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FROM ANIMAL HIBERNATION TO HUMAN'S HYPOMETABOLISM: CELLULAR MECHANISMS OF NATURAL AND ARTIFICIAL HYPOBIOSIS

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Abstract. Objective: to carry out a comparative study of structural and functional responses of rats and hamsters RBCs when the animals entered and left an artificial hypobiosis state under conditions of hypothermia-hypoxia-hypercapnia. **Methods:** RBCs of rats and hamsters: control, in artificial hypobiotic state, 2 and 24 h post-hypobiosis state, and those from winter-hibernating hamsters were investigated. Osmotic resistance was determined by the method of small-angle light scattering. Relative content of main hemoglobin forms (oxy-, deoxy- and metHb) was determined by the method of differential spectrophotometry. Microviscosity of cytosol was evaluated within a range of 37-0°C by EPR spin probe method. **Results:** the artificial hypobiotic state was characterized by body temperature decreasing (down to 16°C), in the same way for both homoiothermal and heterothermal mammals. This was accompanied by changes of osmotic resistance, redistribution of main hemoglobin forms, cytosol microviscosity in RBCs. Increased osmotic resistance and significantly elevated relative content of oxyHb were revealed in 2 h after hypobiotic state. Modifications of the parameters kept changing up to 24 h post-hypobiotic-state. RBC responses to natural and artificial hypobiosis had common as well as different features. Difference in the reaction of RBC cytosol of homoiotherms and heterotherms, associated with the season, was found. **Conclusions:** RBCs response *in vivo* to physiological rearrangements induced by artificial hypobiosis of both heterotherm and homoiotherm mammals. The significant changes of the structural and functional state of erythrocyte kept changing up to 24 h post-hypobiotic-state, while the physiological state of the animals was similar to the control already in 2 hours after hypobiosis. Existence of mammals RBC "aftersensations" in post-hypobiotic state allows to consider the model of artificial hypobiosis perspective for clarification of the cellular mechanisms of controlled induction of hypometabolic state.

Key words: artificial and natural hypobiosis, mammals, decreased body temperature, erythrocyte, osmotic resistance, hemoglobin forms, cytosol microviscosity.

ВІД ГІБЕРНАЦІЇ ТВАРИН ДО ГІПОМЕТАБОЛІЗМУ ЛЮДИНИ: КЛІТИННІ МЕХАНІЗМИ ПРИРОДНОГО ТА ШТУЧНОГО ГІПОБІОЗУ

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Реферат. Мета: проведення порівняльних досліджень структурно-функціональної відповіді еритроцитів щурів та хом'яків на перебування тварин у стані штучного гіпобіозу за умов гіпотермії-гіпоксії-гіперкапнії та вихід з нього. **Методи:** досліджували еритроцити щурів та хом'яків: контрольних, у стані штучного гіпобіозу, через 2 і 24 години після нього, а також зимосплячих хом'яків. Осмотичну стійкість визначали за методом малокутового світлорозсіювання. Відносний вміст форм гемоглобіну (окси-, дезокси- та метНb) визначали за допомогою диференціальної спектрофотометрії. Мікров'язкість цитозолю оцінювали в діапазоні 37-0°С за методом ЕПР спінових зондів. **Результати:** стан штучного гіпобіозу характеризувався зниженням температури тіла (до 16°С), однаковим для гомойотермних і гетеротермних ссавців. Це супроводжувалося змінами осмотичної стійкості, відносного вмісту форм гемоглобіну, мікров'язкості цитозолю еритроцитів. Через 2 години після гіпобіотичного стану фізіологічні показники тварин наближалися до норми, проте спостерігалося значне підвищення осмотичної стійкості та збільшення відносного вмісту оксигемоглобіну. Зміни параметрів еритроцитів зберігалися до 24 годин пост-гіпобіотичного стану. Реакції еритроцитів на природний та штучний гіпобіози мали як спільні, так і особливі риси. Виявлені сезонні відмінності у реакціях цитозолю еритроцитів гомойотермів і гетеротермів. **Висновки:** спостерігалася реакція еритроцитів *in vivo* на фізіологічні перебудови, що викликані станом штучного гіпобіозу як у гетеротермних, так і у гомойотермних ссавців. Виражені зміни структурно-функціонального стану еритроцитів зберігалися протягом 24 годин пост-гіпобіотичного стану, у той час, як фізіологічний стан тварин наближався до норми вже через 2 години. Наявність слідових реакцій еритроцитів ссавців після знаходження у стані гіпобіозу дозволяє розглядати модель штучного гіпобіозу перспективною для прояснення клітинних механізмів індукції контрольованого гіпометаболізму.

Ключові слова: штучний та природний гіпобіоз, ссавці, знижена температура тіла, еритроцити, осмотична стійкість, форми гемоглобіну, мікров'язкість цитозолю.

ОТ ГИБЕРНАЦИИ ЖИВОТНЫХ ДО ГИПОМЕТАБОЛИЗМА ЧЕЛОВЕКА: КЛЕТОЧНЫЕ МЕХАНИЗМЫ ПРИРОДНОГО И ИСКУССТВЕННОГО ГИПОБИОЗА

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Реферат. Цель: провести сравнительные исследования структурно-функционального ответа эритроцитов крыс и хомяков на нахождение в состоянии искусственного гипобиоза в условиях гипотермии-гипоксии-гиперкапнии и выход из него. **Методы:** исследовали эритроциты крыс и сирийских хомяков: контрольных, в состоянии искусственного гипобиоза, через 2 и 24 часа после него, а также хомяков в состоянии зимней спячки. Осмотическую устойчивость оценивали методом малоуглового светорассеяния. Относительное содержание форм гемоглобина (окси-, дезокси-, метHb) определяли методом дифференциальной спектрофотометрии. Микровязкость цитозоля оценивали в диапазоне 37-0°С методом ЭПР спиновых зондов. **Результаты:** состояние искусственного гипобиоза характеризовалось снижением температуры тела (до 16°С), одинаковым для гомойотермных и гетеротермных млекопитающих. Это сопровождалось изменениями осмотической устойчивости, относительного содержания форм гемоглобина, микровязкости цитозоля эритроцитов. Через 2 часа после гипобиоза физиологические показатели животных приближались к норме, однако наблюдалось выраженное повышение осмотической устойчивости и увеличение относительного содержания оксигемоглобина. Изменения параметров эритроцитов сохранялись в течение 24 часов пост-гипобиотического состояния. Реакции эритроцитов на природный и искусственный гипобиоз имели как общие, так и отличительные черты. Выявлены сезонные различия в реакциях цитозоля эритроцитов гомойотермов и гетеротермов. **Выводы:** наблюдалась реакция эритроцитов *in vivo* на физиологические перестройки, вызванные состоянием искусственного гипобиоза как у гетеротермных, так и гомойотермных млекопитающих. Выраженные изменения структурно-функционального состояния эритроцитов сохранялись вплоть до 24 часов пост-гипобиотического состояния, в то время как физиологические состояние животных приближалось к норме через 2 часа. Наличие следовых реакций эритроцитов млекопитающих позволяет рассматривать модель искусственного гипобиоза перспективной для выяснения клеточных механизмов индукции контролированного гипометаболизма.

Ключевые слова: искусственный и природный гипобиоз, млекопитающие, пониженная температура тела, эритроциты, осмотическая устойчивость, формы гемоглобина, микровязкость цитозоля.

1. Introduction

Due to the temperature dependent character of the main physical and chemical processes maintaining the life and providing the cell functional activity, the temperature change initiates the rearrangements which reversibility is determined by both the temperature effect value and time and by the nature and organizing complicity of the system under consideration. Therefore determination of biosystem temperature adaptability limits is of great importance. From the other hand the use of temperature factor for the biopreservation of bioobjects with different organizing level is a cryobiology practice base (Scott et al., 2005).

Recently more and more physiologists and practice physicians have preferred not to re-invent the wheel, but to peer in how the nature solves those or others physiological problems. Hibernating mammals are one of the most blessed objects for this (Bradbury, 2001; Colugnati et al., 2008; Green, 2000).

Interest to the phenomenon of hibernation is mainly determined by ability of hibernating mammals to survive through subzero body temperatures and adapt to abrupt and severe thermal and metabolic shifts during periodic entering and exiting of bouts. But particularly inspiring one is a superresistibility of hibernators. Animals in torpor tolerate lack of oxygen, the action of many poisons, infection with deadly diseases, the effect of lethal doses of radiation, etc. without harm to the body. Moreover cells isolated from animal in torpor are also characterized by increased resistibility (Repina et al., 2008).

The molecular basis of natural torpor in hibernating mammals offers models and applications that are relevant to issues in clinical science (Storey et al., 2010) as well as to solve the problem of a controlled induction of hypometabolic and/or hypothermic states without of pharmacological means (Gorr, 2017).

However, winter hibernation is a genetically fixed adaptive strategy. Usually, the objects of practical medicine and veterinary are organisms, not possessing such adaptive features. Artificially induced hypometabolism in nonhibernating mammals may have considerable clinical implications. At the same time researches indicate the existence of a reason for the poor translatability of artificial hypometabolism to the clinical setting (Dirkes et al., 2015) State of hibernation of homoiotherms can be modeled by introducing them into an artificial hypobiosis under conditions of hypothermia-hypoxia-hypercapnia (Aksyonova et al., 2010;) by Andjus-Bakhmet'ev-Giaya method (Andjus et al., 1955). The state of artificial hypobiosis is similar to the natural hibernation by a wide range of parameters. Thus body temperature of animals in the state of artificial hypobiosis lowers down to (16±1)°C, heart rate decreases (from 380-390 to 80-82 beats/min), and quite complete loss of mobility and pain reflexes are observed (Mel'nychuk et al., 2005). There is still insufficient knowledge about the processes occurring in the organism of an animal introduced into an artificial hypobiosis and leaving this state. Acquiring new knowledge in this area is of great importance for determining the adaptive capacity of mammals and widening of practical application prospects of the hypometabolic phenomenon (Malatesta et al., 2007; Mel'nychuk et al., 2007).

Studies of cellular responses to the deep changes of organism state are necessary for understanding the integral physiological response to the state of hypometabolism. Studies of red blood cells in a transition of animals into hypobiosis are of particular interest. Belonging of RBCs to the blood system which acts also during deep torpor of hibernator (unlike some other systems of the organism) predetermines necessity of erythrocyte adaptive reactions in case of organism's being in hypometabolic state. Moreover, the erythrocyte is used to be a good model system for studies of molecular mechanisms of cellular responses to different extreme conditions both *in vivo* and *in vitro*.

The key link of regulation mechanism of erythrocyte physiology, membrane stability and deformability are the dynamically regulated protein-protein interactions in membrane-cytoskeleton-cytosol system. Modification of erythrocyte's physiological state is accompanied by changing of quantity of cytoskeletal and cytosol (hemoglobin, key enzymes of glycolysis) components in a membrane-bound state (Campanella et al., 2005). It is obvious that such modifications must manifest in changing of erythrocyte cytosol and membrane state. From this point of view the purpose of the present study was to clarify if there is certain erythrocyte response to mammals' being in artificial hypobiosis and leaving it.

We suggest, that comparative studies of RBCs of animals that are genetically adapted to different intervals of body temperature fluctuations (homoiothermal and heterothermal mammals) could be very informative. Particularly the question arises if there are differences in heterotherm and homoiotherm RBC response to the state of artificial hypobiosis. We were also interested in common and different features of cellular response to the states of natural and artificial hypobiosis.

2. Material and methods

2.1. Animals

The study was carried out in autumn-winter period with homoio-(inbred male white rats *Rattus norvegicus,* 180-220 g) and heterothermal (hamsters *Mesocricetus auratus,* 85-95 g, males) mammals. Prior to the experiments, animals had free access to water and food, supplemented with sunflower seeds, kept at 22°C and 12:12 h light-dark cycle.

All procedures for animal maintenance and euthanasia were approved by the Bioethic Committee of the Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv, Ukraine and conform to European Convention on the use of Experimental Animals (Strasbourg, 1985).

2.2. The artificial and nature hypobiosis state

The artificial hypobiosis state was achieved by Andjus–Bakhmet'ev–Giaya method ("closed tank" model) (Andjus and Smith, 1955). An animal was placed to a hermetic vessel (3 dm^3 for rat and 2 dm^3 for hamster) situated in a dark cold room at 2–4°C. 2.5 hours after stay in darkness at hypothermia on the background of enhancing hypoxia and hypercapnia the animals fell into artificial hypometabolic state (ABG-state), characterized by lowered body temperature (down to 16±1°C), decreased heart rate and quite complete loss of mobility and pain reflexes, that is, a state similar to a natural hibernation. The animals rewarm from ABG-state by themselves under the conditions of normal gas composition of air and the average environmental temperature 22–24°C.

In September-October hamsters were placed in a temperature-controlled room at 5°C in constant darkness in order to induce a natural hibernation state. Food and water were removed from a hamster's cage. Hamsters fell into hibernation in 10-14 days. Average bout duration was 3±0.5 days.

2.3. Subjects

Animals were divided into five groups: control, in hypobiotic state (ABG-state), 2 h and 24 h after being in ABG-state, and hamsters in winter hibernation. The body temperature (T_{body}) in control group of animals was at normal range (37°C). Hamsters and rats in ABG-state had T_{body}=16±1°C. In 2 and 24 h after ABG-state physiological parameters of the animals were similar to the control and T_{body} came near 37°C. T_{body} of hibernating hamsters was 4-5°C. Each group has at least 5 animals.

Freshly drawn blood from the animal was prepared with heparin. The blood was washed three times by centrifugation (1800 × g, 5 min) in phosphate-buffered solution (150 mM NaCl, 5 mM NaH_2PO_4–Na_2HPO_4), pH 7.4. Plasma and buffy coat were removed by aspiration.

2.4. RBC osmotic resistance

Osmotic resistance was determined by the method of small-angle light scattering as a part of intact cells in hypotonic NaCl solutions. The most detailed description of methods for analyzing cells based on measuring the light scattering is described in (Mullaney and Dean, 1970). Measurement of light scattering with a wavelength of about 1 micron forwarded at an angle of 9° to the direction of the incident beam was performed on the device, manufactured by "Cryocon". The part of intact cells in hypotonic solutions of non-penetrating substance (NaCl) with the concentration values (%) 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.25, 0.2 was determined according to the small-angle light scattering and the calibration curve. Osmotic fragility curves were obtained at 25°C.

2.5. RBC cytosol state

Erythrocyte cytosol state was evaluated by means EPR spin probe method, using water-soluble TEMPON probe (2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl) and widening agent – potassium ferricyanide, which does not penetrate native erythrocytes. Used concentrations of spin probe and the widening agent (1 and 100 mM, respectively) allow displaying the EPR signal only from probes located inside the cell. Such an approach allows evaluating the cytosol microviscosity and barrier properties of membrane. Dynamic state of cytosol was evaluated by temperature dependences (37-0°C) of probe mobility parameter ($h_0/h\_$) characterizing cytosol microviscosity. We used this parameter instead of common parameter τ_c according to the approach (Minetti et al., 1984). Converging of R^2 (the value of the linear approximation reliability) to unit was considered as an evidence of temperature dependences smoothing. Validity and adequacy of such method for evaluation of aqueous-protein and protein-protein transformations in cytosol are based on ability of TEMPON to create hydrogen bonds with molecules of water. The suspension temperature was varied in the ''Bruker'' ER 100D spectrometer resonator with accuracy of ± 0.5 K.

2.6. Ratio of the main RBC hemoglobin forms

Changes of the ratio of the main hemoglobin forms (oxy-, deoxy- and methemoglobin) were evaluated by differential spectrophotometry (Zwart et al., 1981) using Pye Unicam SP 8000 spectrophotometer.

2.7. Statistical Analyses

The data was expressed as means ± standard error of means. The data analysis was performed using Kruskal-Wallis test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. RBC osmotic resistance

In ABG-state a decreasing of erythrocyte osmotic resistance occurs for both hamsters and rats (Fig. 1, 2). Such osmotic resistance decreasing may be connected with the fact that a temperature is known to affect properties such as enzyme activities, membrane pumps, as well as membrane flexibility.

Erythrocytes osmotic resistance of hamsters and rats at 2 h post-ABG-state was significantly higher in comparison to the control animals (Fig. 1, 2). Such an increased erythrocyte osmotic resistance kept up to 24 h after animals' being in the hypometabolic state (Fig. 1, 2).

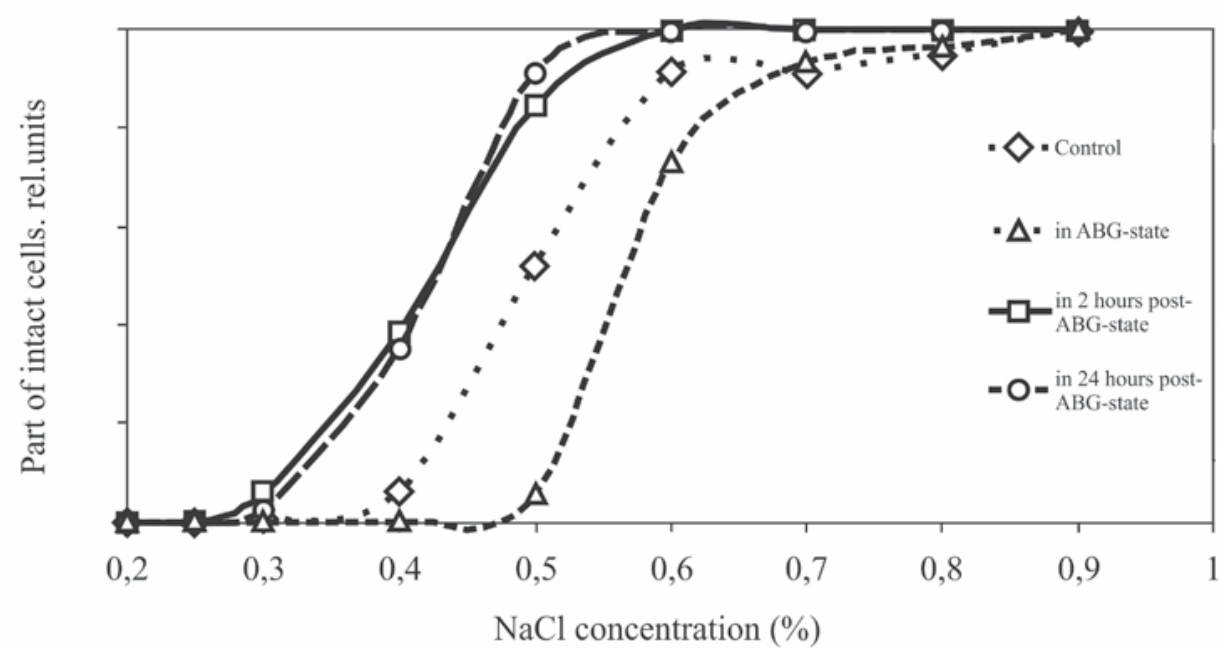

Fig.1. Osmotic fragility curves of hamsters erythrocytes. Each curve is the mean of at least five independent experiments. Measurements were performed in triplicate at each studied NaCl-concentration point.

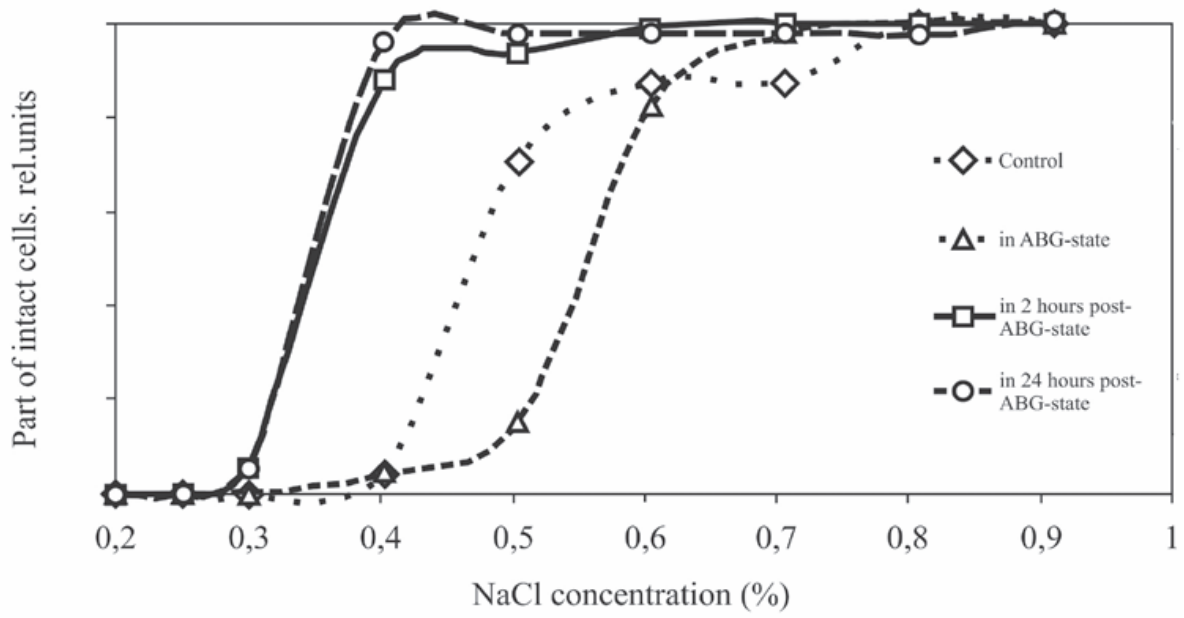

Fig. 2. Osmotic fragility curves of rats erythrocytes. Each curve is the mean of at least five independent experiments. Measurements were performed in triplicate at each studied NaCl-concentration point.

3.2 Erythrocyte cytosol state

Arrhenius dependences of the rotational mobility parameter of the probe in the cytosol of control animals RBCs within the range of 37-0°C are nonmonotonic (Fig. 3, 4). We assume that this reflect structural changes occurring in certain temperature ranges, similar to those registered by the EPR method in membranes (Forte et al., 1985; Minetti et al., 1984).

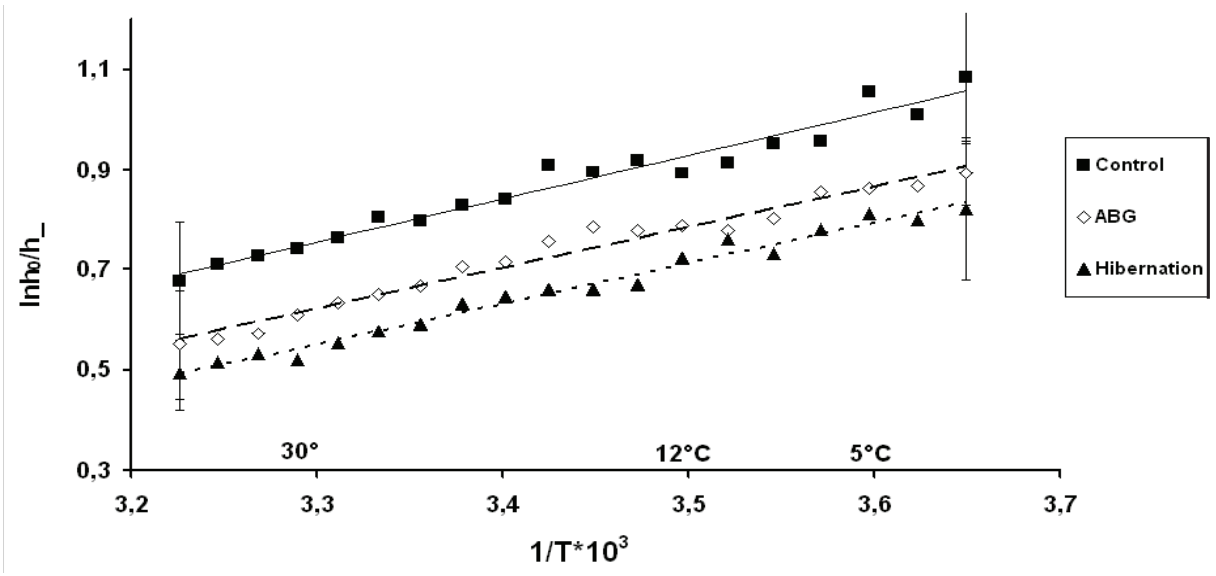

Fig. 3. Arrhenius dependencies for TEMPON mobility in cytosol of hamsters RBCs. Each point represents the mean of three repeats for at least five animals. Means ± S.D. are indicated. Here and below linear approximation is shown.

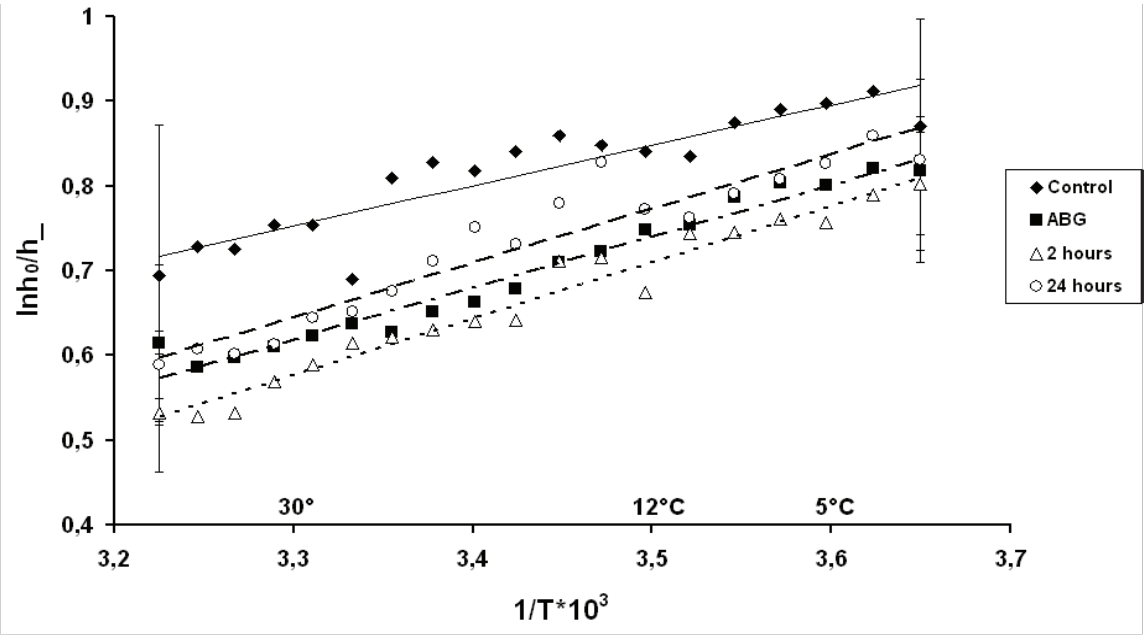

Fig. 4. Arrhenius dependencies for TEMPON mobility in cytosol of rats RBCs. Each point represents the mean of three repeats for at least five animals. Means ± S.D. are indicated.

Both rats' and hamsters' being in the artificial hypobiosis state results in changes of the erythrocyte cytosol dynamic state. This is reflected in significant reduction of cytosol microviscosity for both homoiotherms and heterotherms compared with the control and smoothing of the Arrhenius dependences, more pronounced for rats (Fig. 3, 4). We suppose that an indicator of smoothing could be an increasing of R^2 (the value of the linear approximation reliability) with its converging to unit. In control: $R^2 = 0.959$ (for hamsters), $R^2 = 0.827$ (for rats). In ABG-state: $R^2 = 0.970$ (for hamsters), $R^2 = 0.966$ (for rats). Smoothing of temperature dependencies of cytosol microviscosity indicates the fact of cytosol structure temperature adaptation by analogy with the notions of Willis et al. (1981) about adaptation of RBC membrane structure under hibernation.

Hibernating hamsters (Fig. 3) possess the most reduced cytosol microviscosity and smoothed curves of its temperature dependencies ($R^2 = 0.981$) in comparison to control.

Even 2 h later ABG-state physiological parameters of the animals were similar to the control. However, erythrocyte is telling us its own story.

For hamsters, further changes of cytosol dynamic state were season-dependent. We observed cytosol microviscosity decreasing for up to 24 h post-ABG-state for "winter" hamsters in such a way that the value of cytosol microviscosity and its temperature dependency within a range 37-0°C were approaching these parameters of winter-hibernating animals (Fig. 5). As for "summer" hamsters and rats, cytosol state in 24 h post-ABG-state was close to the control (Fig. 4, 5).

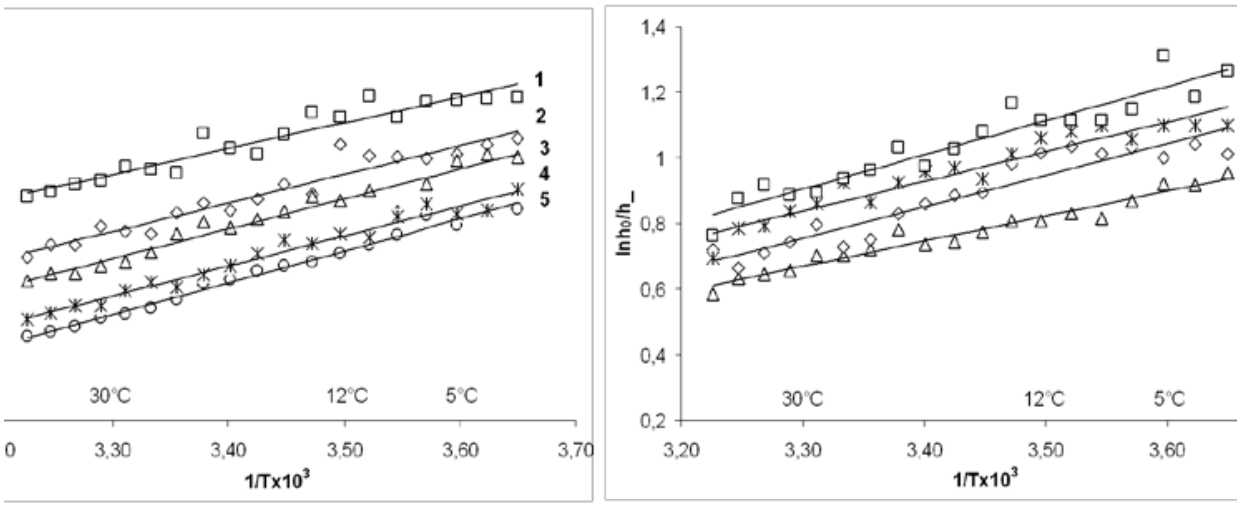

Fig. 5. Typical Arrhenius dependencies for TEMPON mobility in the RBC cytosol of "winter" (at left) and "summer" (at right) hamsters (1 – control, 2 – ABG-state; 3 – in 2 hours after ABG-state; 4 – in 24 hours after ABG-state; 5 – hibernation).

3.3. Relative content of main hemoglobin forms

Rats' and hamsters' being in the artificial hypobiosis state was accompanied by redistribution of hemoglobin forms. An increase in the relative content of oxyhemoglobin was observed (Fig. 6). Elevated level of oxyhemoglobin was also observed in hamsters in a state of hibernation. Important that in 24 hours after animals' being in the artificial hypobiosis the relative content of oxyhemoglobin increases compared with control. We have also observed modifications of the other hemoglobin forms (deoxy- and methemoglobin) in response to hypometabolic state.

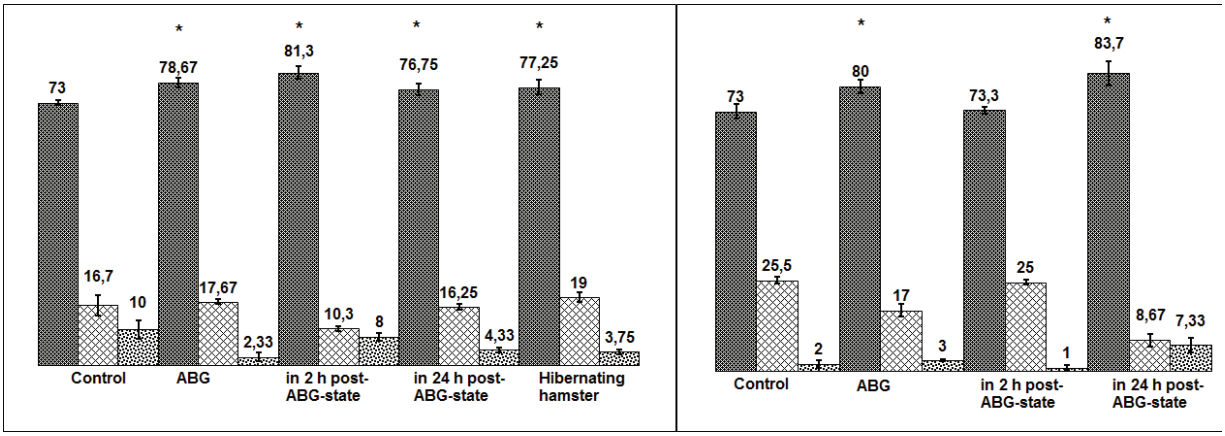

Fig. 6. Relative content of main hemoglobin forms in RBCs of hamsters (at left) and rats (at right) (▩ – oxyhemoglobin, ▩ – deoxyhemoglobin, ▩ – methemoglobin). The error bars represent the standard deviation. * – The changes are significant as compared to control with $p<0,05$.

4. Discussion

Adaptation to environment is an essential vital property of living systems. Ecological impacts of scientific and technical progress, intensive development of medicine, pharmacology, and innovative biotechnology all make it necessary to study the limits of the adaptability of living systems. We mentioned above the fact of the non-specific increase of resistance of mammals organism in the natural hypobiotic state. In this connection, artificially induced introduction of homoiothermal mammals into a state of deep braking of life processes intensity is of a great interest. An important question is whether there are any structural and functional peculiarities of homoiothermal and heterothermal mammals (the cold-sensitive rat and the cold-tolerant hamster) RBCs as a response to this state.

The organisms of both hamsters and rats manifested similar responses to being in ABG-state. Lowering of body temperature down to 16°C occurred in both cases. At RBC level the ABG-state for these two animals was characterized by changes in relative content of the main hemoglobin forms, decreasing of cytosol microviscosity and erythrocyte osmotic resistance. The reduced cytosol microviscosity and smoothing of its temperature dependence, decreasing of osmotic resistance and increase in the relative content of oxyhemoglobin were common for both natural and artificial hypobiotic states.

Both hibernators' and homoioterms' being in the artificial hypobiosis resulted in significant decrease of erythrocytes osmotic resistance. Smoothed temperature dependencies of cytosol microviscosity in erythrocytes of hibernating hamster and of both hamster and rat in ABG-state can serve as a cellular indicator of animal adaptation to artificial and natural hypobiosis by analogy with the notions of Willis et al. (1981) about adaptation of RBC membrane structure under hibernation.

Despite the fact that even 2 h later ABG-state physiological characteristics of the animals were similar to the control, the studied RBC parameters kept changing**.** Osmotic resistance of erythrocytes at 2 h post-ABG-state was significantly higher in comparison to the control animals. Such an increased erythrocyte osmotic resistance kept up to 24 h after animals' being in the hypometabolic state.

Cytosol microviscosity of hamsters RBCs as well as of rats at 2 h post-ABG-state kept decreasing. But 24 h later a difference in the reaction of erythrocyte cytosol of homoiotherms and heterotherms, associated with the season, was found. 'Winter' and 'autumn' hamsters have possessed further lowering of microviscosity, smoothed temperature dependences, in the way that in 24 h after ABG dynamic state of cytosol was close to typical for hibernation state. For rats and 'summer' hamsters it was close to control, testifying to recovery of cellular homeostasis.

Erythrocyte oxygenation-deoxygenating and other physiological stimuli control a balance between membrane-bound and cytosolic state of key glycolytic enzymes and hemoglobin molecules (Campanella et al., 2005). Meaning this we suppose that observed lowering of cytosol microviscosity is a result of free water quantity increasing due to increasing of amount of cytoskeletal and/or cytosol components in membrane-bound state to adapt erythrocyte physiology to the organism's hypobiotic state. Observed redistribution of the main hemoglobin forms can also result in changing of balance of free and bound water, which we evaluate by modifications of water-soluble TEMPON rotational mobility, characterizing cytosol microviscosity.

Osmotic resistance of RBCs is a measure of their stability and ability to withstand varying osmotic gradients, which is particularly important when the body temperature drops. Increase of erythrocytes osmotic resistance, as a result of the animals' being in the artificial hypobiotic state, is probably ensured by membrane reinforcement, in

particular through increased interaction of membrane components with cytoskeleton. Manno et al. (2005) have shown that RBC membrane mechanical properties determining osmotic resistance, deformability, cellular form and erythrocyte state in general are controlled by the dynamically regulated protein-protein interactions of membrane-cytosol-cytoskeleton system with the key role of protein 4.1. This protein is involved in thermoinduced structural transition, detected by EPR spin probe method at 10°C (Forte et al., 1985). This transition smoothing in the erythrocytes of animals in artificial and natural hypobiotic states also gives the evidence in favor of the involvement of cytoskeleton-membrane interactions in tuning of the RBC state to hypobiotic state of the organism. Previously we found change in the relative amounts of protein 4.1 in membrane-bound state, along with spectrin, and hemoglobin in erythrocytes in hibernation conditions (Repina et al., 1998).

5. Conclusions

The cold-sensitive rat and the cold-tolerant hamster react to animals' being in the state of artificial hypobiosis (Andjus-Bakhmet'ev-Giaya model) in the same way by decreasing of body temperature (down to 16°C). Herewith changing of osmotic resistance, redistribution of main hemoglobin forms and cytosol microviscosity in RBCs has been observed.

Experimental data have shown that in 2-3 h after hypobiosis animal's state and behavior were similar to the control. Nevertheless modifications of the studied RBC parameters kept changing up to 24 h post-hypobiotic-state.

Thus, the erythrocyte response to the artificial hypobiosis, resulting in increased erythrocyte functionality (increased osmotic resistance and elevated relative content of oxyhemoglobin) at 24 h postABG-state, is exist. Erythrocyte "aftersensations" to artificial hypobiosis of animals allow considering ABG model as promising for introduction into veterinary practice, particularly for development of approaches for treatment of anemias of various origins. The model of artificial hypobiosis also seems perspective for clarification of the cellular mechanisms of controlled induction of hypometabolic state.

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7. References

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