ISSN 1062-3590, Biology Bulletin, 2011, Vol. 38, No. 5, pp. 518–527. © Pleiades Publishing, Inc., 2011.
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ECOLOGY

Blood System Peculiarities in the Bank Vole (*Clethrionomys glareolus*) under Chronic Environmental Pollution

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Received June 8, 2010

Abstract—The parameters of peripheral blood and hemopoietic organs in mature and immature bank voles inhabiting a chemically polluted area were studied. Variability of the blood system parameters depending on the level of toxic load and the animals' reproductive status was determined. Alteration of the cell composition of erythrocytes and leucocytes, the structure of erythrocytes, and the hemoglobin fractions and leucocyte functions describe the adaptive response to the factors of a changed environment more than the concentration of leucocytes, erythrocytes, and blood hemoglobin.

Keywords: bank vole, blood system, chemical pollution.

DOI: 10.1134/S1062359011050153

The significant release of heavy metals into the atmosphere as a result of mining and processing industries of the Central Urals negatively influences the quality of the environment and exceeds the standard chemical load on the organism of animals and humans (Utkin et al., 2004). Pollution of large territories by industrial emissions raises the problem of applicability of modified natural ecosystems. The questions of the influence of pollutants on the living organism, the relation with the environment, its resistance, and the regularities of functioning in conditions of modified environment are urgent.

At present, significant data on the content of chemical elements of anthropogenic nature in different components of nature ecosystems, their migration along the food chain, and the ability to accumulate in the organism have been collected (Semenov and Tregubenko, 1984; Gil'denskiol'd et al., 1992; Mukhacheva and Bezel, 1995, 2007; Kokhonov, 2005; Mukhacheva, 2005; Bezel et al., 2007; Donnik et al., 2007). Less attention was paid to study of biological effects on animals from nature populations (Dmitrova et al., 1994; Leffler and Nyholm, 1996; Koval'chuk et al., 2002; Rogival et al., 2006; Davydova and Mukhacheva, 2007). To evaluate the status of an ecosystem among numerous physical, chemical, and other tests, the complex biological response of small animals continuously and closely in contact with the environment is most informative. A complex of blood system parameters of the organism, which are sensitive to different shifts in the outer and external environment, and the fine reaction upon alteration of its morphological composition can serve as the test system (Kozinets et al., 1993; Rogival et al., 2006). Investigation of the concentration and composition of blood cells making an accent on investigation of this structure and function can identify on one hand the status of the organism and the mechanism of adaptive reactions and, on the other, influence environmental factors.

A reliable index of the functional cell status, i.e., the activity of the peroxidase–hydrogen peroxide system of leucocytes directly related to the vitality of an organism, which is the molecular basis of nonspecific immunity and possesses antimicrobial, antiviral, and antitumor activity can be used for evaluation of the ecological risk (Mushtakova et al., 2005).

The aim of this study is evaluation of the complex of hematological parameters of the blood system in the bank vole with different reproductive status in the gradient of chemical pollution of the environment in order to evaluate the adaptive mechanisms to modified conditions.

MATERIALS AND METHODS

Animals from territories at various distances from Midle Ural Copper Smolter (MUCS), which has been functioning for more than 70 years, were used in this study. The main ingredients of its emissions are gaseous sulfur compounds and dust particles with sorbed chemical elements (Cu, Pb, Cd, Zn, Zs, Hg, Fe, and others). Zones with different degrees of modification of the ecosystem are formed around the factory as a result of the long term influence of pollutants on the environment (Vorobeychik et al., 1994). Three zones were selected in the western direction from the source

Table 1. Characteristic of territories and objects of study

Parameter	Investigated territory			
	BG1	BG2	B	I
Distance from emission source, km	30	20	5–6	1–2
Total toxic load, in arbitrary units*	1		3.6	6.3–7
Investigated animals	22	15	8	7
Males/females	10/12	8/7	1/7	7/0
Immature	17	15	6	3
Mature	5	0	2	4

Note: BG1, BG2 is background, B is buffer, I is impact territories (for Tables 1–4).

* Mukhacheva, 2005.

of emission against the prevailing wind direction: impact (I), buffer (B), and background (BG2 and BG1, where the technogenic load was similar to the regional background). Capture of animals was carried out using live traps in every zone in same periods in July 2004, 2007, and 2008. The bank vole (*Clethrionomys glareolus* Shreber, 1780), a dominant individuals of small mammalian populations on the studied territories, was the object of study. Animals were delivered to the laboratory, fed with unlimited rich green fodder, and kept for a day to minimize the effect of catching and transportation.

Immature and mature yearlings were used for analysis. Small animals with a body weight of 16–18 g not breed during the birth year were considered as immature, and animals with a body weight of 21–23 g actively growing and reproducing we considered mature. The experimental material and characteristic of areas are shown in Table 1.

The blood for study was sampled from the orbital sinus in narcotized animals, and the concentration of erythrocytes, leucocytes, hemoglobin (*HB*), hematocrit (*HT*), amount (*MCH*) and concentration of hemoglobin (*MCHC*) in the erythrocyte, and average volume of erythrocytes (*MCV*) were determined during the first 10 min after sampling using an Abacus junior vet hemoanalyzer (Austria). Distribution of erythrocytes according to diameter (*D*) in diapason 3.5–8.9 mm at ten points was determined using Celloscope 401 (Lars Yungberg & Co, Sweden). The thickness (*T*), sphericity (*D*/*T*), surface square of erythrocytes of each diameter, concentration of hemoglobin per unit of surface area (Kostelecka-Myrcha, 2002), and the index of erythrocyte deformation (*S*/*MCV* is the ratio of surface are to volume) were calculated. Morphological analysis of the blood cells and the composition of leucocytes was carried out on smears stained according to Pappenheim, the concentration of reticulocytes was done on smears stained by brilliant-cresyl blue, and the activity of peroxidase–hydrogen peroxide of leucocytes was measured on smears using 3,3-diaminobenzidine tetra-hydrochloride dehydrate (Fluka, United Sates) stained by 1% methylene green (Rogovin and But, 1994). Fractioning of blood hemoglobin was carried out by electrophoresis using equipment of the firm BIORAD (United States). The weight of the body and spleen were determined in animals killed by dislocation of neck vertebrae, and the number of spleen and marrow cells in femur were determined using the Goryaev chamber. The concentration of erythrocytes and marrow cells were normalized per body weight (Yushkov et al., 1999).

The concentration of metals (Pb, Cd, Cu, and Zn) in the kidneys was determined by atomic absorption on an AAS 6 Vario spectrophotometer (Analitik Jena AG, Germany) using a flame and electrothermal variant of atomization in an accredited analytical laboratory (no. ROSS.RU0001.515630). Samples were dried, scaled (using scale KERN-770 (Germany) with accuracy 0.00001), and combusted by the method of wet mineralization in 65% nitric acid in a microwave oven MWS-2 (Berghof, Germany). The integral index of the toxic load (Table 1) calculated according to the data of concentration of various elements in the stomach contents was used for evaluation of the influence of pollutants on the animals' organisms (Mukhacheva and Bezel, 1995).

The study results were analyzed using the program Statistica for Windows (discriminant, dispersion, and covariance analysis) and the Tukey-test was used for evaluation of various indexes for different numbers of animals.

RESULTS AND DISCUSSION

The parameters of the blood system of the bank vole inhabiting conditions of chemical pollution of the environment were analyzed in the groups of immature and mature yearlings on the basis of deviations in the adaptive response of the blood system in individuals of the bank vole of different reproductive statuses in the conditions of a modified environment of a seasonal character (Tarakhtii and Davydova, 2007) and different accumulation level of pollutants in them (Mukhacheva and Bezel, 1995, 2007). The difference in the mean values of the blood system indexes in these samples depended slightly on gender ($R\text{-Pao}_{10;\,2} = 6.677$, $p = 0.137$) and the catch year of animals ($R\text{-Pao}_{20;\,2} = 2.801$, $p = 0.296$), where *R*-Pao is the criteria using *F*, i.e., the statistics of Wilks lambda distribution for

Table 2. Results of discriminant analysis

Territory	I	B	BG1	BG2
Immature yearlings*				
I		2.682	2.907	3.603
B	22.094		3.05	5.98
BG1	19.737	9.941		5.884
BG2	24.23	19.103	3.37	
Mature yearlings				
I		22.259*	5.43**	–
B	296.7882*		33.709*	–
BG1	32.027**	421.365*		–

Note: Above the diagonal is the area of the Mahalanobis distance, below diagonal is $F_{9;\,26}$, criteria.

* $p < 0.05$ (for Tables 2–4);

** $p < 0.06$.

approximation that is determined by dispersion analysis. Samples of voles are different in the gradient of environmental pollution, which was determined on the basis of complexes of morphological and hematological indexes using discriminant analysis (Table 2). Differences in the blood system indexes between groups of mature and immature animals in the gradient of environmental pollution were evaluated using dispersion analysis.

The concentration of erythrocytes (0.55–0.74 mln/μl/g of body weight), normalized to body weight, marrow cells (0.57–0.91 mln/μl/g of body weight), and the spleen (1.45–1.64 mln/μl/g of body weight) increase (at $p < 0.05$) of immature individuals from areas BG1, BG2 closer to the pollution source. The number of erythrocytes in individuals from area I decreases and was hardly different from the initial value for marrow and spleen cells, that is, by 16 and 82% lower, respectively, than on BG1 area. The mass of the spleen is lower (31 mg to 73.6 mg) and the index for the spleen (1.97 to 3.98) and number of reticulocytes in the blood was 2.16% to 9.58% and from the B area it is 3.51%, respectively. The concentration of hemoglobin in the blood according to Bogach and coauthors (1988) can serve as the bioindicator of industrial pollution, and, in our case, this index increases (15.5–16.9 mg% at $p < 0.3$) until area I, but the ratio of its fractions changes. The first out of two extracted fractions is the main part (82.7–84.0%) and the second is significantly less (16.0–17.3%) in norm according to decreasing order of electrophoretic activity. A part of the second fraction (from 2.5 to 2.9 g% at $p < 0.05$) increased by 13.8% in the gradient of environmental pollution that can be considered as adaptation of oxygen assimilation (Physiology ..., 1979; Avtsyn and Marachev, 1994). An increase in the fetal hemoglobin content, the affinity of which to oxygen is 15% higher than in hemoglobin A, was observed from the influence of extremal factors or pathological states in adult people (Avtsyn and Marachev, 1994).

The composition of the population of erythrocytes and the cell structure (R-$\mathrm{Pao}_{5;\,19} = 3.972$ at $p < 0.012$) change in the gradient of environmental pollution in immature yearlings, which is important for supply of tissues with oxygen. The part of large erythrocytes decreases with increase in the toxic load, while the part of small erythrocytes increases (Fig. 1). The share of the erythrocytes in diapason 5.4–9.6 μm was 2–40% lower in voles from area I than in voles from area BG1 and higher by 15% of cells with diameter 3.5 μm. The average surface area, section, average diameter, and stability of erythrocytes (Table 3) were lower by 9, 5, 2.5, and 20%, respectively, while the thickness, sphericity, cell volume, content of hemoglobin in erythrocyte per unit of surface area were higher by 24, 19, 19, 22, and 25%, respectively. The last index is more informative and important in oxygenation of tissues than the hemoglobin concentration in the blood and is quite similar in many individuals of mammals (Kostelecka-Myrcha, 2002). The concentration and dimensions of erythrocytes were related to the hematocrit index (correlation coefficient $r = 0.81–0.68$) which in individuals from polluted areas was higher ($p < 0.04$) than from the background (46.7 and 46.2% from areas I and B to 41.9 and 41.5% from BG1 and BG1), which corresponds to the published data (Bogach et al., 1988).

The cell composition (Table 4) and functional activity (Fig. 2) change in the white blood of immature yearlings with growth of the toxic load. The individuals from area I in comparison with area BG1 are characterized by the relative number of neutrophils three times higher (at $p < 0.05$) due to segmented neutrophils, while the number of monocytes and lymphocytes are less increased, and eosinocytes and basocytes were not found. The activity of the peroxidase–hydrogen peroxide system of leucocytes in these voles is minimal and lower than in individuals from territory B where activity coefficient (K) is 27.3% to 18% due to increase of the parts of all types of the cells especially maximally active.

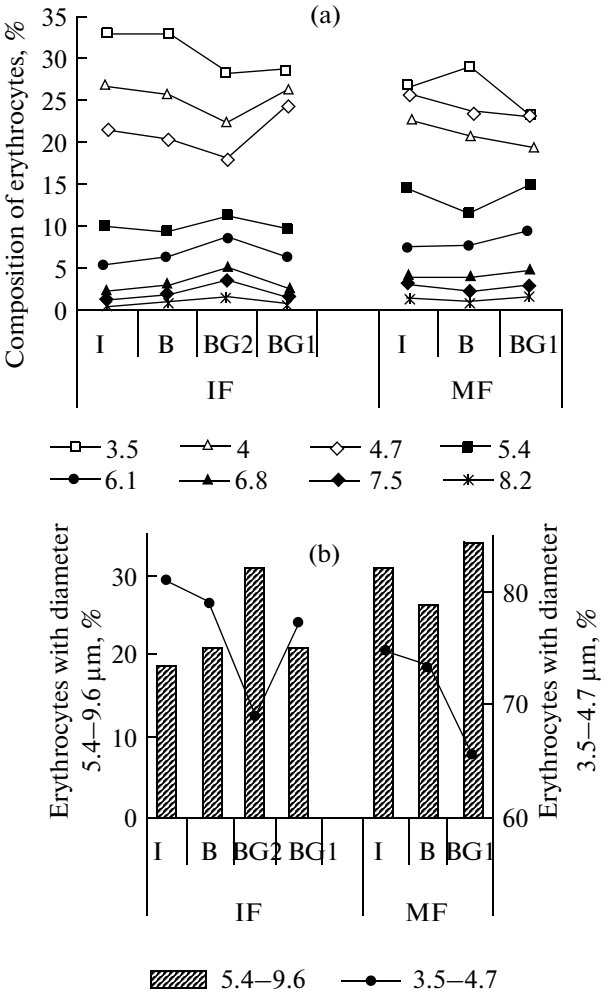

Fig. 1. Composition of erythrocytes with diameter 3.5–8.2 μm (a) and average values in diapason 3.5–4.7 and 5.4–9.6 μm (b) in immature (IF) and mature (MF) yearlings of the bank vole in the gradient of environmental pollution. BG1, BG2 are background, B is buffer, and I is impact territories (for Figs. 1–3).

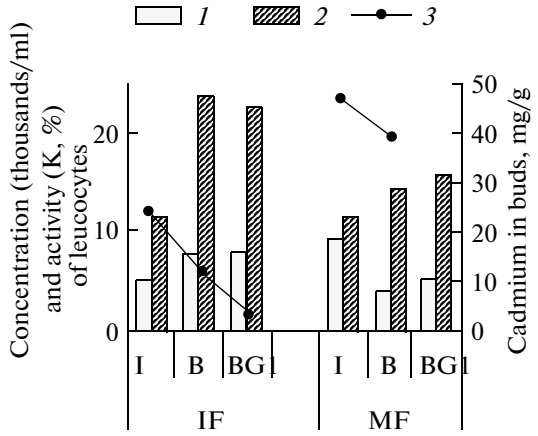

Fig. 2. Concentration of leucocytes (*1*), activity (K) of peroxidase-endogenous hydrogen peroxide of leucocytes system (*2*), concentration of cadmium in buds (*3*) of immature (IF) and mature (MF) yearlings of the bank vole in the gradient of environmental pollution.

The concentration of hemoglobin in the blood (15.1–16.6 g%) and erythrocytes (9.7–11.7 mln/μl) including normalized to body weight (0.43–0.51 mln/μ/g of body weight) is similar in mature yearlings in the gradient of environmental pollution. However, the composition of the erythrocyte population and cell parameters change and differ from the appropriate indexes of immature individuals from the initial values (BG1). The average diameter of erythrocytes (Table 3) is higher in mature individuals from area BG1 due to the greater part (Fig. 1) of erythrocytes with diameter 5.4–6.8 and 8.2 μm and lesser with diameter 4 μm (at $p < 0.05$). The parameters of erythrocytes between samples from area B are less different, and a part of the cells with diameter 3.5–4.7 μm increases in mature individuals, which is comparable with maximal value of hematocrit (45.7%). The average diameter of erythrocytes in area I in these is significantly higher (at $p < 0.05$) due to the greater proportion of large cells (with diameter 8.2 μm at $p < 0.05$ and 8.9, 6.8, 5.4, and 4.7 μm at $p < 0.1$ (Fig. 1a)) and the lesser part of smaller cells (in diapason 3.5–4.7 μm (Fig. 1b)). Erythrocytes of these voles are less spherical than in immature individuals from area I and in mature individuals from area BG1 (height is less by 63 and 14%, respectively). The sphericity of erythrocytes reflects the ability of the cells to deform, and its decrease improves the microcirculation and increases the stability. They are characterized by a greater surface area, and the volume and content of hemoglobin in the erythrocyte and hemoglobin concentration per unit of surface area of erythrocyte are less than in immature individuals (Table 3). An increase in the part of large erythrocytes in individuals from area I is comparable with a slight increase in the cell concentration in marrow (0.74, 0.54, and 0.62 mln/femur/g of body weight) and reticulocytes in the blood (1.96, 0.58, and 7.87% on areas I, B, and BG1, respectively) that can be considered as the response to a large dose of the chronic effect of toxicants. The concentration of the cells in the spleen (3.84, 4.58, and 8.98 to 1.38, 3.41, and 4.71 mln/g of body weight) and its index (4.2, 2.3, and 12.9 to 1.97, 2.07, and 3.55) in mature individuals on areas I, B, and BG1 is higher than in immature individuals.

The concentration and composition of leucocytes change in mature voles based on the gradient of environmental pollution. The number of leucocytes in mature individuals from areas BG1 and B is less than in immature animals and on area I increases by two times, which exceeds the same value for immature individuals (Table 4) and probably compensates for their low functional activity (Fig. 2). The level of neutrophils in white blood of mature animals from area B is higher (at $p < 0.05$) due to segmented and stab neutrophils, and the level of lymphocytes is lower. This

Table 3. Parameters of erythrocytes of mature and immature individuals of the bank vole in the gradient of a polluted environment

Characteristic of erythrocytes	Result of dispersion analysis		Investigated territory							$p < 0.05$
			I (1)	B(2)	GB2 (3)	GB1 (4)	I (5)	B (6)	GB1 (7)	
	MS_{res}	$F_{2; 23}$	Immature				Mature			
Diameter, μm	0.032	2.95*	4.35	4.43	4.71	4.46	4.95	4.59	4.7	1–2, 4, 5
Thickness, μm	0.171	3.422*	3.52	2.78	2.26	2.84	2.16	2.37	2.47	1–5, 6**, 7**
Surface area, μm^2	10.382	3.11**	36.9	38.31	43.61	38.71	47.74	410	43	1–5; 3–4
Section, μm^2	1.689	3.11**	14.88	15.45	17.59	15.61	19.26	16.54	17.35	1–5
Sphericity (D/T)	0.095	3.619*	1.28	1.62	2.19	1.61	2.35	1.94	1.93	1, 2, 4–5
Deformability (S/MCV)	0.018	3.391*	0.72	0.91	1.13	0.89	1.17	1.05	1.02	1–5
Volume, μm^3	23.005	2.369	52.15	42.63	38.54	43.91	41.13	39.19	42.88	1–5**
Hemoglobin in erythrocyte, pg	3.215	2.963*	19.47	14.69	14.52	15.91	15.36	14.2	15.87	1–2, 5, 6
Concentration, pg	13.974	0.141	37.34	34.45	37.68	35.4	37.33	36.38	37.08	
Hemoglobin per unit of surface area, $pg/\mu m^2$	0	2.866*	0.05	0.04	0.03	0.04	0.03	0.03	0.03	1–5, 7**
Number of animals	43		3	6	14	10	4	2	4	

Note: MS_{res} is residual average area, $F_{2, 23}$ is criteria (for Tables 3, 4);
** $p < 0.1$ (for Tables 3, 4)

Table 4. Concentration and composition of leucocytes of the blood from mature and immature yearlings of the bank vole in the gradient of environmental pollution

Index	Result of dispersion analysis		Investigated territory							$p < 0.05$
			I (1)	B (2)	GB2 (3)	GB1 (4)	I (5)	B (6)	GB1 (7)	
	MS_{res}	$F_{2; 23}$	Immature				Mature			
Leucocytes, thousands/μl	12.994	3.131**	5.1	7.71	6.78	7.99	9.33	3.79	4.03	4–1, 5, 6
Neutrophils (N)	25.606	9.779*	23.14	12.67	13.93	6.71	14.5	35	7	5–4, 6, 2, 3
Myelocyte, %	0	0	0	0	0	0	0	0	0	
Metamyelocyte, %	0.784	0.39	1.33	0.33	0.27	0.18	0.63	0	0.2	
Stab (S), %	6.872	6.506*	5.97	3	3.8	2.27	4.5	14	2.2	5–4, 6, 2, 3
Segmentated (S)	12.396	9.477*	15.83	9.33	9.87	4.26	6.88	21	4.6	5–4, 6, 2, 3
Eosinophils, %	0.835	3.103**	0	0.33	1.33	0.91	1.75	0	0.6	
Monocytes, %	44.903	0.026	9.97	1	4.6	3.52	14.25	4	6.4	
Basophils, %	0	0	0	0	0.2	0	0	0	0	
Lymphocytes (L), %	76.59	2.858**	67.22	86.67	79.87	88.86	71.38	62	86	1–2, 3; 5–6
L/N	326.28	0.383	3.31	6.92	8.86	13.24	8.6	1.77	13.09	
S/S	0.35	0.094	0.54	0.4	0.43	0.48	0.5	0.67	0.62	
Number of animals	27		3	3	15	11	4	2	5	

Table 5. Dependence of peripheral blood indexes on cellularity of the spleen (CS, mln/organ) and marrow (M, mln/femur) of the bank vole

Dependent variable	Covariants	b_i	s.e.(b_i)	β_i	$t(34)$	pL
Erythrocytes, mln/μl/g of body weight	CS	0.12	0.04	0.42	2.9	0.007
	M	0.18	0.09	0.28	1.94	0.061
MCV, μm^3	CS	3.76	1.33	0.44	2.88	0.008
Composition of erythrocytes of different diameter, %	CS	3.16	1.69	0.3	1.87	0.071
HT, %	CS	3.28	1.75	0.31	1.88	0.069
HB, g%	CS	1.72	0.74	0.37	2.32	0.027
Leucocytes, thousands/μl	CS	2.03	1.16	0.28	1.75	0.088
Lymphocytes, thousands/μl	CS	1.94	0.82	0.37	2.37	0.024
Monocytes, thousands/μl	M	0.34	0.18	0.29	1.85	0.074

Note: b_i is the regression coefficient (slope angle), s.e.(b_i) is its standard error, β_i is the standardized regression coefficient, t is Students criteria, p is the significance level.

composition of leucocytes in immature individuals was noted on area I. Leucopenia, monocytosis, and eosinopenia are common characteristics for mature individuals on area B and immature individuals on area I. These characteristics are considered as the tensity of reactions and are characterized as stress (Garkavi et al., 1990). An increase in the number of monocytes can influence the proliferation, differentiation, and maintenance of hematopoietic precursors. Their role in cell immunity is increasing (Yagunov et al., 2006).

The value of the ratio lymphocytes/neutrophils decreased in the gradient of environmental pollution in mature and immature voles with distinguishable dynamics of the uniform blood elements, and the value of the ratio stab/segmented increased. The ratio of the considered types of cells characterizes the reactivity of the organism and the blood status with analogy to laboratory animals (Mashneva et al., 1984). The first index significantly changes in the bank vole to a higher degree. Its value is minimal in mature individuals from area B, which is 13% compared to 57% in immature individuals and for individuals from area I was 64% of the initial value for each group.

Pathological forms of the cells such as stomatocytes, acanthocytes, condensation of hemoglobin in the cells, morphologically changed leucocytes, monocyte-like lymphocytes with large azurophilic inclusions in the cytoplasm, and lymphoreticular cells were found together with quantitative, structural, and qualitative changes of the blood cells and hematopoietic organs, in the blood smears of bank vole individuals habituating in the area of the factory.

The alterations of the parameters of the blood and hematopoietic organs are connected, and the spleen is related not only to the cell concentration in the blood and with the dimensions of erythrocytes (Table 5). The connected variability of the indexes reflects one of the regulatory methods of the hematopoietic system, because quantitative changes of any element of the blood system are impossible without involvement other components in the process (Physiology ..., 1979). There is the question of whether or not changes inthe parameters of the blood system of the bank vole are related to the influence of chemical substances.

Variability of the blood system parameters of bank voles (concentration and activity of leucocytes, diameter of erythrocytes, number of the cells in marrow and spleen) was compared with the level of accumulation of pollutants in the organism by the example of cadmium in the buds (Figs. 2, 3). Alteration of these indexes in mature and immature yearlings is different and is characterized by the relation ($r = 0.6–0.8$) to cadmium concentration in buds, which increases closer to the emission source (Fig. 2). The correlation of the accumulation level of cadmium in buds with the hemoglobin concentration in erythrocytes and in the blood was determined to greater degree with its main function ($r = -0.59–0.6$ at $p < 0.07$).

Analysis of variability of the parameters of the blood system of the bank vole and of the concentration of heavy metals in the organism of animals results in the conclusion that voles experience the influence of pollutants. A distinguishable response of immature and mature individuals in the gradient of environmental pollution can be determined by both different levels of accumulation of metals in the organism (Figs. 2, 3) and their different ratios the effect of which can be implemented by combined, synergistic, and antagonistic activity. It is known that abundance of copper and zinc decreases nephrotoxicity of cadmium competing for the binding sites (ATSDR, July 1999. Cadmium ...).

RESULTS AND DISCUSSION

It was determined early that the part of individuals with higher concentrations of heavy metals in the organism increases with an increase in the toxic load in the bank vole population (Mukhacheva, 2005). Comparison of the concentration of pollutants in the stomach contents with the level of accumulation in

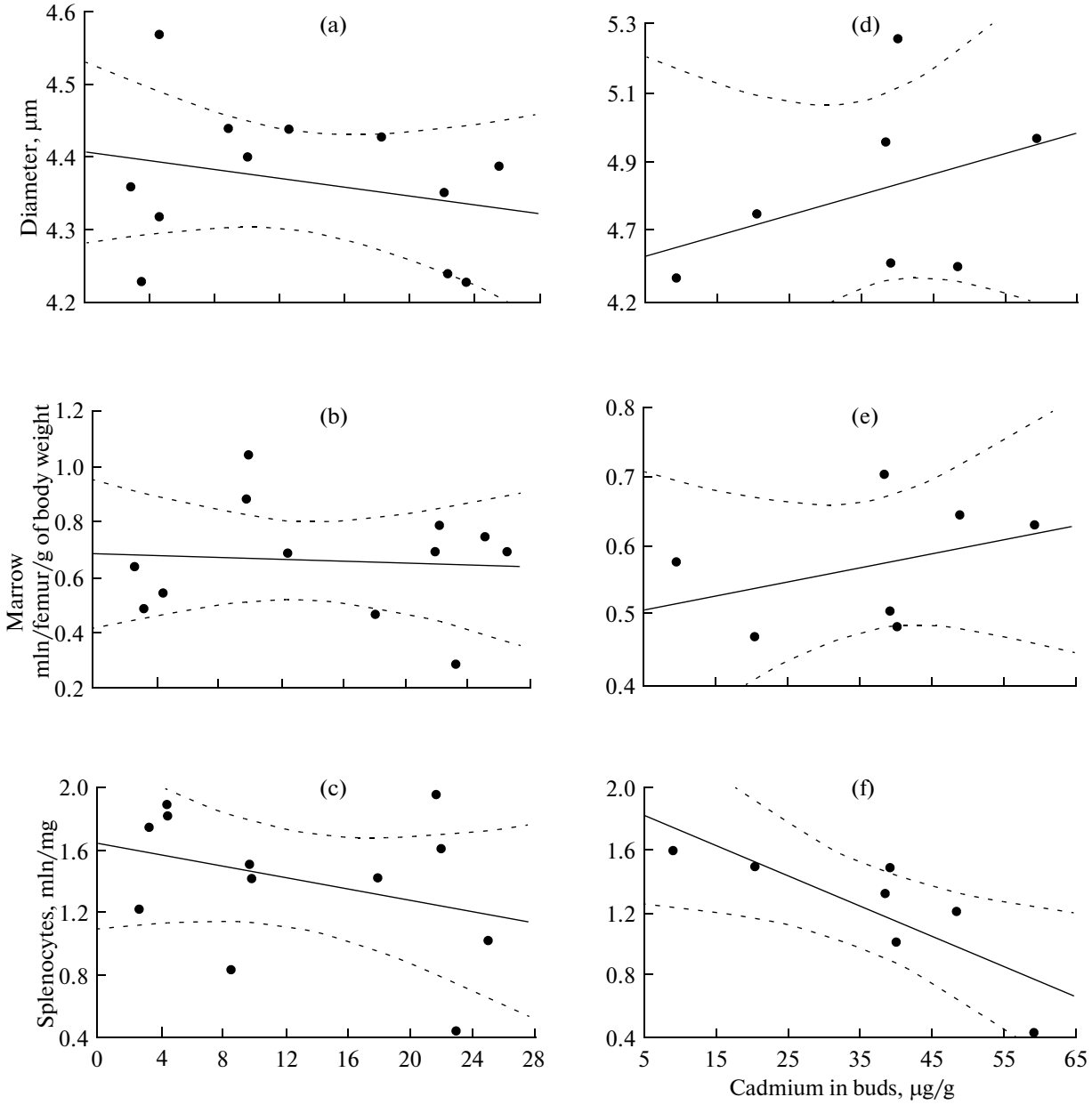

Fig. 3. Variability of diameter of erythrocytes (a, d), cell concentration in the marrow (b, e) and spleen (c, f) of the bank vole relative to the concentration of cadmium in buds of immature and mature yearlings. Pointed line is experimental data, solid line is the regression line, dotted line is the confidence interval (at $p < 0.05$).

organs of voles showed that the barrier function of the gastrointestinal tract supplies homeostasis of physiologically required elements (Cu and Zn) but discriminates in transition of toxic elements (Cd and Pb) to the deposit organ (Bezel et al., 2007). More than 1% of cadmium and 10–20% of lead are absorbed from the gastrointestinal tract (Semenov and Tregubenko, 1984). Direct correlation of the concentration of cadmium in food and the blood was found (Gil'denskiol'd et al., 1992; Rogival et al., 2006). It was shown early that the daily average uptake of cadmium with food in animals from area B is 0.4–0.7 and from area I up to 1.8 μg/g of body weight (Mukhacheva, 2005). Pathological dysfunctions develop in laboratory animals with these parameters. An increase in the number of erythrocytes in immature individuals of the bank vole from area B are hardly considered as redistribution in the blood system because there are changes of cellularity in hematopoietic organs and the share of small erythrocytes increases in mature individuals.

The possibility of physiological adaptation of animals' organisms to conditions of a changed environment is active until a certain level of load on an organism. The concentration of these metals in buds of the bank vole is significantly higher than in cows' buds, and the level of accumulation of metals in organs and

tissues significantly exceeds the maximum permissible concentration (MPC) at comparable concentrations of Pb, Cd, and Cu in the daily food ration of the bank vole and cows from farms located close to the industrial factories (Donnik et al., 2007). As a result, a decrease in the concentration of erythrocytes and the prevalence of their microcytic forms, monocytosis, and decrease in the phagocytic activity of leucocytes was identified by us in the blood of the bank vole. Deviations in the response of the blood of mature and immature animals are especially expressed at a great toxic load (area I) probably also exceeding MPC. The changes described in the bank vole were found also in mouselike rodents from the genus *Apodemus* inhabiting the condition of technogenic environmental pollution. These stress of the red blood sprout in bone marrow and pathological forms of erythrocytes (Baragunova et al., 2003), changes in the parameters of the blood, and cells at concentrations of Cd and Pb in the blood 3–4 times higher than in individuals from clean territories (Rogival et al., 2006). The influence of Cd on quantitative and qualitative changes of erythrocytes was verified in the experiments using rats, and changes were identified already during the first month of chronic treatment of animals at a dose of 1 μg/kg of body weight. It was determined that Cd directly influences both erythrocytes and their precursors in hematopoietic organs (Tugarev, 2003). The main part of cadmium during chronic uptake to the organism first concentrates in the blood plasma as the complex with albumin and later it concentrates in erythrocytes (ATSDR, July 1999. Cadmium ...).

Pathological changes in the organism of mouselike rodents from natural populations were noted at higher concentrations of Cu and Pb (Kokhonov, 2005). Erythrocytes bind up to 90% of cadmium in acute uptake of Pb into the organism, and up to 1250 μg% of metal accumulates in active erythrocytes in workers working with Pb (Semenov and Tregubenko, 1984). The chronic treatment of laboratory animals by Pb in doses 2–3 times higher than MPC resulted in change of the cell structure and decrease of the concentration of lymphocytes and neutrophils (Mashneva et al., 1984). According to the data of these authors, the dysfunction of the enzymatic system, accumulation of intermediate metabolic products, and tissue disintegration precede the morphological changes. They noted a certain relationship between the intensity of accumulation of metabolites and the expression level of morphological dysfunctions.

Cooper and zinc are involved in the composition of the cell structures, many enzymes, hormones, and vitamins. The main part of Zn (85%) is represented in the form of elements of the blood (*Man ...,* 1977), and leucocytes bind zinc more strongly than erythrocytes (Semenov and Tregubenko, 1984).

It is known that Cd, Pb, Cu, and Zn uptaken by the organism of different individuals of animals can be included into the blood cells, directly and indirectly affecting these. The quantitative, structural, and functional changes in the blood system and the presence of pathological forms of erythrocytes and leucocytes in individuals of bank voles inhabiting polluted territories found by us and compared with the literature data are determined by the influence of pollutants for which the concentration increases closer to the emission source as was shown by the example of Cd in buds.

CONCLUSIONS

Variability of the blood system parameters, which depends on the level of toxic load and reproductive status of animals, was determined on the basis of complex investigation of morphophysiological and hematological indexes carried out at the molecular, cellular, and organismic levels.

It can be supposed that the increase of the indexes of the blood and hematopoietic organs in individuals from territories BG1, BG2, and B is related to the activity of pollutants in the organism causing the effect directed to maintenance of the functions of the blood system, which can result in disrupture of the life processes and evidence of adaptive possibilities of the system.

Alterations in the concentration and structure of erythrocytes, number of reticulocytes, hemoglobin fractions, cellularity of hematopoietic organs, and appearance of pathological forms of the cells in the blood of individuals from territory I support the assumption that maintenance of gas-transport function of the blood in immature individuals is determined by production of potentially short-lived erythrocytes with a higher hemoglobin concentration. The changes in mature individuals such as increase in the diameter, surface area, decrease of sphericity of erythrocytes, and enhancement of proliferative activity can be considered as irritation of the marrow by high doses of toxins that together with decrease of the function of leucocytes cause tension in the blood system activity and the beginning features of organism intoxication.

As the result of the chronic effect of pollutants absorbed by the organism of the bank vole, cells with other qualitative properties reflecting the features of adaptation mechanism of mature and immature yearlings and determined by different metabolic levels in these individuals appear in the blood. The data of a complex biological response on the influence of habitat conditions on the territory surrounding the factory evidence the quality of life of the bank vole population requiring a control on the environmental status.

ACKNOWLEDGMENTS

The authors are grateful to Doctor M. N. Sumin for determination of the fractions of hemoglobin in the blood voles carried out in the Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences.

The work is supported by the Russian Foundation for Basic Research, grant no. 10-04-01657.

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