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INFLUENCE OF COPPER AND ZINC ON THE ERYTHROCYTE-METRIC PARAMETERS OF *CARASSIUS GIBELIO* (PISCES, CYPRINIDAE) II. INFLUENCE OF ZINC ON THE ERYTHROCYTE-METRIC PARAMETERS OF *CARASSIUS GIBELIO* (PISCES, CYPRINIDAE)

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Abstract

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Some metric parameters of the erythrocytes (big cell diameter *Dc*, small cell diameter *dc*, big nuclear diameter *Dn*, small nuclear diameter *dn*) in the blood of *Carassius gibelio* after influence on different Zn concentrations were established. The percentage proportion of the various types of *Prise-Jones* curves, preliminary constructed, was also calculated. The beginning of processes of cariopicnoses, hypertrophy and anisocitosis were detected.

Key words: zinc, fish, erythrocytes, metric parameters, *Prise-Jones* curves

Introduction

There are many studies on the ecologic toxicology that are related to clarifying the transport and lodgments of heavy metals, and especially zinc, in fish organisms. (Tishinova-Nanova, 1977; Tompson and Cairus, 1980; Haider, 1985; Bieniarz et al.,1989; Chen Qichen et al., 1988; Papagiannis et al., 2004; Velcheva, 2005; Demirezen and Uruc, 2007; Zhou et al., 2001). The effects of heavy metals on fish are also related to disrupting the biochemical and physiological processes in the organism, and the blood test changes in cases of illness, intoxications and under the influence of various types of stress (Hollis et al., 1999; Vilella et al., 1999; Lionetto et al.., 2000; Oliveira et al., 2005; Celik and Oehlenschlager, 2007).

The morphologic peculiarities of fish blood cells give the opportunity for evaluating the compensatory-adaptive processes in conditions under effects of noxious substances.

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It is assumed that zinc disrupts cell transport through the erythrocytes membranes and it causes confusion of antioxidant functions of the protective system, and the erythrocytes membrane changes its function. (Hawkey et al.,1991; Akahori et al., 1999; Cavas et al., 2005)

We set the task with this *ex situ* study to research the influence of the increasing zinc concentrations on the erythrocyte-metric features of *Carassius gibelio*.

Material and Methods

The stagnant tap water was put in aquariums with capacity of 25 liters. For the aim of the experiment series of increasing concentrations of zinc sulphate ($ZnSO_4.7H_2O$) were used. Test concentrations were respectively 0.1, 0.5 and 1.0 mg/l zinc. The initial concentration (0.1 mg/l) is under the Limited Permitted level of Concentration by Bulgarian Standards for zinc in waters. Stagnant tap water was used as control sample. Water for all experimental and for the control samples were with possessed the following parameters: temperature - 20°C, pH - 7.0-7.5 and water hardness - 9.5 dH.

As experimental fish were used 10 specimens from the species *Carassius gibelio*. The fish were taken from a clean water pond in fish breeding farm. The specimens had no external pathological changes and they were of the same size and age group (length 10-12 cm). The fish were not being fed during the experiment. The duration of the test for each concentration was 96 hours. For each specimen the following erythrocyte indices were determined:

-big diameter of the cell (Dc), small diameter of the cell (dc), big diameter of the nucleus (Dn) and small diameter of the nucleus (dn);

-ratio big cell diameter/small cell diameter (Dc/dc)

-ratio big nuclei diameter/small nuclei diameter (Dn/dn)

-type of Prise-Jones curves of big cell diameter, small cell diameter, big nuclei diameter and small nuclei diameter respectively.

-a width on the bases of the curves.

The blood samples were taken through heart punctures. The samples were collected in monovets with anticoagulant (EDTA). The blood smears were colored for the morphology investigation using a set for instant coloring of blood smears DKK COLOR - 200 (VIVA MT).

An eyepiece- and object-micrometer were used for defining the metric indices (Dc, dc, Dn and dn) (Ibrishimov and Lalov, 1984).

For determination of the anisocytosis and the type of the anemic damages the percentage proportion of the various types of Prise-Jones curves (preliminary constructed) was calculated (Ibrishimov and Lalov, 1984).

The results were variably-statistically processed by methods described by Sepetliev (1986). Test with 95% confidence limited was applied to compared the means whenever the date were significant ($p<0.05$).

Results

Table 1 shows the received results about the sizes of the erythrocytes diameters of Carassius gibelio put under the influence of different zinc concentrations. The big cell diameter gets bigger in a higher zinc concentration in wave tendency (increasing in 0.1 and 1.0 mg/l and decreasing in concentration 0.5 mg/l). The small cell diameter increased by increasing the zinc concentration. A contrary dependence in nucleus sizes was found - the diameters get smaller compared to the control samples in wave tendency - decreasing of the diameters in concentration 0.1 and 0.5 mg/l and increasing of the diameters in concentration 1.0 mg/l up to values that can be compared to the control.

The ratio: big diameter/small is different in the experiment in comparison with the control samples. In the lowest concentration it is higher than this of the control samples but in the other two concentrations it decreases considerably (Table 2). This shows that the erythrocytes are getting slightly sharper in the lowest concentration, and they become slightly rounder in higher concentrations. The same tendency in the ratio of the big and small diameters of the nucleus was found (Table 3).

For the big diameter of erythrocytes, the percent-

Table 1
Metric erythrocytes parameters of *Carassius auratus gibelio* after influence on different zinc concentrations

Metric erythrocytes parameters	Control	0.1 mg/l	0.5 mg/l	1.0 mg/l
Dc	13.3±0.4	14.5 ±0.6***	13.0±0.5	15.8±0.6***
dc	7.9±0.6	8.0±0.7	8.8±0.4***	10.0±0.4***
Dn	7.2±1.0	5.5±0.4	5.5±0.3	6.5±0.3
dn	3.7±0.3	2.8±0.3	3.1±0.2	3.8±0.2

* (p<0.1), **(p<0.02), ***(p<0.001)
Dc-big cell diameter; dc-small cell diameter; Dn-big nuclei diameter; dn-small nuclei diameter

Table 2
Ratio: big cell diameter/small cell diameter, Dc/dc

Control	0.1 mgl^{-1}	0.5 mgl^{-1}	1.0 mgl^{-1}
1.7 (1.60-1.80)	1.82 (1.70-2.00)	1.48 (1.40-1.60)	1.58 (1.50-1.70)

Table 3
Ratio: big nuclei diameter/small nuclei diameter, Dn/dn

Control	0.1 mgl^{-1}	0.5 mgl^{-1}	1.0 mgl^{-1}
1.94 (1.60-2.10)	1.96 (1.80-2.10)	1.8 (1.70-1.90)	1.72 (1.60-1.80)

Table 4
Prise-Jones* curves og the big and small erythrocyte diameteres of *Carassius gibelio

Type of the curves, %	Control, mgl^{-1}		0.1 mgl^{-1}		0.5 mgl^{-1}		1.0 mgl^{-1}	
	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter
One-peak	60	40	80	100	100	100	80	100
Two - peak	0	40	20	20	0	0	0	0
Tree-peak	0	20	0	0	0	0	0	0
Parabolic	40	0	0	0	0	0	20	0
Ascending	0	0	0	0	0	0	0	0
Descending	0	0	0	0	0	0	0	0

age of one-peak Prise-Jones curves increases at the expense of the parabolic curves (Table 4). For the small diameter of erythrocytes, this tendency was even clearer - 100% one-peak curves in all zinc concentrations in comparison with two-peak and parabolic curves for the control specimens (Table 4).

At the curves of the big and small diameters of the nucleus, the anisocytosis decreases when the test concentrations are higher, as in 1.0 mg/l one-peak curves are 100% (Table 5).

The bases of Prise-Jones curves (Table 6) gave the following results: At the big diameter of erythrocytes, no changes in the width of the curves were found

At the small diameter of erythrocytes, there is narrowing of the curves bases

At the big and small diameter of the nuclei, if the concentrations are higher, the narrowing of the curves bases is more clearly expressed.

Table 5
Prise-Jones curves of the big and small nuclear diameters of *Carassius gibelio*

Type of the curves, %	Control, mgl^{-1}		0.1 mgl^{-1}		0.5 mgl^{-1}		1.0 mgl^{-1}	
	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter	big diameter	small diameter
One-peak	40	80	90	100	80	90	100	100
Two - peak	0	0	10	0	0	0	0	0
Tree-peak	20	0	0	0	0	10	0	0
Parabolic	40	0	0	0	20	0	0	0
Ascending	0	20	0	0	0	0	0	0
Descending	0	0	0	0	0	0	0	0

Table 6
***Prise-Jones* curves of the erythrocytes of *Carassius gibelio*. A width on the bases - µm**

Zn concentrations	Dc	dc	Dn	dn
Control	4.8	4.5	5.3	3.8
0.1 mgl^{-1}	4.9	4.3	4.3	2.4
0.5 mgl^{-1}	4.1	3.1	2.9	3.8
1.0 mgl^{-1}	4.8	3.6	3.3	2.6

Dc-big cell diameter; dc-small cell diameter;
Dn-big nuclei diameter; Dn-small nuclei diameter

Discussion

The results allow pointing out some tendencies related to the changes in the metric features of erythrocytes of Carassius gibelio under the influence of different zinc concentrations.

Probably, zinc causes the beginning of compensatory reactions in red blood cells tending to cell hypertrophy. There are confirmations for that in the results in Table 1. As a whole, when zinc concentrations are higher, the cells become bigger and rounder. Meanwhile, the changes in the metric features of the nuclei are contrary - their sizes get smaller.

A change in the shape of the nuclei could be observed - round shapes become pointed. That it may be caused by cario-picnosis due to zinc content in the water.

Analysis of the peaks of Prise-Jones curves showed a clear tendency towards decreasing anisocytosis in higher zinc concentration.

We think that two parallel processes are going - necrosis (picnotic nuclei) and compensatory hypertrophy (increasing sizes of the big and small cell diameters). It is an interesting fact that these processes run in the background of a well-expressed decreasing anisocytosis.

In specialized scientific papers some single similar metric proofs for toxic and compensatory changes in fish erythrocytes under the effects of various toxicants could be found. Witeska and Kosciuk (2003) says that if carps are put for 96 hours in water with zinc sulphate, a stress-reaction can be seen which is expressed by reducing the hematocrit, reducing the frequency of abnormal erythrocytes, compensatory releasing of immature erythrocytes.

Lay and Baldwin (1999) point out that erythrocytes volume correlates more to the oxygen quantity than to the nuclear size and there is no specific explanation for that.

Zelev et al. (2002) specify metric changes in erythrocytes in Rana ridibunda from an industrial region in Bulgaria. As the test has been done with species from another systematic class, in natural conditions and it is related to pollution with nitric oxides, so it is difficult to be juxtaposed to our results.

The present study is a continuation of such preceding studies of the team (Tomova et al., 2008) and its results prove the quantitative patho-morphologic changes found in erythrocytes of *Carassius gibelio* under the effects of different zinc concentrations.

Storelli and Marcotrigiano (2001) as well as Brumbaugh et al. (2005) consider that blood samples of fish may also be used for the purposes of biomonitoring.

The research, done by us about the influence of increasing zinc concentrations on the metric features of fish erythrocytes, gave the opportunity the received results to be used for the purposes of biomonitoring.

Conclusions

In erythrocytes of *Carassius gibelio* under the effects of different zinc concentrations the following tendencies were found:

The processes of compensatory cytoplasm hypertrophy are dominant

As a whole, erythrocytes become bigger and rounder

The nuclei get smaller but their shapes get sharpened, and probably, it is a result of cariopicnosis.

The toxic zinc influence is related to decreasing the variety in cell sizes, i.e. anisocytosis.

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