as a result of which the cytoplasm of such cells took on a honeycomb appearance (Fig. 3, *a*).

Ultrastructural signs of a reduced protein-synthesizing function were detected in the hepatocytes of rodents from regions with a high level of pollution: segregation of the nucleoli into fibrillar and granular components, reduction of the granular ER and Golgi complex. Autophagic processes were enhanced, and residual corpuscles (myelinlike structures) formed, which were released into Disse's space and then appeared in the sinusoidal lumens. Secondary lysosomes with lipofuchsin inclusions were often seen at the biliary pole of the cells (Fig. 3, *b*). Bile capillaries were as a rule dilated, and osmiophilic membranous structures were seen in them.

Kupffer's cells and lymphocytes were observed in the lumens of sinusoids, and fibroblasts occurred

perisinusoidally, but the number of these cells was lower in the animals captured in the Lokot' district. In Disse's space small bundles of collagen fibers were sometimes seen among the hepatocyte microvilli.

In general, the stereological analysis revealed the same direction of changes in the quantitative characteristics of hepatocyte organelles in both mice and voles captured in the regions with an appreciable level of pollution (Tables 1 and 2). Quantitative parameters of the mitochondria and granular ER changed most of all. The volume density of the mitochondria in the field mice of the Lokot' and Uglovskoye districts was reduced 33 and 29%, respectively, in comparison with the animals from the Tyumentsevo district, whereas in voles these values were reduced 46 and 37%, respectively. The surface density of the mitochondria decreased approximately to the same extent, this being conducive to the preservation of their surface-volume ratio.

In field mice from ecologically impacted regions the volume density of granular ER was reduced 32% and the surface density reduced 25%, on average, whereas in voles a decrease (29%) of the volume density of granular ER was recorded only for the Lokot' district (Table 1). By contrast, the surface density of granular ER in hepatocytes of voles was increased 27 and 62% in the animals from the Lokot' and Uglovskoe districts, respectively, this promoting an increase of the surface-volume ratio of this intracellular compartment by 44 and 50%, respectively (Table 2).

The volume density of smooth ER in field mice from the control district was 3.0 ± 0.6 mm^3/cm^3, whereas in the voles it was 8.1 ± 6.3 mm^3/cm^3. In rodents from the Lokot' district, exposed to factors of both a chemical and radiation nature, the volume density of smooth ER was increased approximately 2.5 times, whereas in the animals from the Uglovskoe district this parameter was decreased 70%.

The volume density of the cytoplasm appreciably increased in the rodents from ecologically impacted regions (approximately by 19% in field mice and by 30% in voles). It is noteworthy that the increase of this parameter was mainly due to an increase of the volume density of glycogen, which constituted from 30 to 50% of the volume density of the cytoplasm. In animals from the control region the share of glycogen was 3 to 5% of the cytoplasm volume.

The proportionate decrease of the volume density of mitochondria and granular ER in hepatocytes of field mice from ecosystems subjected to a heavy pollution was conducive to preservation of the volume ratio of these compartments, whereas the more marked reduction of the volume density of the mitochondria in the hepatocytes of voles caused a 42% reduction of the volume ratio of the mitochondria to granular ER. In parallel with this, an appreciable (27- to 35-fold) increase of the volume ratio of glycogen to mitochondria was observed, which was apparently indicative of changes in energy and carbohydrate metabolism in hepatocytes under the ecological conditions under study.

Accumulation of glycogen in hepatocytes is observed in some hereditary diseases [1,10], in patients with chronic hepatitis of different genesis (viral, alcohol, and toxic) [7], and under experimental conditions with excessive intake of fructose [12]. An appreciable reduction of the volume density of the mitochondria and granular ER was found in the hepatocytes of rats fed fructose [13]. Impairment of the enzymatic systems of glycogenolysis or of the enzymes connected with the cytoplasmic membrane, specifically, adenylate cyclase, is believed to be the cause of glycogenoses in the liver [4,7]. As a rule, glycogenoses in hepatocytes involve the redistribution of organelles in the cell, primarily the mitochondria and granular ER [12]. Such a redistribution of organelles, for example, localization of the mitochondria along the lateral and basal plasma membranes, has been observed in other types of damage, specifically, in allergic alteration of the hepatocytes [3], this reflecting some common regularities in the reorganization of these cells during harmful exposures.

The phenomena of the accumulation of large amounts of glycogen and of the fundamental spatial reorganization of hepatocytes in mice and voles from biocenoses with a high level of pollution evidently represent both inherited changes and a reaction to persisting adverse factors of physical and chemical nature.

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