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MORPHOFUNCTIONAL CHARACTERISTICS AND ELEMENTAL COMPOSITION OF RAT LIVER UNDER DIFFERENT NUTRIENT PROVISION

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ABSTRACT: The article presents research of structural and functional changes in the liver in the conditions of modeling of micronutrient deficiencies in the organism of experimental animals with subsequent inclusion of complex minerals into their food ration. The object of the study was the liver of rats «Wistar». The elemental composition of biological substrates has been studied using atomic emission and mass spectrometry (ICP-AES and ICP-MS) in the test laboratory ANO «Center for Biotic Medicine». It has been established that the addition of selenium to the ration increases the concentration of this element in the liver by 15,0% ($p \leq 0,05$) against the background of lowering nickel by 50,0% ($p \leq 0,05$) in the experimental group I relative to control. When you turn on zinc into the ration, the concentration of it increases by 14,3% ($p \leq 0,05$) in the experimental group II relative to the control. Inclusion of iodine in the ration contributed to the increase of the iodine concentration by 4,52% in the experimental group III relative to the control group. Thus, the intake by the animal organism of toxic elements (lead and cadmium salts) leads to a change in the structural elements of the liver, resulting in depletion of hepatocyte in glycogen, or its complete disappearance, in offensive vacuolization and necrosis of hepatocytes and in changes in vessels diameter microvasculature, i.e. structural and functional changes in the liver are identified. Introduction into the ration of complex of essential trace elements (I, Se, Zn) involves an increase in functional activity of the liver.

Keywords: Liver Glycogen, Hepatocytes, Rats, Essential Elements

1. INTRODUCTION

In terms of socio-economic instability and increasing anthropogenic load on territories protection of public health becomes a priority [1]. Hypo - and giperelementozies are one of the major causes of bad health. Deviation in the entry into the body of the macro-and micronutrients, violation of their correlation in the food ration affects the vital activity of the organism. We know a number of diseases, caused by deficiency of essential trace elements, which lead to serious disorder in health status [2], [3].

Xenobiotics are biotransported in the liver involving cellular enzyme systems. For the normal activity of the latter the optimal amount of essential trace elements (zinc, selenium, iodine) [4], [5] must enter the organism. Micronutrients deficiencies in the food ration lead to disruption of the activity of cellular enzyme systems.

Zinc helps to stabilize cell membranes, it is a strong antioxidant protection factor [6], [7]. The activity of more than 40 intracellular enzymes [8] disrupts because of the lack of zinc in food ration.

Iodine deficiency statuses causes the lack of iodine in the environment and food [9].

Selenium is involved in the metabolism of iodine, so selenium deficiency leads to disruption of iodine metabolism [10], [11], because a deficiency of selenium, production of glutathione and glutathioneperoxidase is disrupted and they reduce the sensitivity of hepatocytes to free radical damage [12].

Glutathione is found in high concentrations in the liver (up to 5 millimole), 90 % of it is contained in the cytosol, the rest – in the mitochondria. In the latter glutathione is an antioxidant, it restores hydrogen peroxide and prevents lipid peroxidation by hydroxyl radical. At low concentration of glutathione in a cell, its sensibility to free radical damage increases [13], [14].

In centrolobular hepatocytes higher levels of cytochrome P-450 [15] are noted which is involved in the metabolism of various xenobiotics. Thus, according to the literature data, the process of biotransformation is attributable largely to the activities of centrolobular hepatocytes. However, as studies show, hepatocytes of this zone are more vulnerable to the toxic effects of some products, as glutathione and glutathione peroxidase localize predominantly in periportal cells [16].

Taking into account that the area is a biogeochemical province deficient in the above listed elements, the purpose of our study was to identify the structural and functional changes in the liver under the conditions of creating a model of micronutrient deficiencies in organism of experimental animals with subsequent inclusion in the ration of complex minerals [17]. As the liver is the main organ of biotransformation, in which a significant amount of cellular enzyme systems is actively involved, the normal activity of the latter requires entry of the optimal amount of essential trace elements into the organism. Micronutrient deficiencies in the ration lead to a disruption of the functions of the body's enzyme systems [18].

2. OBJECTIVES

Studies were done in the experimental-biological clinics (vivarium) of the Orenburg State University on the model rats «Wistar». The object of the study was the liver. 100 bimonthly rats – females – were selected for the experiment.

3. MATERIALS AND METHODS

To equalize the status of animals and to create elemental chemical elements deficient state, for three weeks they were nourished by specially prepared polished rice (boiling for 15 minutes in distilled water followed by washing and removing the broth). For prevention of avitaminosis (vitamin deficiency) states in animals, multivitamin complex was administered in the ration in accordance with the recommendations of the Institute of Nutrition (Russian Academy of Medical Sciences) (2001). The animals were watered by twice-distilled water.

The study was conducted as follows (Table 1).

Table 1 The scheme of the experiment

Group	The period of the experiment, weeks	
	Preparatory ration 2-4 week	Basic ration 4-8 week
Control	BR – basic ration (feed, diet);	BR
I	SR	SR + Se
II	SR	SR + Zn
III	SR	SR + I
IV	SR	SR + Se, Zn, I

Note: BR - basic ration (feed, diet); SR - semisynthetic ration (diet).

After a period of equalization the animals were divided into seven groups by the method of analogues pairs: one control and four experimental (n=20) and then transferred to the regimen of the main accounting period. The only difference was that the semisynthetic ration, developed by the Institute of Nutrition (2001), for the animal units of group I was supplemented per os by selenium – (selenopyran – 0,0001 g/head per day); of group II – zinc – (zinc sulfate – 0,042 mg/head per/ day; of the III group – iodine (potassium iodide in an amount of 0,332 g / day head per / day) and of the IV– trace complex I, Se, Zn.

During the experiment, slaughters of animals were practiced by decapitation under ether anesthesia-Rausch. Samples were taken to perform histological studies of the liver. The material was fixed in 10 % neutral formalin, followed by preparation of serial paraffin sections, with the thickness of 5 – 7 microns. Deparaffinized sections were imbued with hematoxylin-eosin. To identify glycogen and glycoproteins histochemical (periodic acid).

Schiff-reaction was used. The material was examined by the method light microscopy. The relative area of sinusoidal capillaries was determined with the help of ocular dot mesh-insert, and the eyepiece – with the help of the micrometer – first the diameter was measured and then the volume of hepatocyte nuclei and nucleoli calculated.

The elemental composition of biological substrates has been studied using atomic emission and mass spectrometry (ICP-AES and ICP-MS) in the test laboratory ANO «Center for Biotic Medicine», Moscow (accreditation certificate – SSES. RU.TSOA.311, registration number in the state register – Ross. RU 0001.513118 from May 29, 2003; Registration Certificate of ISO 9001: 2000, Number 4017 - 05/04/06).

The investigation was fulfilled by the ICP-AES and MS- ICP methods, biosubstrates ashing was carried out using microwave decomposition system MD-2000 (USA). Evaluation of the content of elements in the resulting ash was performed by a mass spectrometer Elan 9000 (Perkin Elmer, USA) and atomic emission spectrometer Optima 2000 V (Perkin Elmer, USA). In total, the content of 14 chemical elements was determined, including Cu, Fe, Li, Mn, Ni, As, Cr, Zn, I, V, Co, Se, Si, B.

The obtained data are expressed as mean values±standard deviation (mean±σ). The results were worked up using the program Statistica 10.

4. RESULTS AND DISCUSSION

Our studies have shown that in the control group of animals the distribution of glycogen,

detected by Schiff-reaction and verified by control pigmentation after treatment with amylase, was little dependent on the internal localization of hepatocytes. The reaction differs among centrolobular and peripheral hepatocytes. The arrangement of cells rich in glycogen is mosaic.

When adding selenium, clear zonal location of cells rich in glycogen was observed. Centrolobular hepatocytes are deprived of glycogen, and Schiff-reaction is significantly expressed in peripheral cells. At this small glycogen granules merge to form large in size clumps of reserve glycogen. Sinusoid capillaries widen significantly near the central veins.

In the number of cellular elements there are many small cells with round nuclei, which are found along clearly oriented hepatic beams, composing the wall of sinusoidal capillaries.

There exists an assumption that these Pit-cells are granular lymphocytes, which possess natural killer activity and simultaneously perform endocrine function.

Due to this, they can (depending on conditions) have opposite effect: when the liver is damaged they as killers destroy the damaged hepatocytes, and during recuperation period they stimulate proliferation of hepatic cells similar to endocrine cells.

The addition of zinc to a balanced ration, after creating in an organism micronutrient deficiency, leads to a significant increase in the intensity of Schiff-reaction in all hepatocytes both in centrolobular, and in peripheral.

Schiff-positive granules in the cytoplasm of the hepatocytes are fine, powder-like indicating that glycogen in these cells is more labile.

The structure of hepatic beams is not broken, but sometimes there is lymphoid infiltration. We can assume that these are Pit-cells.

When you add iodine to a balanced ration the structure of liver beams is not broken, so as it is in the liver of control group of animals, location of rich in glycogen hepatocytes is mosaic, but the intensity of the reaction is more expressed.

In cells, lacking glycogen, vacuolization of the cytoplasm is observed, especially among binucleated hepatocytes.

Cytoplasm of most hepatocytes is enriched with glycogen with the insertion in the ration of the complex of micronutrients. Schiff-reaction was expressed more than in control especially in peripheral hepatocytes.

Although in centrolobular hepatocytes reaction is less, expressed difference in color between centrolobular and peripheral cells is not observed.

Compared with the control the number of cells with medium and large in volume nuclei increases among hepatocytes, the number of nucleoli in them also enlarges, nuclear-nucleolar index reaches the minimum value (Table 2).

Table 2 Indicators of microstructures of centrolobular zone of liver

Group №	Relative area of sinusoi-dal capillary, %	Num-ber of binuc-leated cells	Volu-me of the nucleus, mu³	The volume of the nucleo-lus, mu ³	Nuclear-nucleolar index
Control	10,1 ± 1,30	2,66 ± 0,88	373,9 ± 0,83	1,34 ± 0,33	279,0
I	20,1± 1,26***	5,16 ± 0,40	438,4 ± 0,74	4,59 ± 0,22	95,5
II	14,6 ± 1,02	5,50 ± 0,42 *	447,7 ± 1,00	5,54 ± 0,21	80,8
III	13,7 ± 0,88	4,83 ± 0,87	679,2 ± 0,46*	6,67 ± 0,30	101,8
IV	17,3 ± 1,20	5,30 ± 0,49*	735,6 ± 0,74	9,28 ± 0,47	79,3

Note: * – $p \leq 0,05$, ** – $p \leq 0,01$, *** – $p \leq 0,001$

Morphometric analysis of liver structures denoted the mobility of these indicators depending on the mineral security.

In particular, the introduction of selenium in the ration was marked by the maximum increase of the observed relative area of sinusoidal capillaries by 10 % ($p \leq 0,001$) in the test group I relative the control group.

Additional incorporation of zinc in the ration led to an increase in the number of binucleated cells twice in the experimental group II relative to the control.

The maximum increase in the core was traced with the introduction of iodine in the ration. Thus, the core volume in test group III was 81,7 % ($p \leq 0,001$) higher relative the control. Volume of the nucleolus in the experimental group IV was the maximum value (9,28 mu^3), which was the result of lower nuclear-nucleolar index relative the control group.

This fact indicates an intensification of structural and metabolic processes in cells. Hepatic structure of beams is not broken.

Additional inclusion of the complex of essential elements in the ratio of rats, kept on deficient mineral ration, affects not only the

morphofunctional structure of the liver, but also the quantitative composition of chemical elements in the studied organ (Table 3).

Table 3 Concentration of chemical elements in the rat liver, ug/g DS

Element	Group				
	Control	I	II	III	IV
Essential and conditionally essential microelements					
As	0,207± 0,025	0,205± 0,020	0,203± 0,025	0,200± 0,027	0,201± 0,024
B	0,039± 0,006	0,037± 0,005	0,033± 0,002	0,038± 0,001	0,036± 0,004
Co	0,079± 0,012	0,070± 0,011	0,080± 0,016	0,060± 0,009	0,062± 0,010
Cr	0,103± 0,012	0,102± 0,016	0,103± 0,013	0,100± 0,018	0,102± 0,012
Cu	5,10± 0,51	5,12 ±0,44	5,07± 0,41	5,09± 0,54	5,11± 0,52
Fe	469,0 ± 47,0	463± 51,0	445,9± 48,9	451,3± 50,2	459,3± 48,9
I	0,199± 0,024	0,197± 0,022	0,188± 0,026	0,208± 0,028*	0,207± 0,020
Li	0,0019± 0,00037	0,020± 0,0003	0,0018± 0,00029	0,001± 0,0002	0,00019 ±0,0003 5
Mn	2,98± 0,30	3,00± 0,31	3,04± 0,28	2,88± 0,29	3,00± 0,31
Ni	0,02± 0,003	0,01± 0,002*	0,02± 0,007	0,03± 0,006	0,02± 0,004
Se	0,505± 0,061	0,581± 0,067*	0,506± 0,059	0,502± 0,063	0,597± 0,062
Si	33,77± 3,38	31,1± 3,22	33,2± 3,25	31,7± 2,93	33,4± 3, 19
V	0,0016± 0,00032	0,0014± 0,00025	0,0016± 0,00019	0,0013± 0,00021	0,0014± 0,00028
Zn	29,4± 2,94	29,7± 2,67	33,6± 2,23*	28,3± 3,03	4,3 ± 3,01*

Note: * – $p \leq 0,05$, when comparing the control and experimental groups

It has been established that the addition of selenium to the ration increases the concentration of this element in the liver by 15,0% ($p \leq 0,05$) against the background of lowering nickel by 50,0% ($p \leq 0,05$) in the experimental group I relative to control. When you turn on zinc into the ration, the concentration of it increases by 14,3% ($p \leq 0,05$) in the experimental group II relative to the control.

Inclusion of iodine in the ration contributed to the increase of the iodine concentration by 4,52% in the experimental group III relative to the control group.

Feeding of complex essential elements contributed to increasing concentrations of iodine, manganese, selenium and zinc by 4,02; 3,36; 18,2 and 16,7% ($p \leq 0,05$) against the background of lowering concentrations of arsenic and vanadium by 2,90 and 12,5%.

5. CONCLUSION

Thus, the intake by the animal organism of toxic elements (lead and cadmium salts) leads to a change in the structural elements of the liver, resulting in depletion of hepatocyte in glycogen, or its complete disappearance, in offensive vacuolization and necrosis of hepatocytes and in changes in vessels diameter microvasculature, i.e. structural and functional changes in the liver are identified. Introduction into the ration of complex of essential trace elements (I, Se, Zn) involves an increase in functional activity of the liver. Additional administration of a complex of essential elements in the ration increases the concentration of iodine, manganese, selenium and zinc in the liver against the background of lowering nickel, vanadium and arsenic.

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