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ИЗУЧЕНИЕ МИГРАЦИОННОЙ АКТИВНОСТИ ЯДЕРНЫХ ЭРИТРОЦИТОВ И ЛЕЙКОЦИТОВ OREOCHROMIS SP

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A STUDY OF THE MIGRATORY ACTIVITY OF OREOCHROMIS SP ERYTHROCYTES AND LEUKOCYTES

Introduction

Under the action of extreme factors on the organism, which include temperature, homeostatic constants will be changed. Hemocytes, especially white blood cells, having a high reactivity, are quickly included in the adaptation reactions. They are capable of non-specific response to alternating exposure. There are studies which report on the positive impact of elevated temperature on the factors of nonspecific resistance and immunegenesis. In recent years, much attention has been paid to the study of migration and phagocytic activities of vertebrate animal blood cells [1, 5, 6, 9, 14]. The features of spontaneous migration and migration, stimulated by different substances of leukocytes under the changed functional and pathological conditions of organism were studied. The migration is one of the phases of the protective functions in the phagocytic blood cells [2–4, 7]. It’s known that the low vertebrate erythrocytes are capable of

absorption of alien particles [12, 13]. However, scientific research regarding the features of migration reactions in nuclear erythrocytes and leukocytes remains little studied.

The purpose of our study was study of the migratory activity of *Oreochromis* sp. leukocytes and erythrocytes under the action of temperature factor.

Material and methods

The study was carried out on fish *Oreochromis* sp. (15 individuals). Erythrocytes and leukocytes were served as the objects of the study. The peripheral blood, collected from ether-narcotized fish, was used. The blood samples were taken from the heart. Heparin at 10 U/mL was used as an anticoagulant [10]. The obtained blood samples were centrifuged for 10 min at a relative centrifugal force equal to 400g [9]. The leukocyte-rich lower part of the plasma and the leukocyte ring were collected. The washed and re-suspended red and white blood cells were counted in Goryaev chamber. In this work, an isotonic solution (0.8% solution of NaCl) was used.

Spontaneous locomotion activity of hemocytes was evaluated in a test migration under agarose. Three microliters of hemocyte suspension containing about 300 thousand cells was placed into the well cut out in the agarose gel applied on the object glass. The object glasses with erythrocytes and leukocytes were incubated at anaerobic conditions at temperatures of 20°C in the refrigerator, 37°C in thermostat, control at room temperature (28^{o}C). One day later the cells were fixed for one hour with 10% glutaraldehyde. The spontaneous migration areas of blood cells were determined using the software ImageJ 1.47v.

The obtained results were processed using statistical variation methods with the use of special software on a computer. The level of statistical significance was $p \leq 0.05$.

Results and discussion

The results show that after 24 hours of incubation the blood cells are distributed around the edges of the well. While the cells form pseudopodia for amoeboid motility. It is known that for the formation of pseudopodia in red blood cells (except mammals), white blood cells and platelets at amoeboid movement necessary to have the presence and a certain value of the "membrane reserve", embedded in the folding of plasma membrane [9, 10]. At the expense of membrane folds, passing through the narrow capillaries, phagocytes are deformed with an increase in surface area at constant volume [8-10]. The value of membrane reserve of mammal leukocytes (and especially, of human) was well studied. In nuclear red blood cells of fish also was revealed the presence and studied the value of "membrane reserve" that allows to implement the migration reaction and phagocytosis [11]. In studies conducted by us revealed that red blood cells of *Oreochromis* sp. migrate.

The migratory activity of blood cells was evaluated by area of their distribution after 24 hours of incubation. Indicators of the area of spontaneous migration of *Oreochromis* sp. hemocytes at different incubated temperatures are shown in the table.

Table – Area of the distribution of *Oreochromis* sp. blood cells after 24 hours of incubation at different temperatures, mm^2

Type of cells	Incubation temperature, ^{o}C		
	20	28	37
Erythrocytes	3.16±0.25 $^{a\,A}$	3.81±0.18 $^{b\,A}$	4.35±0.19 $^{c\,A}$
Leukocytes	2.83 ±0.07 $^{a\,B}$	2.38±0.10 $^{b\,B}$	2.24±0.18 $^{b\,B}$

Notes: $^{a,\ b,\ c}$ – significant difference of indicator in a single row; $^{A,\ B}$ – significant difference of indicator in a single column ($p \leq 0.05$)

The table shows that the area of distribution of erythrocytes was significantly changed by different incubated temperatures. In comparison with incubation at 28^{o}C the area of spontaneous migration of red blood cells at elevated incubation temperature to 37^{o}C was 14.17% higher than that. At reduced incubation temperature to 20^{o}C, migration area was 20.57% lower than that at temperature of 28^{o}C.

For leukocytes is characterized by the opposite pattern. At elevated temperature of the incubation, the changes of migration area of leukocyte were not observed (see the table). When

temperature of the incubation reduces to 20°C, the area of spontaneous migration increases by 18.91% compared with room temperature.

It can be assumed, that enhancement of spontaneous locomotion of cells with increasing incubation temperature is a consequence of the activation of plasmalemma caused by thermal factor. Decrease or increase in temperature for the blood cells of functionally active animals is a factor that contributes to the activation of plasmalemma and increased motor activity of the cells. An indirect confirmation of this is the work [4], which stated that the increase in hemocyte activity occurs not only in inflammation, but can be caused by various by nature agents.

Comparative analysis of the migration area of erythrocytes and leukocytes is shown that at incubated temperatures of 20, 28 and 37°C, migration area of red blood cells was 11.66, 60.08 and 94.19%, respectively, higher than that of white blood cells. This is confirmed by the work of Chernyavskikh, which states that under the same conditions of incubation, the red blood cells migrated edge of the incubation well more active than white blood cells [8].

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