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THE EFFECT OF CHRONIC IMMOBILIZATION STRESS ON THE CONTENT OF MICRO- AND MACROELEMENTS IN THE PANCREAS AND BLOOD SERUM OF RATS AND THEIR NEWBORN OFFSPRING.

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Abstract. *Have been considered the role of macro- and microelements - Ca, Mg, Zn, Fe, and Cu imbalance in the blood serum and pancreatic tissue homogenate of rats, whose pregnancy took place against the background of chronic immobilization stress and their newborn offspring, compared to the control, where it is established: heterogeneity changes in the level of macro- and microelement composition as a whole, can be traced regularity in the direction of deviations from the control values of indicators in the tissue of the pancreas, which consists in a decrease in the level of Ca and Mg and Zn and Cu in female rats and their offspring. A more stable reduction of Zn and Cu is determined in the blood serum of female rats and their newborn offspring. Changes in macro- and microelement composition in pancreatic tissue and blood serum are not identical, which manifests organ specificity regarding their balance in different organs, particularly in the pancreas. Such changes in the composition of macro- and microelements with a predominant deficiency of Ca, Mg, Zn, and Cu are an essential link in the pathogenesis of pancreatic damage, which occurs during stereotypic events inherent in the body's neurohumoral response to stress as a result of animal immobilization.*

Keywords: *macro- and microelements, immobilization stress, pancreas, blood serum*

Introduction.

Diseases of the digestive organs and the pancreas, among them, are the most important problem today. According to official statistics, the rate of prevalence of diseases associated with damage to the pancreas will significantly exceed the rate of growth of the general prevalence of diseases of the digestive organs [1]. The pancreas plays a leading role in the activity of the body as a whole, and therefore when it is damaged (intoxication, autoimmune diseases) due to endo- and exocrine dysfunction, systemic metabolic disorders occur [2, 3, 4, 5].

Chronic stress has been shown in many studies to increase levels of corticosterone, blood glucose, and decrease insulin levels, which is associated with pancreatic β-cell damage, metabolic shifts, and abnormalities in the prenatal development of offspring that are observed up to the postpubertal period of life [3,5]. Currently, the literature is actively considering the impact of vital macro- and microelements on the functioning of the body of adults [7] and children [8,9]. This group today includes potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), iodine (I) and many others. All of them enter the body with food and drinking water, and their biological activity can be preserved even in very low concentrations, while in elevated, relative to the norm, concentrations, even irreplaceable microelements are capable of exhibiting a toxic effect [7,10]. Macro- and microelements are activators of many enzymes, have

a multifaceted effect on all links of immunity, regulate the processes of proliferation and differentiation of cells of the immune system, are able to directly block oxidative processes, influence the state of proteins, membrane excitability, muscle contraction, energy accumulation [7, 9, 11, 12, 13]. Thus, the question of the dynamics of changes and the balance of macro- and microelements of elements in the tissue of the pancreas and in the blood of animals that were under the influence of stress during pregnancy [13,14], as well as the negative impact of their imbalance on the functional state of the pancreas is relevant as for both theoretical and practical medicine and needs further clarification.

The purpose of the study was to determine the specifics of the content of macro- and microelements - Ca, Mg, Zn, Fe, Cu in the blood serum and pancreatic tissue homogenate of rats pregnancy which takes place against the background of chronic immobilization stress and their newborn offspring.

The volume of the study included experiments on 30 nonlinear rats (WAG/G Sto population) aged 4.5-5 months and their offspring. The animals were divided into the groups: the rats that received chronic immobilization stress, their newborn offspring, and the rats of a control group (C-group). Rats of the control group were kept in a vivarium in comfortable living conditions during the experiment (30±1.1 days). The content of micro- and macroelements was studied by the spectrophotometric method (by color intensity, at a wavelength of 540-580 nm) on a biochemical analyzer Stat Fax (USA). The level of Ca, Mg, and Fe in blood serum and pancreas homogenate was determined with the help of reagent kits from the company "Filisit-Diagnostika" (Dnipro) according to the instructions. Color intensity was measured photometrically at 540 nm - proportional to Ca concentration; 540-560 nm is proportional to the concentration of Mg. The content of Zn and Cu in the PZ homogenate and blood serum was determined using Zn-DAC. Lq (companies DAC-Spectro-Med, Moldova) according to the attached instructions. The color intensity, measured at a wavelength of 550±10 nm, is directly proportional to the concentration of Zn. The color intensity, measured at a wavelength of 580±10 nm, is directly proportional to the concentration of Cu.

Taking into account the normally distributed population, the research results were processed using the method of variational statistics with the calculation of the arithmetic mean value (M), the mean error of the arithmetic mean value (m), and the probability value (P). Statistical processing of the obtained results was carried out using the analysis package of the Microsoft Excel-2003 program, the computer program Biostat.exe-2008, and STATISTICA-10. One-way analysis of variance and the U Mann-Whitney test was used to determine the reliability of differences between experimental groups. Differences were considered significant at $p<0.05$ [15, 16].

The results of the study and their discussion. In the pancreatic tissue of female rats, a decrease in the content of four out of five (80%) studied micro- and macroelements: Ca, Mg, Zn, and Cu (by 41.5%, 40.0%, 25.6%, and 26 .7% ($p<0.01$) respectively) (Table 1). Only the level of Fe was insignificantly increased (by 7.4%, $p>0.05$). The Ca/Mg ratio indicator does not differ from the average statistical value of animals of the control group (K gr.), which indicates the balanced effects of these macroelements. Less pronounced changes in the balance of micro- and macroelements

were found in blood serum than in the pancreas (Table 1) the normal content of not only Fe, but also Ca is noted (the level of the indicator is 38.7% higher than the indicator in the pancreas); the content of Mg and Zn is reduced by 32,5% and 18,8% ($p<0.01$) compared to the norm and is greater than in the pancreas by 7,5% and 6,8% ($p<0.01$) respectively, and the Cu level is 31,3% lower than the norm, which is not significantly different from that in the pancreas.

Table 1. The content of macro- and microelements (M±m) in the tissue of the pancreas and blood serum of rats whose pregnancy took place against the background of chronic immobilization stress.

Indicators		Rats	
		Pancreatic homogenate (n=7)	blood serum (n=7)
Ca (мг/100 gr.of tissue (pancreas) (мМ/L – blood serum)	Main gr.	3,44±0,36**	2,4±0,05 (p_{panc} <0,05)
	Contr. gr.	5,88±0,15	2,47±0,04 (p_{panc} <0,01)
Ca pancreas / blood serum (un.)	Main gr.	1,44±0,14**	
	Contr. gr.	2,35±0,05	
Mg (мg/100 gr.of tissue (pancreas)) (мМ/L – blood serum)	Main gr.	1,04±0,06**	0,76±0,03** (p_{panc} <0,01)
	Contr. gr.	1,95±0,04	1,12±0,02 (p_{panc} <0,01)
Mg pancreas / blood serum (un.)	Main gr.	1,38±0,08**	
	Contr. gr.	1,72±0,04	
Ca/Mg (un.)	Main gr.	3,33±0,32	3,23±0,09**
	Contr. gr.	3,02±0,07	2,21±0,02 (p_{panc} <0,01)
Fe (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr.	22,49±0,74	13,06±0,31 (p_{panc} <0,01)
	Contr. gr.	20,94±0,43	13,74±0,29 (p_{panc} <0,01)
Fe pancreas / blood serum (un.)	Main gr.	1,73±0,07*	
	Contr. gr.	0,52±0,04	
Zn (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr..	4,3±0,17**	14,76±0,2** (p_{panc} <0,01)
	Contr. gr.	5,78±0,13	18,17±0,29 (p_{panc} <0,01)
$Zn_{panc/s}$ (un.)	Main gr.	0,29±0,01	
	Contr. gr.	0,32±0,01	
Cu (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr.	5,5±0,23**	7,33±0,26** (p_{panc} <0,01)
	Contr. gr.	7,5±0,18	10,68±0,16 (p_{panc} <0,01)
$Cu_{panc/s}$ (un.)	Main gr.	0,76±0,02*	
	Contr. gr.	0,69±0,02	

*Notes: 1. **- $p<0.01$; * - $p<0.05$ – comparison with the control group (C. group).*
2. P_{panc} - comparison with indicators in the pancreas.

The values of Ca $_{panc/s}$, Mg $_{panc/s}$, Fe $_{panc/s}$, Zn $_{panc/s}$, and Cu $_{panc/s}$ show the nature of the difference in the content of micro- and macroelements in the pancreas and blood serum (decrease in comparison with the norm by 38,6% and 19,7% ($p<0.01$), respectively, Ca $_{panc\ /s}$ and Mg $_{panc\ /s}$, by 8.9% ($p>0.05$) – Zn $_{panc\ /s}$ and an increase of 14.0% and 10.6% ($p<0.05$) respectively Fe $_{panc\ /s}$ and Cu $_{panc\ /s}$) (see Table 1).

In the pancreatic tissue of newborn offspring from rats the pregnancy of which took place against the background of chronic immobilization stress, in comparison with animals of the control group (KN group), only the content of Cu was found to be without significant changes (-2.6%, $p>0.05$). The content of trace elements - Ca, Zn, and Fe was significantly reduced (by 44.8%, 35.9%, and 8.3% ($p<0.01$), respectively), and Mg - increased by 13.7% ($p<0.01$) (see Table 2).

Table 2. The content of macro- and microelements (M±m) in the tissue of the pancreas and blood serum of newborn offspring of rats, the pregnancy of which took place against the background of chronic immobilization stress.

Indicators		Newborn rat offspring
		Pancreatic homogenate (n=9)
Ca (мг/100 gr.of tissue (pancreas) (мМ/L – blood serum)	Main gr.	1,98±0,03** $p_{frat}<0,01$
	Contr. gr.	3,59±0,09 $p_{frat}<0,01$
Ca pancreas / blood serum (un.)	Main gr.	-
	Contr. gr.	-
Mg (мg/100 gr.of tissue (pancreas)) (мМ/L – blood serum)	Main gr.	1,64±0,02** $p_{frat}<0,01$
	Contr. gr.	1,44±0,03 $p_{frat}<0,01$
Mg pancreas / blood serum (un.)	Main gr.	-
	Contr. gr.	-
Ca/Mg (un.)	Main gr.	1,21±0,02** $p_{frat}<0,01$
	Contr. gr.	2,43±0,09 $p_{frat}<0,01$
Fe (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr.	20,16±0,13** $p_{frat}<0,05$
	Contr. gr.	21,98±1,18 $p_{frat}<0,05$
Fe pancreas / blood serum (un.)	Main gr.	-
	Contr. gr.	-
Zn (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr..	2,56±0,03** $p_{frat}<0,01$
	Contr. gr.	4,0±0,1 $p_{frat}<0,01$
Zn $_{panc/s}$ (un.)	Main gr.	-
	Contr. gr.	-
Cu (мg/100 gr.of tissue (pancreas)) (мкМ/L – blood serum)	Main gr.	2,98±0,03 $p_{frat}<0,01$
	Contr. gr.	3,06±0,05 $p_{frat}<0,01$
Cu $_{panc/s}$ (un.)	Main gr.	-
	Contr. gr.	-

*Notes: 1. **- $p<0.01$; * - $p<0.05$ – comparison with the control group (C. group).*

2. P_{frat} - comparison with female rat indicators

This led to a significant decrease in the value of the ratio of Ca and Mg more than twice ($p<0.01$) increased by 52.8% ($p<0.01$), and Cu $_{panc\ /s}$ decreased by 8.1% ($p<0$, 01). In general, the comparison of indicators of the content of micro- and macroelements in the tissue of the pancreas allows us to state that in all newborn rats that have experienced prenatal stress, a stably low level of Ca, Zn, and Cu is observed; the level of Mg and Fe, although it has a predominant tendency to decrease, is not so stable, as its deviation towards a moderate increase is noted in certain age groups.

The effect of chronic immobilization stress on rats during pregnancy causes in females and their newborn offspring a violation of the balance of micro- and macroelements both in the tissue of the pancreas and in the blood serum, which mainly consists in reducing their content in the specified biological environments. The content of individual micro- and macroelements in the tissue of the pancreas and blood serum in female rats and newborn offspring has reliable differences, but in general, the amount of micro- and macroelements, the level of which exceeds the limits of normal fluctuations, has unidirectional deviations in the tissue of the pancreas and in the serum blood [14,17,18,19].

Characteristic feature of the balance of micro- and macroelements in the pancreas due to chronic stress in female rats and prenatal stress in newborn rats is a low level of Ca, Zn, and Cu content, which occurs in almost all animals and is not prognostically favorable. Changes in the level of micro- and macroelements in the tissue of the pancreas and blood serum are not absolutely identical, which indicates the presence of organ specificity regarding the balance of macro- and microelements, which means the relative informativeness of blood serum indicators for determining the state of metabolic processes in individual organs, in particular in the pancreas gland

Probably, the negative effect of chronic stress on the composition of macro- and microelements is realized through an increase in the blood serum levels of precursors and metabolites of catecholamines, which contribute to a decrease in their content due to the stimulation of the secretion of such hormones as parathormone and cortisol, the absorption of Ca in the intestines is inhibited and its content in the body decreases. increases excretion of Mg-magnesiumuria, which regulates the transmembrane transfer of calcium and sodium ions, independently participates in many metabolic reactions related to the production, accumulation, transfer, and utilization of energy, free radicals, and their oxidation products, transmembrane transfer of calcium and sodium ions, affects the function of the nervous system and the coordination of all processes, including the formation of adequate adaptive and adaptive reactions and stress resistance [12,15,20,21]

There are data that during stress in the organism, there are phase changes in the content of Zn in the homogenate of the PZ tissue: the accumulation of Zn levels in the PZ cells with an increase in corticosterone and corticotropin in the blood passes into a phase of a decrease in Zn concentration with a decrease in the levels of these hormones [22]. In a healthy pancreas, Zn is concentrated in granules and is contained in β-cells, and is necessary for the normal production and storage of insulin, Zn exhibits an insulin-mimetic and antidiabetic effect [23], is a coenzyme of many enzymes (carbonic anhydrase and lactate dehydrogenase), as well as superoxide dismutase, which protects the body from the products of peroxidation of lipids, the damaging effect of which is

also realized during stress. It shows similar effects and Cu, which also affects carbohydrate metabolism by accelerating glucose oxidation processes, inhibits the breakdown of glycogen in the liver. Cu is part of many enzymes: cytochrome oxidase, tyrosinase, etc. It is a cofactor of the enzyme superoxide dismutase, which plays an important role in the neutralization of free radicals.

Taking into account the importance of micro- and macroelements in the activation of all functions of mitochondria, DNA and RNA replication, cell division, the inflammatory process, immune cells, regulation of carbohydrates (secretion of glucagon and insulin), lipid and protein metabolism, the release of hormones and neurotransmitters, secretion of glands, regulation of protective function AOS, participating in both the exocrine and endocrine functions of the pancreas (including the processes of activation of digestive enzymes) [5, 61], it can be noted that stress-induced disturbances in their balance can be one of the links in the pathogenesis of pancreatic damage during pregnancy and prenatal development of rat offspring, which can contribute to the emergence of various pathologies of the pancreas in the future.

The given data have substantiated the feasibility of further research aimed at clarifying the possibilities of restoring the functional state of pancreatic cells with the help of magnesium and zinc preparations, which should probably be included in the pathogenetic therapy of its damage because, unlike various organic compounds, macro- and microelements in the body are not synthesized.

Summary and conclusions

Were received the experimental data obtained are indicative of the fact that the immobilization stress of rats during pregnancy is a significant risk factor for the development of organic pathology of the pancreas in their offspring.

1. The pregnancy of rats, which takes place against the background of chronic immobilization stress, is characterized by a violation of the balance of the macro- and microelement composition of the pancreatic tissue and blood serum with a predominant decrease in their content in the specified biological environments.

2. Changes in the composition of macro- and microelements in pancreatic tissue and blood serum are not identical, which manifests organ specificity regarding their balance. Despite the heterogeneity of the above-mentioned changes in general, there is a regularity in the direction of deviations of indicators from control values in the tissue of the pancreas, which consists in a decrease in the level of Ca and Mg and Zn and Cu, and a more stable decrease in Zn and Cu in blood serum in both female rats and their offspring.

3. Violation of the balance of macro- and microelement composition with a predominant deficiency of Ca, Mg, Zn, and Cu, which occur during stereotypic events inherent in the implementation of the body's neurohumoral response to stress, the cause of which is animal immobilization, is an important link in the pathogenesis of pancreatic damage.

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