THE SEASONALITY EXPRESSED IN MOTILITY OF FROG *RANA RIDIBUNDA* PALL 1771 ERYTHROCYTES AND LEUKOCYTES IN AGAROSE GEL UNDER THE INFLUENCE OF THE TEMPERATURE

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ABSTRACT

The agarose migration test revealed the influence of temperature on seasonal fluctuation of locomotional activity of nuclear hemocytes of frogs of the genus Rana ridibunda Pall. It's been shown that at different incubation temperatures, the spontaneous migration area of blood cells changed in spring and summer, and did not change during autumn and winter. It's been found that the migratory activity of erythrocytes and leukocytes in the spring increased only at low incubation temperature, in the summer - both at low and at high temperatures.

Keywords: blood red cells, white cells, locomotion activity.

TÓM TẮT

Tính mùa vụ thể hiện ở tính vận động của hồng cầu và bạch cầu Ếch Rana ridibunda Pall 1771 trên gel agarose dưới ảnh hưởng của nhiệt độ

Phương pháp kiểm định sự di cư dưới gel agarose đã xác định sự ảnh hưởng của nhiệt độ lên sự biến đổi theo mùa của hoạt tính di cư của tế bào máu có nhân ở ếch Rana ridibumda Pall. Đã chỉ ra rằng, trong những nhiệt độ nuôi ủ khác nhau diện tích của sự di cư tự phát đã thay đổi trong mùa xuân và mùa hè và đã không thay đổi trong mùa thu và mùa đông. Đã đưa ra rằng, trong mùa xuân, hoạt tính di cư của hồng cầu và bạch cầu chỉ tăng trong điều kiện giảm nhiệt độ nuôi ủ , trong mùa hè – tăng cả trong điều kiện tăng và giảm nhiệt độ nuôi ủ.

Từ khóa: tế bào hồng cầu, bạch cầu, hoạt tính di cư.

1. Introduction

To understand the different physiological states, in clinical and scientific practice, the indices of blood – major body tissues of vertebrates, are widely used [9, 10, 15]. Correct knowledge of the blood system helps accurately identify normal and pathological processes in the body [7]. For many indices of blood and the immune system in mammals and humans seasonal fluctuations, which are a form of organism's adaptive reactions to cyclical changes in the environment and are common to all levels of biological organization [1], were identified and described [11, 16], Circannual

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changes of many functions of organisms are defined by temperature fluctuation. The general picture of the changes occurring in the body of mammals and humans with acute overheating was adequately described [5].There are studies that report on the positive effect of high temperature on the factors of nonspecific resistance and immunegenesis [14]. However, in chronobiological investigations, there is almost no information on the effect of temperature on seasonality of locomotion activity of leukocytes, and the migratory activity of erythrocytes of the lower vertebrates has not been studied. Particular interest is a class of amphibian living in different environments, which undoubtedly affect the functional activity of blood cells [8].

Purpose of study: To study the influence of temperature factor on seasonal fluctuations of locomotion activity of hemocytes of the frog *Rana ridibunda* Pall.

2. Materials and methods

Experiments were carried out on frogs *Rana ridibunda* Pall. (30 individuals), which were caught from the Vezelka river of Belgorod city. Nuclear red blood cells and white blood cells served as the objects of the study.

Blood samples were taken from frog heart after giving a light ether anesthesia. Heparin at 10 U/ml was used as an anticoagulant. The obtained blood was centrifuged for 4 min at 400g. The leukocyte-rich lower part of the plasma and the leukocyte ring were collected. The washed and re-suspended leukocytes and erythrocytes were counted in Goryaev chamber. In this work an isotonic solution (0.6% solution of NaCl) was used.

Spontaneous locomotion activity of hemocytes was evaluated in a test migration under agarose. Classic method described in many transactions [4, 13] was used as the basic method (M. Z. Fedorova and V. N. Levin modification [5]). Three microliters of hemocyte suspension containing about 300 thousand cells (were diluted with isotonic solution) were placed into the well cut out in the agarose gel applied on the object glass. The object glasses with red blood cells and white blood cells were incubated at anaerobic conditions at temperatures of 8°C - in the refrigerator, 37°C - in thermostat, control - at room temperature (22^oC). One day later the cells were fixed for one hour with 10% glutaraldehyde. Then agarose was removed and hemocytes were stained with azure-eosin. The spontaneous migration areas of blood cells were determined at the low magnification microscope with an ocular micrometer.

The obtained data were processed using statistical variational methods. The average arithmetic sample (M) and the standard error of the mean (m) were calculated with computer programs Excel 7.0 and Statistica 6.0. The significance of differences between the characteristic values of the compared groups was determined using unpaired (two-sample) Student's t-test. Changes were taken at the level of statistical significance at $p < 0.05$.

3. Results and discussion

Comparative analysis of the data obtained at different incubation temperatures of blood cells revealed the presence of seasonal migratory activity of erythrocytes of *Rana ridibunda* Pall. (table 1).

Table 1. *Indices of migration area of frog erythrocytes (mm^2)*

Study period	Incubation temperature		
	8°C	**22°C**	**37°C**
Spring	3.24 ± 0.15	2.82 ± 0.11 ©	2.70 ± 0.12 ©
Summer	3.16 ± 0.27	2.78 ± 0.48 ©	3.39 ± 0.35 * ®
Autumn	2.95 ± 0.47 *	3.16 ± 0.32 *	3.02 ± 0.17 * #
Winter	3.13 ± 0.16	3.07 ± 0.22 *	3.11 ± 0.20 * #

Note: here and in the table 2: Significant difference in comparison by Student's t-criterion ($p < 0.05$): * - with the spring period, # - with the summer period, b - with the autumn period, © - with a temperature 8°C, ® - with a temperature 22°C.

At low incubation temperature, migration area of erythrocytes in the autumn was 9.0% lower than in the spring. At room temperature, the values of the studied parameter in the autumn and the winter were 10.8% and 8.1% higher than in the spring, respectively. At elevated temperatures, locomotion activity of red blood cells in the summer, autumn and winter was 20.4%, 10.6% and 13.2%, respectively, higher than in the spring. In the autumn and winter, at this temperature, indices of migration area of red blood cells were 10.9% and 8.3% lower than in summer. In the spring, the highest migration activity of red blood cells was noted at 8°C, and the lowest – at 37°C. The decrease of migration area of red blood cells in the spring at 22 and 37°C compared to 8°C was 13.0% and 16.7%, respectively. In the summer, the highest migration activity of red blood cells was detected at 37°C, while the lowest - at 22°C. The decrease of migration area at 22°C compared to 8°C in the summer was 12.0%, while the increase of that value at elevated temperature compared to room temperature was 18.0%. In autumn and winter, incubation temperature change had no influence on migration activity of erythrocytes.

At low incubation temperature, seasonal changes of the migration area of leukocytes were not observed (table 2).

Table 2. *Indices of migration area of frog leukocytes (mm^2)*

Study period	Incubation temperature		
	8°C	**22°C**	**37°C**
Spring	3.13 ± 0.15	2.69 ± 0.13 ©	2.68 ± 0.14 ©
Summer	3.05 ± 0.19	2.71 ± 0.23 ©	2.98 ± 0.11 * ®
Autumn	3.14 ± 0.47	3.08 ± 0.30 * #	2.74 ± 0.28 #
Winter	3.04 ± 0.22	3.01 ± 0.22 * #	3.14 ± 0.16 * # &

At room temperature, the migratory activity of white blood cells in the autumn and winter periods was 12.7% and 10.6% higher than in the spring; and 12.0% and 10.0%, higher than in the summer. At elevated incubation temperature, in the summer and winter, locomotion activity of leukocytes was 10.1% and 14.7% higher than in the spring. The migration area of white blood cells at 37°C in the autumn was 8.1% lower and in the winter - by 5.1% higher than in the summer. At elevated incubation temperature, locomotion activity in the winter was 12.7% higher than in the autumn. In the spring, the highest value of leucocytes spontaneous locomotion area was detected at 8°C. Increasing the temperature to 22 and 37°C contributed to the decline of migratory activity of white blood cells by 14.1% and 14.4% compared to migratory activity under low temperature. In summer, at 22°C index of the migration area of leukocytes was 11.2% lower than at lower temperatures, while at 37°C this index was 9.1% higher than at 22°C. In the autumn and winter, the incubation temperature change of white blood cells had no effect on the migration.

It is known that the frog during the hibernation reduces body temperature and its motional activity is sharply limited. These processes can be observed not only in the coldest period of hibernation, but also in period between the breeding season and leaving to the hibernation [2]. Thus, our data on the migratory activity of blood cells in the autumn and winter periods are consistent with the functional activity of the animals. In the spring, an increase of incubation temperature of hemocytes over the body temperature of the animal is a factor, contributing to the decline the functional activity of blood cells. In the summer, the average ambient temperature corresponds to room temperature [3]. The increasing or decreasing temperature of hemocytes in functionally active animals contributes to the activation of plasmalemma and to the increase of motional activity of cells. The work of Mayansky A. N. [12], which states that an

increase in the hemocyte activity occurs not only at inflammation, but can be caused by the different nature of the agents, is an indirect confirmation of this fact. All stimulants somehow interact with the plasmalemma, changing its molecular topography. A number of authors [14], studying the influencing mechanisms of the thermal factor on the body, showed that under thermal influence, an increase in permeability of the lysosomal membrane and the release of proteolytic enzymes into the bloodstream occur. Some of these enzymes have ability to modify the structure of the surface membrane of red blood cells, which stimulates their activation. The so-called "weak" interactions between molecules is determined by ambient temperature, which regulates microviscosity of the lipid bilayer, lipid phase distribution, microenvironment proteins, protein-lipid interactions, and other characteristics of the structural organization of the membrane [6].

4. Conclusion

- The locomotion activity of *Rana ridibunda* Pall. nuclear red cells and white blood cells at low (8°C) incubation temperature increases in the spring and in the summer whereas at high temperature (37°C) it increases only in the summer.
- In the autumn and the winter, when the exposing frog blood cells in hypo-or hyperthermia, the indices of spontaneous migration area do not change.

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