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Energetic Metabolism and the Blood System of Small Mammals Living under Different Climatic and Geographical Conditions

L. A. Koval'chuk and S. V. Tsvirenko

Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences, ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia

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Abstract—Studies at the level of phenotypic and genotypic adaptations of small mammals under extreme environmental conditions have exceptional significance for the estimation of the nature of tissue and cell transformations in the organism. Comparative analysis of adaptive abilities of the energy metabolism and blood in small mammals from different ecological zones is presented.

Investigation of mechanisms of injury and adaptation under the influence of extreme factors on organisms is one of the major biological problems that are closely related to understanding the general regularities of the maintenance and restoration of energetic homeostasis in organisms. Studies on animals from natural populations living in different climatic and geographical regions and possessing evolutionarily optimally regulated systems of adaptation to environmental specificity allow us to obtain information on the regularities and mechanisms of emergency regulation and stable compensatory reactions that are formed under the long-term influence of extreme factors.

Our studies were motivated by the lack of a common opinion concerning concrete mechanisms of injuring action of extreme environmental factors. Characteristics of structural and metabolic processes in the period of adaptation to low temperatures in animals living in the Far North may be considered to be an optimal variant of adaptive transformation.

MATERIALS AND METHODS

We studied stable adaptations of animals to extreme environmental conditions (influence of cold) characterized by the formation of expressed distinctions of regeneratory and metabolic processes in tissues of male mice SVA (weight 19–22 g, 56 specimens) and red voles (*Cleithrionomys rutilus* Pall., weight 18–22 g) captured from natural populations of the Kurgan oblast (60 specimens) and the Yamal Peninsula (43 specimens), as well as Siberian lemmings (*Lemmus sibiricus* Kerr., weight 40–50 g, 41 specimens) from the Yamal Peninsula. The influence of cold on animals was determined in a VT-1000 climate chamber (East Germany), during 23 h daily at 0°C with an interruption for animal feeding. Total oxygen consumption by animals was measured by means of an MN-5130 gas analyzer. Patterns of the respiration of homogenate and mitochondria excreted by liver and marrow were used to estimate the character and intensity of oxygenation energetic processes in tissues. Tissue respiration was recorded by means of the polarographic method (Kovalenko, 1975). Counts of erythrocytes, reticulocytes, and leucocytes, as well as marrow cells content, hemoglobin content, and myelogram were used to estimate regeneratory processes in the marrow. Incorporation of ^{3}H-thymidine in DNA, myelokaryocytes measured with liquid scintillation radiometry by an SVS-2 counter was used to estimate the intensity of DNA synthesis (Epifanova, 1977).

The content of diene conjugates of higher unsaturated fatty acids was measured to estimate the condition of peroxidal oxidation of lipids (POL) and the antioxidative activity (AOA) (Stalnaya, 1977). The content of uronic acids (acidic glycosaminoglycans (aGAG) and neutral glycosaminoglycans (nGAG)) in the tissues were determined by the methods of Bitter and Muir (1962) and Warren (1959). Protein was analyzed by the method of Lowry (1951). The data obtained were statistically treated using an Elektronika D3-28 PC.

RESULTS AND DISCUSSION

We found significant differences in the initial levels of metabolic processes in small mammals depending on their geographical distribution. Total oxygen consumption was lower in red voles from the northern population than in those from the southern population (−12.2%, $p < 0.05$). A minimal level of basal metabolism was recorded in lemmings. Oxygen consumption increases earlier in the northern animals at low temperatures, and especially in lemmings: 38% ($p < 0.01$) in a day. This may indicate a higher reactivity of functional systems of the organism undergoing the effect of an extreme factor (Fig. 1).

We observed a significant increase in oxygen consumption in the voles from the temperate zone (southern population) at cooling days 7 (+28%, $p < 0.05$) and 9 (+24.8%, $p < 0.05$). Depression in oxygen utilization

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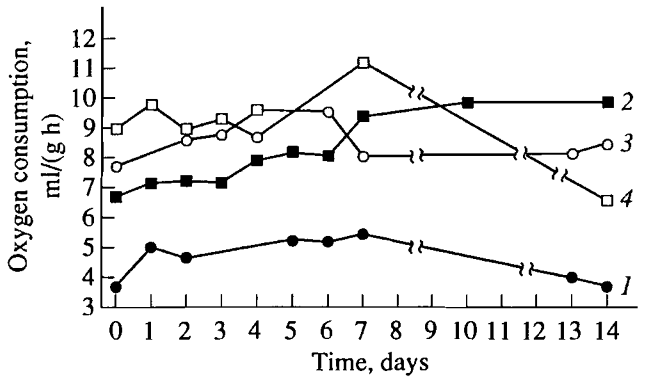

Fig. 1. Basal metabolism of small mammals in the experiment under the cold influence (0°C); (*1*) lemmings ($n = 41$); (2) SVA mice ($n = 56$); (*3*) red voles of the northern population ($n = 43$); (*4*) red voles of the southern population ($n = 60$).

occurs later, and the value falls to 23.1% ($p < 0.01$) below the level of intact animals by day 14 (Fig. 1). We observed a different situation for laboratory mice. Cold caused an increase in the parameter until day 10 (+52.6%, $p < 0.01$). Two and four weeks later, it remained at the level exceeding the control by 1.5 times.

A decrease in metabolic intensity after its increase at the early period of cooling is a peculiarity of oxygen metabolism in the voles from the natural population (Fig. 1). The significance of this increase in metabolism is well-understood: this is an increase in energy and heat production under intense heat loss, whereas the decrease apparently indicates the switching on of special mechanisms to conserve heat and increase the economy of nutrients and oxygen. This mechanism seems to be more effective in lemmings who display total normalization in oxygen use.

It is notable that regenerative processes play an important role in the development of compensatory and adaptive reactions to extreme influences (Kryzhanovskii, 1974; Yastrebov *et al.*, 1988), because they not only ensure regeneration of injured tissue but represent a structural basis for reinforcement of certain tissue and cell functions as well (Meerson, 1967; Sarkisov, 1987). Modern theoretical views allow us to assume that the intensity, character, and reserve facilities of regenerative processes significantly influence the resistance of organisms to extreme factors, the action of which is accompanied by functional and structural changes and, at a prolonged influence, also with participation in the formation of adaptations to new conditions. Data on mechanisms of observed transformations are limited and only represent the results of laboratory experimentation on animals (Kirillov, 1977; Heroux, 1970; Ottani and Mulhtradt, 1980).

The intensity of oxidation processes and the content of endogenous energetic substrates $V_{end} + V_{dnf}$, including succinate and NAD-dependent (at a certain decrease in lipid concentration), are higher in the liver

Table 1. Specific distinctions of liver metabolism in small mammals

Parameters		Lemming $n = 29$	Red vole		Mice SVA
			Northern population $n = 31$	Southern population $n = 28$	$n = 46$
Energetic processes in mitochondria, nAO_2/min/mg protein	V_{nc}	5.0 ± 0.7	22.8 ± 1.9*	14.9 ± 2.1	13.3 ± 1.2
	V_3	8.6 ± 1.3	81.1 ± 6.0*	51.0 ± 5.1	50.2 ± 4.9
	ADP/O	1.35 ± 0.03	0.87 ± 0.02	0.91 ± 0.02	1.48 ± 0.09
	$V_{end} + V_{dnf}$	2.7 ± 0.6	9.4 ± 0.8*	4.5 ± 0.8	3.0 ± 0.3
Condition of the processes of peroxyde oxidation of lipids (POL) and antioxidative activity (AOA)	h	4.5 ± 0.4	4.2 ± 0.3*	11.4 ± 0.9	3.0 ± 0.3
	l	36.0 ± 3.1	34.3 ± 0.3*	30.1 ± 0.4	40.0 ± 1.1
	tan α	0.15 ± 0.01	0.64 ± 0.09	0.59 ± 0.11	0.45 ± 0.05
	H	11.8 ± 0.6	16.7 ± 0.8*	13.5 ± 0.9	21.0 ± 0.8
	h'	37.7 ± 0.6	45.7 ± 1.6*	49.5 ± 0.3	50.0 ± 0.7
Functional condition of the lysosome apparatus, $mkNR_n$/g/s	Total phosphatase activity (AP)	1.67 ± 0.26	4.69 ± 0.83	1.72 ± 0.19	3.17 ± 0.48
	Free activity of acidic phosphatase (AP),	0.89 ± 0.09	2.65 ± 0.36*	1.46 ± 0.21	2.17 ± 0.36
	% from free activity	63.0 ± 4.4	55.3 ± 4.3*	83.8 ± 2.8	73.3 ± 5.1
aGAG—acidic glycosaminoglycans		62.8 ± 7.9	84.8 ± 9.0	84.3 ± 10.8	40.5 ± 5.6
nGAG—neutral glycosaminoglycans		1.02 ± 0.04	0.57 ± 0.06	0.73 ± 0.08	0.80 ± 0.10

* $p \leq 0.05$ when the vole populations are compared.

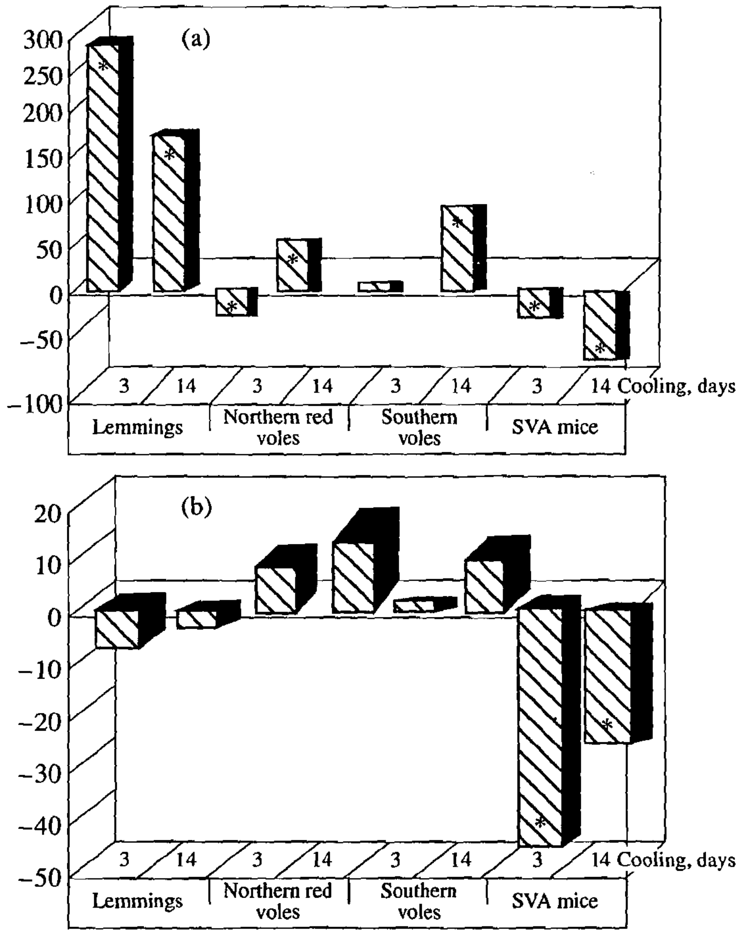

Fig. 2. Parameters of oxidative metabolism in the liver mitochondria of lemmings, red voles, and laboratory mice under the influence of cold for 3 and 14 days, in % to initial level: (a) V_3—rate of oxidation of energetic substrates during the ATP-synthesis ($p \leq 0.05\%$); (b) ADP/O—coefficient of effectiveness of oxidative phosphorylation ($p \leq 0.05$).

of voles from the northern population (Table 1). They have more active lysosomic enzymes, processes of peroxide oxidation, processes of antioxidation protection, and the oxidability of lipids. In red voles from the temperate zone, cooling induces the activation of acidic phosphatase by the 14th day: free phosphatase +186% and total phosphate +18%. However, the proportion of free and total activity does not differ from the control (+4.0%, $p > 0.05$), which indicates the stability of organelle membranes which seem to be under reliable control. A similar situation was observed for voles from the North but even by the 3rd day after cooling. Two weeks later, they display labilization of lysosome membranes (+42%, $p < 0.05$) leading to some increase in free activity (+30.3%, $p < 0.05$). Lemmings display a tendency toward a decrease in acidic phosphatase three days later (KF-free 36%, total 22%, at $p < 0.05$). After 14 days of cooling, free and total activity increase significantly at a slightly changing lysosome stability (+23.4%, $p > 0.05$). No significant changes in parameters of the liver lysosomal apparatus under low temperatures were noted in laboratory mice. The data obtained indicate that pathways of transformations of the lysosomal apparatus are different in the widespread vole species. Adaptive reaction of lysosomes is typical for the northern populations, which coincides with an increase in the total liver metabolic rate. Low organelle activity is more expedient for the southern populations, which corresponds with the general tendency of economy of functions and metabolism in these animals.

A comparison of the two populations of voles and lemmings indicated different directions in liver oxidative metabolic changes compared to the initial condition (Fig. 2). First of all, the character and intensity of the reaction of the energetic apparatus of the liver depend on the initial functional and metabolic conditions (Bol'shakov *et al.*, 1984): the lower the activity in intact animals is, the earlier and more prominent the stimulation of oxidative processes is. In lemmings, autochthons of the Subarctic, the low oxidative activity in the liver is formed with characters of higher effectiveness of oxidation energy use: higher ADP/O (Table 1). The stimulation of respiration in these animals under cooling is accompanied with adequate mobilization of

Table 2. Parameters of regenerative processes in the tissues of animals dwelling in different climatic and geographical zones

Parameters	Mice SVA $n = 38$	Siberian lemming $n = 18$	Red voles	
			Southern population (Kurgan oblast) $n = 25$	Northern population (Yamal Peninsula) $n = 31$
Liver				
DNA, mg/g of dry weight	8.7 ± 1.8	11.1 ± 1.1	13.0 ± 2.0	18.0 ± 1.6*
Incorporation of ^{3}H-thymidine, Bk/mg DNA	150.9 ± 21.9	63.7 ± 9.8	181.8 ± 31.6	76.4 ± 14.8*
Binuclear cells, %	45.5	23.1	39.7	53.0*
Mean hepatocyte ploidy	8.142	5.445	7.186	5.614
Marrow				
Hemoglobin, g/l		151.5 ± 2.77	146.6 ± 1.7	156.6 ± 3.7*
Erythrocytes, t/l		10.2 ± 0.1	9.2 ± 0.5	10.6 ± 0.5*
Reticulocytes, g/l		177.7 ± 6.3	239.0 ± 23.9	143.1 ± 3.5*
Myelocytes			9.6 ± 0.4	7.2 ± 0.6*
Erythrokaryocytes		1.6 ± 0.2	2.4 ± 0.1	1.9 ± 0.2*
Index of erythroblast maturation		0.75 ± 0.03	0.71 ± 0.02	0.62 ± 0.02*

* $p \le 0.05$ when the populations of voles are compared.

substrates. However, the higher metabolic activity in the liver is fixed in voles from the northern population. Along with the insurance of higher reactivity of metabolic and structural plastic processes, this creates a threat of breach and depletion of the energetic apparatus.

The influence of low temperatures for 14 days did not cause a significant increase in antioxidative activity (AOA) in the liver: $\tan\alpha = 26.7\%$, $H = 19.4\%$, and $l = 29.9\%$ at significant decrease of these parameters ($p < 0.05$). An identical but clearer reaction is displayed by the northern voles: $\tan\alpha = 81.3\%$, $H = 93.0\%$, and $l = -29.9\%$. The complex of sharp shifts in these parameters indicates a significant increase in AOA and lipid oxidability. Some decrease in AOA at the same conditions ($h = 42.9\%$, $h' = +18.2\%$, $p < 0.05$) is displayed by the voles from the temperate zone.

The results described above allow us to conclude that animals adapting to conditions of the North have evolved mechanisms which provide them with more reliable antioxidative protection for maintenance of the optimal properties of biomembranes that are realized both in the rest condition and under low temperatures (Archakov, 1975; Novikov, 1985). Our data for small mammals from natural populations indicate an important role of the formation of the new morphofunctional state of tissue. Apparently, the peculiarities of adaptive growth of animals living in the North may be considered to be the optimal variant of adaptive transformation.

Differences in the parameters indicative of special traits in physiological regeneration of the liver were found in the intact animals studied. Compared to the voles from the temperate zone, red voles from the northern population have a higher DNA content in their liver, lower intensity of incorporation of ^{3}H-thymidine in DNA, and lower hepatocyte mean ploidy due to a decrease in the quality of polyploid cells at some increase of binuclear cells (Table 2). Lemmings displayed a low rate of DNA synthesis in their liver, lower hepatocyte ploidy, and the content of binuclear cells lowest among all animals studied.

High levels of hemoglobin and erythrocytes at low counts of reticulocytes have been found in lemmings and red voles from the northern population. The total pool of marrow hemopoietic cells, number of DNA-synthesizing erythroid cells, and index of erythroblast maturation are smaller in the marrow of these animals. Such a combination of parameters might indicate an increase of erythropoiesis activity in northern species due to a higher proliferative activity of erythrokaryocytes and erythroid cell maturation rate.

Lemmings and red voles from the northern populations, which have initially different states of regeneratory processes, also show different reactions to the influence of cold. Proliferation activates earlier and more significantly in the liver of these animals, which is expressed in the increase of incorporation of ^{3}H-thymidine in DNA, change in the proportions of classes of hepatocyte ploidy, and processes of intracellular regeneration (hypertrophy) that reflect the tissue DNA content. In laboratory mice and the voles from the temperate zone, an alternative shift takes place three days later, apparently due to catabolism of structural and deposed substances. Apparently, the events of hypertrophy develop to the 14th day in SVA mice but are absent in voles. Changes in regenerative processes under the influence of cold on lemmings and red voles from the Yamal Peninsula are more pronounced and retained onger. The tendency to restoration of the initial level of the parameters of intact animals was not observed in the northern individuals even 14 days later. This indicates that the reactivity to cold is higher in animals adapted to conditions of the North and possessing fixed special mechanisms to make them more tolerant of low temperatures.

A similar situation was observed in hemopoietic tissue. A higher regenerative activity was measured in the northern voles. It manifested itself in an increase of peripheral blood reticulocyte count (+147.2%, $p \leq 0.05$) and marrow erythroid elements (+19.3, $p \leq 0.05$) three days later, which remained two weeks after the exposure to cold. No significant differences in erythron parameters were recorded in the voles from the temperate zone after 3 and 4 days of cooling. However, taking into account the dynamics of erythrocyte and hemoglobin content, one can suppose that the reaction developed later than the first period and finished before the second period of the study.

CONCLUSION

Our results indicate that the means by which animals adapt to conditions of the North have different mechanisms of emergency regulation within a background of common tendencies which have similar directions.

We demonstrated two variants of animal adaptations to the adverse natural factors: "mobilizational," related to emergency stimulation of reserve facilities of tissue metabolic systems; and "economical," characterized by an increase in the potential facilities of tissue systems due to their current activity in the rest condition.

The functional state of the energetic apparatus, lysosomes, POL–AOA system, and GAG content play the role of universal metabolic processes that can change the sensitivity of cells and the activity of energetic and plastic processes in tissues. Influences of metabolic factors on regenerative processes depend on the expression of the shift and on the time of the effect. Targeted influences on these metabolic systems cause changes in intensity and character of compensatory and adaptive reactions of organism.

It is reasonable that the tissue transformations at stable adaptation, ensuring the maintenance of basic homeostatic parameters, create at the same time a new background in the organism where physiological processes will have a different character.

Our results, combined with fragmentary data of other authors, would allow us to outline the regularities of the changes in the state of animal energetic system and regenerative processes under conditions of a permanently changing environment, as well as to find approaches to the creation of a biological model for the analysis of causal relationships between regulatory systems and responding tissues.

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