Karyometric Analysis of Hepatocytes of Common Field Mice from Regions with Different Levels of Technogenic Contamination

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Increased polyploidy of hepatocytes is shown using karyometric analysis in common field mice from different Altai regions with high levels of technogenic contamination of radiation and chemical nature. A higher degree of polyploidy is noted in animals with more marked alterations in hepatocytes classified as dystrophic and necrobiotic.

Key Words: *technogenic contamination; common field mice; hepatocytes; karyometry*

The massive increase in the influence of modern technology on biogeocenoses during recent decades has spawned a wide rariety of biological effects induced by diverse physical and chemical factors on different levels of structural and functional organization [6,9,16]. The possible impacts of such influences may be assessed by a study of the peculiarities of regeneratory reactions in vital organs and tissues of animals inhabiting regions with high levels of technogenic contamination [13,14]. As a central organ of metabolism, the liver plays a major role in the development of adaptive-compensatory processes and is therefore a prime focus of attention in studies of the impacts of various adverse factors. Nonetheless, many aspects of liver regeneration remain unknown for wild animals dwelling in heavily contaminated regions.

The goal of the present investigation was to perform a karyometric analysis of the hepatocyte population in field mice from Altai regions with various ecological conditions.

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MATERIALS AND METHODS

The studies were carried out on the adult male common field mice *Apodemus sylvaticus* caught in 1993 in three regions of the Altai Territory. These regions varied in level of technogenic contamination as follows: two regions (Uglovskii and Loktevskii) were situated in the zone of passage of radioactive clouds after atmospheric nuclear explosions in the Semipalatinsk nuclear test site exposed to radioactive contamination over several years (1949-1962) [5]. The density of soil contamination in these regions was over 120 mCi/km^2 for ^{137}Cs and 70 mCi/km^2 for ^{90}Sr [11,15]. Furthermore, a significant contribution to the ecologic situation in the Loktevskii region was made by the intensive development of the mining industry and the abundance of heavy metal salts in the soil [15]. The third region (Tyumentsevskii) was examined for comparison.

A total of 86 rodents were studied (29 in the Uglovskii, 31 in the Loktevskii, and 26 in the Tyumentsevskii region). The animals were decapitated at one and the same time. The liver was promptly removed, weighed, and examined visually. For light microscopy samples of the large lobe were fixed in 10% neutral formalin and then processed to obtain paraffin sections. Other samples were concurrently fixed in 4%

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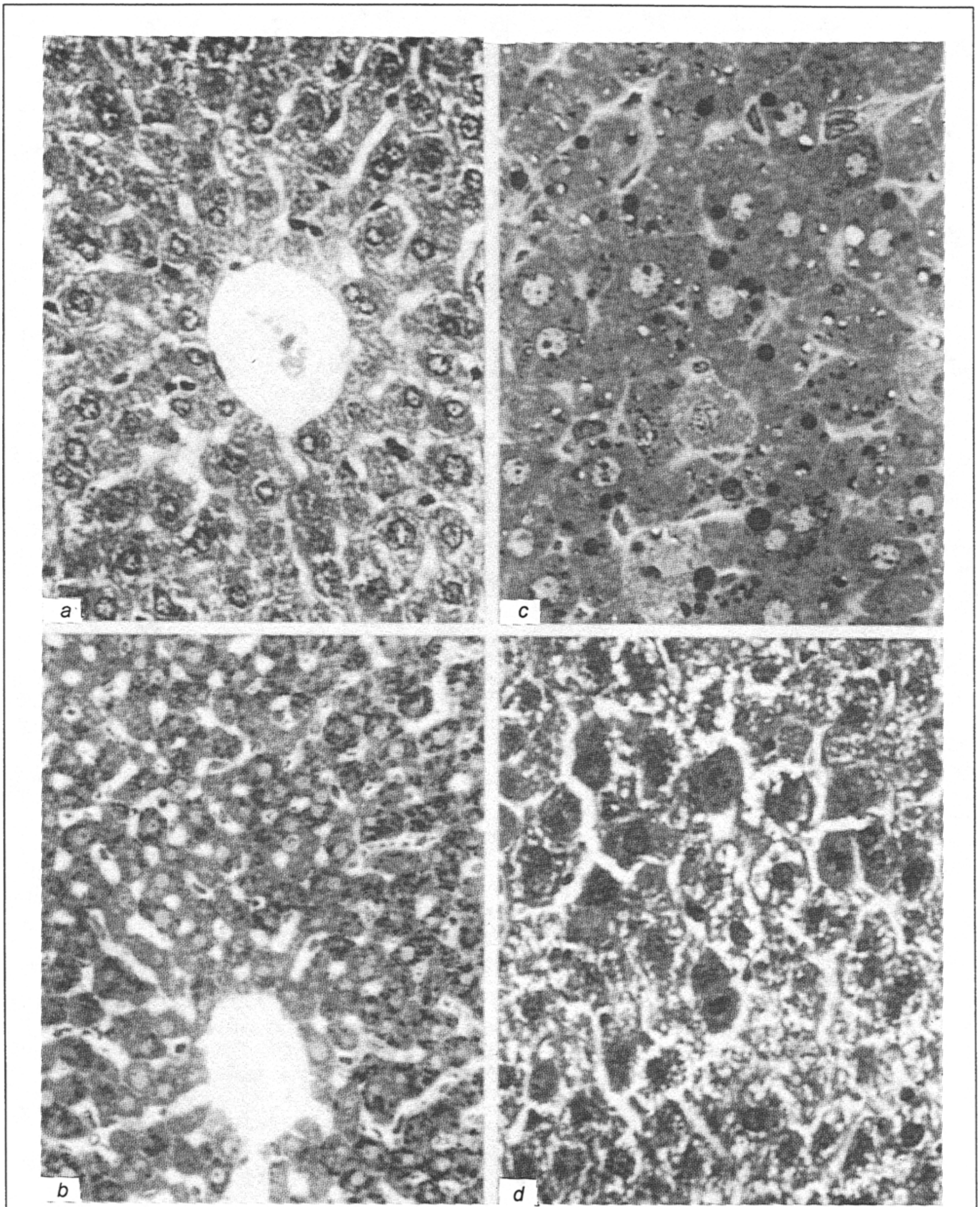

Fig. 1. Liver of field mice from regions with different levels of technogenic contamination. *a*) normal liver structure in animals from the Tyumentsevskii region. ×500; *b*) glycogen accumulations in hepatocytes at periphery of lobule. ×800; *c*) nuclear polymorphism and lipid inclusions in hepatocytes of an animal from the Uglovskii region. ×625; *d*) hepatocytes at different stages of the mitotic cycle, marked dystrophy of parenchymatous cells. ×500. *a, d*) hematoxylin and eosin staining; *b*) semithin section, PAS-reaction; *c*) semithin section, Azure II staining.

TABLE 1. Body and Liver Weight in Adult Male Field Mice from Different Regions of the Altai Territory (*M*±*m*)

Index	Regions studied		
	Uglovskii	Loktevskii	Tyumentsevskii
Body weight, g	17.7±0.4	17.4±0.5	17.4±0.7
Liver weight, g	0.86±0.03	0.82±0.04	0.90±0.05
Relative weight of liver, mg/g	49.0±1.3	47.3±1.7	52.2±2.0

paraformaldehyde, postfixed in 1% osmium tetroxide, and, after dehydration, embedded in an Epon-Araldite mixture. The semithin sections were stained with 1% Azure II and the PAS-reaction was performed.

The diameter of hepatocyte nuclei was measured with the aid of a MOV-1-15^x ocular micrometer under an MBI-11 light microscope. One thousand hepatocyte nuclei were assessed for each experimental group (one region of examination). The data were subjected to statistical treatment [12].

RESULTS

Adult *A. sylvaticus* from the three regions did not differ in body weight (Table 1), whereas the values of absolute and relative liver mass were 10% higher in mice from the Tyumentsevskii region.

The microscopic structure of the liver in mice from the Tyumentsevskii region did not differ from normal. Hepatocytes stained uniformly with acid dyes (Fig. 1, *a*); just solitary cells or small clusters exhibited eosinophilic cytoplasm and intensely basophilic nuclei. Glycogen accumulations were predominantly demonstrated with the PAS-reaction in hepatocytes situated at the periphery of the lobules (Fig. 1, *b*). Small lipid inclusions were found in hepatocytes at the periphery of the lobules in 27% of animals. Hepatocyte nuclei were euchromic, as a rule, and contained several nucleoli; their polymorphism was noted.

Some heterogeneity of hepatocytes due to a large number of eosinophilic cells often situated around the central veins was found in animals from the Uglovskii and Loktevskii regions. Marked dystrophic alterations of hepatocytes manifested in heavy lysis of the cytoplasmic matrix were noted in some cases. Such changes were more commonly found in animals from the Loktevskii region, which also exhibited small foci of hepatocyte necrosis in some cases. It should be noted that the trabecular structure and general pattern of the hepatic tissue were preserved. Pronounced polymorphism of nuclei was found in hepatocytes of practically all mice from these two regions (Fig. 1, *c*). Cells with giant nuclei were frequently encountered. Mitoses of hepatocytes were recorded in about one-third of animals from the Uglovskii region (Fig. 1, *d*).

In 65% of cases disturbances in hemodynamics presented as venous and sinusoidal plethora. Lymphostasis and moderate periportal sclerosis were noted in some cases. The portal tracts often contained foci of mononuclear infiltration.

The above morphological changes are nonspecific and have been found in toxic and ionizing damage of the liver as well as in *Muridae* inhabiting the vicinity of Chernobyl [7,8,10,13].

Analysis of the size distribution of hepatocyte nuclei (Fig. 2) reveals a polymodality of distribution due both to heterogeneity of the cell population as a result of polyploidization and to the fact that different

TABLE 2. Results of Karyometric Analysis of the Hepatocyte Population in Field Mice from Different Regions of the Altai Territory (*M*±*m*)

Index		Regions studied		
		Uglovskii	Loktevskii	Tyumentsevskii
Diameter of nuclei, μ:	2n	5.16±0.044	5.16±0.043	5.07±0.050
	4n	6.51±0.020	6.52±0.021	6.50±0.018
	8n	8.00±0.025	8.00±0.021	8.05±0.027
	16n	9.50±0.060*	9.66±0.082**	9.27±0.042
Number of nuclei, ‰	2n	92	80	113
	4n	468	397	513
	8n	375	460	321
	16n	65	63	53
Number of hepatocytes, ‰				
	mononuclear	865±5	866±9	849±5
	binuclear	135±5	134±9	151±5

Note. *n* - number of nuclei; *$p<0.01$, **$p<0.001$ as compared with the data on the Tyumentsevskii region.

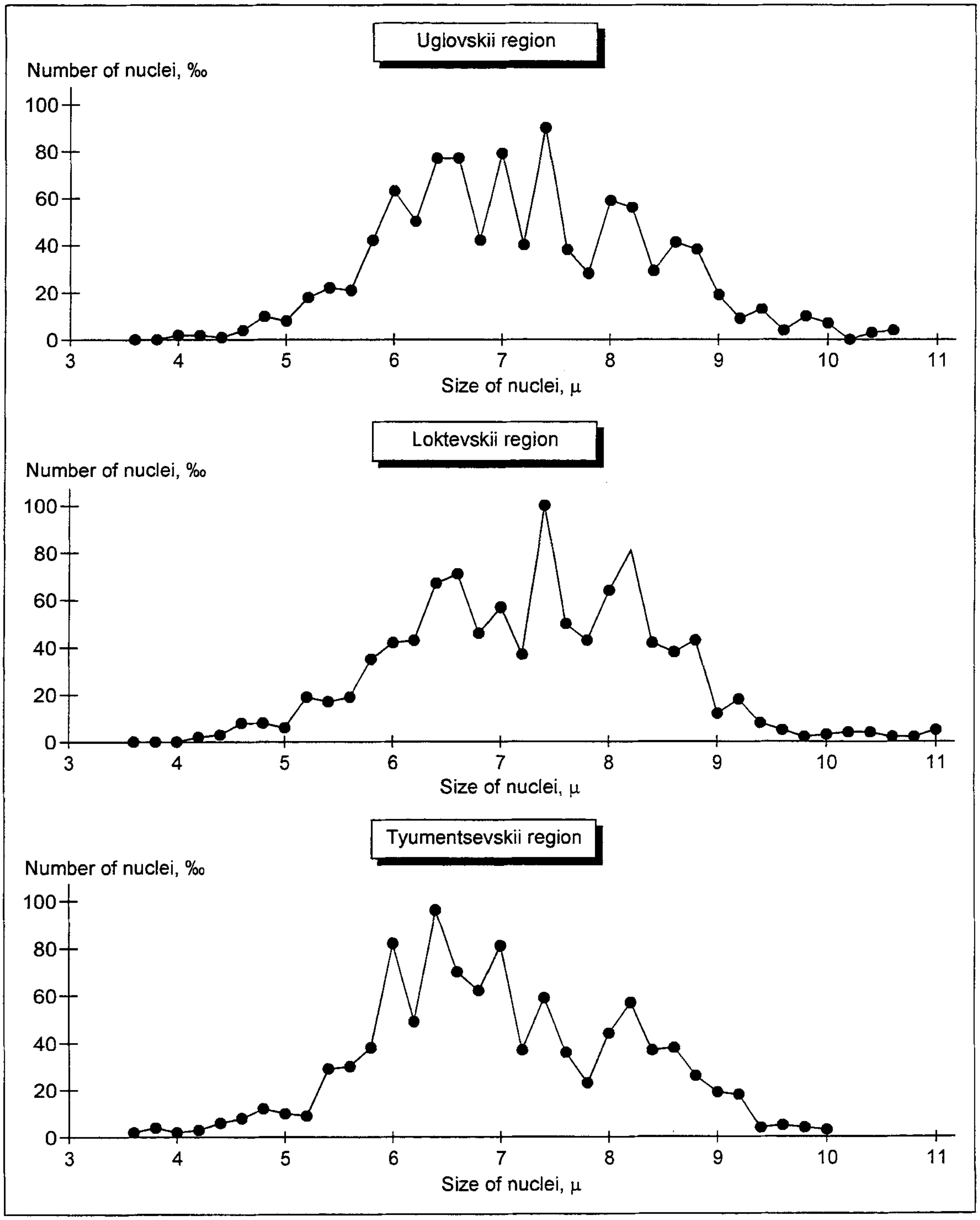

Fig. 2. Size distribution of hepatocyte nuclei in field mice from regions with different levels of technogenic contamination.

nuclear profiles were found on slides but referred to one size class. A paired comparison of the empirical distributions using the χ^2 test revealed reliable differences (for the Tyumentsevskii and Uglovskii, and Loktevskii and Uglovskii regions, $p<0.05$, and for Tyumentsevskii and Loktevskii, $p<0.01$).

Comparison of each of the empirical distributions with the theoretical normal distribution also revealed reliable differences for each case ($p<0.01$). Assuming that the deviation of each of the distributions from normal was due to heterogeneity of the studied populations, we divided each of them into 4 subpopulations and determined size and statistical parameters for each. The size intervals for the subpopulations were 4.0-5.6, 5.6-7.2, 7.2-9.0 and 9.0-10.4 μ in all three cases. Points 5.6, 7.2, and 9.0 μ were chosen because they harbored the maximal differences between neighboring frequencies in all distributions studied.

The computed mean values of nuclear diameter in hepatocytes practically did not differ for the 4 subpopulations in each of the size distributions (Table 2). Calculation of the nuclear volume (on the basis of a spherical model) yielded values for each size class as follows: 70.6, 144.3, 269.8, and 445.6 μ^3, where each ensuing volume was about double the preceding one. Since numerous cytophotometric studies of the liver have established a relationship between ploidy and mean sizes (volumes) of hepatocyte nuclei [1], namely, a match of the multiplicity of volume changes to nucleus ploidy, it may be considered that the subpopulations characterize nuclei with 2, 4, 8, and 16 sets of chromosomes, respectively.

The results testify that the complex of technogenic factors does not induce significant alterations in the size of hepatocyte nuclei of different classes of ploidy except that with 16 sets of chromosomes. In this case a small, but reliable increase of mean nuclear diameter was noted in animals from the Uglovskii and Loktevskii regions (Table 2). Since a reliable change in the ratios of mono- and binuclear hepatocytes was not recorded in animals from the regions studied, an increase of the number of nuclei with 8 and 16 sets of chromosomes in mice from the Uglovskii and Loktevskii regions corresponds to a rise of the number of high-ploidy hepatocytes. Even a slight decrease (by 12%) of the number of binuclear cells was found in mice from regions with heavy technogenic contamination. It should be noted that the boost of polyploidization of hepatocytes in animals from the Uglovskii and Loktevskii regions correlated with a decrease of the liver weight. These processes are most pronounced in the Loktevskii region, characterized by combined effects of radiation and chemical pollution.

Increased polyploidy in hepatocytes is found in different hepatic lesions, particularly in radiation damage [2-4]. It has been shown that the increase of the mean ploidy of hepatocytes with an increase of the ionizing radiation dose is comparable to that in the process of natural aging [3]. The polyploidy phenomenon is variously interpreted in the modern literature, either as a peculiar gene mutation [3] or a specific regeneratory reaction [2]. It is also assumed that polyploid cells are more radioresistant and that the polyploidy phenomenon is a nonspecific protective mechanism acting on the tissue level [4]. The extent of hepatocyte polyploidy, regardless of the causes and mechanisms of its development, reflects the intensity of regeneratory processes and correlates with the severity of adverse impacts.

It has been experimentally established that the regenerating liver loses one of its important functions - that of metabolizing xenobiotics [10]. Since polyploidization is an irreversible process [2] and the constant stimulation of hepatocytes leads to their further polyploidization, a hepatocyte population with an attenuated or depressed detoxication function becomes progressively predominant in the liver.

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