Parenchyma-Stroma Interactions in the Liver of Muridae from Ecologically Contrasting Areas

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Changes in the spatial organization of hepatic tissue are demonstrated in Muridae caught in biocenoses with heavy technogenic pollution. The hemocapillary to hepatocyte volume ratio and, consequently, the stroma to parenchyma volume ratio are found to be significantly decreased. The changes are more pronounced in field-mice, which makes them a more preferable model for the assessment of long-term consequences of anthropogenic interventions, specifically, of radiation pollution.

Key Words: *technogenic pollution; long-term consequence; Muridae*

Intensive anthropogenic transformation of natural ecosystems during recent decades markedly impaired and destabilized the living conditions of numerous biological species and caused adaptive-compensatory rearrangements of their vital morphofunctional systems [8,9-11]. Study of wild animal populations from the regions with a high level of anthropogenic influences is necessary for the estimation of potential long-term consequences of such influences and of the intensity and direction of structural and functional changes [4,12,13].

The objective of this study was to assess spatial microorganization of the liver tissue in Muridae from ecologically contrasting regions.

MATERIALS AND METHODS

Liver samples were obtained from mature male long-tailed mice (*Apodemus sylvaticus*) (n=86) and field-mice (*Microtus arvalis*) (n=62) captured in three ecologically contrasting regions of the Altai territory [12]. Two regions (Uglovskoe and the Lokot') over several years were exposed to radioactive pollution resulting from atmospheric nuclear explosions on the Semipalatisk nuclear testing area [5]. The third region (Tyumentsevo) served as a control.

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For histological studies liver samples from the large lobe were fixed in 10% neutral Formalin and routinely processed to prepare paraffin sections. The sections were stained with hematoxylin and eosin, Schiff reagent, and after Van Gieson. In parallel, liver samples were fixed with 4% paraformaldehyde, postfixed with 1% OsO_4, dehydrated, and embedded in Epon-Araldite to prepare semithin sections. The sections were stained with 1% azure II and Schiff reagent.

Stereological analysis was performed on semithin sections using the short-line multipurpose test system [9]. Volume and surface densities of hepatocytes, their nuclei, sinusoidal hemocapillaries, and endothelial cells as well as that of cells, fibers, and ground substance of the connective tissue were evaluated. Secondary stereological parameters (surface-volume ratios of major liver tissue components, volume and surface-volume ratios of hemocapillaries to hepatocytes, and the stroma-parenchyma volume ratio) were calculated from primary parameters.

The data were analyzed using Student's t test.

RESULTS

Animals of both species from the three regions did not differ significantly with respect to their body weight, while the relative weight of the liver was decreased in long-tailed mice and increased in field-

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mice from ecologically unfavorable regions (Tables 1 and 2).

Light microscopy revealed no appreciable structural changes in the liver of long-tailed and field-mice from the Tyumentsevo region. The hepatocytes formed trabeculae and stained uniformly with acid dyes; occasional eosinophilic cells were generally located at the portal tracts. Glycogen was found primarily in hepatocytes located at the periphery of the lobule (Fig. 1, *a*). Sometimes hepatocytes contained small lipid inclusions. Hemodynamic disorders occurred in some animals.

Considerable changes in liver microstructure occurred in mice of both species captured in the Lokot' region. Marked heterogeneity of hepatocytes due to the presence of eosinophilic cells located predominantly near the central veins and due to cells with large empty spaces located primarily at the periphery of the lobule was observed in most of the animals (Fig. 1, *b*). In 30% of the animals, these cells contained massive glycogen deposits, which were revealed by PAS reaction. It should be noted that the nucleus and cytoplasmic organelle occupied a minor part of these hepatocytes, and the tinctorial properties of glycogen were changed: PAS-positive staining of flocculent cytoplasm was less intense. In 30% of the animals, hepatocytes contained lipid inclusions, their size and number increasing from the peripheral part of the lobule (Fig. 1, *c*).

Hemodynamic disturbances (sinusoidal and venous plethora, Fig. 2, *a*) occurred in 40% field-mice and 65% long-tailed mice. Lymphostasis and moderate periportal sclerosis were observed in some animals. Mononuclear infiltration of the portal tracts

TABLE 1. Stereological Analysis of the Liver Tissue of Long-Tailed Mice from Ecologically Contrasting Regions of the Altai Territory (*M±m*)

Parameter	Regions		
	Tyumentsevo	Uglovskoe	Lokot'
Body weight, g	17.4±0.7	17.7±0.4	17.4±0.5
Liver weight, g	0.90±0.05	0.86±0.03	0.82±0.04
Relative liver weight, mg/g	52.2±2.0	49.0±1.3	47.3±1.7
Volume density, mm^3/cm^3:			
hepatocytes	799.2±8.9	791.9±10.5	843.1±7.5***
hepatocyte nuclei	57.4±2.6	57.5±3.7	52.8±2.4
hemocapillaries	125.8±6.7	132.7±7.8	90.6±5.5***
endothelial cells	1.6±0.5	2.2±0.4	2.1±0.2
connective tissue cells	8.8±0.4	10.5±0.6	5.6±0.5**
ground substance and fibers of connective tissue	7.2±1.0	5.2±0.6	5.8±1.0
Surface density, m^2/cm^3			
hepatocytes	0.3511±0.0063	0.3183±0.0079	0.3374±0.0090
hepatocyte nuclei	0.0634±0.0012	0.0601±0.0036	0.0594±0.0032
hemocapillaries	0.1216±0.0033	0.1246±0.0041	0.1156±0.0033
endothelial cells	0.0033±0.0009	0.0028±0.0007	0.0033±0.0007
connective tissue cells	0.0133±0.0009	0.0160±0.0016	0.0075±0.0015**
Surface-volume ratio, m^2/cm^3:			
hepatocytes	0.409±0.007	0.376±0.012	0.378±0.012
hepatocyte nuclei	1.107±0.029	1.047±0.033	1.125±0.038
hemocapillaries	0.974±0.041	0.949±0.053	1.291±0.075**
endothelial cells	2.027±0.165	1.255±0.246	1.684±0.372
connective tissue cells	1.502±0.063	1.525±0.120	1.317±0.136
hemocapillaries to hepatocytes	0.142±0.005	0.146±0.005	0.129±0.004*
Volume ratio, number:			
stroma to parenchyma	0.168±0.009	0.178±0.010	0.116±0.008**
hemocapillaries to hepatocytes	0.147±0.009	0.156±0.010	0.101±0.007**
nucleus to cytoplasm (of hepatocytes)	0.072±0.004	0.073±0.005	0.063±0.003

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the corresponding parameter of mice from the Tyumentsevo region.

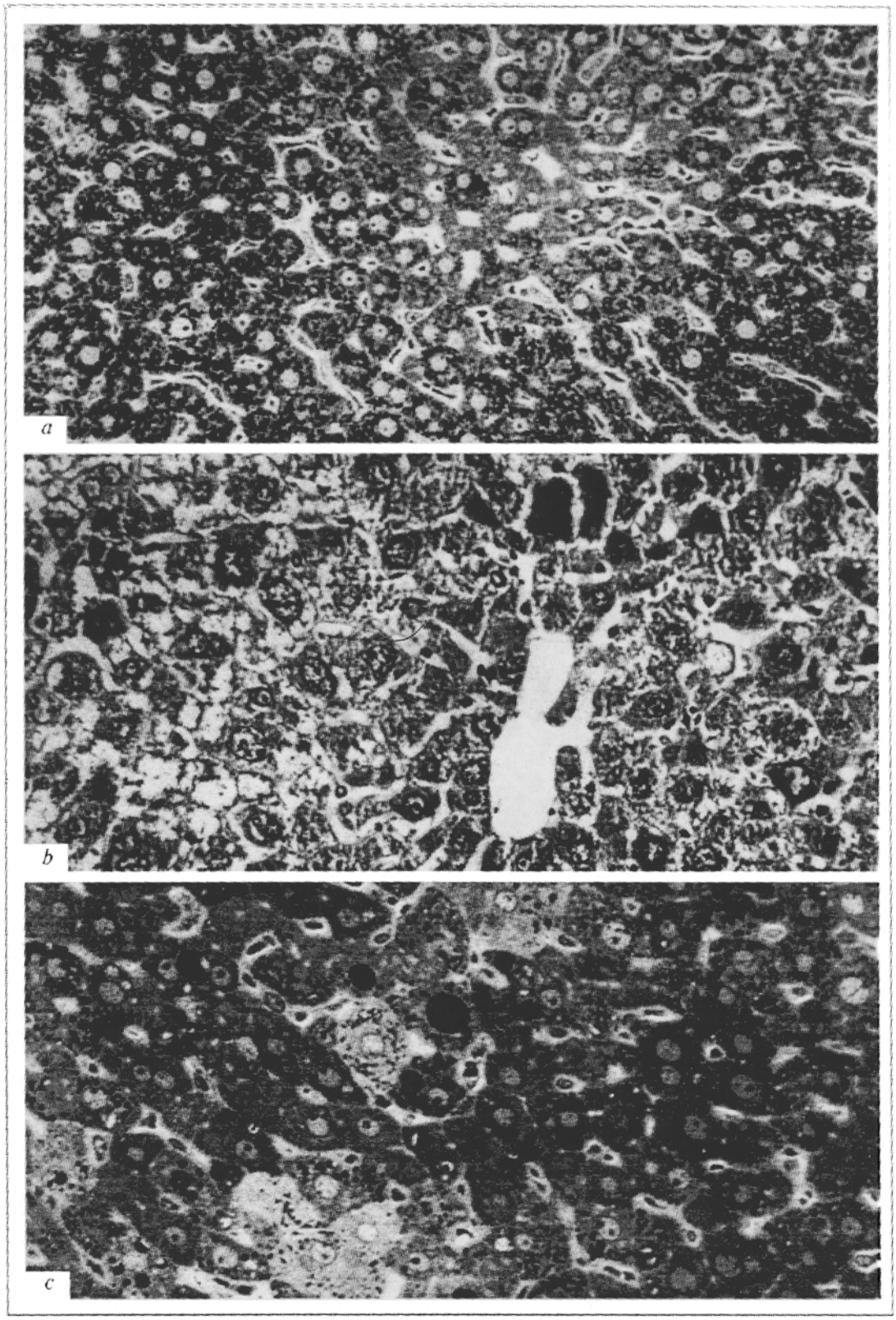

Fig. 1. Liver of Muridae from ecologically contrasting regions. *a*) glycogen distribution in hepatocytes from long-tailed mouse from the Tyumentsevo region. × 312; *b*) pronounced heterogeneity of hepatocytes of long-tailed mouse from the Lokot' region, × 400; *c*) large lipid inclusions in hepatocytes of field-mouse from the Lokot' region, × 500. *a*) and *c*) semithin sections, PAS reaction; *b*) hematoxylin and eosin staining.

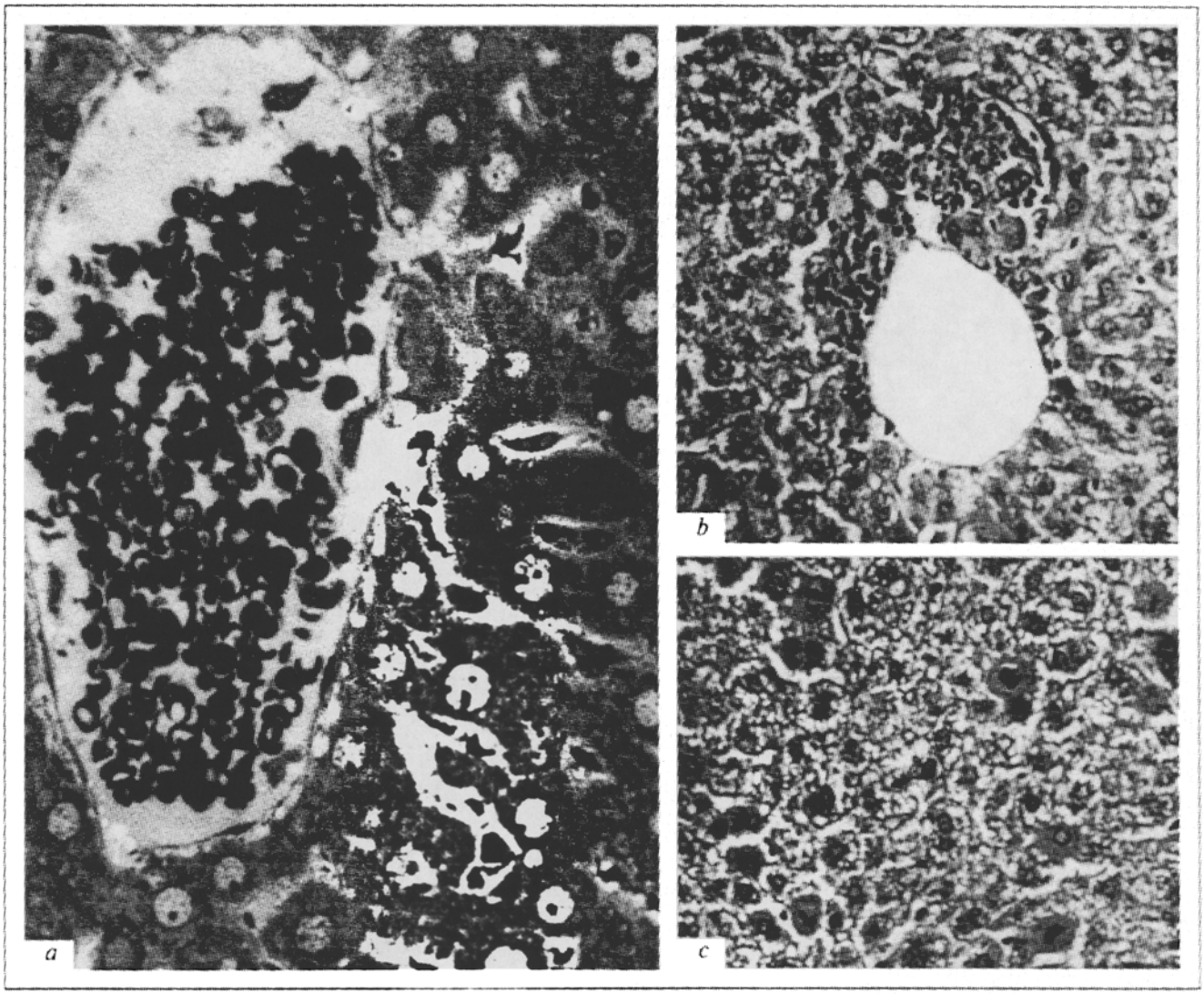

Fig. 2. Liver of long-tailed mice from regions with high level of technogenic pollution. *a*) venous plethora, × 625; *b*) mononuclear infiltration of portal tracts, × 300; *c*) hepatocytes at different stages of the mitotic cycle, × 312; *a*) semithin sections stained with azure II, *b*) and *c*) hematoxylin and eosin staining.

(sometimes pronounced) occurred in about 30% of them (Fig. 2, *b*).

In Muridae from the Uglovskoe region, the general pattern of morphofunctional changes was similar to that in mice from the Lokot' region; however, the intensity of some changes was different. Hepatocyte heterogeneity was recorded in 40% of the animals. Massive glycogen deposits were revealed in 1/3 of cases; however, glycogen was evenly distributed in the cell, and its tinctorial properties were little altered. Hepatocyte mitoses were revealed in about 30% of long-tailed mice (Fig. 2, *c*). Half of the animals developed hemodynamic disorders. Moderate mononuclear infiltration of hepatocytes occurred in 60% of mice.

Stereological analysis revealed the most pronounced changes in the liver architectonics in long-tailed mice from the Lokot' region and in field-mice from the Uglovskoe and Lokot' regions (Tables 1 and 2).

In long-tailed mice from the Lokot' region, the volume density of hepatocytes was significantly increased compared with that in animals from the Tyumentsevo and Uglovskoe regions (Table 1). The volume density of sinusoidal hemocapillaries was markedly (30%) decreased. The decrease in the surface density of these structures was smaller, which resulted in a significant 33% increase in the hemocapillary surface-volume ratio. Opposite changes in the volume density of hepatocytes and hemocapillaries led to a significant drop (by 30%) of their volume ratio, while their surface-volume ratio was slightly lowered (by 9%).

In long-tailed mice from the Lokot' region, the volume and surface densities of the connective tissue

cells were also lowered by 36 and 44%, respectively ($p<0.01$), while the volume density of the ground substance and fibers of the connective tissue dropped by 20%. This results in a significant 31% decrease in the stroma-parenchyma volume ratio.

Changes in the spatial microorganization of the liver of field-mice from the Lokot' and Uglovskoe regions were unilateral and consisted primarily in decreased volume density of hemocapillaries (by 24 and 19%, respectively, Table 2).The volume density of endothelial cells increased by 42 and 114%, respectively) and that of connective tissue cells by 31 and 51%, respectively.

In these animals, the volume density of the ground substance and fibers dropped by 26 and 23%, respectively. Together with reduced volume density of hemocapillaries this led to a decrease in the stroma-parenchyma ratio (by 24 and 18%). The volume density of hemocapillaries to hepatocytes declined practically to the same extent (28 and 21%).

Thus, changes in the liver tissue architectonics occurring in Muridae from the regions with high level of technogenic contamination were unilateral and consisted primarily in a decrease in the volume and surface-volume ratios of hemocapillaries to hepatocytes. This markedly contributed to the drop in the stroma-parenchyma ratio. Long-tailed mice from the Uglovskoe region, which in major stereological parameters did not differ from the mice captured in the control region, are the only exception in this respect. The different degree of changes in the liver tissue architectonics of different species of Muridae probably results from the differences in the habits of these animals. Being subterranean animals, field-mice eat both roots, seeds, and green foliage [2,3], which makes them more vulnerable to radia-

TABLE 2. Stereological Analysis of the Liver Tissue of Field-Mice from Ecologically Contrasting Regions of the Altai Territory ($M\pm m$)

Parameter	Regions		
	Tyumentsevo	Uglovskoe	Lokot'
Body weight, g	28.2±1.0	30.3±1.7	22.7±2.2
Liver weight, g	1.2±0.1	1.4±0.2	1.2±0.2
Relative liver weight, mg/g	42.6±1.2	47.4±6.5	52.9±5.7
Volume density, mm^3/cm^3:			
hepatocytes	796.4±19.9	829.1±7.4	820.7±6.9
hepatocyte nuclei	54.4±2.1	53.4±5.1	52.6±1.8
hemocapillaries	134.1±9.5	101.8±5.9*	108.9±5.8
endothelial cells	0.7±0.1	1.0±0.2*	1.5±0.2**
connective tissue cells	7.1±1.4	9.3±1.0	10.7±1.1
ground substance and fibers of connective tissue	7.3±1.6	5.4±0.4	5.6±0.5
Surface density, m^2/cm^3:			
hepatocytes	0.3696±0.0152	0.3808±0.0113	0.3736±0.0118
hepatocyte nuclei	0.0623±0.0070	0.0694±0.0083	0.0670±0.0051
hemocapillaries	0.1360±0.0016	0.1248±0.0067	0.1307±0.0052
endothelial cells	0.0017±0.0003	0.0020±0.0003	0.0021±0.0006
connective tissue cells	0.0131±0.0018	0.0161±0.0018	0.0181±0.0016
Surface-volume ratio, m^2/cm^3:			
hepatocytes	0.436±0.009	0.432±0.011	0.428±0.013
hepatocyte nuclei	1.142±0.098	1.292±0.057	1.267±0.062
hemocapillaries	1.051±0.078	1.232±0.067	1.205±0.038
endothelial cells	2.373±0.173	2.202±0.444	1.318±0.294
connective tissue cells	2.092±0.454	1.717±0.030	1.705±0.055
hemocapillaries to hepatocytes	0.160±0.004	0.144±0.008	0.150±0.006
Volume ratio, number:			
stroma to parenchyma	0.177±0.030	0.134±0.007	0.145±0.009
hemocapillaries to hepatocytes	0.159±0.008	0.115±0.007*	0.125±0.008*
nucleus to cytoplasm (of hepatocytes)	0.068±0.002	0.065±0.006	0.064±0.002

tion, since most of radionuclides with long half-life (^{137}Cs and ^{90}Sr) are accumulated in soil [6,10]. In other words, these animals are more exposed to external and internal chronic irradiation.

In contrast to field-mice, long-tailed mice populate biotopes with trees and bushes, spending most of their active life overground [1]. Therefore, they are more exposed to other technogenic factors, such as atmospheric chemical contaminants. The populations of these rodents are open and not isolated; however, *Microtus* mice are less inclined to migration than *Apodemus* mice [7]. Therefore, field-mice are more preferable as a model for evaluating the effects of local technogenic contaminants.

Nevertheless, it should be noted that both field-mice and long-tailed mice from the studied regions significantly differ in the ratio of hepatocytes with various ploidy. Among the Muridae from heavily contaminated regions the number of octa- and hexadecaploid (long-tailed mice) and di- and tetraploid hepatocytes (field-mice) increased in comparison with those in mice from the Tyumentsevo region. This shift in the quantitative ratio of hepatocytes in line with changes in tissue architectonics probably represents genetically determined adaptive reorganization of the liver (a kind of regenerative strategy) caused by environmental factors and is a marker of long-term consequences of ionizing radiation on the animal organism.

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