ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 9, pp. 1126-1131. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © G. E. Aksyonova, O. S. Logvinovich, L. A. Fialkovskaya, V. N. Afanasyev, D. A. Ignat'ev, I. K. Kolomiytseva, 2010, published in Biokhimiya, 2010, Vol. 75, No. 9, pp. 1257-1264.

Ornithine Decarboxylase Activity in Rat Organs and Tissues under Artificial Hypobiosis

G. E. Aksyonova, O. S. Logvinovich, L. A. Fialkovskaya, V. N. Afanasyev, D. A. Ignat'ev, and I. K. Kolomiytseva*

Institute of Cell Biophysics, Russian Academy of Sciences, Institutskaya ul. 3, 142290 Pushchino, Moscow Region, Russia; fax: (4967) 330-509; E-mail: kolomiytseviskra@rambler.ru

Received August 4, 2009
Revision received December 18, 2009

Abstract—The influence of hypothermia–hypoxia–hypercapnia on ornithine decarboxylase (ODC, EC 4.1.1.17) activities in rat organs and tissues and also on the thymocyte distribution throughout the cell cycle stages was studied. The state of artificial hypobiosis in rats on decrease in the body temperature to 14.4-18.0°C during 3.0-3.5 h was accompanied by drops in the ODC activities in the neocortex and liver by 50-60% and in rapidly proliferating tissues (thymus, spleen, and small intestine mucosa) by 80% of the control value. In kidneys the ODC activity raised to 200% of the control level. Twenty-four hours after termination of the cooling and replacing the rats under the standard conditions, the ODC activities in the neocortex, liver, kidneys, spleen, and intestinal mucosa returned to the control values, but remained decreased in the thymus. Forty-eight hours later the ODC activities in the thymus and spleen exceeded the normal level. The distribution of thymocytes throughout the cell cycle stages did not change in rats in the state of hypothermia (hypobiosis); 24 and 48 h after termination of the cooling the fraction of thymocytes in the S stage was decreased and the fraction of the cells in the G_0+G_1 stage was increased. The normal distribution of thymocytes throughout the cell cycle stages recovered in 72 h. Thus, in the thymus the diminution of the ODC activity preceded the suppression of the cell proliferation rate. The tissue-specific changes in the ODC activity are suggested to reflect adaptive changes in the functional and proliferative activities of organs and tissues during the development of hypobiosis under conditions of hypothermia–hypoxia–hypercapnia.

DOI: 10.1134/S0006297910090051

Key words: artificial hypobiosis, rats, ornithine decarboxylase, thymocytes, cell cycle

Studies on biochemical and physiological mechanisms of artificial hypobiosis in non-hibernating mammals are interesting for biology and medicine. Under conditions of hypoxia–hypercapnia, rats can be cooled to 14-15°C and retain the ability for returning without assistance to normothermia in the normal gas medium [1, 2]. The resistance of animals to chemical and physical factors, including ionizing radiation, increases in the state of artificial hypothermia (cold anesthesia, hypobiosis) [1-4]. Notwithstanding a certain similarity, the artificial hypobiosis of non-hibernating mammals is different from hibernation [5]. Stress reactions can contribute to the response of the organism of non-hibernating mammals to low environmental temperatures.

Changes in the lymphoid organs and in the gastrointestinal tract mucosa are characteristic features of the organism's nonspecific reaction to environmental factors (stress reaction according to the definition by Selye) [6, 7]. Changes in the level of tissue metabolism and in the rate of cell proliferation are important components of adaptive reactions of the organism [8-10]. The metabolic and proliferative activities of cells and tissues can be assessed by intensity of the metabolism of polyamines that are involved in syntheses of DNA, RNA, and proteins, in membrane and cytoskeleton stabilization, in membrane permeability, etc. [11-13]. The intensity of polyamine metabolism can be adequately characterized by determination of activity of ornithine decarboxylase (ODC, EC 4.1.1.17), which is the first enzyme in the chain of synthesis of polyamines (putrescine, spermidine, and spermine) and limits the rate of their generation; ODC is regulated by many signaling pathways. The activity of ODC sharply increases in response to action of mitogenic stim-

Abbreviations: DTT, dithiothreitol; ODC, ornithine decarboxylase.

* To whom correspondence should be addressed.

uli and growth factors. This enzyme is a marker of cell activation and proliferation [11, 14]. The hormonal regulation of physiological functions of different organs is associated with changes in the ODC activity in them [15-17]. Thus, activities of ODC in organs and tissues are sensitive to central neuroendocrine and also to paracrine influences and can serve a functional biochemical test in studies on the organism's adaptation to external influences. The involvement of ODC in adaptation of mammals to low environmental temperatures and in transition to the hypobiosis state was discussed in [18].

Cultivation of mammalian cells at low temperatures is associated with growth delay and accumulation of cells in the G_1 or G_2 phase [19, 20]. It is interesting to study the proliferative activity of organ and tissue cells of mammals after hypothermia.

The purpose of the present work was to determine the influence of rat hypothermia on the ODC activity in the neocortex, liver, kidneys, thymus, spleen, and small intestine mucosa and also on the proliferative activity in the thymus assessed by the distribution of thymocytes throughout its cell cycle stages.

MATERIALS AND METHODS

Labeled L-[1-^{14}C]ornithine from Amersham International (USA), dithiothreitol (DTT), L-ornithine, and Tris from Sigma (USA), pyridoxal-5′-phosphate from Ferak (Germany), and Na_2-EDTA from Acros (Belgium) were used. Other reagents were of domestic production with at least chemical purity qualification.

All procedures with the animals were performed in accordance with requirements of the Institute's Commission on Ethics and of the European Convention for Protection of Vertebrates used for experimental and other scientific purposes (1986/609/EEC).

The animals were maintained under standard laboratory conditions (20-21°C, 12-h day–night cycle, 65% humidity) on food and water *ad libitum*. The standard diet was balanced in proteins, vitamins, minerals, etc. The body weight of the animals was 180-220 g. The hypothermia state of rats was obtained using a closed vessel method that allowed us to reach a deep and reversible cold anesthesia without premedication [2]. The rats were kept in a 5-dm^3 hermetic chamber at the temperature of 1-2°C for 3-3.5 h that under conditions of hypoxia–hypercapnia increasing with respiration resulted in the state of cold anesthesia with body temperature lowering to 14.4-18.0°C. Upon replacing under standard conditions, the rats recovered themselves to normothermia within 3-4 h and later manifested no changes in behavior. After the cooling and taking the rats from the chamber, their body temperature and heart rate (HR) were determined. The body temperature was measured rectally at the accuracy of 0.2°C with a TEMP-60 electrothermometer sensor placed into the colon at the depth of 6 cm; the HR was recorded with an EEG-4-02 electroencephalograph, with lead ECG needle electrodes placed subcutaneously on the animal's left front leg and above the left scapula. The body temperature of the animals taken from the chamber was on average 16.2 ± 0.2°C (from 14.4 to 18.0°C) compared to the normal 38°C; HR was 60 ± 3 bpm (from 36 to 100 bpm) compared to the normal rate 340-360 bpm. The animals in the state of normothermia were decapitated at the same time of day (between 12 a.m. and 1 p.m.), the animals subjected to hypothermia were decapitated immediately after being taken from the chamber and 24, 48, and 72 h after termination of the cooling. All further procedures were performed on ice. The neocortex, liver, left kidney, thymus, spleen, and a scrape of the small intestine mucosa were frozen in liquid nitrogen and stored for no longer than three weeks.

To determine the ODC activity, the tissues were weighed, minced, and homogenized in a glass homogenizer with a fluoroplastic pestle for 1 min at 2°C in two-four volumes of 0.1 M Tris-HCl buffer (pH 7.5) supplemented with 5 mM DTT, 0.5 mM Na_2-EDTA, and 40 μM of previously neutralized pyridoxal-5′-phosphate. The homogenate was centrifuged at 20,000*g* for 30 min at 2°C. The activity of ODC was determined in the supernatant by the release of $^{14}CO_2$ from labeled L-[1-^{14}C]ornithine using a published method [21] with some modifications [22]. The reaction was performed in the volume of 0.5 ml, which contained 0.1 M Tris-HCl buffer (pH 7.2), 5 mM DTT, 0.3 mM EDTA, 0.2 mM pyridoxal-5′-phosphate, 0.16 mM L-ornithine, 1.85 kBq (0.05 μCi) L-[1-^{14}C]ornithine with the specific radioactivity of 2 GBq/mmol, and 3-15 mg of the sample protein. To absorb the released $^{14}CO_2$, filter paper strips saturated with 50 μl of 0.4 M $Ba(OH)_2$ in 20% NaOH were used. The incubation was performed for 1 h at 37°C, and the reaction was stopped by addition of 0.5 ml of 40% TCA. The reaction was performed twice for each specimen. The radioactivity of the filters was determined with a SL-30 scintillation liquid counter (Intertechnique, France). The enzyme activity was determined against a null sample supplemented with an aliquot of the homogenization medium instead of the supernatant. The results were expressed in pmol CO_2/h per mg protein. Four to eleven animals were used for every experimental point, with a corresponding control for each. The results are presented as the mean ± standard error. The significance of differences was assessed by Student's *t*-test.

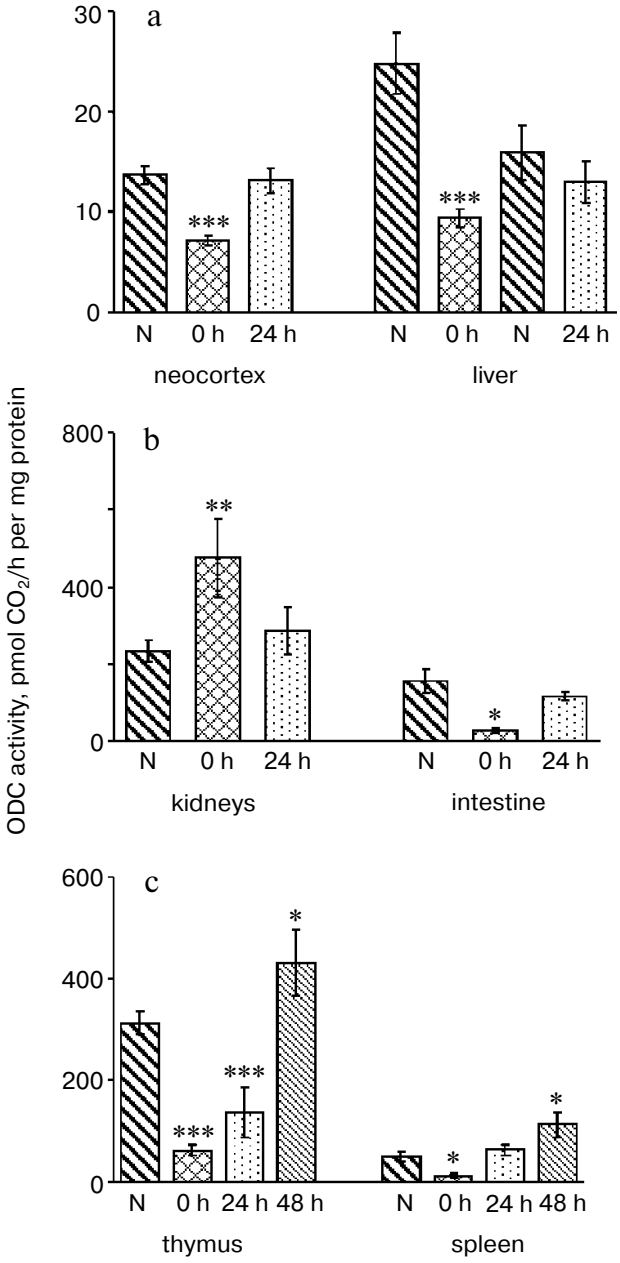
The protein content was determined by the Lowry method [23] with BSA as a standard.

The intensity of thymocyte proliferation was assessed by flow cytofluorimetry, which allowed us to characterize the cell distribution throughout the cell cycle stages. The thymocyte suspension was prepared by rubbing the thymus through a nylon fabric in calcium-free Hanks' medium. The cells in the suspension were fixed by addition of

cold 96% ethanol, stored at –20°C for at least 24 h, and stained with a DNA-specific fluorescent dye Hoechst-33258 (1 μg/ml in 0.1 M Tris-HCl buffer, pH 7.4, supplemented with 0.1 M NaCl) for 30 min at 37°C. The analysis was performed with a LAKS-1 apparatus produced at the Institute of Cell Biology, Russian Academy of Sciences [24].

RESULTS

Under conditions of normothermia the ODC activities in rat tissues and organs were as follows: 13.7 ± 0.9 in the neocortex (n = 9, n is the number of animals), 20.4 ± 2.3 in the liver (n = 16), 234.4 ± 28.4 in the kidney (n = 18), 156.0 ± 31.1 in the intestinal mucosa (n = 10), 48.8 ± 7.1 in the spleen (n = 20), and 312.8 ± 22.0 pmol CO_2/h per mg protein (n = 19) in the thymus. Cooling of the rat body to 14.4-18.0°C under conditions of hypoxia–hypercapnia was accompanied by a decrease in the ODC activity in the neocortex to 53%, in the liver to 46%, in the intestinal mucosa to 18%, in the spleen to 24%, and in the thymus to 20% of the corresponding control (figure). In the kidneys the ODC activity increased to 203% of the control (figure, panel (b)). Twenty-four hours after termination of the cooling and replacing the animals under standard conditions, the enzyme activities were recovered in the neocortex, liver, kidneys, intestinal mucosa, and spleen, but in the thymus the ODC activity remained decreased (figure). Forty-eight hours after termination of the cooling the ODC activities in the thymus and spleen became higher than in the control (figure, panel (c)). Twenty-four and forty-eight hours after termination of the cooling the fraction of thymocytes in stage G_0+G_1 was increased and the fraction of cells in stage S of the cell cycle was decreased. The initial level was recovered 72 h after termination of the cooling (Table 1). Hypothermia under conditions of hypoxia–hypercapnia caused no significant change in the thymus weight, the weight of spleen slightly decreased, and the weight of kidneys significantly increased (Table 2). Twenty-four hours after termination of the hypothermia–hypoxia–hypercapnia weights of the spleen and kidneys returned to the control values.



ODC activities in neocortex and liver (a), in kidneys and small intestine mucosa (b), and in thymus and spleen (c) at different times after termination of cooling of rats. N, norm; 0, 24, and 48 h, time after termination of cooling. *, **, ***, differences from control are significant at $p < 0.05$, $p < 0.005$, and $p < 0.001$, respectively

DISCUSSION

On the organismal level agents of certain intensity induce in mammals activation of the sympathoadrenal system and of the hypothalamus–hypophysis–adrenals axis with a development of a systemic nonspecific adaptive reaction of the organism, or stress [6]. Hormones of the adrenals, catecholamines and glucocorticoids, released into blood during the first hour of the animal's exposition to close to zero temperatures in normal gas medium [1, 25, 26] influence the ODC activity in different organs. Glucocorticoids and their synthetic analogs in active normothermal animals decrease the ODC activities in the thymus and spleen and increase them in the brain, liver, kidneys [15-17, 27-29], and in the intestinal mucosa [30]. Noradrenaline increases the ODC activity in the liver [31], and β-adrenoreceptor agonists increase it in the heart [32]. This reflects the strategy of the organism's

adaptive reaction represented by a glucocorticoid-dependent involution of the thymico–lymphatic system [6] and mobilization of vitally important organs.

Damage of the gastrointestinal mucosa under stress conditions is not caused by the action of adrenocortical hormones [6]. On the contrary, glucocorticoids promote the recovery of these damages by a mechanism including activation of polyamine synthesis [33]. The decrease in the ODC activity in the lymphoid organs (figure, panel (c)) and its increase in the kidneys (figure, panel (b)) observed in hypobiosis correlated with changes in the ODC activities in these organs caused by glucocorticoids [15, 16, 28] and by immobilization, which is a classical model of stress [27, 34]. Immobilization also induces an increase in the ODC activity in the neocortex [27], liver [27, 34], and intestinal mucosa [33].

In the stress situation caused by 2-h exposure to the environmental temperature of 4°C in the normal gas medium without development in the animals of the hypobiosis state, the ODC activities also increased in the liver and kidneys [34]. The hypobiosis state in rats under conditions of hypoxia–hypercapnia and the decrease in the body temperature to 14.4-18.0°C was accompanied by a decrease in the ODC activity in the neocortex, liver, and in the small intestine mucosa (figure, panels (a) and (b)), as differentiated from its increase in response to glucocorticoids and in the above-considered models of stress. An exception is presented by maternal deprivation of infant rats, which is accompanied by a decrease in the ODC activity in the brain, heart, and liver [34, 35]. Other stress agents (including their combining with the deprivation), such as immobilization or low environmental temperature, induced in infant rats a typical stress reaction with an increase in ODC activities in the above-mentioned organs [34]. The deprivation effects were reversible with a certain tactile stimulation of the infant rats.

The works [34, 35] present a brilliant example of inducing two types of an organism's adaptive reactions depending on the external agent. It seems reasonable to suppose a similarity between adaptation of animal infants to the maternal deprivation and hypobiosis.

A phenomenon of cold diuresis under low environmental temperatures is specific for mammals as a result of a decrease in water reabsorption in kidney channels asso-

Table 1. Distribution of thymocytes throughout cell cycle stages at different times after termination of cooling of rats

Time after termination of cooling, h	*n*	Thymocytes, % of total cell number		
		$G_0 + G_1$	S	$G_2 + M$
Control	33	88.4 ± 0.3	6.7 ± 0.2	4.9 ± 0.2
0	9	88.1 ± 0.4	7.2 ± 0.2	4.7 ± 0.2
24	8	90.9 ± 0.6*	4.8 ± 0.4*	4.3 ± 0.3
48	10	90.0 ± 0.5**	5.3 ± 0.3*	4.7 ± 0.4
72	5	88.2 ± 0.4	7.2 ± 0.3	4.6 ± 0.2

* Difference from control is significant at $p < 0.001$.
** Difference from control is significant at $p < 0.005$.

Table 2. Weights of kidney, thymus, and spleen at different times after termination of cooling of rats

Time after termination of cooling, h	Weight, g					
	kidney	*n*	thymus	*n*	spleen	*n*
Control	0.786 ± 0.018	17	0.584 ± 0.019	49	1.358 ± 0.067	46
0	0.976 ± 0.034*	9	0.554 ± 0.022	19	1.118 ± 0.069**	18
24	0.779 ± 0.017	10	0.560 ± 0.020	19	1.228 ± 0.104	22
48	–		0.550 ± 0.026	21	1.592 ± 0.155	16

* Difference from control is significant at $p < 0.001$.
** Difference from control is significant at $p < 0.05$.

ciated with a decrease in secretion of the antidiuretic hormone vasopressin [36]. The cold diuresis is also observed in rats under artificial hypothermia at the decrease in body temperature to 28°C [37]. The increase in kidney weight by 24% observed by us in the state of hypothermia seems to be associated with this phenomenon. On the contrary, a sharp decrease in kidney blood flow, the glomerular filtration, and in uropoiesis are characteristic for hibernating animals in the torpor state [38]. The loss of water by the organism must be considered as a factor limiting the duration of artificial hypothermia of non-hibernating mammals.

Regulatory pathways of ODC activity are various and depend on the type of cells, tissues, and influence. The enzyme quantity is regulated on the level of transcription, degradation of mRNA, translation of mRNA, and the degradation of the enzyme [39]. The lifetime of ODC in mammalian tissues is 10-30 min [11], and high absolute values of ODC activity in kidneys under conditions of hypothermia (figure, panel (b)) can be maintained on supporting the balance between enzyme synthesis and degradation. Under conditions of hypothermia the rate of transcription and translation is decreased, and the most sensitive is cap-dependent translation initiation, which in hibernating animals is terminated at temperatures below 18°C [38]. However, under hypothermia mRNA in the cells is stabilized [19] and the rate of protein degradation is sharply decreased [40]. Under conditions of cellular cold stress mainly mRNAs are translated with internal ribosome entry sites (IRES) on the 5′-terminus, and these sequences are responsible for the cap-independent translation [19]. The presence of IRES-sequences in mRNA of ornithine decarboxylase has been shown [39]. In organs and tissues some ODC molecules are inactive because they form a complex with a specific ODC-inhibiting protein, antizyme, the production of which is regulated by polyamines on the level of translation [39]. In the thymus about one third of the total amount of ODC is in the inactive complex with the antizyme [15]. Inhibition of ODC activity in the thymus by dexamethasone is associated with an induction of the antizyme through a polyamine-independent mechanism. The induction of the antizyme results in an increase in the relative amount of the ODC–antizyme complex and in a decrease in the total amount of ODC, because the inactive complex undergoes accelerated proteolytic degradation [15, 39]. The decrease in ODC activity in the lymphoid organs observed by us on cooling can also be associated with the induction of the antizyme by glucocorticoids. Note that the rate of ODC *in vitro* interaction with the antizyme increases with a decrease in the temperature [11].

Hypothermia–hypoxia–hypercapnia induced more prolonged decrease in ODC activity in the thymus than in the spleen (figure, panel (c)). The thymus is a primary lymphoid organ where T-lymphocytes are produced from bone marrow precursors [41]. Possibly, the proliferation and differentiation of thymocytes and rearrangement of the genes of T-receptors need the greater rate of polyamine metabolism and recovery of these processes takes more time.

The sharp decrease in ODC activities in actively proliferating tissues of thymus, spleen, and small intestine mucosa (figure, panels (b) and (c)) can be associated with adaptive reactions on the level of cells and tissues. Studies on mammalian cell cultures show that a decrease in the cultivation temperature (cold stress, or cold shock) cause growth delay with a prevalent accumulation of cells in the G_1 phase, a decrease in the level of metabolism, modification of transcription and translation, disassembling of the cytoskeleton, and synthesis of specific cold-shock proteins [19]. The cell responses to cold shock can also be caused by a decrease in the contents and metabolism of polyamines, which are involved in the cell entrance and passing the cell cycle [14], stabilization of the cytoskeleton [13], and regulation of transcription and translation [11, 14].

The thymocyte distribution throughout the cell cycle stages suggests a decrease in the proliferative activity and accumulation of cells in the G_1 phase of the cell cycle (Table 1). The decrease in the proliferation associated with accumulation of cells in the G_1 phase was observed in cell cultures subjected to deep hypothermia after returning to the optimal temperature [20] and also on a decrease in the intracellular pool of polyamines caused by a long-term inhibition of ODC activity [42]. The decrease and recovery of the fraction of thymocytes in S phase of the cell cycle are 24 h delayed compared to the corresponding changes in ODC activity (figure, panel (c) and Table 1). This is in agreement with the data that the duration of the cell cycle of thymocytes is ~24 h.

An acute stress leads to decrease in cell number and involution of lymphoid organs due to apoptosis of glucocorticoid-sensitive lymphoid cells and cell migration from the lymphoid organs [43]. In our experiments hypothermia under conditions of hypoxia–hypercapnia did not cause significant changes in the thymus weight. A decrease in ODC activity and in the intracellular pool of polyamines caused growth delay but not always led to apoptosis [42]. The spleen weight in the state of hypobiosis was slightly decreased (Table 1). A decrease in spleen weight manifested soon after the action of stress factor could be caused by a reduction of its capsule caused by adrenalin [43]. Thus, hypothermia, hypoxia, and hypercapnia did not lead to involution of lymphoid organs – thymus and spleen.

A decrease in the intracellular pool of polyamines along with inhibition of ODC was earlier shown [44-46] to cause, along with cell growth delay, induction of anti-apoptotic factors and protect cells against apoptosis under damaging influences.

The development of hypobiosis in rats under conditions of hypothermia–hypoxia–hypercapnia is accompanied by changes in ODC activities in all organs and tissues

under study. These changes are different from those observed in experimental models of acute stress. The decrease in ODC activity in the thymus correlates with suppression of thymocyte proliferation. The decrease in ODC activities in the animal's organs and tissues under conditions of hypothermia is likely to be associated with systemic adaptive reactions to cooling and hypoxia–hypercapnia developed on the cell and organismal levels and directed to coordinated decrease in metabolism with saving of viability.

This work was supported by the Russian Foundation for Basic Research (project No. 09-04-00993a).

REFERENCES

1. Timofeev, N. N., and Prokop'eva, L. N. (1997) *Neurochemistry of Hypobiosis and Limits of the Organism's Cryoresistance* [in Russian], Meditsina, Moscow.
2. Ignat'ev, D. A., Fialkovskaya, L. A., Perepelkina, N. I., Markevich, L. N., Kraev, I. V., and Kolomiytseva, I. K. (2006) *Radiats. Biol. Radioekol.*, **46**, 705-712.
3. Maistrakh, E. V. (1964) *Hypothermia and Anabiosis* [in Russian], Nauka, Moscow-Leningrad.
4. Konstantinova, M. M. (1961) *Dokl. Akad. Nauk SSSR*, **138**, 223-226.
5. Lyman, C. P., Willis, J. S., Malan, A., and Wang, L. C. H. (1982) *Hibernation and Torpor in Mammals and Birds*, Academic Press, London.
6. Selye, H. (1960) *Essays about Adaptation Syndrome* [Russian translation], Medgiz, Moscow.
7. Garkavi, L. Kh., Kvakina, E. B., and Kuzmenko, T. S. (1998) *Antistress Reactions and Activating Therapy. Activation Reaction as a Pathway to Health via Self-Organization* [in Russian], IMEDIS, Moscow.
8. Meerson, F. Z. (1993) *Adaptation Medicine: The Concept of Long-Term Adaptation* [in Russian], Delo, Moscow.
9. Kolomiytseva, I. K., Kulagina, T. P., Markevich, L. N., Potekhina, N. I., Slozhenikina, L. V., and Fialkovskaya, L. A. (2002) *Biofizika*, **47**, 1106-1115.
10. Kolomiytseva, I. K., Fialkovskaya, L. A., Afanas'ev, V. N., and Slozhenikina, L. V. (2005) *Biophysics*, **50**, Suppl. 1, S18-S25.
11. Berdinskikh, N. K., and Zaletok, S. P. (1987) *Polyamines and Tumorigenesis* [in Russian], Naukova Dumka, Kiev.
12. Schuber, F. (1989) *Biochem. J.*, **260**, 1-10.
13. Pohjanpelto, P., Virtanen, I., and Holtta, E. (1981) *Nature*, **293**, 475-477.
14. Wallace, H. M., Fraser, A. V., and Hughes, A. (2003) *Biochem. J.*, **376**, 1-14.
15. Peng, T., Rotrakarn, D., Janzen, A., and Richards, J. F. (1989) *Arch. Biochem. Biophys.*, **273**, 99-105.
16. Zahner, S. L., Prahiad, K. V., and Mitchell, J. L. A. (1986) *Cytobios*, **45**, 25-34.
17. Chideckel, E. W., Rozovsci, S. J., and Bellur, E. R. (1980) *Biochem. J.*, **192**, 765-767.
18. Melnichuk, D. O., Mikhailovskii, V. O., and Melnichuk, C. D. (2000) *Ukr. Biokhim. Zh.*, **72**, 70-80.
19. Al-Fageeh, M. B., and Smales, C. M. (2006) *Biochem. J.*, **397**, 247-259.
20. Rieder, C. L., and Cole, R. W. (2002) *Cell Cycle*, **1**, 169-175.
21. Janne, J., and Williams-Ashman, H. G. (1971) *J. Biol. Chem.*, **246**, 1725-1732.
22. Slozhenikina, L. V., Fialkovskaya, L. A., and Kolomiytseva, I. K. (1999) *Int. J. Radiat. Biol.*, **75**, 193-199.
23. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
24. Afanasyev, V. N., Korol, B. A., Matylevich, N. P., Pechatnikov, V. A., and Umansky, S. R. (1993) *Cytometry*, **14**, 603-609.
25. Timofeev, N. N. (2005) *Hypobiosis. The Past*, *the Present*, *the Future* [in Russian], Inform-Znanie, Moscow.
26. Popova, N. K., Koryakina, L. A., and Kolokoltsev, A. A. (1979) *Genetika*, **15**, 715-719.
27. Gilad, V. H., Rabey, J. M., Kimiagar, Y., and Gilad, J. M. (2001) *Biochem. Pharmacol.*, **61**, 207-213.
28. Richards, J. F. (1978) *Life Sci.*, **23**, 1619-1624.
29. Cousin, M. A., Lando, D., and Moguilewsky, M. (1982) *J. Neurochem.*, **38**, 1296-1304.
30. Wang, J. Y., and Johnson, L. R. (1990) *Am. J. Physiol.*, **258**, G942-G950.
31. Mikhailovsky, V. O., Tsyakalo, L. V., Stognii, N. A., and Gulyi, M. F. (1982) *Biomed. Khim.*, No. 6, 71-75.
32. Flamigni, F., Stefanelli, C., Guarnieri, C., and Caldarera, C. M. (1986) *Biochim. Biophys. Acta*, **882**, 377-383.
33. Wang, J. Y., and Johnson, L. R. (1991) *Gastroenterology*, **100**, 333-343.
34. Kuhn, C. M., Grignolo, A., and Schanberg, S. M. (1983) *Pharmacol. Biochem. Behav.*, **18**, 669-672.
35. Butler, S. R., Suskind, M. R., and Schanberg, S. M. (1978) *Science*, **199**, 445-447.
36. Morgan, M. L., Anderson, R. J., Ellis, M. A., and Berl, T. (1983) *Am. J. Physiol. Renal. Physiol.*, **244**, F210-F216.
37. Broman, M., Kallskog, O., Nygren, K., and Wolgast, M. (1998) *Acta Physiol. Scand.*, **162**, 475-480.
38. Carey, H. V., Andrews, M. T., and Martin, S. L. (2003) *Physiol. Rev.*, **83**, 1153-1181.
39. Pegg, A. E. (2006) *J. Biol. Chem.*, **281**, 14529-14532.
40. Roobol, A., Carden, M. J., Newsam, R. J., and Smales, C. M. (2009) *FEBS J.*, **276**, 286-302.
41. Yarilin, A. A. (1999) *Fundamentals of Immunology* [in Russian], Meditsina, Moscow.
42. Zhang, A. H., Rao, J. N., Zou, T., Liu, L., Marasa, B. S., Xiao, L., Chen, J., Turner, D. J., and Wang, J. Y. (2007) *Am. J. Physiol. Cell Physiol.*, **293**, 379-389.
43. Gorizontov, P. D., Belousova, O. I., and Fedotova, M. I. (1983) *Stress and the Blood System* [in Russian], Meditsina, Moscow.
44. Zhang, H. M., Rao, J. N., Guo, X., Liu, L., Zou, T., Turner, D. J., and Wang, J. Y. (2004) *J. Biol. Chem.*, **279**, 22539-22547.
45. Bhattacharya, S., Ray, R. M., and Johnson, L. R. (2005) *Biochem. J.*, **392**, 335-344.
46. Kucharewska, P., Welch, J. E., Svensson, K. J., and Belting, M. (2009) *Biochem. Biophys. Res. Commun.*, **380**, 413-418.