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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Seasonal Activity of Frog Erythrocytes by Data of Electrophoretic Mobility

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Abstract—The peculiarities of frog erythrocyte electrophoretic mobility, coupled to the seasonal course of temperatures, have been studied. At the periods of anabiosis and of burst of hemopoiesis, in the vascular bed there increases the portion of functionally young erythrocytes (up to 22%) with increased values of the cell membrane surface charge. Preparation to winter is accompanied by a rise of the number of circulating functionally worn-down blood cells (up to 60%) with low values of the superficial charge and low mobility in electrical field. Use of the cell microelectrophoresis method of evaluation of seasonal activity of frog erythrocytes allows obtaining objective data about the cellular surface charge and its depending functional cell activity without submitting the erythrocytes to modifying actions.

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Key words: ξ-potential, erythrocytes, hemopoiesis, anabiosis, seasonal activity.

INTRODUCTION

The annual frog life cycle is subordinated to the temperature seasonal course and is divided into two contrast phases—the active including reproduction, nutrition, and preparation to winter and the inactive, when the animal, according to data of electrophysiological studies [1], is in one of three sleep forms. The adaptation allowing to amphibians to inhabit area with low winter temperatures is anabiosis. The anabiosis state is accompanied by complex reconstructions of the qualitative and quantitative compositions of the basic regulatory systems with involvement of this process of intracellular water and cryoprotectors (glucose and glycerol) [2, 3].

The large temperature diapason (from 0 to 38°C) of the functional activity of the frog biochemical systems implies the characteristic thermotropic properties of the cellular membranes, which provide a change of the ionic transport at adaptation to the seasonal temperature course [4]. The goal of this work was to study peculiarity of the frog erythrocyte electrophoretic mobility in relation to the seasonal course of temperatures.

MATERIALS AND METHODS

The study was carried out on the frog *Rana ridibunda* Pall at the period of physiological anabiosis (January–February), during the spring–summer burst of hemopoiesis (May–June), and extinction of hemopoiesis (September–October). The number of animals in experimental studies for each season was 20. The blood was obtained by the heart puncture and stabilized with heparin (5 units/ml). The erythrocyte electrophoretic mobility (EEM) was determined by the method of microelectrophoresis in a horizontal microcamera [5, 6]. The heparinized blood was centrifuged for 15 min at 1000

Seasonal dynamic of the parameters of the erythrocytes electrophoretic mobility

Physiological state	EEN values (μm cm B^{-1} s^{-1})	Time of movement of erythrocytes (s)	Distance passed by erythrocytes (μm)
Physiological anabiosis	0.05 ± 0.007	104.28 ± 2.7*	2.14 ± 0.41
	0.44 ± 0.06	26.67 ± 0.5*	4.28 ± 0.49*
	1.57 ± 0.08	7.73 ± 0.68*	7.12 ± 0.86*
	2.89 ± 0.37*	4.5 ± 0.56*	8.01 ± 0.89
Spring-summer burst of hemopoiesis	0.55 ± 0.06	10.91 ± 2.7	1.82 ± 0.27
	1.69 ± 0.16	2.93 ± 0.56	2.56 ± 0.41
	4.21 ± 0.28	1.18 ± 0.12	2.85 ± 0.29
	9.42 ± 0.51	1.16 ± 0.16	6.33 ± 0.88
Extinction of hemopoiesis	0.03 ± 0.001	68.03 ± 2.6*	0.66 ± 0.01
	0.39 ± 0.09	19.23 ± 0.2*	3.44 ± 0.51
	1.40 ± 0.01	6.00 ± 0.2*	5.00 ± 0.2

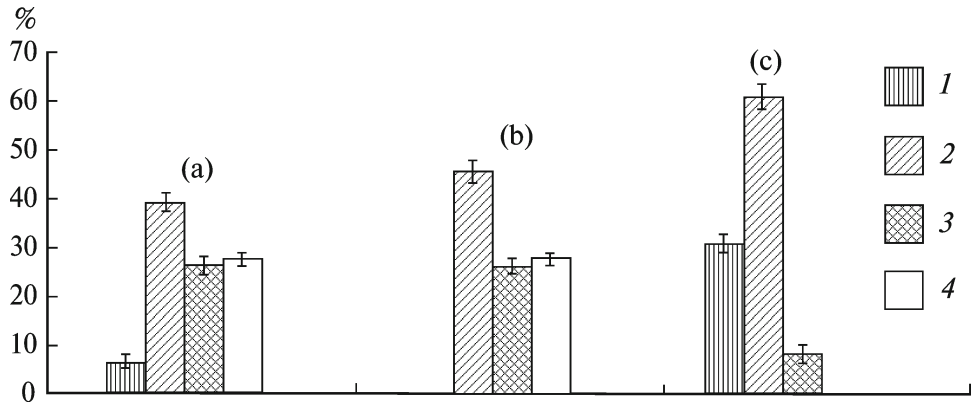
Note: Asterisk designates statistically significant differences as compared with data obtained at the period of the spring-summer burst of hemopoiesis at $p \le 0.05$.

rev/min and plasma was collected. The obtained erythrocytic mass was washed out three times by using Hanks' medium (pH 7.9). Aliquots were prepared by dilution of the erythrocytic mass with Hanks' medium in ratio of 1 : 1000. From each aliquot, ξ-potential was measured in 200 erythrocytes. The micromethod of the ξ-potential determination is based on measurement of the erythrocyte electrophoretic mobility that is equal to the rate of the cell movement appearing under effect of the external electric field. The EEM measurement was performed in the horizontal microcamera under the standard conditions: the current strength 5 mA, voltage 100 V, temperature of the suspension medium 23°C. The control of the process of EEM measurement, the calculation of distances of the cell image translocation, the collection of the obtained data were performed by using the complex of the apparatus-software visualization of morphological preparations, analysis and recording of "VideoTest" optic and morphological parameters. The time of the cell translocation in the electric field was measured with a stopwatch. The viscosity of the suspension medium measured by using a VK-4 medical viscosimeter was 8.94 mPaUcm^2. The correlation between the EEM parameters and ξ-potential was calculated by Smolukhovskii's formula [7]:

$$\xi = \frac{4\pi\eta Sd}{tu\varepsilon}$$

where ξ—potential (μm cm B^{-1} c^{-1}); η—viscosity coefficient; *S*—the path passed by blood cells (μm); *d*—distance between electrodes (cm); *t*—time of movement (s); *u*—potential difference between electrodes (B); ε—dielectric constant of the dispersion medium.

The erythrocyte functional activity was evaluated by the ξ-potential value in the applied electrical field. The young cells are moved in the electrical field faster than the old ones; the ξ-potential value depends only on the cell surface property—on the amount of carboxyl groups of sialic acids and the medium [8], but does not depend on the shape and size of particles [7]. The EEM normal indices were considered the values obtained at the period of the spring–summer burst of hemopoiesis. The statistical significance of differences was determined by using Student's criterion.



The percent ratio of erythrocytes in groups by ξ-potential values (*vertical axis, %*) at different season periods. (a) Anabiosis, (b) activation, (c) extinction. (*1*) Old erythrocytes, (*2*) functionally worn-out, (*3*) functionally mature cells, (*4*) young cells.

RESULTS AND DISCUSSION

In the performed experiments, we established peculiarities of the erythrocyte electrophoretic mobility at different season periods. The presence in the blood flow of four different functional erythrocyte groups are detected for the physiological anabiosis and for the spring–summer burst of hemopoiesis, while at the period of extinction—of three groups. The statistical difference of ξ-potential values were detected in the group of functionally immature (young) erythrocytes—the values higher by 69.32% ($p < 0.05$), as compared to the period of the spring–summer burst of hemopoiesis. The immature erythrocyte forms in the blood flow are not detected at extinction of hemopoiesis (see table). The charge of the cellular surface can be of different origin and is due to the carboxyl and amino groups of the cell surface proper and to the medium ions absorbed on the surface.

The uniform dependence of the erythrocyte electrokinetic parameters is detected at the studied season periods. The table data demonstrate that the distance passed by the young erythrocyte forms with high ξ-potential values is longer than in the old worn-out cell forms. It is established for the physiological anabiosis period that the young erythrocyte forms pass the distance, on average, of 8.01 ± 0.89 μm for 4.5 ± 0.56 s, while the old erythrocyte forms—the distance of 2.14 ± 0.41 μm for 104.28 ± 2.7 s (see table). Hence, the functionally young erythrocyte forms are characterized by the higher movement rate in the applied electrical field as compared to the old ones that lose at aging the negatively charged sialic acids of the cell surface. It is established that maximal EEM values of the functional subpopulations are characteristic of the period of the spring–summer burst of hemopoiesis.

By analysis of the cell percent ratio in the groups, a decrease of the part of the functionally mature forms was revealed at the period of physiological anabiosis (see figure).

At the periods of the burst of the hemopoiesis and anabiosis the number of the functionally young cells is practically equal (~22%). From the point of view of the organism adaptation to the seasonal temperature course, the appearance in the blood flow of erythrocytes with increased values of ξ-potential and voltage of the electrical field in the membrane produces the optimal conditions for an increased oxygen permeability of the erythrocyte membrane and, hence, for an increase of intensity of processes of oxygenation and deoxygenation [9]. The appearance in the blood flow of the functionally young cells at low temperatures is connected with preservation of the high Na^+,K^+-pomp activity and with a decrease of intensity of the passive input and output of cations [10]; as a result, the asymmetrical ion absorption produced on the membrane the charge determining effectiveness of the gas-transport erythrocyte system.

At the period of preparation to winter, about 60% of cells with low values of ξ-potential circulate in the blood, while in the state of anabiosis, 39% of cells

are in this group (see figure). It cannot be ruled out that the appearance in the blood of erythrocytes with low values of ξ-potential is due to an enhancement of the macrophage phagocytic activity at this period. According to data of morphological studies of the hemopoietic tissue [11], at the autumn period the macrophages with large particles of the yellow-brown color predominate in the spleen, the density of mitoses and the number of free elements decrease, there is observed the presence of stromal cells and pulp strands. The bone marrow hemopoietic function is extinguishing by the end of May, while its degeneration begins from the middle of July; therefore, the bone marrow does not participate in hemopoiesis at the summer–autumn period. The established data of the cell distribution by values of the superficial charge is connected with the seasonal cyclicity of hemopoiesis in foci of the spleen-bone marrow and liver hemopoiesis [12].

As a result of this study, it has been shown that the frog adaptation both to the low and to the high temperatures is accompanied by the appearance in the blood flow of erythrocytes with a maximal movement rate in the electrical field and with increased values of the superficial charge of cellular membranes. The period of preparation to anabiosis is characterized by circulation in the blood flow of worn-out cells with the low values of the superficial charge and low mobility in the electrical field. It cannot be ruled out that a decrease of the functional activity of the erythrocyte membranes is due to the high cell wear and loss of the sialic acid carboxyl groups at the season of elevated temperatures.

Use of the parameters of the cell distribution by the electrophoretic mobility allows determining functional cell activity at different season periods. The applied method has several advantages, as in the course of measurements the cell is not submitted to the modifying actions, while the obtained information is related only to the cell surface, on the charge of which the intensity of the gas exchange depends in the cell-tissue system.

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