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THE EFFECT OF TOXIC AND HELIOPHYSICAL FACTORS ON THE BIOCHEMICAL PARAMETERS OF THE EXTERNAL MUCUS OF CARP, (*CYPRINUS CARPIO* L.)

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ABSTRACT. The dynamics of the biochemical parameters of the external mucus of carp *Cyprinus carpio* L. exposed to 1 μM concentrations of heavy metal salts ($CuSO_4 \cdot 5H_2O$, $Pb(NO_3)_2$ and $ZnSO_4 \cdot 7H_2O$) were studied. The biochemical parameters of external mucus (specific weight, pH, total protein, hemoglobin, ketones) were measured during exposure at 1, 3, 6, 24 h and regularly during a 21-day post-exposure period. Significant changes in hemoglobin and total protein concentrations were determined during the period of exposure to heavy metals and after it. Correlations of changes in biochemical parameters of mucus with the intensity of some parameters of sun activity and radio radiation flow were found during the post-exposure period. It was concluded that it is necessary to assess the possible complex effects of various origin and the magnitude of environmental factors on fish.

Key words: *CYPRINUS CARPIO*, BIOCHEMICAL PARAMETERS, HELIOPHYSICAL FACTORS

INTRODUCTION

Animals in natural conditions often experience the general effects of stress which cause a temporary disturbance in homeostasis (Kovalskii 1982, Slonim 1971, Shilov 1981). Our previous studies demonstrated the rhythmic character of changes in biochemical parameters as healthy carp were subjected to different stressors – starvation, isolation and chemicals of biotic origin (Lebedeva et al. 2000). Correlations of these rhythmic alterations with changes in heliophysical factors, such as intensity of sun activity and radio radiation flow, were determined (Lebedeva and Golovkina 1998). The data obtained allowed us to conclude that metabolic changes in fish induced by stressors of various origin, i.e. heavy metals, will also be related to the effects of heliophysical factors.

The metabolic processes that occur in fish are reflected in changes of the biochemical parameters of various organs and tissues (Lebedeva et al. 1988, 1993). Environmental factors can also induce changes in skin mucus (Lebedeva and Golovkina 1998,

Lebedeva 1999), as this tissue forms an additional barrier to potentially harmful substances and protects the animal from external effects (Fletcher and Grant 1968, Pickering 1974, Mattey et al. 1979, Coello and Khan 1996). Thus, the composition of skin mucus may serve as a criterion of the physiological status of fish, and detecting alterations in this fluid may allow the particular effects different factors have on it to be identified.

The aims of the present study were to determine the effect of exposure to heavy metals on the biochemical parameters of carp mucus and to ascertain whether heliophysical factors, specifically intensity of sun activity and level of radio radiation flow, are related to the effect of exposure to heavy metals.

MATERIAL AND METHODS

One-year-old carp, *Cyprinus carpio* L., weighing about 20 g each, were acclimated under laboratory conditions for at least one week until they started feeding. The fish were kept in groups of five individuals in aquaria with a 20 l capacity supplied with well-aerated and filtered water. The water temperature ranged from 18.5 to 21°C. A group consisting of ten fish was exposed to each chemical. The fish were exposed to a 1 μM concentration of heavy metal salts - $CuSO_4 \cdot 5H_2O$, $Pb(NO_3)_2$ and $ZnSO_4 \cdot 7H_2O$ for 24 h. The biochemical parameters of fish mucus were determined at 1, 3, 6 and 24 h from the initiation of exposure. After the 24-h exposure period, the fish were transferred to aquaria with metal-free water and the study was continued for 21 days. Parameters were measured on days 1, 3, 6, 9, 12, 15, 18 and 21 during the post-exposure period. The water in the aquaria was changed daily. The carp were fed with commercial fish food pellets ad libitum. To conduct the analysis, every fish was caught and analytical film was gently pressed to fish. The biochemical composition of the skin mucus was determined with reagents immobilized on a solid carrier of multi-layer Ames film (produced by Ames Division, Miles Laboratories, Ltd., England) using the method described by Lebedeva and Golovkina (1990). This method allows the skin mucus composition and quantitative changes in its parameters during a long-term study to be evaluated without damaging the fish. The basic parameters of the skin mucus, i.e. total protein (g l^{-1}), ketones (mM), hemoglobin (μg l^{-1}), pH and specific weight (g cm^{-3}), were determined.

The biochemical data obtained were compared with the intensity of the following heliophysical factors: changes in sun activity measured as the index of magnetic per-

turbation (Ak); sunspot intensity evaluated in Wolf numbers (W); radio radiation flow intensity (F). The evaluation of the intensity of heliophysical factors was performed by the Russian Scientific Center of Rehabilitation and Physiotherapy. The biochemical data represent the mean ± SE of the parameter measured for ten fish. The significance of all data obtained was determined using Student's t-test at $P \leq 0.05$ and $P \leq 0.01$, and the Spearman correlation coefficient r was calculated.

RESULTS

An increase in the hemoglobin concentration in the mucus of fish exposed to heavy metal salts was detected after 1 h from initiation until the end of exposure. This parameter increased most significantly (three-fold) in fish exposed to zinc ($P \leq 0.01$; Table 1).

TABLE 1

The effect of exposure to heavy metals on biochemical parameters of external mucus of carp

Parameters	Control	Exposed fish			
		Duration of exposure (h)			
		1	3	6	24
Specific weight ($g\ cm^{-3}$)	1.018	a. 1.014	1.017	1.015	1.017
		b. 1.014	1.015	1.015	1.022
		c. 1.015	1.016	1.017	1.022
pH	6.4±0.01	a. 6.4	6.6	6.5	6.2
		b. 6.6	6.2	6.3	6.5
		c. 6.5	6.5	6.7	6.6
Total protein ($g\ l^{-1}$)	0.70±0.04	a. 0.50±0.04*	0.50±0.04*	0.40±0.03**	0.70±0.07
		b. 0.70±0.07	0.80±0.07	0.70±0.07	0.80±0.08
		c. 0.80±0.07	1.20±0.08**	0.90±0.08*	0.90±0.08*
Hemoglobin ($\mu g\ l^{-1}$)	100.0±5.5	a. 150.0±9.5**	170.0±10.5**	193.0±12.5**	250.0±12.5**
		b. 166.0±10.5**	185.0±11.0**	217.0±13.0**	258.0±15.5**
		c. 175.0±11.5**	193.0±12.1**	231.0±13.2**	304.0±15.8**
Ketones (mM)	0.50±0.05	a. 0.50±0.05	0.50±0.03	0.50±0.04	0.50±0.04
		b. 0.50±0.04	0.50±0.03	0.50±0.04	0.50±0.03
		c. 0.50±0.03	0.50±0.04	0.50±0.05	0.50±0.04

*a – 1 μM concentration of $CuSO_4 \cdot 5H_20$; b – 1 μM concentration of $Pb(NO_3)_2$, c – 1 μM concentration of $ZnSO_4 \cdot 7H_20$. Asterisks * denote values significantly different from controls $P \leq 0.05$, **- $P \leq 0.01$*

The hemoglobin concentration in the mucus of exposed fish had returned to control values within 21 days after transferring fish to metal-free water (Fig. 1). Protein concentration in the mucus of exposed fish changed in different ways: copper induced a short-term decrease; lead had no effect; zinc resulted in a significant elevation in protein level ($P \leq 0.05$; Table 1). No significant differences in protein concentrations were found in the mucus of fish exposed to copper and lead at the end of exposure as compared to controls, although an increase in protein concentration induced by zinc was detected by the end of exposure. The protein concentration in the mucus of the transferred fish fluctuated over the 21-day period (Fig. 2), and the magnitude of these fluctuations was greater than that observed during exposure. The concentration of protein in the mucus of fish exposed to lead and zinc returned to the control level by the end of the study, while that of carp exposed to copper increased at the end of the post-exposure period.

No significant alterations in pH level were measured during the 24-h period of exposure to heavy metals. The most pronounced decrease in this parameter was determined during the post-exposure period on critical days 14 and 15 when the mucus was the thickest. Ketone concentration did not change during the 24-h expo-

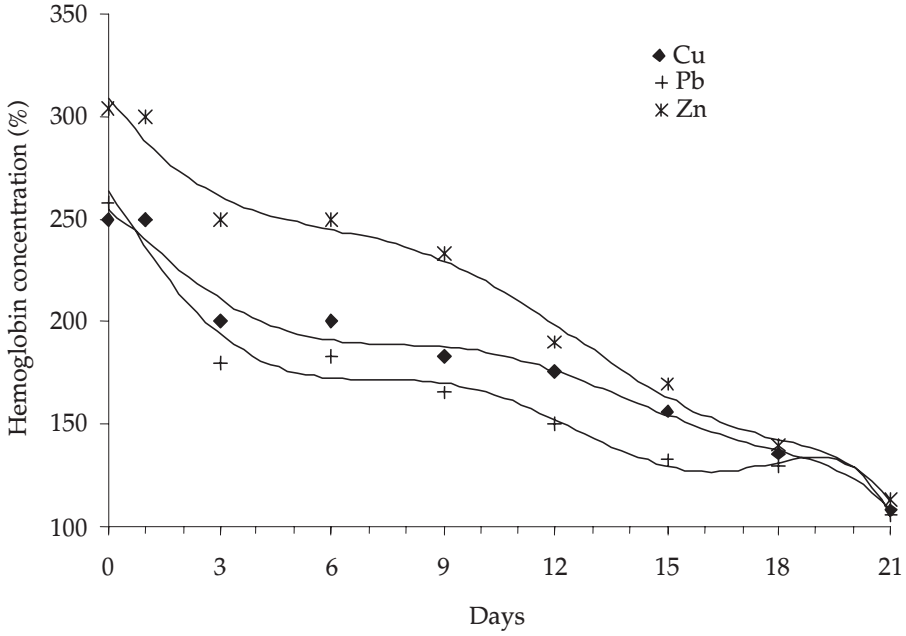

Fig. 1. Fluctuations in the hemoglobin concentration (%) in the mucus of carp during the post-exposure period. Control level 100%.

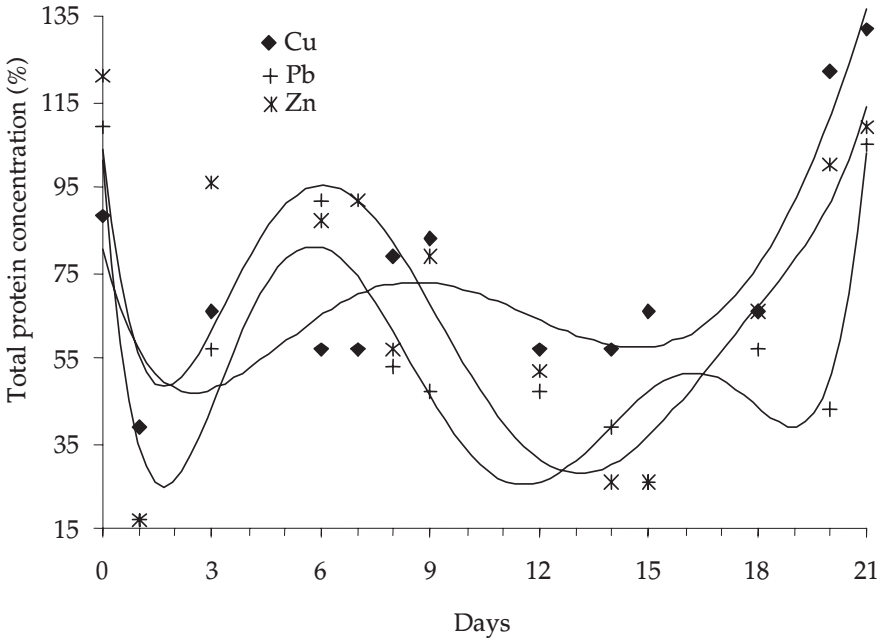

Fig. 2. Fluctuations in total protein concentration (%) in the mucus of carp during the post-exposure period. Control level 100%.

sure period to heavy metals, but during the post-exposure period this parameter decreased to a nearly undetectable level. A slight increase in the specific weight of mucus of fish exposed to heavy metal salts was determined at the end of exposure.

A correlation was detected between the dynamic alterations of biochemical parameters in the mucus of fish during the post-exposure period, in comparison with control fish, and the intensity of the following heliophysical factors: changes in sun activity; sunspot; radio radiation flow intensity (Table 2). The hemoglobin concentration in the mucus of fish exposed to copper and zinc correlated significantly with the index of magnetic perturbation and radio radiation flow intensity on the day the parameter was registered ($P \leq 0.05$), while changes induced by lead correlated only with radio radiation flow intensity ($r = - 0.70$; $P \leq 0.01$). Only the hemoglobin concentration in the mucus of control fish correlated significantly with sunspot intensity ($P \leq 0.05$), and in exposed fish it correlated with radio radiation flow intensity ($P \leq 0.05$). Only the protein content in the mucus of control fish correlated with the index of magnetic perturbation ($P \leq 0.05$).

TABLE 2

Correlation coefficient (r) of the dynamics of heliophysical factors and biochemical parameters in the external mucus of carp

Heavy metal salts/ heliophysical factors	r	
	Hemoglobin	Total protein
$CuSO_4 \cdot 5H_2O$		
Sunspot intensity W	-0.30	0.15
Magnetic perturbation Ak	-0.52*	0.46
Radio radiation flow intensity F	-0.61*	0.23
$Pb(NO_3)_2$		
Sunspot intensity W	-0.20	0.11
Magnetic perturbation Ak	-0.41	0.23
Radio radiation flow intensity F	-0.70**	-0.43
$ZnSO_4 \cdot 7H_2O$		
Sunspot intensity W	-0.37	0.12
Magnetic perturbation Ak	-0.58*	0.08
Radio radiation flow intensity F	-0.70**	-0.29
Control		
Sunspot intensity W	-0.80*	0.36
Magnetic perturbation Ak	0.45	0.77*
Radio radiation flow intensity F	0.76*	0.32

*Asterisks * denote values significantly different from controls $P \leq 0.05$, ** $P \leq 0.01$*

DISCUSSION

The exposure of fish to heavy metals induced alterations in the physiological parameters of carp mucus. Our previous studies also demonstrated changes in metabolic processes of fish effected by lower than threshold concentrations of factors of biogenic origin (Lebedeva et al. 2001). Rainbow trout (*Parasalmo mykiss*) exposed to a heavy metal model mixture (Cu, Zn, Cr, Ni, Fe) at concentrations similar to those found in unpolluted Lithuanian rivers also induced alterations in the physiological parameters of the fish (Vosylienė et al. 1999). The specific peculiarities of the effect of similar concentrations of the same metals on feeding behavioral responses of fish were not found; however, 24-48 h exposure of fish to these chemicals induced changes in feeding behavior, i.e. they refused to feed (Kasumyan and Morsi 1998). However, the aim of our study was to ascertain whether a new, even low-magnitude factor (heliophysical) can induce changes in recovery processes and their duration which occur in an animal following the effects of toxicants.

Differences in alterations of hemoglobin and total protein concentrations in the mucus of fish exposed to metals were probably related to the specific character of their action. Hemoglobin emergence in mucus has been shown in many species of studied fish (Lebedeva 1999). It serves as the criterion of stress reaction, while hemoglobin concentration depends on the magnitude of the applied stressor (Lebedeva et al. 1993, 1998). Our previous studies showed that with the development of the stress syndrome in silver carp, *Hypophthalmichthys molitrix* (Val.), the concentration of blood creatinine increased three-fold and the number of erythrocytes in the mucus increased 100-fold (Lebedeva and Golovkina 1993). An increase in hemoglobin concentration determined in the mucus after one hour of exposure in the present study might be regarded as the beginning of the stress reaction in fish which continued throughout the period of exposure. The decrease of the hemoglobin concentration in the mucus of fish transferred into metal-free water occurred gradually, and the shape of the decreasing curves were similar for all metals. The decrease of hemoglobin concentration in the mucus probably reflected the compensatory processes occurring in fish after exposure to toxicants of the same nature – heavy metals.

The exposure of fish to metals also induced changes in protein concentrations of various magnitudes and duration. Fish in the lead nitrate solution were shown to start secreting mucous glycoproteins from epidermal cells of the body, and the magnitude of this protective reaction depended on the fish species (Coello and Khan 1996). Electron microscopic studies showed significant disorders in the release of mucus within 24 h of carp exposure to heavy metals. The fish secreted excessive amounts of mucus whose physical and chemical composition was different in comparison to that of the controls (Devitsina 2001). The recovery of protein concentrations in fish exposed to heavy metals in our studies took place differently as compared to hemoglobin, and the complete or partial return to the control fish level occurred only at the end of the study. The different periods of recovery could be connected with the specific action that the studied metals have on the fish.

Alterations in pH level were not expressed as the parameters discussed above, nor did heavy metals induce significant changes in the thickness (specific weight) of the mucus. The low magnitude of these parameters and their alterations did not allow the dynamics of changes in these parameters to be precisely calculated or discussed. However, the changes that occurred in total protein and pH concentrations could modify mucus properties. Structural changes in mucus may increase its permeability and reduce its protective function. No significant changes were determined in ketone

concentration during exposure to toxicants, although the starvation of the fish induced an increase in ketone concentration (Lebedeva et al. 2001). The reduction of ketone concentration to undetectable levels was found in the mucus of the transferred fish; therefore, it was impossible to calculate the correlation of this parameter with heliophysical factors.

The composition of fish mucus, as with other biological fluids, reflects the physiological and biochemical alterations occurring in an animal (Natochin 1984, Lebedeva 1999). Changes in the biochemical parameters of mucus found in the present study can also be attributed to the direct interaction of toxicants with mucus components. However, we have reservations concerning this idea based on the following suggestions: (I) the mucus is released and removed from the skin constantly so its composition is constantly renewed; (II) the duration of fish exposure to toxicants lasted 24 h, while after exposure the fish were kept in metal-free water for a 21-day period during which the altered parameters returned to pre-exposure levels; (III) the concentrations of toxicants used for exposure did not induce damage to fish skin and could not significantly change the function of mucus cells or the composition of mucus. Our data proves that changes in the biochemical composition of mucus reflected the stress effect metals had on the metabolic processes of the fish when they entered their bodies. The dynamics of parameters during the exposure period indicated that changes in metabolic processes took place in the fish, and the nature of their return to control levels demonstrated the recovery process. This study showed that this process was long enough. Alterations in the biochemical parameters of carp were determined during a period that was 20 times longer than the duration of exposure (24 h). It should also be noted that the alterations of biochemical parameters during the post-exposure period to some extent could be connected with the processes of depuration of heavy metals from the fish. The studied concentrations of heavy metals were rather low; however, according to Jezierska and Witeska (2001), the rapid uptake of the metals in the skin and scales is followed by a relatively gradual release of them. Metals can enter the skin and scales not only from the environment but also from the bloodstream, and surface mucus is one way of excreting metals.

The effects of heliophysical factors on biota have not yet been sufficiently studied (Gulyajeva 1998, Shnol et al. 1998). The significant relationship of the intensity of some sun activity parameters and radio radiation flow with alterations of biochemical parameters may explain the fluctuations in the mucus of exposed and control fish in response to alterations of heliophysical factors. The rhythms and relationship of cor-

relations found in the control fish probably reflected the physiological status of healthy fish (hemoglobin concentration correlated significantly with sunspot and radio radiation flow intensity and protein did so with magnetic perturbation). Meanwhile, alterations in the physiological status of exposed fish are reflected only by correlations of hemoglobin concentration with radio radiation flow intensity and the index of magnetic perturbation. These peculiarities could reflect the desynchronization of endogenic rhythms in comparison to control fish.

The present data confirmed the necessity of studying the combined effect of various low intensity factors on fish, especially in model studies. The magnitude of the effect of different types of environmental factors - chemical, physical, heliophysical and ecological - is very often low; however, the sum of the effects could induce remarkable changes in the physiological status of fish.

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STRESZCZENIE

WPŁYW SUBSTANCJI TOKSYCZNYCH I CZYNNIKÓW HELIOFIZYCZNYCH NA WŁAŚCIWOŚCI BIOCHEMICZNE ŚLUZU KARPIA (*CYPRINUS CARPIO* L.)

Badano dynamikę zmian parametrów biochemicznych śluzu karpia *Cyprinus carpio* L. poddanego działaniu soli metali ciężkich ($CuSO_4 \cdot 5H_2O$, $Pb(NO_3)_2$ i $ZnSO_4 \cdot 7H_2O$) o koncentracji 1 mM. Właściwości biochemiczne śluzu (masa właściwa, pH, białko ogólne, hemoglobina, ketony) określano po 1, 3, 6, 24 godzinach i po 21 dniach ekspozycji. Stwierdzono istotne zmiany koncentracji hemoglobiny i białka ogólnego w trakcie i po poddaniu śluzu działaniu metali ciężkich. Stwierdzono zależność pomiędzy zmianami właściwości biochemicznych śluzu a nasileniem wybranych parametrów aktywności słońca i promieniowania słonecznego po ekspozycji śluzu na działanie metali ciężkich. Postawiono wnioski, że konieczne jest zbadanie skutków złożonego działania na ryby rozmaitych czynników środowiskowych o różnym natężeniu.

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