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INTERRELATIONS OF ESTRON WITH HYPOPHYSIS' PEPTIDES IN REGULATION OF BILE FORMATION IN THE RATS OF DIFFERENT SEX

Influence of the intraportal injected estron on both the processes of bile acids conjugation, and hydroxylation of the bile acids and the level of the hypophysis hormones in the blood of different sex rats has been studied. It was shown, that the changes in efficiency of the liver enzymes functionation coincide with the such of the prolactin level in blood.

Key words: estron, bile, peptides bilious lipids

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INFLUENCE OF HEAVY METALS IONS ON THE CONTENT OF PROTEINS AND NUCLEIC ACIDS IN THE ORGANISM OF FRESHWATER FISH

From the launched research we obtained the aggregate data that confirm and broaden our concept of the important role of protein and nucleic metabolism in the processes of detoxication of heavy metals ions, formation of resistance to them and also allow making an integrated estimation of biochemical reaction of carp organism to chronic intoxication.

Key words: freshwater fish, proteins, nucleic acids, heavy metals

Contamination of water reservoirs by heavy metals is one of the limiting factors of aquatic ecosystems functioning and their biological productivity. Being part of many organic substances, or engaging them in the interaction, they influence many biochemical processes in aquatic organisms. The ions of metals can form strong connections in the tissues along with various biologically active centres, including the sulphur-containing ligands that may be enclosed in proteins and amino acids. Their activity is related to the enzymes that contain metal ions in their composition or are actuated by them [6, 10].

One of the basic principles of biochemical adaptation of an organism is to maintain the structural and functional integrity of macromolecules. Much of this is applied to proteins and nucleic acids – biopolymers that perform an extremely important role in the adaptation of aquatic lives to environmental conditions [10].

Materials and methods

The object of the given research was carp – *Cyprinus carpio* L. For the experiment the 2 year old fish with the mass of 250-300 grams were rummaged from the natural stews of Ternopil region (Zalistsi

fish-breeding complex). The experiments were carried out in 200 litre aquariums filled with the precipitated water from the local water supply system under constant gas and temperature operating conditions. During the process the fish were not fed. The effect of Mg, Zn, Cu and Pb ions in two concentrations that complied with 2 and 5 maximum permissible concentrations (MPC) [1]. The period of acclimation was 14 days.

The total content of protein in tissues was determined by a biuretic method with some modifications [3], while in nucleic acids fractions – by Lowry and co-authors [11]. Nucleic acids were fixed spectrophotometrically by Tsanev R.H. and Markov G.G. [8] in accordance with the authors' recommendations [2]. For the protein fractions of fish blood serum the diagnostic set "Cormay gel protein 100" (Austria) was used. To determine the significant difference the obtained data underwent certain statistic processing.

Research results and their discussion

In our studies, under the influence of higher concentrations of ions of metals significant deviations from the control indices of content as for aggregate proteins and proteins combined with nucleic acids. Some increase in the total number of proteins in the liver may indicate about an active part of this organ in the synthesis of adaptive proteins.

The slightest deviation from the control indexes of the total protein content (table 1) was found in the muscles of carp, suggesting that along with the increased activity of lysosomal proteases and the rising content of free amino acids, the aggregate protein content remains constant. The latter speaks rather about the deep restructuring of protein metabolism in the body of fish influenced by heavy metals than of their not being used in energy processes by amino acids oxidation.

Table 1

Effect of heavy metals on the content of total protein in carp tissues mg%, M ± m, n = 5

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	9,84±0,72	9,40±0,42	10,86±0,52	11,22±0,40
2 MPC	10,35±0,73	9,67±0,62	9,94±0,45	11,45±0,33
5 MPC	10,36±0,93	9,03±0,27	12,85±1,26	12,17±0,69
Muscles				
Control	12,58±0,83	12,73±0,38	15,50±0,32	15,08±0,24
2 MPC	13,55±1,49	13,70±0,58	13,81±0,27*	14,84±0,79
5 MPC	13,22±0,39	13,55±0,54	14,19±0,34*	14,92±0,27
Blood				
Control	13,03±0,59	11,21±0,54	13,86±1,29	12,26±0,78
2 MPC	14,58±0,26*	13,94±0,63*	15,56±0,47	12,94±0,64
5 MPC	13,63±1,05	11,09±0,86	17,81±0,73*	12,22±0,81

The change of the content of proteins in the structure of nucleoprotein complexes is probably related to the functional characteristics of these complexes. It is a well known fact that some proteins can act as the repressors of genome. Therefore, their number in the tissue may be an indicator of the size of the protein blockade of nucleic acids molecules. In our studies we could not find any statistically significant deviations from the control indices of protein content in the fractions of RNA and DNA (tables 2, 3). It is possible that under these experimental conditions the body of fish does not undergo any significant functional changes at the genetic level and its adaptation passes on the level of phenotype through modification of the quantitative and qualitative composition of molecules.

Table 2

Effect of heavy metals on the content of RNA in carp tissues mg% P, M ± m, n = 5

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	64,23±1,56	47,33±4,18	43,76±2,99	45,72±1,69
2 MPC	69,84±1,56*	50,49±4,05	40,67±1,09	53,01± 4,80
5 MPC	67,04±3,72	59,95±2,99*	52,45±1,86*	73,49±6,19*
Muscles				
Control	14,44±0,48	13,67±1,68	6,73±0,52	13,04±0,36
2 MPC	13,88±0,81	15,01±1,12	5,76±0,14	12,48±0,81
5 MPC	14,55±1,05	12,34±0,57	6,17±0,46	15,00±0,52*
Blood				
Control	27,49±2,20	17,18±2,09	23,28±1,69	26,65±0,99
2 MPC	28,40±2,01	18,51±1,56	28,89±1,30*	21,32±1,90*
5 MPC	23,49±2,88	12,27±1,20	28,61±1,05*	22,16±0,69*

Changing of the chemical structure of water environment inevitably leads to the changes in protein composition of fish blood. The obtained data proves the alteration of the total protein concentration and the ratio of protein fractions in the serum of carp, its body exposed to higher concentrations of heavy metal ions. Thus, the total protein content in the blood serum of fish increases when affected by manganese, zinc, lead, and especially copper. Deviations of this index from the control indices increase along with the rise of metal concentration in water.

Table 3

Effect of heavy metals on the content of DNA in carp tissues mg% P, M ± m, n = 5

Group	Manganese	Zinc	Copper	Lead
Liver				
Control	23,20±3,14	21,00±1,29	15,00±1,73	20,50±0,96
2 MPC	26,40±2,31	22,00±1,67	13,20±1,02	23,00±1,73
5 MPC	22,80±1,03	21,60±1,17	22,40±1,17*	29,50±1,26*
Muscles				
Control	9,25±0,75	8,00±0,32	3,40±0,51	6,40±0,24
2 MPC	6,60±0,87*	7,80±0,20	3,00±0,32	6,00±0,00*
5 MPC	6,00±0,45*	8,40±0,40	2,60±0,24	7,40±0,24*
Blood				
Control.	51,60±3,06	51,20±3,38	36,00±4,97	31,60±0,98
2 MPC	52,00±3,74	56,00±1,41	37,00±4,36	32,00±2,53
5 MPC	56,67±3,71	45,20±5,98	44,00±2,28	42,50±1,71*

An increase of the total protein content in the serum of carp due to heavy metals, in our opinion, should be considered as a result primarily of the synthesis of the acute phase of proteins, growth of the level of blood haemolyse in the experimental fish, augmentation of the number of transporting proteins which bind and transfer the ions of metals, and also of blood coagulation. Besides, the higher content of proteins in the blood serum of experimental fish may be caused by the enhanced dissolution of proteins in tissues, resulting from the risen activity of proteolytic enzymes under intoxication.

An important diagnostic value has the determination of the fractional composition of carp blood serum exposed to the influence of heavy metals. Thus, under both of the studied metals concentrations the content of albumin in blood serum of fish increases (figure 1). The only exception is lead at 2 MPC of metal in water. This protein plays an important role in maintaining the osmotic pressure in the blood and transport of a number of substances, including amino acids and inorganic ions [9]. Therefore, the increase of quantity of albumin, which due to intoxication leads to the active

proteolysis of tissue proteins and the transport of free amino acids become clear. The largest growth of albumin concentration in the serum of fish is observed under the influence of copper ions. This phenomenon is consistent with the data that albumin carries the fast-exchange fractions of copper, while the slow-exchange fractions of this metal are transported by α_2–globulins [9]. The ability of albumin to bind calcium ions and magnesium is also well known [4]. It is possible that according to the similar principle this protein binds other divalent ions, thereby reducing their toxicity to the body. On the other hand, the ions of the investigated metals may exhibit a stimulating effect on the biosynthesis of albumin.

Because of intoxication the carp blood serum globulins undergo certain changes. These proteins are involved in the transportation of lipids, hormones, vitamins, metal ions, form important complexes of blood coagulation, while γ-globulins fraction contains antibodies of the immune system. It is logical to assume that the change of globulins in the content of blood serum leads to the violation of performance of the described functions by them.

Higher concentrations of ions of the investigated metals in particular, caused a slight increase in the content of α_1-globulin at 2 MPC of metals in water, while the α_2-fraction responded in the same way to 5 MPC of the investigated metals. Zinc was considered to be an exception, for the action of which the reduction of the content of fractions α_1– and α_2–globulins was observed in both cases. Taking into account that zinc inhibits the activity of certain proteases, the decrease of α_1-globulins containing antitrypsin and antichimotrypsin might be a response of the carp serum protein system to the increased level of zinc in water. One should also admit the growth of the content of α_2-globulins for the effect of copper ions at the concentration of 5 MPC, which is consistent with the data [4] that exactly this fraction contains ceruleoplasmin – an acute phase protein which actively transports the ions of copper.

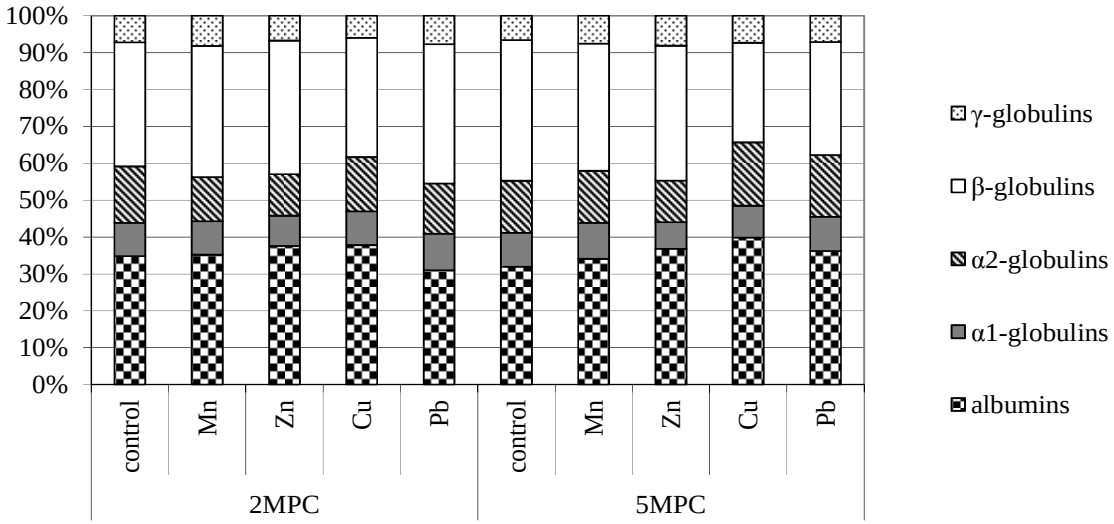

Figure. 1. Relative content of carp blood serum proteins in control groups and under intoxication (% of total proteins)

Somewhat different dynamics was detected as for the *β*-globulins of carp blood serum under the influence of ions of the investigated metals. At the level of 2 MPC of metals in water we have noticed the growth in the total proteins of this fraction under the effect of ions of manganese, zinc and lead and only copper ions were reducing that index. At 5 MPC of metals in water all of the investigated chemical elements caused the reduction of *β*-globulins content in the blood serum of fish. Thus the more significant deviations from the control group were observed due to the influence of copper ions and lead.

One of the main functions of *β*-globulins is the transportation of iron ions [4], which is part of haemoglobin structure, and thus participates in the processes of oxidation. Decreasing of the amount

of this metal in the blood leads to a decrease of oxidation processes in the whole organism, which we observe in the conditions of intoxication when anaerobic ways of energy formation is dominated over the aerobic.

Because of the action of ions of the investigated metals the content of γ-globulins, especially at the concentration of 5 MPC grows in the carp blood serum. With this fraction of proteins that contains antibodies, the protective properties of the body are mainly associated and therefore it is clear that their number increases under intoxication.

A very important diagnostic value has the determination of fish blood serum lipoproteins content — complexes of proteins and lipids, whose roles in the process of adaptation of the body of fish to the environmental conditions aux rather significant. Our studies revealed α- and β-lipoprotein fractions: the α-fraction accounted for 72-78% of the proteins, and β – for only 22-28% (Table 2). The dynamics of changes in these fractions under the influence of ions of metals investigated was also different. While the number of α-lipoproteins in the blood serum of experimental fish at both studied concentrations of metals in water was increasing, the amount of β-lipoprotein, in contrast, declined. The only exception was the indicator of the impact of lead ions at 2 MPC of metal in water.

The reduction of the content of β-lipoproteins, which is a low-density lipoprotein fraction, is probably happening due to the fact that these protein-lipid complexes are absorbed by tissues and undergo disintegration in lysosomes [7]. Therefore, the fortified catabolism of β-lipoproteins and the decrease of their content may be the result of the increased activity of lysosomal enzymes in the studied tissues of fish under intoxication.

Table 4

Dynamics of the content of lipoproteins in the carp blood serum under the influence of heavy metal ions, %, M ± m, n = 5

Group	α- lipoproteins		β- lipoproteins	
	2 MPC	5 MPC	2 MPC	5 MPC
Control	72,22±0,74	77,63±1,3	27,77±0,74	22,37±1,3
Manganese	86,18±0,83	89,31±0,7	13,82±0,66	10,69±0,7
Zinc	86,18±0,06	89,48±1,5	11,82±0,07	10,52±1,15
Copper	85,68±1,02	90,73±0,7	14,32±1,02	9,27±0,7
Lead	68,47±1,27	83,08±1,88	31,53±1,27	16,92±1,88

The growth of α-lipoproteins may be explained by the fact that this fraction is quite easily formed from very low density lipoproteins and chylomicrons, decay of which is accompanied by the increase in the number of phospholipids, free cholesterol and apolipoproteins [7]. Biosynthesis of α-lipoproteins takes place in the liver and small intestine and the main function of this fraction is to maintain the transformation processes of lipids. Alongside with this the level of high-density lipoproteins (α-lipoprotein) in blood serum is an integral indicator of lipoproteins exchange and characterizes the efficiency of the transport systems functioning and transformation of lipids in the body as a whole.

Conclusions

So, the study of carp proteins and nucleic acids system under intoxication of its body by heavy metal ions, made it possible to learn the mechanisms of functional homeostasis and adaptive responses of fish organism, which can serve as a prerequisite for identifying of the integrated indicators that point to the key changes in the aquatic organisms under the extreme conditions.

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ВПЛИВ ІОНІВ ВАЖКИХ МЕТАЛІВ НА ВМІСТ БІЛКІВ ТА НУКЛЕЇНОВИХ КИСЛОТ В ОРГАНІЗМІ ПРІСНОВОДНИХ РИБ

В дослідженнях одержано сукупність даних, які підтверджують і розширюють уяву про важливу роль білкового та нуклеїнового обмінів у процесах детоксикації іонів важких металів, формуванні стійкості до них, а також дають можливість здійснити комплексну оцінку біохімічної реакції організму риб на хронічну інтоксикацію.

Ключові слова: прісноводні риби, білки, нуклеїнові кислоти, важкі метали

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ВЛИЯНИЕ ИОНОВ ТЯЖЕЛЫХ МЕТАЛЛОВ НА СОДЕРЖАНИЕ БЕЛКОВ И НУКЛЕИНОВЫХ КИСЛОТ В ОРГАНИЗМЕ ПРЕСНОВОДНЫХ РЫБ

В исследованиях получено совокупность данных, которые подтверждают и расширяют представление о важной роли белкового и нуклеинового обменов в процессах детоксикации ионов тяжелых металлов, формировании устойчивости к ним, а также дают возможность осуществить комплексную оценку биохимической реакции организма рыб на хроническую интоксикацию.

Ключевые слова: пресноводные рыбы, белки, нуклеиновые кислоты, тяжелые металлы

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