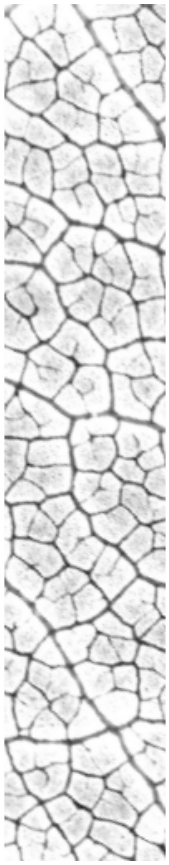

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Leukocytes phagocytic activity under moderate hypotension conditions in some representatives of bony fish, amphibians and reptiles

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Abstract
The leukocytes phagocytic activity against *Bacillus subtilis* and agromerulated latex particles in representatives of bony fish, amphibians and reptiles under reduced medium osmolarity conditions was studied. It was found that in moderate hypotension compared with isotonia, the white blood cells absorption capacity of the fish was not changed, but it was reduced for amphibians and reptiles.

Keywords: leukocytes, phagocytosis, hypotension, lower vertebrates

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INTRODUCTION

The influence of various factors of both external and internal environment, which include osmotic blood pressure, differently affects the hemocytes absorption capacity, due not only to phylogenetic (Galkin 1997, Trubkin AND Kharitonov 2011), but also to ecological features of animals (Pronina AND Koryagina 2011). The ions concentration in the environment determines the osmoregulation mechanisms in animals of different ecological groups (Natochin 2002). The transition from water to land lifestyle is associated with deep restructuring of the body in general and the blood system in particular (Zhitenyova et al. 2001). In this case the system of cell volume self-regulation plays an important role in maintaining the blood cells functional activity (Orlov and Gurlo 1991).

In scientific literature there are many works devoted to the evaluation of the white blood cells phagocytic activity of vertebrates under normal medium osmolarity conditions (Douglas and Cui 1983, Forlenzaa et al. 2011, Lipunova and Skorkina 2007, Taruna et al. 2005, Underhill and Goodridge 2012). The effect of hypoosmotic load on the leukocytes absorptive capacity of lower vertebrates has not been studied enough. Thus, research of the nuclear hemocytes phagocytic activity in some representatives of bony fish, amphibians and reptiles under low medium osmolarity conditions is relevant and can be used for comparative analysis and evaluation of evolutionary mechanisms of the organism adaptation to environmental factors.

MATERIALS AND METHODS

We used peripheral blood of the Bony fish class representatives, such as white amur (*Ctenopharyngodon idella*), white carp (*Hypophthalmichthys molitrix*) and crucian carp (*Carassius gibelio*), of the Amphibians class, such as fire-bellied toad (*Bombina bombina*), gray toad (*Bufo bufo*) and lake frog (*Rana ridibunda*), of the Reptiles class, Such as nimble lizard (*Lacerta agilis*), red-turtle (*Trachemys scripta*) and marsh turtle (*Emys orbicularis*). The object of the study was leukocytes. Before blood sampling the animals were anesthetized by ether. Fish and reptiles blood was taken from the tail vein; amphibians blood was taken from the heart (Zapadnyuk et al. 1983). To prevent blood clotting, heparin was used in the amount of 10 units per 1 ml of blood (Douglas and Cui 1983). The received blood was centrifuged for 10 min at 400 *g*4; the layer of leukocytes and the part of leukocytes enriched plasma were collected. In order to study the absorption capacity, hay stick (*Bacillus subtilis*) and agromerulated latex particles with a diameter of 0.8 μm were used as objects of phagocytosis (Alexandrov et al. 1988, Eeden et al. 1999, Potapova et al. 1977). The leukocyte suspension was diluted with isotonic (0.8% NaCl for fish and reptiles, 0.6% NaCl for amphibians) and moderately hypotonic (0.4% NaCl for fish and reptiles, 0.3% NaCl for

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Table 1. Indicators of leukocytes phagocytic activity in the representatives of the Pisces superclass under different NaCl osmolarity conditions, %

Type of animal	Incubation conditions	
	Isotonic environment	Hypotonic environment
Bacillus subtilis		
Ctenopharyngodon idella	21.40±1.86	25.25±1.44*
Hypophthalmichthys molitrix	24.00±1.00	26.50±0.50*
Carassius gibelio	24.00±0,91	22.67±0,88
Latex particles		
Ctenopharyngodon idella	31.33±1.20	29.33±0.88
Hypophthalmichthys molitrix	29.50±1.51	29.52±0.50
Carassius gibelio	29.33±1.20	30.33±1.20

Note: * – significance of differences compared to incubation under isotonic conditions by Student t-test ($p < 0.05$)

Table 2. Indicators of leukocytes phagocytic activity in the representatives of the Amphibia class under different NaCl osmolarity conditions, %

Type of animal	Incubation conditions	
	Isotonic environment	Hypotonic environment
Bacillus subtilis		
Bombina bombina	26.75±1.75	13.25±2.01*
Bufo bufo	26.01±1.52	17.67±1.96*
Rana ridibunda	23.52±1.51	9.67±0.33*
Latex particles		
Bombina bombina	25.25±0.85	11.51±0.86*
Bufo bufo	18.33±1.66	8.25±1.79*
Rana ridibunda	26.50±1.71	8.01±0.01*

Note: * – significance of differences compared to incubation under isotonic conditions by Student t-test ($p < 0.05$)

amphibians) solutions in a ratio of 1:10. The resulting solutions together with the phagocytic reaction objects in a ratio of 1:50 were placed in 2 ml tubes and incubated at room temperature for 30 min.

Then smears were made, cells were fixed with ethanol, stained with azur-eosin according to Romanovsky, phagocytic activity (FA) of leukocytes was calculated. To avoid inaccuracies in calculation of absorbed particles associated with difficulties in determining their localization (inside or on the surface of the cell), immersion magnification (МИ 90 lens, x 15 eyepiece) was used (Menshikov and Baidullaeva 2001).

The obtained digital material was processed by methods of variation statistics using special programs on a personal computer. To determine the reliability of differences between groups, Student argument and Fisher-Snedecor criterion validation calculating was used. The results were considered as reliable starting from $p \leq 0.05$.

RESULTS

The results of leukocytes phagocytic activity research in representatives of the Bony fish class under different medium osmolarity conditions are presented in **Table 1**.

According to the table, during incubation under hypotension conditions (compared to isotonia conditions) the *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* leukocytes absorption capacity against Bacillus subtilis is increased by 15.25% and 9.43%, respectively; in case of latex particles this capacity is not changed. Moderate reduction of NaCl concentration has no effect on the phagocytic activity of *Carassius gibelio* white blood cells, regardless of the phagocytosis object.

Table 3. Indicators of leukocytes phagocytic activity in the representatives of the Reptiles class under different NaCl osmolarity conditions, %

Type of animal	Incubation conditions	
	Isotonic environment	Hypotonic environment
Bacillus subtilis		
Lacerta agilis	22.50±1,84	3.51±0,29*
Trachemys scripta	29.00±0,11	18.21 ±0,41*
Emys orbicularis	22.00±0,91	15.20±0,80*
Latex particles		
Lacerta agilis	18.75±0.47	8.67±0.33*
Trachemys scripta	28.65±0.65	16.05±0.41*
Emys orbicularis	19.23±0,78	15.05±0.27*

Note: * – significance of differences compared to incubation under isotonic conditions by Student t-test ($p < 0.05$)

Under reduced medium osmolarity conditions (compared to normal osmolarity conditions) the leukocytes phagocytic activity of the Amphibians class representatives against Bacillus subtilis and latex particles decreases: by 50.47% and 54.42% for *Bombina bombina,* by 32.06% and 54.99% for *Bufo bufo,* by 58.89 and 70.72% *Rana ridibunda*, respectively (see **Table 2**).

Reptiles have a similar decrease of the studied parameter in the context of hypotension compared with isotony (**Table 3**). Thus, during incubation with Bacillus subtilis, the decrease in phagocytic activity of *Lacerta agilis*, *Trachemys scripta* and *Emys orbicularis* leukocytes with reduced environmental osmolarity is 84.40%, 37.21% and 30.91%, for latex particles it is 53.76%, 43.98% and 21.74%, respectively, compared with normal osmolarity.

DISCUSSION

The revealed changes in the leukocytes phagocytic activity of the lower vertebrates in response to hypoosmotic load, indicate the dependence of the white blood cells absorption capacity on the environment and are determined by the animals ecological status (Zhitenyova et al. 2001).

The decrease in the medium osmolarity does not have a negative impact on the leukocytes phagocytic activity in representatives of the Bony fish class and leads to decrease of the studied indicator in representatives of the Amphibians and Reptiles classes. It is known that for the fresh water fish, the risk of excessive body hydration is high, that explains the corresponding adaptations both at the cellular and systemic levels (Romer and Parsons 1992). Considering hypoosmolarity as a stress factor, it can be assumed that under extreme conditions, hemocytes of primary water animals are able to quickly include compensatory reactions (Prokopenko and Yakhontov 1981), which are

associated with the plasma membrane reorganization, cytoskeleton elements restructuring (Bennett 1985, Smirnova et al. 1987) and cell ion transport systems activation (Mc Manus 1995). Based on the fact that the above structures and systems are involved in the cell phagocytic activity ensuring (Houde et al. 2003, Mc Manus 1995), it can be assumed that there are mechanisms that agree osmoregulation and phagocytosis. The differences in the absorption capacity of fish and other lower vertebrates blood cells may be due to the hemocytes osmoregulatory mechanism peculiarities of animals studied groups.

CONCLUSION

Thus, a moderate decrease in osmolarity has no negative effect on the phagocytic activity of fish leukocytes. A significant decrease in the absorptive capacity of amphibians and reptile leukocytes in the hypotonic environment can be explained by the osmoregulatory mechanisms peculiarities of their hemocytes.

SUMMARY

1. Under hypotension conditions, the absorption capacity of the white blood cells of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* against Bacillus subtilis is increased by 15.25% and 9.43%, respectively, in case of the latex particles is not changed.

2. The leukocytes phagocytic activity of Amphibians class representatives under reduced osmolarity conditions is decreased by 70.72%. Similar results were obtained for representatives of the Reptiles class.

3. The results can be explained by cellular adaptations to the environment, in particular, the presence of compensatory mechanisms in bony fish.

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