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MORPHOLOGICAL AND FUNCTIONAL STATE OF EXTRAORGANIC BLOODSTREAM OF PINEAL GLAND OF RATS UNDER THE CHRONIC STRESS AND ILLUMINATION

Abstract. *The results of the study of the morphological and functional state of extraorganic bloodstream of pineal gland of rats under the chronic stress and illumination are presented in the work. It has been found out that there is complete absence of blood corpuscles in the lumens of venous and arterial vessels. Thus, the blood circulation abnormality and exsanguination of the vessels are accompanied by the hypoxia, edema of the surrounding tissues and atrophy of the parenchymatous elements of pineal gland.*
Keywords: *pineal gland, chronic stress, bloodstream.*

Introduction. The problem of study of the structure, functioning and pathology of pineal gland is still relevant, as the pineal gland is one of the most significant parts of the neuroendocrine system, and takes part in a wide range of the regulatory effects on numerous vital activities [1-4]. As the hypothalamus and pituitary gland, pineal gland is the central regulatory element, which controls activity of the endocrine glands. The deep connection of the pineal gland with the reactive and adaptive processes, which are the result of stressors, is explained by the extremely wide range of regulatory effects, in which the pineal gland is involved, and by its hypersensibility to changes in external and internal environment.

It is well known, that the stress, which can be characterized as the non-specific component of the organism response to an irritant, is implemented involving the neuroendocrine system. The neurosecretory complex of hypothalamus and pituitary gland is the higher neuroendocrine transmitters of the organism, it coordinates endocrine control of metabolism with the work of the autonomic nervous system and integrated emotional and behavioral reactions. And while the role of the complex is studied in detail, the significance of the pineal gland in these processes is not studied enough. Many aspects of this problem, especially those connected with the bloodstream reaction on the different stresses, are still not solved [5-8].

Objective: to find out the features of the morphological and functional state of extraorganic bloodstream of pineal gland of rats under the chronic stress and 30-days illumination.

Materials and methods. Study was performed with 24 pubertal male Wistar rats with the body weight of 220-240 g. The animals were kept in vivarium under the day and night illumination. The intensity of illumination was 1000-1500 lux and was carried out with two lamps, which were located on both sides of the cage within 30 days. Starting from the 21st day of the experiment the rats were placed in a tank

with some water for 1 hour for the forced swimming. The disposable trainings were conducted during 10 days.

At the end of the experiment the animals were subjected to euthanasia in strict accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "General Ethical Principles of Animals Experiments", ratified on the First National Congress of bioethics (Kyiv, 2001).

After removing the pineal gland with the adjoining blood vessels, the obtained complex was immersed into the fixing solution of 10% neutral formalin. Using standard methods the samples were placed in paraffin blocks. The slices with thickness of 4 mm were produced from the blocks and stained with hematoxylin and eosin. Obtained samples were studied with the microscope «PrimoStarZeiss» at different magnifications with the following photography of micropreparations with DSLR «Canon».

Results and discussion. As a result of study of the tissue specimens it was found out, that when the diameter of the extraorganic venous vessels becomes bigger, the distribution of blood corpuscles in them changes considerably. It was revealed, that in rather large venous vessels there is a predominance of plasma component of blood, and a small amount of blood corpuscles mainly takes the central position. Red cell mass may contain the separately located spherocytes. But mostly there are the clods of red blood cells in the lumen. Some venous walls are thickened. The dissection of the vascular wall is observed frequently. In this case there is a formation of two layers of cell structures, which in some cases is accompanied by the spasm of the vascular wall.

In some places there is a destruction of the integrity of the vascular wall, which accompanied by the formation of extravasates, i.e. the appearance of red blood cells outside the bloodstream (Fig. 1).

There is also another more common type of distribution of blood, when the extraorganic

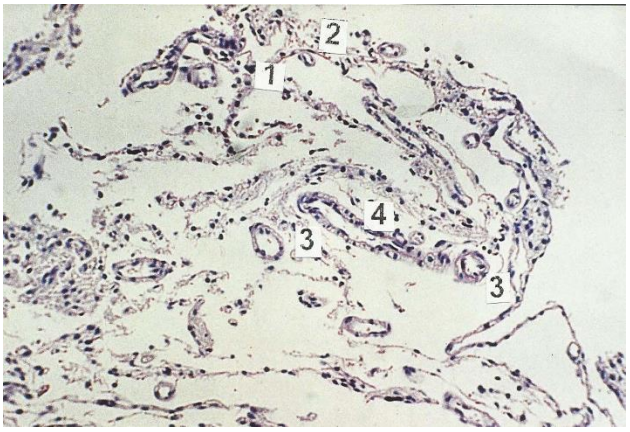

Fig. 1. Photomicrography of the bloodless extraorganic blood vessels of the pineal gland of the rat under the chronic stress and 30-days illumination. Magnification: eyelens×10, objective ×10. Coloration: hematoxylin and eosin. 1 - lumen of the small venule; 2 - isolated red blood cells outside the vessel; 3 - anemied small arterioles; 4 - perivascular space.

venous vessels look like completely bloodless (Fig. 1). These changes were found out in the samples of the large venous vessels. The feature is that their walls are mostly thinned, and in some places there are small defects between the neighboring endotheliocytes. Thereby, the edema of the surrounding tissue of various degrees is developed.

It was established, that in the arterial system of the extraorganic blood vessels of the pineal gland there are also reactive changes in response to the stressors. The feature of the arterial vessels of all dimensions is the complete absence of the blood corpuscles in their lumens. It was revealed, that transverse sections of the small arterioles are roundish-shaped, and their lumens are narrowed. This state is the result of the thickening of the vessels wall. The hypertrophy of endothelial cells, which is shown as the increasing volume of nuclei, is the main reason of the thickening of the vessel wall. The cells become ovoid or round-shaped and appear in the lumen of the vessel, causing its narrowing.

In the arterial vessels of the larger dimensions the feature is the spasm of their vessel wall. The result of this spasm is a modified form of the lumen. The lumen became the ellipse-shaped with sinuous surface instead of the round-shaped, which is typical for these

vessels. Restructuring of the coat of the arteriole wall leads to the contraction of the myocytes. Thus, endothelial cells lose their typical shape and order of location. The nuclei of these cells are usually hypertrophied. Their chaotic location relatively to the surface of the endothelial lining is the feature. The elastic membrane of the vessel wall acquires well marked but disordered sinuosity. The muscular layer of the vessel wall is thickened and structureless in some places with the signs of homogenization (Fig. 2).

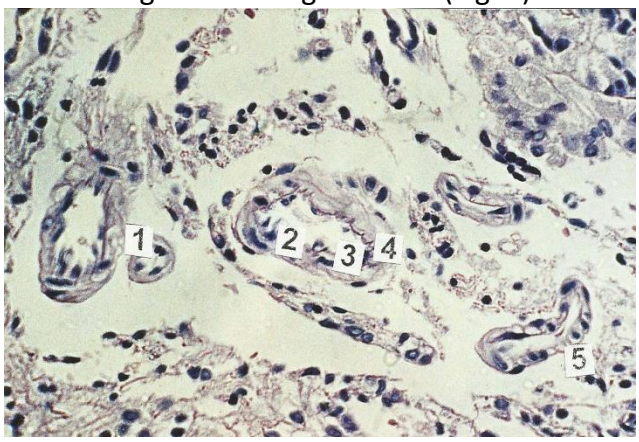

Fig. 2. Photomicrography of the bloodless extraorganic blood vessels of the pineal gland of the rat under the chronic stress and 30-days illumination. Magnification: eyelens×10, objective ×10. Coloration: hematoxylin and eosin. 1 – hypertrophy and destruction of the arteriole wall; 2 – spasm of the arteriole wall; 3 – sinuosity of the elastic membrane of the arteriole wall; 4 – homogenization and thickening of the muscular coat of the arteriole; 5 - perivascular space.

It was revealed, that the blood vessels, which are adjacent to the capsule of the pineal gland, look like the hollow tubes of different caliber without the blood cells. A small amount of red blood cells, which occupy a peripheral position in the lumen of the blood vessel, can be found in some samples.

Thus, the blood circulation abnormality and exsanguination of the vessels are accompanied by the hypoxia, edema of the surrounding tissues and atrophy of the parenchymatous elements of pineal gland.

Conclusions: Morphological state of extraorganic bloodstream of pineal gland indicates that the chronic stress accompanied with the abnormal photoperiod, caused by the day and night illumination of animals during 30 days, leads to the inhibition of its functional activity due to the emerging hypoxia.

Prospects of further research. According to the results of the experiments it is planned to perform the further study of the intraorganic bloodstream of pineal gland under the chronic stress and 30-days illumination.

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INFLUENCE OF SENSITIZATION WITH BRAIN ANTIGEN SENSITIZATION ON THE CONDITION OF CEREBRAL CORTEX SENSOMOTOR NEUROGLIAL ELEMENTS OF THEIR IMMUNOHISTOCHEMICAL DETECTION

Abstract. *Conducted studies in rats revealed, that sensitization with the brain antigen is the factor, which can lead to the brain damage and induce the neuroinflammation, gliosis and neurodegenerative processes on its own. Evaluation of expression of GFAP, S100 and Iba-1 in the cerebral cortex makes it possible not only to determine the change in number of glial cells, but also, to some extent, to estimate their functional activity.*
Key words*: brain, sensitization, GFAP, S100, Iba-1.*

Introduction. Normally, cerebral tissue is isolated from immune system with blood-brain barrier [15]. But, due to the data of several authors, from 5 to 92% of people have antibrain antibodies in their blood [5,9]. Their presence can lead to the brain damage, initiate or intensify neurological processes [3, 4, 5, 9, 13], which can be accompanied by toxicity and glial neuroinflammation [12]. Under these conditions both degenerative changes of neurons and multidirectional reactive changes of glia may appear [8, 13, 14].

Objective: to find out influence of sensitization with brain antigen on the state of glial cells in sensomotor cerebral cortex at their immunohistochemical detection (S-100, GFAP, iba-1).

Materials and methods. Study was performed with 35 white pubertal male Wistar rats with the body weight of 260-290 g. The animals were held in vivarium, fed a standard diet and housed 5 animals in a cage with free access to food and water and under constant light conditions due to the «Principles of Laboratory Animal Care». Study was performed in strict accordance with «Guide for the Care and Use of Laboratory Animals» (NIH publ. No. 93 23, revised 1985). The male rats were used in the study as the estrogen level influences the course of the brain damage considerably [11]. The experimental animals were sensitized with 20% water-salt extract of the homologous brain tissue, prepared by the standard procedure [2], and containing of 0,33-0,5 mg/ml protein by Lowri. The extract was subcutaneous intro-duced into the rats as follows: 1st day - in amount of 0.5 ml; 2nd day - 1 ml; 3d day - 1.5 ml [1]. The control group is 10 animals, which were not under any action.

The brain was studied in 12, 15, 22, 42 and 102 days after the start of the experiment with the introduction of the sodium thiopental (200 mg/kg) in animals. During up to 1 minute the skull was opened and the brain was removed and sectioned frontally into three pieces, then the middle piece was placed in the 10% buffered formalin (pH 7.4, 40oC) for 24 hours. The samples were placed into the paraffin, and the slices with thickness of 4 µm were produced with the following staining with azure II-eosin and phosphotungstic hematoxylin.

Immunohistochemical (IHC) reactions were performed according to the manufactures` protocols with the antibodies against: protein S-100 (Polyclonal Rabbit Anti-S100 (Dako, Denmark)), Iba-1 (rabbit polyclonal, 1:750, Molecular Probes Inc., USA), GFAP (Dako, Denmark). In order to visualize the products of the IHC-reaction the system EnVisionTM FLEX, (Dako, Denmark) was used. The slices were stained with hematoxylin Gill. The samples of the

rats brain with defined reactivity were used as a positive control. As a negative control were used the samples, obtained without using the primary antibodies.

Tissue specimens were studied and photographed with the microscope Ni-kon Eclipse 80 and camera DS-5SMc/L2 (Nikon, Japan) under standardized con-ditions. GFAP+ and Iba-1+ cells were counted at 10 areas 430x320 µm. For densitometry evaluation of the expression of S100 in digital images (x400, 1280x960 pixels RGB) the system of analysis ImageJ 1.46 was used. Obtained digital data were processed by standard statistical methods.

Results and discussion. Conducted studies showed, that in 12, 15 and 22 days after the sensitization the mild perivascular edema takes place in the cerebral cortex. Neuron contours are usually irregular. Often the phenomena, which can be described as hypercondensation of chromatophilous substance is observed. Furthermore, there are some cells of chromatolysis in the cytoplasm of neurons. Degenerative hyperchromic neurons occur rather often, necrotic neurons occur less frequently, and sometimes the shrunken neurons with clarified cytoplasm occur. In some cases there are cells of small spongy degeneration. Later on, these effects become less expressive, but the increase in the number of the glial cells appears. In 102 days after immunization small clusters of glial cells sometimes appears in the cerebral cortex.

Evaluation of the expression of GFAP in the sensomotor cerebral cortex of rats of the control group revealed the cytoplasmically marked cells with processes. They have little nuclei, surrounded with a thin layer of cytoplasm, and thin processes, which moderately branch and gradually thin. Such cells structure, revealed by the expression of GFAP, most closely matches the macroglia of CNS [6, 7]. At the same time numerous small granular and filamentous GFAP + formations are also detected in neuropile. These formations can be considered as the fragments of astrocytes, which are in the slice.

For the sensitized animals there is some increase in the number of the GFAP+ cells in 12-15 days after the start of the experiment. At the same time their bodies and processes often seem edematous. Usually there is a higher level of expression of GFAP in these samples compared to the control ones. In addition, the thinnest terminal branches of processes were visualized something worse than in control samples. Under these conditions there is smaller number of the GFAP+ cells in the neuropile. Later on, with a general increase in the number of glial cells in the sensomotor cerebral cortex, there is an increase in the number of the GFAP+ cells, that is approved by evaluation of their specific number (Figure). Usually the cells are something hypertrophied.

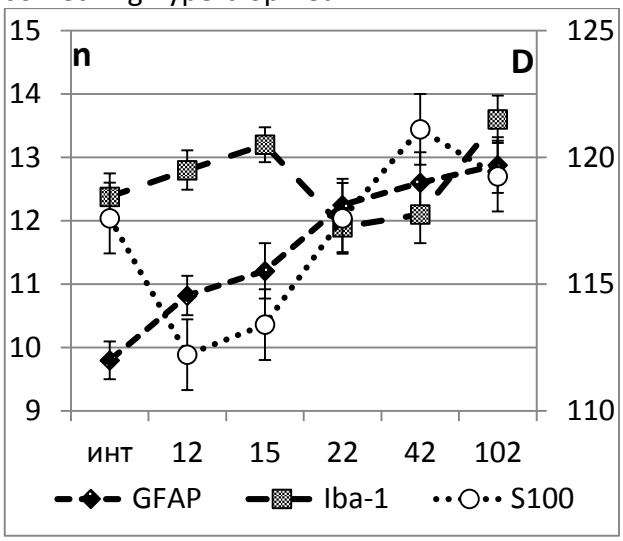

Figure. Change of number of GFAP+ (astrocyte), Iba-1+ (microglia) of cells (n – average number at the area 430x320 µm) and intensity of the expression of S100 (D, arb. units) in the sensomotor cerebral cortex of rats for different time after sensitization with the brain antigen.

For the rats of the control group the expression of the S100 appears as a small granulation, which is almost equally distributed in the neuropile. Neurons of the cerebral cortex don`t express the protein and have sharp contours, some-times they have clearly visible axon hills and apical axons. Glial cells are visual-ized just sometimes, and their bodies and processes can be seen, while their color intensity is almost as the color intensity of the surrounding neuropile. This was the reason for densitometric evaluation of the optical density of the expression of S100, instead of counting of cells.

Generally, there is a decrease in the expression of S100 in the cerebral cor-tex under

the sensitization in 12-15 days comparatively to the control samples (Figure). Marked glial cells with a small amount of the slightly branched processes are clearly seen. On the 22nd day of the experiment level of the expression of S100 is equal to the initial level (Figure). But in this case in 102 days after the start of the experiment there are marked glial cells, that indicates the higher ac-cumulation of S100 in their bodies and large processes than in terminal branches of the processes.

Revealed Iba-1+ cells of rats of the control group have rather small nuclei and processes and typical structure of microglial cells. They had small amount of the slightly branched processes with thin branches. Sometimes these cells are ad-jacent to blood microvessels or neurons along the perimeter.

Under the sensitization number of the Iba-1+ cells in 12 and 15 days after start of the experiment is something higher than in control samples (Fig. 1). But intensity of their marking is something lower and appears less intensively than for their processes. On 22nd and 43d days of the experiment number of the microglial cells decreases and does not differ from control values significantly. At the same time there is some increase in the intensity of the expression of the Iba-1. In 102 days after the start of the sensitization in the sensomotor cerebral cortex there is much higher number of the microglial cells with the much higher level of the expression of Iba-1 comparatively to the control samples. Also the cells usually have bigger dimensions and higher number of the processes than in the con-trol samples.

Thus, the studies that were carried out, can be considered as analogues of the sensitization with brain antigens under some pathological states [4, 5, 9, 13]. It was shown, that mild neurodegenerative processes take place and expressive reaction of glia develops even at the absence of the direct vascular brain damage. The reaction has the character of neuroinflammation with the increase in amount of Iba-1+-microglia and its hypertrophy. At the same time there is a gliosis with the gradual and dynamically stable increase in the number of astrocytes and their hyperplasia, which is clearly revealed by the expression of GFAP. Mostly this process coincides with the increase in the antibrain antibodies number in the blood of the experimental rats [3]. The decrease in the expression of S100, which allows estimating of the degree of differentiation and functional activity of the glial cells [10], and also, to some extent, of GFAP in the cells of microglia of the sensomotor cerebral cortex at the early stage of the development of sensitization (during the first month), indicates the decrease in their activity. The increase in the number of the cells of microglia as a response to the sensitization is nonlinear, and mostly coincides with the dynamics of changes in circulating immune com-plexes in the blood of experimental animals [3].

Conclusion. Sensitization with the brain antigen is the factor, which can lead to the brain damage and induce the neuroinflammation, gliosis and neurodegenerative processes on its own. Evaluation of expression of GFAP, S100 and Iba-1 in the cerebral cortex makes it possible not only to determine the change in the number of glial cells, but also, to some extent, to estimate their functional activity.

Prospects of further research. Further studies in this area may be aimed at the understanding of the ideas on the role of the immune system in the development of morphological and functional changes of the gangliar layer of the sensomotor cerebral cortex.

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THE EFFECT OF VEGETATIVE DENERVATION ON PHOTOPERIODIC CHANGES IN THE MORPHOLOGICAL STRUCTURE OF RATS' SEX GLANDS

Abstract. *We have studied the features of photoperiodic changes in the weight and structure of the testes and their epididymides and accessory sex glands (seminal vesicles, prostate) in nonpubertal male white rats after surgical bilateral lumbar sympathectomy and bilateral pelvic neurectomy. Photoperiodic changes in animals had been modelled for 7 days using continuous illumination, constant darkness and natural light in the spring and summer. It was established that the vegetative denervation disturbs adequacy of photoperiodic changes in the gonads in the puberty period.*
Key words: *testes, epididymides, seminal and prostate glands, photoperiodism, bilateral pelvic neurectomy, bilateral lumbar sympathectomy, male rats.*

Introduction. It is well known that both in male sex glands and in female ones there are seasonal changes due to the illumination rate (photoperiod): with increasing photoperiod in the spring and summer time, gametogenesis and hormonogenic function of the gonads get activated, which is accompanied by an increased morphofunctional activity of the accessory sex glands. As the photoperiod becomes shorter during the autumn-winter season there are reverse changes associated with changes in the activity of the pineal gland and hormone melatonin production [5, 8, 9].

At the same time, it has been undoubtedly proved that there is a multiple efferent innervation of the sex glands [6] and an influence of the vegetative innervation on the changes in the gonads [2]. However, there is no information in the literature on possible participation of the vegetative innervations in the photoperiodic changes in the gonads, despite the fact that the techniques of surgical peripheral denervation of the gonads are used in clinical practice, particularly for patients with chronic orchialgia [4].

Objective: The purpose of our study was to establish the role of the sympathetic and parasympathetic innervation in the photoperiodic changes in the gonads of laboratory rats.

Materials and methods. The study was conducted in the spring and summer on 155 nonpubertal male white rats aged 4–5 weeks weighing 40–60 grams. We studied the features of photoperiodic changes in the animals' weight and structure of the testes and their epididymides as well as accessory sex glands (seminal vesicles, prostate gland), morphometry parameters of these glands after surgical bilateral lumbar sympathectomy and bilateral pelvic neurectomy which were performed separately. Pentobarbital (40 mg/kg) injected intraperitoneally, and then laparotomized. For the bilateral pelvic neurectomy group (n=24), the pelvic nerves were retrieved and bilaterally dissected as described in detail by Carlson and De Feo [1]. The nerves were cut with approximately 1–2 mm removed from the nerve to avoid regeneration [3]. For the lumbar sympathetic denervation group (n=47), the chain was dissected from L2 to the aortic bifurcation [7]. Sham surgery (n=23) involved exposing, but not sectioning, the nerves.

Photoperiodic changes in animals' bodies were simulated after surgical manipulation for 7 days using continuous illumination, constant darkness and natural lighting during the spring and summer season [10]. The sex glands were

embedded in paraffin wax and blocks were prepared. The sections was stained with hematoxylin and eosin and analyzed under a binocular light microscope by using screw ocular-callipers MOB-1-16x (LOMO, Russia). We measured diameters of convoluted seminiferous tubules and that of the epididymis, height of the epithelium in the epididymis tubule, of seminal vesicles and prostate on the sections of histologic specimens. Statistical analysis of the results was performed using the software Statistica 10 (StatSoft, USA). Whether the continuous variables were normally distributed or not was determined by using the Kolmogorov-Smirnov test. Descriptive statistics were represented as mean ± SD. The differences among groups in terms of mean values were evaluated by using the parametric Student's t-test, and those in terms of median values were evaluated by using the non-parametric Mann-Whitney U-test. A p value of ≤ 0.05 was considered to be statistically significant.

Results and discussion. It was established that in intact and false-operated animals constant light causes acceleration whereas constant darkness slows down the development of the gonads. Bilateral sympathectomy (tabl. 1, 3) under natural lighting leads to severe atrophic changes in the gonads. However sympathectomy under conditions of constant darkness and constant lighting only slightly suppresses the development of the sex glands at puberty.

After the pelvic neurectomy (tabl. 2, 4) under

Table 1

The average weight of reproductive organs in immature male rats under different lighting conditions following bilateral lumbar sympathectomy in the spring and summer season (mg per 100 g of body weight, M±m)

Nature of exposure	Number of animals	Testes	Epididymides	Seminal vesicles and PG
Intact, daylight	22	1146,0±71,21	114,8±6,60	101,3±5,04
Pseudo-operated, daylight	7	1185,1±132,29	120,7±20,43	100,8±9,12
Sympathectomy, daylight	15	736,7±65,17 *, ^	82,4±7,58 *, ^	72,5±3,74 *, ^
Intact, permanent light	22	1265,9±70,48	125,1±5,45	120,0±11,39
Pseudo-operated, permanent light	9	1199,8±151,63	127,0±19,07	93,4±11,44
Sympathectomy, permanent light	15	1010,9±82,50 #	109,2±10,68 #	87,7±6,27 **, #
Intact, permanent darkness	17	873,1±94,50 *, **	87,7±9,56 *, **	85,9±8,84 **
Pseudo-operated, permanent darkness	7	906,7±118,06	87,8±11,17	83,4±7,47
Sympathectomy, permanent darkness	17	793,3±58,26 ##	90,8±6,79	71,1±6,29 ##

*Notice. * – p≤0,05 relatively to the values in the intact animals under standard light; ** – p≤0,05 relatively to the values in the intact animals under permanent light; # – p≤0,05 relatively to the values in the animals after sympathectomy under standard light; ## – p≤0,05 relatively to the values in the animals after sympathectomy under permanent light; ^ – p≤0,05 relatively to the values in the pseudo-operated animals under standard light; PG – prostate gland.*

Table 2

The average weight of reproductive organs in immature male rats under different lighting conditions following bilateral pelvic neurectomy in the spring and summer season (mg per 100 g body weight, M±m)

Nature of exposure	Number of animals	Testes	Epididymides	Seminal vesicles and PG
Intact, daylight	22	1146,0±71,21	114,8±6,60	101,3±5,04
Pseudo-operated, daylight	7	1185,1±132,29	120,7±20,43	100,8±9,12
Pelvic neurectomy, daylight	8	1033,6±83,54	97,6±6,83	95,7±5,04
Intact, permanent light	22	1265,9±70,48	125,1±5,45	120,0±11,39
Pseudo-operated, permanent light	9	1199,8±151,63	127,0±19,07	93,4±11,44
Pelvic neurectomy, permanent light	8	1195,0±40,75 #	102,8±5,49 **	81,0±3,53 **, #
Intact, permanent darkness	17	873,1±94,50 *, **	87,7±9,56 *, **	85,9±8,84 **
Pseudo-operated, permanent darkness	7	906,7±118,06	87,8±11,17	83,4±7,47
Pelvic neurectomy, permanent darkness	8	1018,8±84,22 ##	100,1±7,27	84,9±7,88

*Notice. * – p≤0,05 relatively to the values in the intact animals under standard light; ** – p≤0,05 relatively to the values in the intact animals under permanent light; # – p≤0,05 relatively to the the animals after pelvic neurectomy under standard light; ## – p≤0,05 relatively to the the animals after pelvic neurectomy under permanent light; PG – prostate gland.*

natural lighting conditions there is only a slight inhibition of the development of gonads. Under conditions of constant light pelvic neurectomy suppresses the development of gonads less than under usual lighting, not significantly affecting the signs of photoperidism in morphofunctional development of gonads. However, after the pelvic neurectomy under conditions of constant darkness instead of the expected further suppression of gonads we did not find a significantly decrease in the identified values.

Thus, bilateral sympathectomy causes disorders in the development of the sex glands in puberty more than bilateral pelvic neurectomy, and the parasympathetic nerves provide photoperiodic changes in the gonads during puberty better than the sympathetic ones. Thus, the maintaining of vegetative innervation of the gonads contribute to the adequacy of photoperiodic changes in the gonads.

Conclusions. 1. Bilateral sympathectomy causes disorders in the development of the sex glands in puberty more than bilateral pelvic neurectomy.

2. The parasympathetic nerves provide photoperiodic changes in the gonads during puberty better than the sympathetic ones.

3. The maintaining of vegetative innervation of the gonads contribute to the adequacy of photoperiodic changes in the gonads.

Prospects of further research. To determine the role of the central divisions of the autonomic nervous system in the implementation of photoperiodic changes in the male gonads.

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Table 3

Morphometric values of the reproductive organs in male immature rats under different modes of light after the bilateral lumbar sympathectomy in the spring and summer season (microns, M±m)

Nature of exposure	Number of animals	Diameter:		Height of epithelial cells:		
		tubuli seminiferi contorti	epididymis canal	epididymis canal	seminal vesicles	prostate gland
Intact, daylight	22	120,4±6,82	105,4±6,11	13,8±0,55	8,4±0,57	9,4±0,70
Pseudo-operated, daylight	7	123,2±4,15	107,3±5,02	12,2±0,68	8,4±0,45	9,6±0,62
Sympathectomy, daylight	15	82,3±4,12 *, ^	93,5±3,83 ^	11,2±0,49 *	7,1±0,31 *, ^	7,8±0,42 *, ^
Intact, permanent light	22	141,4±9,92	110,6±8,28	13,6±0,53	11,8±0,72 *	11,4±0,84
Pseudo-operated, permanent light	9	136,8±5,67	112,1±7,35	12,4±0,69	11,2±0,50 ^	11,0±0,77
Sympathectomy, permanent light	15	118,7±4,51 **, ^^, #	106,8±4,76 #	12,3±0,62	8,3±0,37 **, ^^, #	9,2±0,40 **, ^^, #
Intact, permanent darkness	17	104,3±6,22 *, **	96,8±6,21	10,5±0,71 *, **	8,2±0,42 **	8,9±0,72 **
Pseudo-operated, permanent darkness	7	115,7±4,89 ^^	98,9±9,43	10,8±0,58	8,6±0,35 ^^	9,0±0,50 ^^
Sympathectomy, permanent darkness	17	95,8±8,97 ##	98,6±4,02	11,9±0,51	7,6±0,35	9,1±0,49 #

Notice. ^^ – p≤0,05 relatively to the values in pseudo-operated animals under permanent light;; other symbols are the same as in table 1.

Table 4

Morphometric values of the reproductive organs in male immature rats under different modes of light after the bilateral pelvic neurectomy in the spring and summer season (microns, M±m)

Nature of exposure	Number of animals	Diameter:		Height of epithelial cells:		
		tubuli seminiferi contorti	epididymis canal	epididymis canal	seminal vesicles	prostate gland
Intact, daylight	22	120,4±6,82	105,4±6,11	13,8±0,55	8,4±0,57	9,4±0,70
Pseudo-operated, daylight	7	123,2±4,15	107,3±5,02	12,2±0,68	8,4±0,45	9,6±0,62
Pelvic neurectomy, daylight	8	118,6±5,18	98,5±3,41	11,9±0,58 *	7,5±0,30 ^	8,0±0,38 ^
Intact, permanent light	22	141,4±9,92	110,6±8,28	13,6±0,53	11,8±0,72 *	11,4±0,84
Pseudo-operated, permanent light	7	136,8±5,67	112,1±7,35	12,4±0,69	11,2±0,50 ^	11,0±0,77
Pelvic neurectomy, permanent light	8	134,5±5,82 #	103,7±4,37	12,8±0,64	8,5±0,41 ^^, **	9,3±0,52 #, **
Intact, permanent darkness	17	104,3±6,22 *, **	96,8±6,21	10,5±0,71 *, **	8,2±0,42 **	8,9±0,72 **
Pseudo-operated, permanent darkness	7	115,7±4,89 ^^	98,9±9,43	10,8±0,58	8,6±0,35 ^^	9,0±0,50 ^^
Pelvic neurectomy, permanent darkness	8	117,3±5,26 ##	101,9±3,28	12,1±0,63	8,1±0,33	7,6±0,43 ##, ^^^

Notice. ^ – *p≤0,05 relatively to the values in pseudo-operated animals under standard light; ^^ – p≤0,05 relatively to the values in pseudo-*

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CHANGES OF CONCENTRATION OF MIDDLE MASS MOLECULES IN BLOOD SERUM IN THE EARLY DAYS OF ISOLATED ORTHOPEDIC TRAUMA COMPLICATED BY BLOOD LOSS

Abstract. *Isolated orthopedic trauma (fracture of thigh bone) at the early period of traumatic disease is accompanied by an increase in the content of the middle mass molecules in blood with a maximum level on the 3-d day and normalization in 7 days. Under these conditions, additional blood loss enhances the level of endogenous intoxication, and is accompanied by a significant accumulation of fractions of middle mass molecules in the blood, and in 7 days their content does not reach the control level.*

Key words: *isolated orthopedic trauma, blood loss, endotoxicosis.*

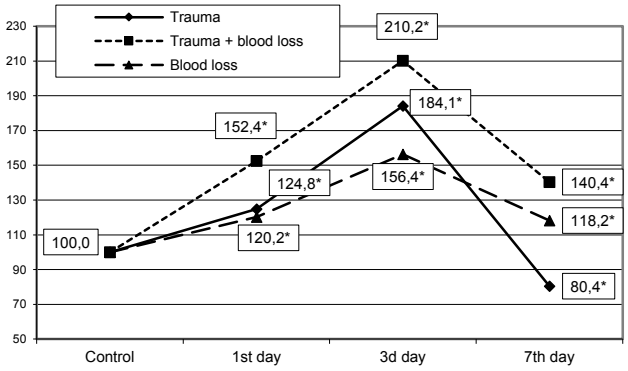
Introduction. There is a considerable increase in the level of traumatism complicated by blood loss for last years. In the pathogenesis of critical states, accompanied by metabolic disorder and natural detoxification, endotoxemia, caused by traumatic shock, hypovolemia, systemic hypoxia and lipid peroxidation, plays a significant role [1]. Middle-molecular products of proteolysis, which in modern literature are called middle mass molecules (MMM), are the general biochemical marker of physiologically active components. Evaluation of content of these molecules in blood nd other biological fluids is the sensitive indicator of syndrome of multiple organ failure and systemic organism response to inflammation [2,3]. These molecules are peptides, glycopeptides, degradation products of fibrinogen, albumin, thrombin, collagen fragments, other protein substances and derivatives of lipids, phospholipids, etc. Manifestations of biological activity of middle-molecular peptides are quite numerous. They have vazo-, cardio, neuro- and immunosuppressive properties, inhibit such metabolic processes as mitochondria respiratory, DNA synthesis in hepatocytes, synthesis and utilization of glucose, hemoglobin synthesis, activity of some enzymes. Action of MMM disturb transport of amino acids, lipid peroxidation in the brain [4,5]. MMM accumulation supposed to be the result of insufficient activity of exopeptidases, which normally are responsible for degradation of peptides in blood [14].

Objective: to study the features of changes of concentration of MMM at the acute phase and at the early days of traumatic disease on a background of isolated orthopedic trauma, and their dependence on the intensity of blood loss.

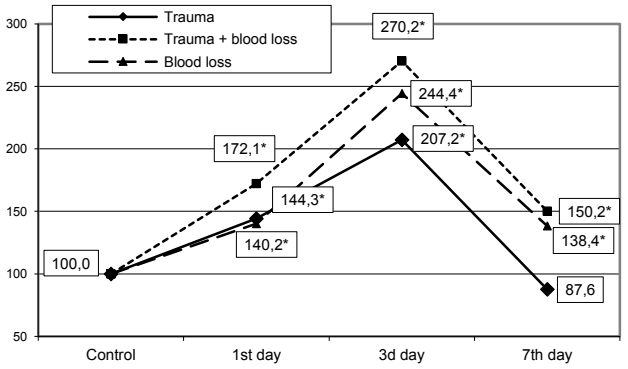
Materials and methods. The study was performed on 80 nonlinear white male rats, which were fed a standard diet in a vivarium. The animals were divided into 4 groups of 8 each. The first group consisted of rats with simulated isolated orthopedic trauma, which was the closed fracture of thigh bone by dosed kick with specially designed device. The second group consisted of the rats with the blood loss of 20-22% of the circulating blood volume by intersection of the femoral vein. The rats with both these damages were in the third group. And the intact rats were in the forth control group. All traumas were made under the thiopental sodium anesthesia (40 $mg \cdot kg^{-1}$ of body weight). The animals were taken out of the experiment in 1, 3 and 7 days after the start of post-traumatic period by a total bloodletting from heart under the same anesthesia. Content of MMM fractions in blood serum were determined at the wavelengths 254 and 280 nm (MMM_{254}, MMM_{280}) [4]. Statistical significance

of differences between experimental and control groups was evaluated using the program STATISTICA 10.0 ("StatSoft, Inc.", USA).

Results and discussion. Studies showed (Figure), that under the effect of trauma the content of the studied MMM fractions in the blood serum increases considerably and reaches the maximum level on the 3^{d} day: MMM_{254} is higher on 84.1% than in control group, MMM_{280} - on 107.2% ($p<0.05$). In 7 days the indexes decrease and become smaller than the control level (on 19.6%, $p<0.05$ and on 12.4%, $p<0.05$ respectively).



MMM_{254}



MMM_{280}

Figure – Changes of content of MMM_{254} i MMM_{280} in the blood serum (percent of the control level) on a background of the isolated orthopedic trauma, blood loss and them together at the acute phase and at early days of traumatic disease (- differences compared to the control group are statistically significant, $p<0.05$).*

At the blood loss the indexes increase considerably too compared to the control group, reaching the maximum level on the 3^{d} day. In one day their values were nearly equal to the ones of the animals with isolated orthopedic trauma ($p>0.05$). In 3 days the content of the MMM_{254} is much smaller, and the content of the MMM_{280} is much higher than the ones of animals with isolated orthopedic trauma ($p<0.05$). In 7 days the contents of both fractions are much higher than the ones of animals with isolated orthopedic trauma ($p<0.05$).

Under the combination of isolated orthopedic trauma with blood loss the studied indexes are statistically significantly higher than the ones of control group and groups with separate traumas ($p<0.05$) during all the observation time. Dynamics of their change is the same: maximum level is on the 3^{d} day (in 2.1 and 2.7 times respectively, $p<0.05$) with the following decrease, which does not reach the control level. Under these conditions in 7 days the content of MMM_{280} is on the same level of the animals with blood loss ($p>0.05$).

Obtained results show, that all the traumas are accompanied by metabolic disorder, which leads to the accumulation of the products of protein proteolysis – MMM, that indicates the consistency of the revealed disorder. The dynamics of their changes under the each studied trauma are the same: manifestation in 3 days and following decrease in 7 days, which indicates the intensification of the processes of detoxification. Under these conditions on a background of the isolated orthopedic trauma the content of the MMM fractions becomes lower than control, which is the sign of the excessive adaptation and compensatory response of the traumatized organism, aimed at the restore of homeostasis. The phenomenon of such excess is obviously a general biological phenomenon in case of damage or irritant of moderate intensity. Attention should be paid to the fact, that the amplitude of the disorders of the content of the MMM_{280} fraction is higher. According to literature data, this fraction contains substances with aromatic groups, which detoxification occurs in the liver and lungs, where they are biologically transformed by monooxygenase system or change in conjugation reactions with following removal through the kidneys, skin, gastrointestinal tract [5]. Blood loss intensifies the accumulation of the MMM in blood and even compared to the isolated orthopedic trauma in 3 days the content of the MMM_{280} fraction is higher, and in 7 days

the same occurs to the both fractions. So, the leading role in the genesis of their appearance is played by the development of systemic hypoxia, which is not compensated to the 7th day of the post-traumatic period. Under the conditions of the combination of both traumas the amplitude of the disorders is higher, that points to the effect of summation of both traumatic injuries, where getting of molecules damaged cells into the bloodstream, hypercatecholaminemia, stimulation of metabolism, accumulation of inflammatory cytokines with impaired blood flow in the microvasculature play the leading role, which in its turn deepens systemic hypoxia [5,6]. Additional "vicious circle" closes and deepens the pathological process.

Conclusions. Additional blood loss deepens the level of the endogenous intoxication at the isolated orthopedic trauma, which is accompanied by the considerable increase in the content of the MMM254 and MMM280 fractions with the maximum level on the 3d day and the decrease in 7 days, which does not reach the control level.

Prospects for further research. The deepening of the pathological process at the combination of isolated orthopedic trauma with blood loss require for searching for adequate methods of systematic correction at the acute period of traumatic disease.

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DIAGNOSIS OF ATRESIA IN THE ILEUM OF NEWBORNS

Abstract. *The most pronounced degenerative changes of the structure of the wall can be observed in the area of the ileal atresia and in the preatresic segment; they are less pronounced in the postatresic segment. In the area of atresia there were multiple foci of necrosis and fibrosis, separation of ileal membranes, desquamation of epithelium, disorders in angiogenesis.*
Key words: *atresia, preatresic segment, postatresic segment, ileum.*

Introduction. Ileal atresia incidence is 1: 1600 newborns. This malformation is equally common in males and females. The proportion of ileal atresia and that of the jejunum is approximately equal, the proximal part of the small intestine and distal portion of the ileum are mainly affected (31 and 36% of cases respectively). In approximately half of the cases the intestinal atresia occurs in the form of free diverticula; atresia with taenia occurs in less than 40% of cases; membrane form of atresia in this place as opposed to the duodenum can be observed only in 13-20% of cases. Intestinal stenoses occur by almost 20 times less frequently than atresia. In 6% of cases atresia is characterized by its multiple nature [1-5]. Ileum atresia is a topical issue of Gastroenterology, requiring surgery in infants and young children.

Objective: to establish morphological changes in the wall of the ileum in newborns with atresia.

Materials and methods. The study was conducted on 14 fragments of the ileum in newborns with atresia using morphometry, microscopy of histological sections and statistical analysis.

Results and discussion. To systematize the results of the study we examined changes in the area of atresia, in preatresic and postatresic segments of the ileum. In atresia the muscular membrane of the ileum underwent a complete fibrous transformation (Fig. 1). There are a lot of fibroblasts in the circular muscular layer as well as lymphocytic infiltration. The nuclei of smooth myosites are enlightened, eosinophilia of the cytoplasm is reduced. The blood vessels of the

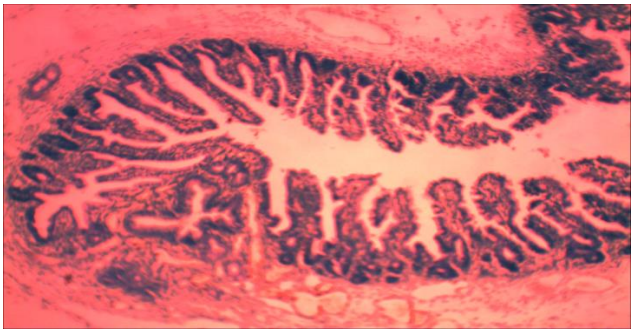

Fig. 1. A transition from the preatresic segment to atresia area of the terminal part of the ileum. Staining with hematoxylin-eosin. Mount. r. 8, Magn.7

ileum dramatically dilated, erythrocyte adhesion to the vessel walls can be observed. In atresia areas fibrosis and necrosis occur alternately, so the intestinal parameters are not possible to determine. Dilation in the ileum atresia can be explained by a larger amount of intestinal contents than in the colon. The wall of the colon is thinner, the diameter is larger, and the colon dilates less with the same volume of the content.

According to the literature and the results obtained, researchers are practically unanimous on the question of etiology and pathogenesis of the membrane form of atresia (type I according to the classification). At the dissection of the ileum the membrane looks like a thin filmy foramen that resembles a fold in the intestinal mucosa. The membrane of the ileum reaches 0.6 mm thick.

As to the multiple bowel atresia (type IV), developing as a result of apple peel syndrome there is no unanimous opinion regarding the occurence of atresia. However, researchers

assume a significant disorder in the development of mesenteric arteries, which is associated with genetic disorders – "vascular" theory and the emergence of atresia due to disturbances in rotation of the intestinal tube. The areas of the intestine look like segmented multiple mesenteric taeniae, the unaffected segments are compensatory dilated and thickened, some areas are narrowed, indicating their functional unloadedness.

In atresia with fibrous taeniae the preatresic area of the ileum ends with diverticula, in certain areas there are some intestinal segments on both sides. The closed intestinal segments are connected with a mesentery and its fibrous taeniae (type II), which are thin stringlike formations, going from one enclosed segment of intestine to another. These taeniae form a free edge of the mesentery and is actually a thickened free edge of the visceral peritoneum duplication. In complete atresia (type III) the segments of the bowel are completely apart, not only along the intestinal tube, but also along the mesentery. In this case we can observe some disorders in both the intestinal angiogenesis and in the dorsal mesentery.

In the preatresic segment of the ileum on the side of its mucosa, shortening of villi and crypts enlargement are observed. In some areas of the mucous membrane there is no epithelium. The cells become cubic, the epithelial cells get shorter. There is a significant hypertrophy of the muscular layer in the muscular membrane of preatresic segment of the ileum.

In morphological studies of the preatresic segment of the ileum on the side of mucous membrane the villi become shorter and the crypts get larger. In the preatresic segment of the ileum in the muscular membrane, there is a significant hypertrophy of the circular muscular layer compared to longitudinal one, hyperplasia of smooth myosites, areas of fibrosis, areas of polymorphonuclear leukocyte infiltration along the blood vessels. (Fig. 2). There is a dissection of muscular membranes, its swelling, disintegration of muscular layers into individual muscular cells. Compared with the structure of the intestine of a newborn in a normal muscular membrane of the preatresic segment of the

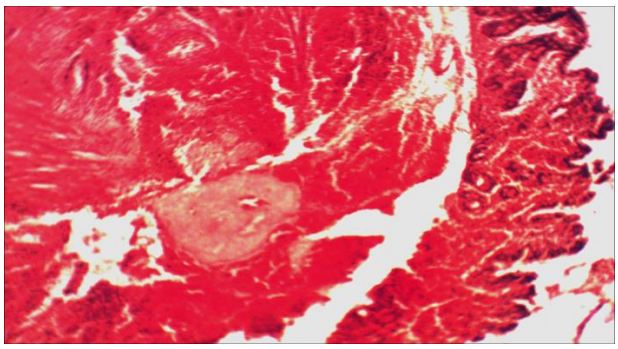

Fig. 2. Atrophy of the mucous and muscular membranes of the preatresic segment of the ileum. Staining with hematoxylin-eosin. Mount. r. 10^x, magn. 10^x

ileum a dilation of small vessels of muscular type with hypertrophy of the wall and the dilation of capillaries can be observed. In the intervals between the circular and longitudinal muscular layers a significant amount of cellular elements of connective tissue was found. The changes in the ileum muscular membrane are confirmed by morphometry findings.

In preatresic segments of the ileum there are significant changes in the nerve cells and their processes. There is an increase and change in shape of neurocytes, thickening of their processes, significant thickening at the ends of dendrites was found too. Nerve fibers that have not undergone decay, have rare residual effects of dyschromia and local edema. Around the middle third of the ileum segment the vascular glomeruli of ganglia are formed with a dense grid.

The areas with atresia undergo the most destructive changes both in the nervous system and in hemocirculatory stream. In the segments of the ileum that has undergone them, significant changes in hemomicrocirculatory stream and its intramural nerve plexuses were found. Intramural arteriolar diameters in most cases are narrowed to 16.70 mm. Their contours are winding. The capillaries that surround nerve cells near the atresia segment are dilated and tortuous. Compared with the norm, diameter of the venules in the preatresic and postatresic segments increased by almost twice. The number of arteriolovenulary anastomoses increases. The ileum atresia areas with multiple foci of necrosis and fibrosis indicate directly the possible primacy of blood vessels disorders due

to ischemia in this area with the development of fibrosis (Fig. 3).

In the postatresic segment of the ileum morphological changes occur, such as: mucous membrane with submucosa base is in a state of edema, it is peeled off in some areas. There are some multiple areas of epithelial desquamation. The muscular membrane is thinned, there is a decrease in myositis sizes when their number increased, which is indicative of muscular layer hyperplasia (Fig. 4).

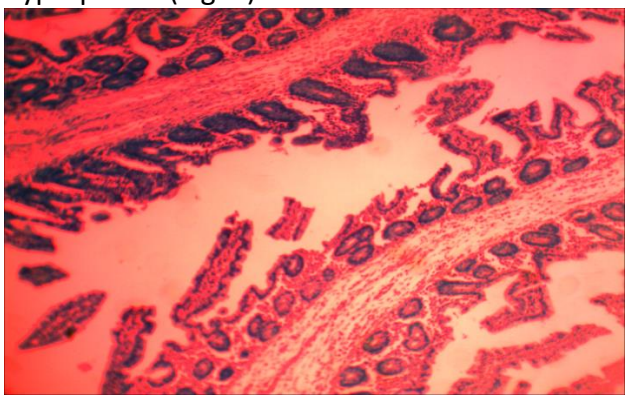

Fig. 3. Partial atresia of the ileal wall. Staining with hematoxylin-eosin. Mount. r. 10x, magn. 10x

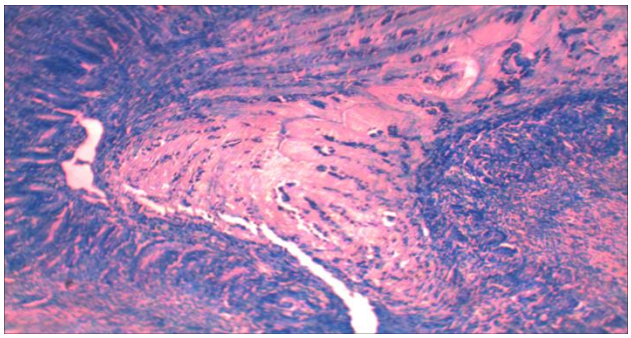

Fig. 4. Blood vessels in atresia of the terminal portion of the ileum. Staining with hematoxylin-eosin. Mount. r. 10x, magn. 10x

Morphological findings in the postatresic segment indicate its functional unloadedness, for myocyte hyperplasia is not accompanied by increasing their size; they are only divided.

The ileum tends to develop a pathogenic type with primary disorders in mesenteric vessels corresponding to atresia with fibrous taenia and complete form of atresia with a vascular link in the pathogenesis. We revealed morphologically visually some intact areas of the intestine - before preatresic and after postatresic segments at the level of mesenteric vessels. In pathogenic type of ileal atresia with primary disorders of vascular development, it is recommended to resect visually intact portion of the intestine above the preatresic and below the postatresic segments of the intestine near the closest ileal arteries.

We can observe a dilation of the ileum up to 13-14 mm: by twice compared to unaffected areas. In the ileum atresia with fibrous taenia the atresic section ends with a diverticulum. Enclosed segments of the intestine are connected to the mesentery and its fibrous taenia, and are thin stringlike taeniae. On the side of the ileal mucous membrane the villi become shorter, the crypts get wider and shorter and the number of goblet cells increases. The height of the epithelium reduces compared with normal areas, and in some places there is no membrane epithelium. There is a partial detachment of the epithelium from its own layer of the mucous membrane. There are numerous subepithelial swellings and inflammations in the mucosa and submucosal layers. Epithelial cells change their shape from cylindrical to cubical one and they sometimes become flat.

Morphological characteristics of the ileum mucosa: the mucous membrane is 490±12 micrometers thick (normal), 310±8 micrometers thick(atresia); the villi are 280±14 highmicrometers (normal) 317±9 micrometers high (atresia); The villi are 46±1 micrometers thickthick (normal) 46.9±2 micrometers thick (atresia); the crypts are 217± 10micrometers deep (normal) 216±10 micrometers deep(atresia); the epitheliocytes are 28±2 micrometers high (normal), 28.3±3 micrometers high (atresia); diameter of the epitheliocyte nuclei is 3.3±0.1 micrometers (normal) 2.9±0.1 (atresia).

Morphological characteristics of the muscular layer of the ileal preatresic segment: the muscular layer is 37±6 micrometers thick (normal) 160±26 micrometers thick (atresia); the circular muscular layer is 22±1 micrometers thick (normal) 102±10 micrometers thick (atresia); longitudinal muscle layer is 16±0.6 micrometers thick (normal) 47±2 micrometers thick (atresia); diameter of smooth myositis is 8.6±0.2 micrometers (normal) 7.2±0.8

micrometers (atresia).

Areas of ileal atresia. In the areas of ileal atresia there were significant degenerative changes in the structure of the wall, separation of the mucous membrane, there are also multiple forms of degenerative neuroblasts, their number reduces, the hemocirculotary changes.

The changes in the hemomicrocirculatory stream are more pronounced in the ileal preatresic segment. Around the middle third of the segment the vascular glomeruli of ganglia are formed with a dense grid. The capillaries, that surround nerve cells, are dilated and winding near the atresic segment. The capacity of the ganglia blood vessels increases.

The postatresic segment of ileum is characterized by multiple degenerative changes in all membranes of the intestinal wall. In mucosa we observe a severe destruction of the villi and a desquamation of their apical epithelium.. In the lamina propria mucosa we can often observe oa polymorphonuclear leukocyte infiltration, more pronounced in the area of the villi. Crypta tunicae mucosae have irregular narrow space. There are multiple areas of epithelial desquamation. The muscular layer is thinned, its hyperplasia was found.

Conclusions. 1. The degenerative changes in the structure of the ileal walls are most pronounced in the area of atresia and preatresic segment whereas they are less seen in the postatresic segment. In the area of atresia there are multiple foci of necrosis and fibrosis, separation of the ileal membranes, desquamation of the mucous membrane epithelium, disorders in angiogenesis. 2. In the postatresic segment we can observe the thinning of the mucous membrane, muscular layer atrophy, disintegration of the neurovascular plexuses, and reducing blood flow to the nerve nodes resulting from the functional unlodedness of this this segment.

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PROOXIDANT-ANTIOXIDANT BALANCE OF THE LUNGS IN CARRAGEENAN PLEURISY AND SHORT-TERM PINEAL GLAND HYPERFUNCTION

Abstract. *We have studied free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under10-day epiphysis hyperfunction. It was established that melatonin did not cause significant changes in the formation of superoxide in animals with carrageenan pleurisy but it promoted a reduction of peroxidation processes in the experimental organs. In this case there were no significant changes in the activity of antioxidant enzymes.*
Key words: *melatonine, epiphysis, carrageenan pleurisy, prooxidant-antioxidant system.*

Introduction. Lipid peroxidation (LPO) is an essential element of vital activity of any cell and it is a variant of free radical oxidation by its chemical nature. [5, 6, 9, 16]. Normally, all tissues of living organisms undergo a constant generation of reactive oxygen species(ROS), which, as signaling molecules, ensure the preservation of normal metabolic background necessary for functional activity of cells and are a component of nonspecific host defense against pathogens, microorganisms, tumor cells. As ROS represent a serious threat to the functioning of cells, there is quite a complex multilevel system of protection against them. Physiological prooxidant-antioxidant system (PAS) has a protective (effector) and regulatory functions.

Disorders in prooxidant-antioxidant balance in favor of the first one, lead to a potential damage, commonly called oxidative stress [20]. Oxidative stress occurs in the normal course of life too, being a kind of obligatory byproduct, caused by the function of oxidoreductases (cyclooxygenases, lipoxygenases, xanthine oxidases) multienzymatic ensembles of mitochondria, microsomes, resulting from immune responses (phagocytosis, immune observation) and non-enzymatic auto-oxidation reactions that occur due to a contact of gaseous (lungs, skin) and dissolved oxygen with the lipid membrane structures that are easily oxidized.

LPO and its regulation are of particular importance for the respiratory system, due to the great intensity of lipid metabolism in the lungs and close dependence of aero-hematic barrier function on the structure of alveolar phospholipids [15].

Neurohormone melatonin (MT) is a compound, possessing an antioxidant activity. Its protective effect in lipid oxidation is carried out by two mechanisms, which include direct inactivation of free radicals and / or inhibition of their generation in the cell and regulation of the activity of antioxidant enzymes by the impact on the genetic apparatus of cells, meaning it acts both as a straight and as a secondary antioxidant. [3]. But the impact of the MT on PAS of the lungs remains poorly studied.

Objective: to study free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under 10-day epiphysis hyperfunction.

Materials and methods. We used Wistar rats weighing 240-260 g, which were kept under standard vivarium conditions. We selected males for the study because melatonin levels in blood plasma of females depends on the menstrual cycle phase [12]. The animals were randomized into four groups with 7 animals in each one: an intact group, 10 day epiphysis hyperfunction, carrageenan pleurisy, 10 day epiphysis hyperfunction against the background of carrageenan pleurisy.

Pineal gland hyperfunction was modeled by

keeping animals in conditions of constant darkness and intragastric administration of melatonin solution (Sigma, USA) at a dose of 1.0 mg / kg [18].

To create a non-immune model of acute inflammation, we used 1% solution of carrageenan (Sigma, USA) [13, 19]. The experimental pleurisy was induced in anesthetized animals by intrapleural injection of 0.1 ml of carrageenan. The solution was injected on the 8th day of the experiment, and 48 hours later euthanasia of the animals was carried out.

Euthanasia of the rats was conducted under the rules of bioethics in accordance with the "European Convention for the Protection of vertebrate animals used for scientific experiments and other scientific purposes" (Strasbourg, 1986) and "General ethics of animal experimentation," approved by the first national bioethics congress (Kyiv, 2001).

The ROS products were evaluated by superoxide content. Superoxide products in tissue homogenates were determined by the reaction with nitro blue tetrazolium under the influence of NADH, NADP and pirogenal [17].

To evaluate the intensity of peroxidation in homogenates of organs, we determined the contents of primary and secondary products: diene conjugates (DC) oxidienes, trienes and TBA-active products. The effectiveness of antioxidant activity was assessed by lung superoxide dismutase (SOD), catalase (CT), glutathione peroxidase (GPO) and the concentrations of vitamins A, α-tocopherol and β-carotene.

The concentration of diene conjugates was determined by I.D. Stalna technique (1977) [14]. The concentration of TBA-active products was determined by I.D. Stalna, T. Harishvili method (1977) by reaction with 2- thiobarbituric acid [14]. The catalase activity was determined by M.O. Koroliuk with co-authors technique (1988 [7]. Superoxide dismutase activity was determined kinetically by the reaction of adrenaline autooxidation in the alkaline environment with the generation of superoxide-anion (radicale) [4]. Glutathione peroxidase activity was determined by the method of co-V.A. Pakhomova using tert-Butyl hydroperoxide as a substrate [11].

The total proteolytic activity was determined by hydrolysis of casein [8]. The concentration of trienes, oxidienes, α-tocopherol, vitamin A and β-carotene was determined by a modified method based on the molar extinction coefficient [10].

Statistical analysis was performed using Microsoft Office Excel 2003.

The test for the normal distribution was performed using Shapiro-W Wilkie criterion. Evaluation of reliability differences between groups with normal distribution of characteristics was performed using Student's t-test. When comparing the two groups with the free distribution of signs, we used nonparametric Wilcoxon (Mann-Whitney) U-test. The difference was considered statistically significant at $p < 0.05$ [1, 2].

Results and discussion. In assessing superoxide generation sources we found out that short-term light deprivation and exogenous MT contributed to the activation of generation of superoxide-anion (radicale) of mitochondrial ETL by 16% ($p < 0.01$) (Table. 1).

The rats with pleurisy had a significant increase of superoxide in their lungs from all sources of its generation. For instance, its content increased by 63% ($p < 0.001$) due to the mitochondrial electron transport chain by 50% ($p < 0.001$) – from microsomal electron transport chain and NO-synthase and by 26% ($p < 0.001$) – from phagocytes.

The rats with pleurisy that were in conditions of light deprivation and received MT when compared with normal active sources of superoxide radicals had both the mitochondrial chain (73%, $p < 0.001$) and microsomal one (66%, $p < 0.001$) as well as tissue phagocytes (26%, $p < 0.001$). When compared to the rats with epiphysis hyperfunction, the growth of superoxide generation turned out to be from two sources - mitochondrial (49%, $p < 0.001$) and microsomal (48%, $p < 0.001$) respiratory chains. The generation of superoxide anion-radicale in the lungs of experimental rats remained at the level of values, typical for pleurisy control.

The study of influence of MT on PAS in the lungs of rats (Table. 2) showed a slight decrease of peroxidation processes in animals with epiphysis hyperfunction compared with the norm which was illustrated by a reduced trienes

Table 1

The content and sources of superoxide-anion (radicale) generation in rats' lung tissue homogenate (M ± m, n = 7)

Group / Value	Intact	10-day epiphysis hyperfunction	carrageenan pleurisy	10dayepiphysis hyperfunction combined with carrageenan pleurisy
$\bullet O_2^-$ from mitochondrial electron-transport chain (nmol $\bullet O_2^-$/g·sec.), induced with NADH	19,900±0,224	23,110±0,102 p_1<0,01	32,382±0,752 p_1<0,001	34,525±1,086 p_1<0,001 p_2<0,001
$\bullet O_2^-$ from microsomal electron-transport chain and NO-synthase (nmol $\bullet O_2^-$/g·sec.), induced with NADP	21,000±0,845	23,530±0,967	31,525±1,846 p_1<0,001	34,906±0,777 p_1<0,001 p_2<0,001
$\bullet O_2^-$ from tissue phagocytes (nmol $\bullet O_2^-$ /g·sec.), induced with pirogenal	4,018±0,090	4,743±0,260	5,044±0,360 p_1<0,001	5,047±0,209 p_1<0,001

Notes: it is statistically reliable compared to p_1 – intact group; p_2 – epiphysis hyperfunction.

Table 2

Biochemical parameters of PAS in rats' lungs (M±m, n=7)

Group / Value	Intact	10-day epiphysis hyperfunction	carrageenan pleurisy	10-day epiphysis hyperfunction combined with carrageenan pleurisy
Diene conjugates (mmol/kg)	10,140±0,810	9,816±0,308	13,370±1,160 p_1<0,05	11,350±0,699
Trienes (mcmol/kg)	216,757±43,374	78,517±28,016 p_1<0,05	238,787±23,902	99,894±24,881 p_1<0,05 p_3<0,001
Oxidienes (mcmol/kg)	531,231±71,307	175,714±42,295 p_1<0,01	628,453±51,621	343,766±59,224 p_2<0,05 p_3<0,01
TBA-active products (mcmol/g)	8,423±0,354	10,700±1,345	10,220±0,170 p_1<0,001	10,070±0,155 p_1<0,01
Catalase activity (mAbs / kg)	4,691±0,017	3,931±0,142 p_1<0,01	6,374±0,333 p_1<0,001	6,407±0,431 p_1<0,01 p_2<0,001
SOD activity (st.un./g)	0,091±0,021	0,182±0,023	0,255±0,019	0,291±0,043

		p_1<0,05	p_1<0,001	p_1<0,01 p_2<0,05
GPO activity (mAbs / kg)	5,500±0,431	5,756±0,396	4,307±0,453	5,924±0,167 p_3<0,01
Total proteolytic activity (mAbs / kg))	57,320±10,160	59,390±11,640	68,210±6,180	85,330±4,512 p_1<0,05
VitaminA (mcmol/kg)	337,578±22,139	257,735±42,906	364,437±55,042	373,598±79,363
β-carotene (mcmol/kg)	73,439±13,187	90,058±15,963	84,384±17,532	134,696±35,382
α-tocopherol (mcmol/kg)	525,685±40,563	305,592±67,051 p_1<0,05	600,154±56,119	367,125±62,779 p_3<0,05

Notes: it is statistically reliable compared to p_1 – intact group; p_2 – epiphysis hyperfunction; p_3 – carrageenan pleurisy.

and oxidienes content in homogenates of the experimental organs by 3 times (p <0.05 and p <0.01). The changes in the prooxidant rate took place against the background of divergent changes in antioxidant system, namely the reduction of catalase activity by 16% (p <0.01) and rising by twice (p <0.05), SOD activity and a decrease in the concentration of α-tocopherol almost 2 fold (p <0.05).

In epiphysis hyperfunction the rats with pleurisy underwent some changes in the processes of peroxidation and antioxidant protection. MT contributed to the reliable reduction of trienes (p <0.001) and oxidienes (p <0.01) by almost 2-fold and increased GPO b activity by 38% (p <0.01) in the carrageenan rats, but at the same time the content of α-tocopherol reduced significantly (by 61%, p <0.05).

The findings show that the MT during the short epiphysis hyperfunction only inhibits the generation of superoxide radicals in carrageenan pleurisy; it also reduces the intensity of peroxidation in animals with pleurisy, and without it. In carrageenan inflammation the changes due to the activity of antioxidant enzymes were much less significant, since MT, perhaps, acted as a direct antioxidant. This may explain the fact that there was no significant reduction of antioxidant vitamins in the lungs of animals.

Conclusions. 1. Melatonin reduces the free radical processes under conditions of oxidative stress.

2. In carrageenan inflammation melatonin acts primarily as a direct antioxidant.

Perspectives of further investigations. It is planned to study free radical and antioxidant processes in the lungs of rats with carrageenan pleurisy under 10-day decrease in the carrageenan activity.

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ASPECTS OF DEVELOPING PROFESSIONAL COMPETENCE IN MEDICAL STUDENTS DUE TO MODERNIZATION OF MEDICAL SCIENCE

Abstract. *The development of higher medical education involves training of future medical practitioners as qualified and competent specialists, competitive on the labour market. Modern science provides person's capability to perform certain activities manifested through their knowledge, ways of thinking, comprehension, implementation of particular actions within specific field. In the process of training at higher medical institution, future medical specialists develop their professional competence, intellectual and personal values, in order to be able to further advance and improve them in the process of professional activity at work.*
Key words: *medical education, teaching and learning process, practical skills, medical students.*

Modernization of our national higher education system is a purposeful movement towards modern mechanism of educational activities. In order to increase opportunities of students' mobility, to achieve interoperability of training programs and qualifications, to provide training for students on an individual part of the educational and professional programs, to improve the quality of training and competitiveness of the graduates, to enable an access to labor markets, to enhance the prestige of higher education in Ukraine, we suggested the idea of organizing the educational process. The integrity of the educational process, providing professional training, based on the unity of theoretical and practical classes, continuity in the formation of knowledge and skills in the classroom and self-study and a system of knowledge assessment. The modernization of the educational process includes a significant increase in self-learning time, individualized training that needs proper scientific and medical support of the educational process and the corresponding material base. Continuous improvement of teaching methods should be accompanied by a comprehensive assessment of the quality of organization and conduction of classes.

Microbiology as an academic discipline provides guidelines for the development of skills to apply knowledge of the subject in future careers and fundamentals of diagnosis, treatment and prevention of major infectious diseases. Students' workload is expressed in credits that are recorded as a result of the successful mastering the module. The curriculum provides some lectures, highlighting the main themes on Microbiology and solving problems, summarizing and structuring the theoretical material [4].

Practical classes are aimed at consolidation and deepening the knowledge that students received in the theoretical teaching, imparting the necessary practical skills in the discipline. Methods of educational practice are the explanation, demonstration, modeling a production situation, work in small groups, role-playing, self practical skills and abilities according to the sequence of actions, discussing mistakes. Practical exercises include individual work of students in the classroom, practical skills in cultivation and identification of microorganisms, choosing the right method of diagnosis and prevention as well as basic principles of treatment of infectious diseases. Each practice session provides high organizational work, which means a diligent preparation for the class by both the teacher and their students, selection of forms and methods, as well as a rational distribution of time for practical sessions. An important psychological aspect of classes: an atmosphere

of goodwill, interest and emotion should prevail at a class. Arousing students' interest for their practical work is one of the possibilities to improve the theoretical knowledge and practical skills in general, and optimal visualization of educational material contributes to a better understanding and mastering. By training medical students it is important not only to understand and learn information, but also to master the ways of its practical application and decision-making [2].

Taking into consideration the fact that the amount of information that students need to learn is quite impressive, most of the students learn the material on their own. In this regard, unsupervised work of the students becomes a leading part of the learning process, which must have its notional characteristics, should be controlled, checked and evaluated. Independent work of students consists of their praparation for practical knowledge, consideration of topics not included in practical classes, training in review of scientific literature etc. In the classroom and in the process of unsupervised work the students master some practical skills. Therefore such training has many positive aspects, encourages students to learn systematically, contributes to the intensification of the educational process, provides a complete learning of the program material on subjects, of relevant practical skills, additional scientific information and promotes self-education.

The final stage of self-training is self-assessment. Students are offered a list of issues that need to be answered. Considering the psychological characteristics of students, in addition to self-assessment we also carry out real evaluation of mastering self-training material, by including tests on the subject to determine the initial and final level of knowledge at practical classes and a final module control [1].

Repeated reviews ensure the emergence and consolidation of conditioned reflex connections that are the physiological basis of skills. This phase requires the teacher to use an individual approach: every student is given the possibility to repeat an action as many times as they actually need in order to reach the stage of automated performance. While mastering practical skills they discuss physician's participation in the implementation of laboratory diagnostics, performing serological tests that promotes professional thinking and allows medical students to avoid the idea of doctor's activity as the mechanical performance of tasks, consolidating their knowledge and skills, uniting them in a clear system [3].

Conclusions. Thus, the students' learned skills give them the opportunity to prove themselves in training, to find their place in scientific research work, careers, profession-oriented projects. The obtained skills are important in improving the quality of learning. Promoting independent thinking and maximum mental performance of students in all stages of a class provides a detailed analysis of the information received and stimulates the creative development of personality.

Prospects for further research. The main tasks set by the teachers of our department today are improving existing materials, multimedia presentations, development and implementation of e-learning in teaching aids, reference books that will be available to medical students.

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DYNAMICS OF THE ONSET OF PATHOLOGICAL CHANGES IN THE RETINAL LAYERS AT THE END OF THE FIRST WEEK OF OPIOID EXPOSURE

Abstract. *Objective of our work was to find out the fundamental issues relating to the dynamics of early changes in the reticular layers when the body was exposed to opioid. We conducted research on 15 sexually mature outbred male rats weighing 160 g, aged 4.5 months. The animals were injected intramuscularly with a medication nalbuphine, once a day in the same period of time (at 10-11 a.m.) for 7 days. The initial dose of nalbuphine was 0.212 mg / kg. By doing so we created conditions of chronic opioid effect. Histological specimens were prepared by the conventional method using dyes of hematoxylin, eosin and asan by Heidenhain method All animals were in vivarium conditions and the work that dealt with issues of maintenance, care, labeling and all other manipulations, was carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other scientific purposes" [Strasbourg, 1985]. During the experimental study we established changes in the structure of the reticular layers after a week injection of opioid. As a result of our experimental opioid exposure during the first week we recorded some pathological changes, which manifested themselves in the appearance of the initial effects of vacuolar degeneration of the pigment epithelium, signs of microcystic degeneration of outer reticular layer with symptoms of vacuolar degeneration of retinal ganglion cells.*

Key words: *microstructure, layers, retina, opioid, early stages, rats.*

Introduction. The results of the paper correspond to the research plan of Danylo Halytskyi Lviv National Medical University and are a part of the scientific - research theme of the department of General Anatomy "Structural organization of angioarchitectonics and anthropometric features of organs in intra and extrauterine development period, influenced by exo - and endopathogenic factors" (the number of state registration 0115U000041) for 2015 – 2019.

Today 100 million addicts have been registered all over the world [1, 2], including 2,897 people in Ukraine, who, according to statistical data of the Ministry of Health, use opioid drugs uncontrollably [3]. Prolonged use of surrogate medication opioids, according to the literature, contributes to pathological changes in a number of organs. In particular, there is a number of publications that cover the processes of pathological manifestations in the cardiovascular system [1, 4, 5, 6, 7]. Some authors also covered the problem of changes in the iridocorneal angle as well as the pathotransformation of optic nerves resulting from toxic damage by opioids [8, 9]. In the available literature we found rare and disconnected data on pathological changes of the vision in particular those, concerning the retina.

Neurovascular factor creates a base for pathogenesis of opioid retinopathies. To date there are no studies that can answer the question: what is affected primarily – the layers

of the retina, due to trophic disorders, or the retinal vessels as an object of neurotrophic effect.

Therefore, I believe that this study is important both in terms of experimental and morphological studies, and from a practical point of view.

Materials and methods. We conducted our research on 15 sexually mature outbred male rats weighing 160 g and aged 4.5 months. The animals were injected intramuscularly with a medication nalbuphine, once a day in the same period of time (at 10-11 a.m.) for 7 days. The initial dose of nalbuphine was 0.212 mg / kg. By doing so, we created conditions of chronic opioid effect. [10].

The animals were divided into 2 groups. The first group of animals received nalbuphine for 7 days, followed by a collection of experimental material (the end of the 1st week of experimental opioid exposure); the 2nd group was a control one that received injections of saline intramuscularly in the same period of time (10-11 a.m.). All animals were in vivarium conditions and the work that dealt with issues of maintenance, care, labeling and all other manipulations, was carried out in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other scientific purposes" [Strasbourg, 1985], "General ethics of animal experimentation, "approved the first National Congress of bioethics [Kyiv, 2001]. Law of Ukraine № 3447 - IV «On protection of animals against cruel treatment." Commission on bioethics of Danylo Halytskyi Lviv National Medical University revealed that the research met the ethical requirements under MHP of Ukraine number 231 of 01. 11. 2000 (protocol № 10 dated 26.12. 2011.) (protocol № 2 on February 20, 2012). Before taking biopsy material the animal were put to sleep by intraperitoneal administration of thiopental (at 25 mg / 1 kg). As a material for microstructure studies we used rats' eyeballs obtained by postmortem enucleation with further consideration of conservation of topographic correlation of eye membranes by making histological sections 5-7 microns thick. Histological specimens were prepared by the conventional method using dyes of hematoxylin, eosin and asan by Heidenhain method [11]. Microscopic studies and photography of the specimens were performed with a microscope IBI – 1 and a digital camera Nicon D 3100.

Results and discussion. As a result of our microscopic examination of the structural layers of the rats' retina after a week of the experiment the retinal pigment epithelium in the experimental animals in the interval from the optic nerve to the dentate line is discontinuous. In some areas of the retina the pigment epithelium consists of elongated cells which are arranged with their longitudinal axis parallel to the basement membrane and are firmly connected to the inner layer of the latter. We found some spaces in some areas of the retina where the epithelial layer is absent. In other parts of the retina we found epithelial cells with swollen cytoplasm which contains semitransparent vacuoles (vacuolar degeneration), as shown in Fig. 1. The nuclei of such epithelial cells are usually saved and located closer to the basal pole. The basement membrane is integral, homogeneous and weakly basophilic. In the lumen of the capillaries of the choroid there are some signs of stagnation and plethora. Cytoplasmic processes of photoreceptor cells lie somewhat loose and sparse in the layer of rods and cones. There were not any structural changes in the outer boundary layer at this stage of the experiment. The outer nuclear layer sometimes contains irregularly shaped or pyknotic nuclei. In some parts there are significant spaces between the nuclei, as shown in Fig. 2. In the disk of optic

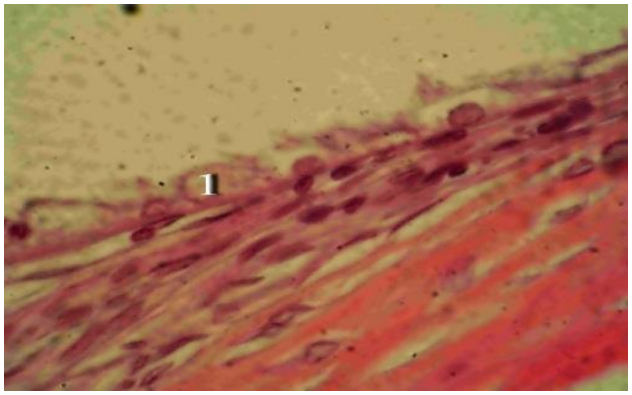

Fig 1. Rat's retina a week after a daily opioid injection. Stained with hematoxylin and eosin. Magn.: x 1000. 1– vacuolization of pigment epithelium

nerve area the outer nuclear layer is thinner, the spaces between the nuclei are slightly larger,the number of rows of nuclei is 5-8. In the area of the dentate line the outer nuclear layer is also thinner, it contains 3-5 rows of nuclei. The nuclei of photoreceptor cells move into the outer reticular layer in some places. Nuclei of bipolar cells sometimes occur in the external reticular layer.

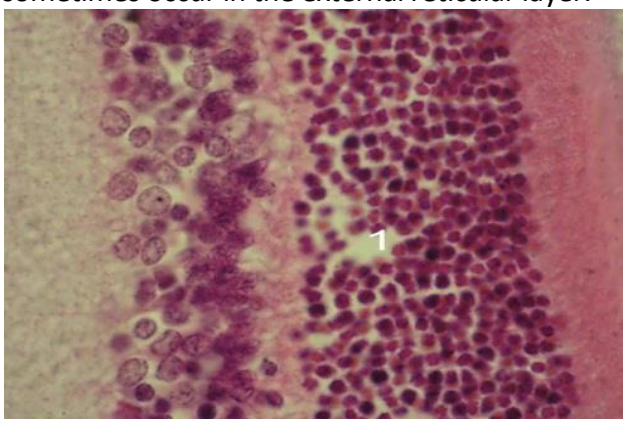

Fig. 2. Rat's retina a week after opioid injection. Stained with asan. Magn.: x 1000. 1 – nuclear-free areas in the outer nuclear layer.

Quite often clear spaces of the basic substance occur in the outer reticular layer, often forming microcysts (microcystic degeneration), as shown in Fig. 3 and Fig. 4. In some areas microcysts reach the inner nuclear layer, and sometimes they contact with the outer nuclear layer.

The inner nuclear layer within the specified term of the experiment has structural organization similar to the control one, but the ground substance around some nuclei of the

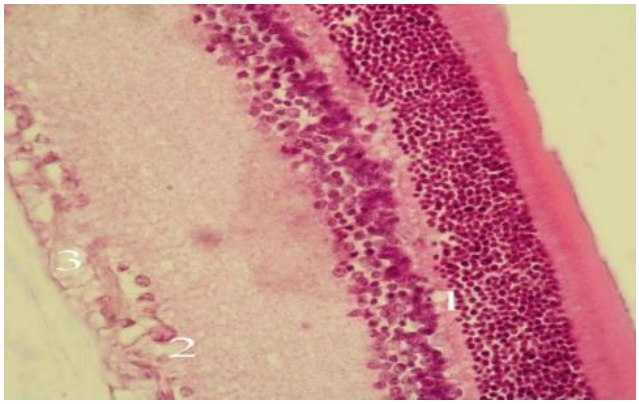

Fig. 3. Rat's retina a week after opioid injection. Stained with hematoxylin and eosin. Magn.: x 400. 1– microcystic degeneration of the outer reticular layer; 2 – pericellular swelling of ganglion cells; 3 – microcystic degeneration of the nerve fiber layer.

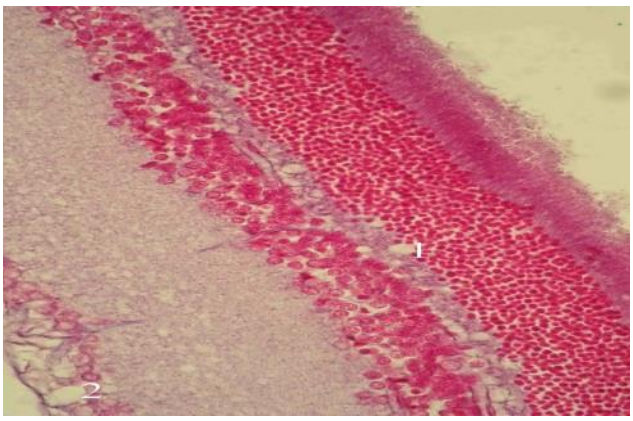

Fig. 4 Rat's retina a week after opioid injection. Stained with asan. Magn: x 400. 1– microcystic cavities in the outer reticular layer; 2– microcystic cavities of the nerve fiber layer.

bipolar cells is cleared, there are some signs of a slight pericellular swelling, as shown in Fig. 5 and Fig. 6. In some places the nuclei of amacrinal cells immersed in the inner reticular layer.

There were not any pathological changes in

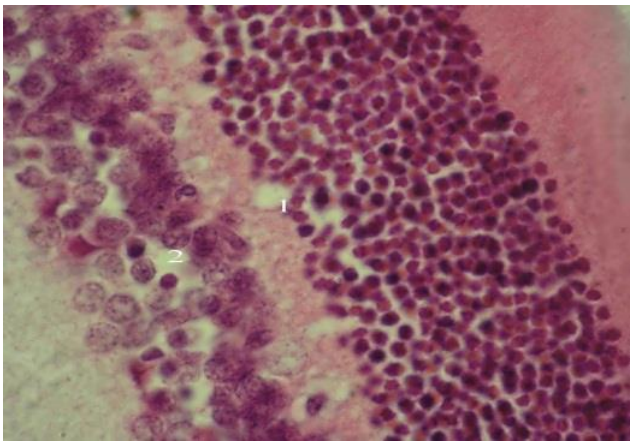

Fig. 5. Rat's retina a week after opioid injection. Stained with asan. Magn.: x 1000. 1– microcystic cavities in the outer reticular layer; 2– Cleared areas in the inner nuclear layer.

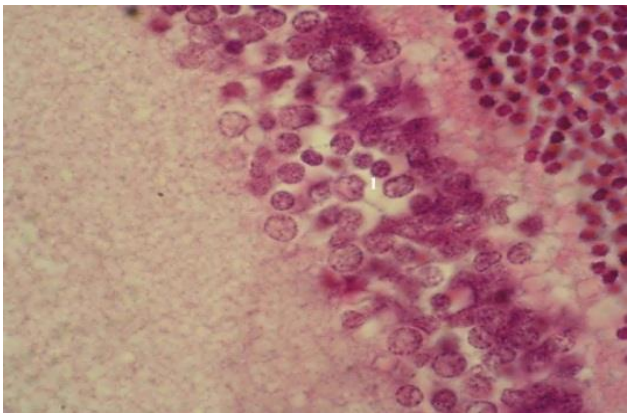

Fig. 6. Rat's retina a week after opioid injection. Stained with asan. Magn.: x 1000. 1– pericellular swelling in the inner nuclear layer.

the structure of the inner reticular layer within the specified period of the experiment. In some cells of the ganglionar layer the nuclear center is cleared, its chromatin is located near the shell of the nucleus. The cytoplasm of some ganglionar cells is cleared and contains vacuoles. The endothelium of some vessels, located on the edge of the layer of nerve fibers is slightly swollen. The ground substance of the nerve fiber layer is often cleared, containing microcysts in such areas, as shown in Fig. 7. This layer becomes much broader towards the optic nerve and containes extensively shaded thick purple fibers when stained with asan. There were not any structural changes in the inner boundary layer at the end of the first week with opioid exposure.

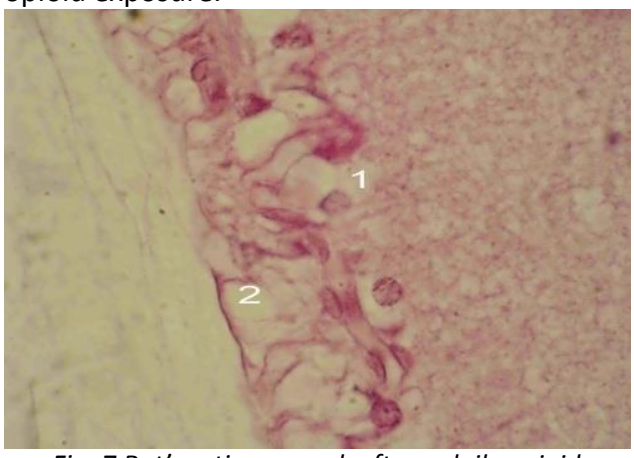

Fig. 7 Rat's retina a week after a daily opioid injection. Stained with hematoxylin and eosin. Magn.: x 1000. 1– vacuolization of some ganglionary cells; 2– microcystic degeneration of nerve fibers.

Conclusions. As a result of our experimental opioid exposure, during the first week we recorded some pathological changes, which manifested themselves in the appearance of the initial effects of vacuolar degeneration of the pigment epithelium, signs of microcystic degeneration of outer reticular layer with symptoms of vacuolar degeneration of retinal ganglion cells.

Perspectives of further investigations. The pathomorphological manifestations in the layers of the retina during experimental opioid exposure that have been found, can serve as a morphologic basis for further study of the manifestations of opioid retinopathy in the experiment. Our results can later be used for comparative characteristics concerning the dinamics of pathological changes in the layers of the retina in cases of short and long term exposure to low doses of opioids.

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CHANGES OF LIPIDOGRAM AND MORPHOLOGICAL ASPECTS OF ENDOTHELIAL DYSFUNCTION AT PHYSICAL ACTIVITY OF VARYING INTENSITY

Abstract. *It was found out, that physical activities of high intensity are accompanied by development of severe endothelial dysfunction, which is manifested by atherogenic damage of the femoral artery. Use of physical activity of moderate intensity is characterized by anti-atherogenic changes in the blood serum of the animals and the normal structure of the femoral artery wall.*
Key words: *physical activities of various intensity, hypercholesterolemia, endothelial dysfunction, femoral artery.*

Introduction. Motor activity is an essential part of everyday life of each person and a base of active functioning of the body and its resistance to adverse actions. Accordingly, dynamic compliance between the needs of the body, which are caused by the load of physical activity, and admission of oxygen and various substrates to the organs of the musculoskeletal system needs to be achieved. This adaptation to physical activity (FA) is provided by optimal interaction of body systems [5]. Structural ensure of adaptation to FA is manifested at different hierarchical levels of its organization – from molecular to systemic. Although the adaptive capabilities of the organism are significant, they are limited, and beyond them there is the state of dysadaptation, which causes development of destructive reversible and irreversible pathological processes. In the process of adaptation to increased muscle activity cardiovascular system is one of the first to adapt to these conditions. At the same time, FA of varying intensity affects the structure and function both the heart with various vessels and blood system significantly [3,4]. The relevance of this problem is caused by both the practical use of physical activity of varying intensity and the need to understand the key mechanisms through which it affects the processes of atherogenesis [1].

Despite there is a large amount of studies devoted to vascular endothelial dysfunction, many questions are still unclear. In particular, the features of the development of endothelial dysfunction and the damage of other layers of femoral artery wall at a background of various physical activities are not studied.

Objective: to study changes of lipid spectrum of blood and degree of damages of femoral artery wall at physical activities of varying intensity.

Materials and methods. Study was performed with 30 white male outbred rats with the body weight of 180-240 g. Physical activities of high and moderate intensity (FAHI and FAMI) were simulated on 20 male rats by running in a treadmill at speeds of 36 and 24 m/min during 1 hour daily for 2 months according to the methodology. 10 animals were the control group. Experiments were carried out according to “General Ethical Principles of Animals Experiments”, ratified on the First National Congress of bioethics (Kyiv, 2001). Blood samples for experiments were taken fasting from the abdominal aorta under nembutal anesthesia.

For determination of total cholesterol (TCS), cholesterol of low density lipoproteins (LDL-C),

cholesterol of high density lipoproteins (HDL-C) in blood serum sets of firm "Olveks Diagnosticum" were used, and for determination of triglyceride (TG) and total lipids (TL) the sets of PLIVA-Lachema a.s. were used. Cholesterol of low density lipoproteins (LDL-C) was calculated by the Fridvald formula: LDL-C=TCS - HDL-C/2.2 [1], and the atherogenic factor (AF) was calculated by the formula of A.N. Klimov: AF = (TCS - HDL-C)/HDL-C [6].

Macroscopic evaluation of atherosclerotic changes of femoral artery carried out by the method of G.G. Avtandilov, and microscopic one was carried out on histological samples stained with hematoxylin-eosin and fuchsin. For statistical processing of the digital data modern computer program "STATISTICA 5.0" was used.

Results and discussion. Analysis of research works shows that different types of physical activities, in particular, of moderate or high intensity, both dynamic and static, influence the level of various lipid fractions in blood serum and the state of the endothelium of blood vessels significantly [1,2].

Studying the influence of physical activities of high and moderate intensity on the level of some lipid of blood system (Table) and on the structure of the femoral artery wall, it was noticed, that FAHI influence the mentioned parameters negatively, and FAMI influence positively. Thus, under the action of FAHI the level of TL increases on 26% ($p<0.001$), the level of TCS increases on 61% ($p<0.001$), the level of LDL-C increases on 52% ($p<0.001$), the content of triglycerides increases in 2.56 times ($p<0.001$), and the concentration of HDL-C increases on 141% ($p<0.001$). At the same time the atherogenic factor increases in 3.76 times ($p<0.001$) compared to the control group.

Due to some researches [2,5,6], the increased level of TG and TCS at FAHI is the compensatory reaction, which is aimed at power supply for skeletal muscles, working at the maximum tension. There is intensive mechanism of mobilization of fats from fat depots and their oxidation in skeletal muscle. This quickly leads to the formation of oxygen debt, development of the tension hypoxia and switching to anaerobic way of energy supply (anaerobic glycolysis) with development of metabolic acidosis due to accumulation of lactic and pyruvic acids. In such conditions fatty acids are not fully oxidized. At the metabolic acidosis the activity of lipoprotein lipase, which catalyzes hydrolysis of lipoproteins in the bloodstream, decreases significantly. The excess of fatty acids and lipoproteins in the circulating blood continues until the body returns to aerobic energy supply and capture of lipids in the liver is over.

Table

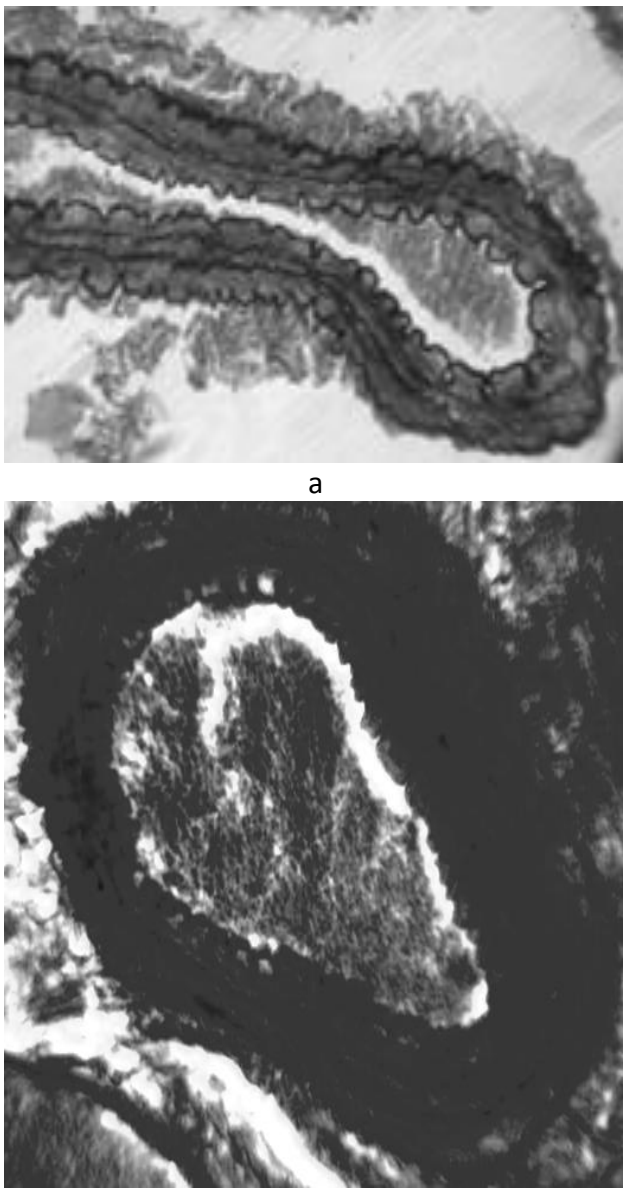
Content of lipids in the blood serum of various groups of the experimental animals (M±m)

Indexes	Control (n=10)	FAHI (n=10)	FAMI (n=10)
Total lipids, g/l	3,88± 0,13	4,90 ± 0,02*	3,75 ± 0,15
Total cholesterol, mmol/l	1,27 ±0,02	2,05 ±0,30*	1,20 ± 0,04
Triglycerides, mmol/l	1,21 ±0,03	3,10 ±0,01*	1,24 ± 0,04
LDL-cholesterol, mmol/l	1,14 ±0,01	1,73 ± 0,02*	0,80 ±0,01*
HDL-cholesterol, mmol/l	0,29 ±0,02	0,70 ± 0,08*	0,88 ±0,01*
Atherogenic factor	0,51 ±0,03	1,92 ±0,07*	0,36 ±0,05*

*Note: * - the changes are statistically significant (P <0.05) relatively to controls*

Increase in the content of atherogenic lipids in the blood serum causes damage of the inner vascular membrane, including the one of femoral artery, accompanied by development of endothelial dysfunction and significant violations of hemodynamics. The results of histological study show, that the main structural manifestations of damage of the intima are local destruction of the endothelial edema and separation of the inner elastic membrane, edema of the muscle membrane, local destruction of the external elastic membrane. These changes are well observed when comparing the damaged sample with the normal structure of femoral artery wall (Fig. 1a, b), which agrees to the literature data [4,6].

In contradistinction to FAHI, at FAMI there is a decrease in the concentration of TL in the



a

b

Fig. 1. Structure of the femoral artery wall of the control rat (a) ant its local damage at FAHI (b). Hematoxylin and eosin stain. Magnification: 8x10.

blood serum on 3,4%, of TCS - on 6%, LDL-C - on 30%, while the contents of TG and HDL-C increase on 2% and 203% accordingly. At the same time AF decreases in 1.42 times compared to the control. It is well known, that at FAMI red muscles with slow contraction type and dense capillary net, which fibers contain a lot of mitochondria, are mainly involved in the process of contraction. This provides a high level of aerobic oxidation in the muscles and reliable maintenance of homeostasis of the blood [3], which provides for optimal conditions for the transport processes. In this connection, moderate physical activities have antiatherogenic properties and provide normal structure of the walls of the blood vessels, which is confirmed by histological studies of femoral artery.

Thus, physical activity of moderate intensity have antiatherogenic character in the lipid spectrum of the blood serum. This is the result of enhancement of the blood flow through the capillaries of the muscles and activation of the endothelial lipoprotein lipase, which helps to eliminate atherogenic forms of lipids from the blood and to increase the content of lipoproteins with antiatherogenic properties in the blood [2,4,6]. Hence, physical activities of moderate intensity can be recommended to apply in order to correct the lipid changes of the blood system.

Conclusions. 1. Physical activities of high intensity are accompanied by the significant changes of lipid transport system of blood, which results in an increase in the content of TL, TCS, TG, LDL-C in blood serum, increase in the atherogenic factor and decrease in the concentration of HDL-C.

2. On the contrary physical activities of moderate intensity lead to an increase in the concentration of HDL-C and a decrease in the content of TL, TCS, TG, LDL-C and atherogenic factor.

3. Structural reorganization of the femoral artery wall at FAHI is characterized by the destruction and detachment of the endothelial layer, edema and stratification of the inner elastic membrane and muscle membrane, whereas there is no of these changes at FAMI.

Prospects for further research. It is planned to study the correlative relationships between the production of substances with multidirectional properties (NO and endothelin-1) by endothelium, and to study blood lipid spectrum at hypercholesterolemia and physical activity of varying intensity with its following confirmation by electron microscopy.

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INFLUENCE OF REDUCED GLUTATHIONE ON THE INTENSITY OF OXIDATIVE STRESS IN PATIENTS WITH ULCER OF STOMACH AND DUODENAL WITH FIXED METAL DENTURES

Abstract. *The efficiency of restored glutathione (hepaval) in the therapy of the patients with gastric and duodenal ulcer with fixed metal dentures has been studied. It was established that the use of hepaval is significantly more effective compared to the use of vitamin E on the stable inhibition of the lipid peroxidation and oxidative modification of proteins. Combined enteral use with local applications on the oral mucosa of restored glutathione showed higher efficiency on the rebalancing of oxidant-antioxidant system at the both levels: systemic and local, as a result of elimination of negative prooxidant effect of fixed metal dentures in patients with gastric and duodenal ulcer compared to enteral use of restored glutathione.*
Key words: *gastric ulcer, fixed metal dentures, restored glutathione.*

Introduction. Relevance of the study is caused by the high incidence of combination of dental disease and acid diseases (AD) of gastric (GU) and duodenal ulcers (DU), leading to tooth loss [2,5]. This process is caused by anatomical and physiological proximity of oral cavity and digestive tract (DT), community of innervation and humoral regulation, that create preconditions for the involvement of periodontal tissues in pathological process at AD [2,5,6]. Pathology of periodontal tissues is diagnosed in 92% of patients with GU and DU, and is mostly represented by generalized periodontitis, which can cause tooth loss [2].

Particularly, activation of lipid peroxidation (LPO) and of oxidative modification of proteins (OMP) in gastroesophageal acid reflux, inflammation of periodontal tissues, is associated with pathogenic influence of hydrochloric acid, proteolytic enzymes, microorganisms of plaque. This activation is manifested by the development of inflammation, tissue hypoxia, apoptosis acceleration and intensified desquamation of the epithelium of the mucous membranes both of the digestive tract and the mouth, significant imbalance of generation of elements of connective tissue, which fix the tooth, which in turn accelerates the loss of teeth [2,3,4]. Probably, the situation is complicated by the use of fixed metal containing dentures (FMD), as they provoke LPO and OMP and promote the intensifying of all known ways of progression of pathological states.

The development of ulcer and inflammatory disease of periodontium is a result of an imbalance between defense mechanisms and factors of aggression with a predominance of the latter [2,5]. Among the protective factors of periodontium and mucous membrane of gastroduodenal area the state of antioxidant system (AOS) is essential [1,6].

There are significant intensity of oxidative stress and significant imbalance AOS at the presence of FMD in patients with GU and DU. Thus, it would be logical to study the efficacy of antioxidants in order to test the working hypothesis about their positive influence on reducing of intensity of LOP and OMP.

Objective: to find out the efficacy of reduced glutathione (GW) in combined therapy of GU and DU in acute phase in the presence of fixed metal dentures by study of intensity of LPO and OMP in the blood and oral fluid.

Materials and methods. 45 patients with GU and DU in the acute phase of FMD were examined. For determination of efficiency of therapy 3 groups of patients were formed. The patients were randomly assigned by the age, sex, stage of ulcer and its current phase. The first group of patients (control 1-14 people) in addition to the traditional therapy of the underlying disease received 100 mg of vitamin E once a day for 30 days. The second group of patients (study 2-16 people) in addition to the traditional therapy of the underlying disease received restored glutathione (hepaval) enterally in a dose of 250 mg once a day for 30 days. The third group of patients (study 3 - 15 people) in addition to the traditional therapy of the underlying disease received 250 mg of RG (hepaval) enterally 1 time a day, and 250 mg RG locally as application to gums 1 per day (at night) for 30 days.

Content of molecular products of LPO – isolated double bonds (IDB) in compounds, diene conjugates (DC) – In the blood was studied by I.A. Volchehorsky et al., content of malonic aldehyde (MA) in oral fluid was studied by U.A. Vladymyrov, A.I. Archakov. Intensity of OMP in blood serum was determined by the method of Dubinina O.Ye. et al. in modification of the I.F. Meshchyshen. The activity of catalase was studied by M.A. Koroliuk et al. The enzyme activity was calculated per 1 g of Hb. Statistical analysis of data was performed using modern methods of variation statistics.

Results and discussion. Before therapy there is considerable intensity of oxidative stress in patients with GU and DU with FMD (Table. 1), which is significantly higher compared to those in patients with ulcer with intact tooth row ($p < 0.05$) and fixed metal-ceramic dentures ($p < 0.05$).

At the proposed therapy in patients with ulcer there is a significant decrease in the intensity of LPO (Table. 1). As a result of antioxidant action RG there is a more significant decrease in content of LPO products in the blood serum of patients of 2 and 3 groups already on the 30th day of therapy ($p < 0.05$): content of MA in the blood serum in 2 group decreased on 27.9% compared to initial values ($p < 0.05$), and the same content in 3 group decreased more significantly: on 30.7% ($p < 0.05$). Instead effect of vitamin E in the control group on parameters of intensity of LPO by the content MA in the blood serum reveals just a downward trend ($p > 0.05$). In one month after therapy MA content in the blood serum in patients of 2 and 3 groups are significantly lower (in 38.9% and 40.0% respectively ($p<0.05$)) compared to the content on the 30th day of therapy and compared to the content in patients of the control group ($p < 0.05$).

As to the influence of just enteral and enteral with local impact of RG, the analysis of the content of MA in the blood serum after therapy, and at all times of observations indicates the absence of significant changes ($p > 0.05$) (Table. 1). It should also be noted that the achieved stabilization of parameters in patients of 2 and 3 groups is stable and it can be observed even in 6 months after therapy ($p < 0.05$).

Contents of intermediates of LPO in the blood change more significantly under the influence of the therapy (Table. 1). Thus, content of IDB in the blood in patients of 1 group decreased in 1.4 times ($p<0.05$), in patients of 2 group – in 2.0 times ($p<0.05$), in patients of 3 group – in 2.1 times ($p<0.05$), with the existence of statistically significant intergroup differences only compared to group 1 ($p < 0.05$). The decrease in the content of DC in blood during therapy is the less: in patients of 1 group - on 11.4% ($p<0.05$), 2 group - on 39.1% ($p<0.05$), 3 group - on 39.8% ($p<0.05$) with the existence of statistically significant intergroup difference only compared to the parameters of 1 group ($p<0.05$). Thus, the efficacy of the proposed therapy with the inclusion of RG is higher compared to the use of natural antioxidants (vit. E) due to the intensity of effect on LPO.

The study of the effectiveness of combined therapy that includes RG showed a significant effect on the intensity of oxidative modification of proteins (Table 1). Thus, content of R-AKDNPH in the blood of patients in all groups decreased significantly compared with 1, 2, 3 groups, in 1.3 times ($p<0.05$), in 1.6 times ($p < 0.05$) and in 1.7 times ($p<0.05$) respectively with the reliable intergroup differences

Table 1

Parameters of intensity of lipid peroxidation and oxidative modification of proteins in blood of patients with GU and DU FMD during therapy (M±m)

Time of observation	Parameters, measurement unit	Groups of examined patients		
		Group 1 (n=14)	Group 2 (n=16)	Group 3 (n=15)
HI	MA, µmol/l	2.53±0.07		
	IDB, E220/ ml.bl.	2.62±0.03		
	DC, E232/ ml.bl.	1.46±0.02		
	R-AKDNPH	1.37±0.02		
	N-AKDNPH	14.13±0.15		
Before therapy	MA, µmol/l	4.52±0.13 *	4.48±0.12 *	4.50±0.14 *
	IDB, E220/ml.bl.	6.48±0.06 *	6.49±0.07 *	6.48±0.06 *
	DC, E232/ ml.bl.	2.70±0.06 *	2.74±0.05 *	2.69±0.06 *
	R-AKDNPH	2.68±0.03 *	2.70±0.02 *	2.69±0.03 *
	N-AKDNPH	21.51±0.45 *	22.18±0.39 *	21.29±0.43 *
After therapy	MA, µmol/l	4.03±0.12 *	3.23±0.18 */**/#	3.12±0.21 */**/#
	IDB, E220/ ml.bl.	4.50±0.14 */**	3.18±0.13 **/ #	3.07±0.14 **/ #
	DC, E232/ ml.bl.	2.39±0.07 */**	1.67±0.05 **/ #	1.62±0.02 **/ #
	R-AKDNPH	2.11±0.06 */**	1.72±0.04 */**/ #	1.57±0.03 */**/ #/ ##
	N-AKDNPH	19.21±0.54 */**	16.10±0.37 */**	15.32±0.32 */**/ #
In 1 month after therapy	MA, µmol/l	3.93±0.41 *	2.75±0.15 **/#	2.67±0.13 **/ #
	IDB, E220/ ml.bl.	4.12±0.17 */**	3.08±0.11 */**/#	2.85±0.02 **/ # / ##
	DC, E232/ ml.bl.	1.96±0.13 */**	1.59±0.07 **/ #	1.48±0.06 **/ #
	R-AKDNPH	1.93±0.09 */**	1.50±0.02 */**/ #	1.39±0.01 **/ # / ##
	N-AKDNPH	18.53±0.48 **	15.45±0.12 */**/ #	14.67±0.11 **/ # / ##

*Notes. 1. * - the difference is statistically significant compared to the parameters in healthy individuals (P <0.05); ** - the difference is statistically significant compared to the parameters before treatment (P <0.05);*

 # - the difference is statistically significant compared to the parameters in group 1 patients after therapy (P <0.05);

- the difference is statistically significant compared to the parameters in group 2 patients after therapy (P <0.05).

2. Measurement units of content in of R-AKDNPH and N-AKDNPH blood - opt.un.g / l of protein.

between all groups (p <0.05). The content of N-AKDNPH during therapy also decreased significantly: in 1, 2, 3 groups, in 10.7% (p<0.05), in 27.5% (p <0.05) and in 28.1% (p<0.05) respectively with the reliable intergroup differences between all groups (p<0.05). The mentioned indicates not only higher efficiency of RG compared to vitamin E, but also significantly higher efficiency of combined enteral and local use of RG on intensity of systemic oxidative stress in relation to impact on OMP. Moreover, the decrease in intensity of OMP is stable only in patients of 2 and 3 groups throughout all the observation period.

While study of the contents of studied parameters in oral fluid the significant decrease in the intensity of LPO in the oral mucosa at the proposed therapy is was found out (Table 2). The result of antioxidant action of RG is higher statistically significant decrease in content of LPO products in the oral fluid, especially in patients of 3 group already after 30 days of

therapy (p<0.05). Thus, the content of MA in group 1 patients is not statistically significant. In group 2 MA content decreased on 27.5% compared to initial values (p<0.05) and similar parameters in the 3rd group decrease even more, on 45.2% (p<0.05), with the intergroup differences when compared among all groups (p <0.05).

In 30 days after treatment MA contents in oral fluid in patients 2 and 3 groups are significantly lower (on 32.9% and 46.2% (p<0.05)), compared to the parameters before therapy, and compared to the parameters in the control group of patients (1) (p<0.05).

Table 2

Parameters of intensity of lipid peroxidation and antioxidant system state in the oral fluid of patients with GU and DU with FMD during therapy (M±m)

Time of observation	Parameters, measurement unit	Groups of examined patients		
		Group 1 (n=14)	Group 2 (n=16)	Group (n=15)
HI	MA, µmol/l	1.50±0.23		
	DC, µmol/l	12.21±1.15		
	Catalase, mmol/min·l	2.72±0.19		
Before therapy	MA, µmol/l	4.23±0.21 *	4.25±0.17 *	4.23±0.19 *
	DC, µmol/l	39.42±1.63 *	39.79±1.57 *	40.08±1.66 *
	Catalase, mmol/min·l	1.46±0.07 *	1.44±0.09 *	1.43±0.08 *
After therapy	MA, µmol/l	4.15±0.24 *	3.08±0.13 */**/ #	2.32±0.12 */**/#/ ##
	DC, µmol/l	32.50±2.14 */**	25.18±2.19 */**/ #	14.22±1.14 **/ #/ ##
	Catalase, mmol /min·l	1.89±0.04 */**	2.37±0.03 **/ #	2.62±0.02 **/ # /##
	MA, µmol/l	3.97±0.35 *	2.85±0.15 */**/#	2.27±0.13 */**/ #/ ##
	DC, µmol/l	31.86±2.42 */**	18.53±1.17 */**/#	12.85±1.08 **/ # / ##
	Catalase, mmol /min·l	1.96±0.15 */**	2.49±0.05 **/ #	2.68±0.04 **/ #/##

*Notes. * - the difference is statistically significant compared to the parameters in healthy individuals (P <0.05); ** - the difference is statistically significant compared to the parameters before treatment (P <0.05); # - the difference is statistically significant compared to the parameters in group 1 patients after therapy (P <0.05); ## - the difference is statistically significant compared to the parameters in group 2 patients after therapy (P <0.05).*

As to the influence of just enteral and enteral with local impact of RG, the analysis of the content of MA in oral fluid after therapy, and at all times of observations indicates the presence of significant changes (p<0.05) (Table. 1). Increase in the content of intermediates of LPO (DC) in oral fluid during therapy is higher than in the content of the final products (MA).

Thus, there is a decrease in DC content in 1.2 times in patients of Group 1 (p<0.05), in 1.6 times - in group 2(p <0.05), in 1.8 times - in group 3 (p<0.05) with the statistically significant intergroup difference not only compared to group 1 (p<0.05), but when comparing parameters between 2 and 3 groups (p<0.05). Thus, the efficacy of the proposed therapy with the inclusion of RG is higher not only in comparison with the use of natural antioxidants

(vit. E) by the intensity of influence on parameters of lipid peroxidation, but also at the combined use of RG enterally and locally by application to the oral mucosa in comparison with the only enteral use. The confirmation is considerable increase in the activity of the factors of LPO, particularly to treat oppressed catalase in oral fluid of patients with ulcer under the influence of combined therapy with RG (Table 2). During therapy the activity of catalase in patients of 1 group increases in 1.3 times ($p<0.05$), 2 group - in 1.6 times ($p<0.05$), 3 group - in 1.8 times ($p<0.05$), that is the maximum of groups. Probably, these results may indicate the patients obtained the double dose of RG, as applied locally, RG eventually absorbed into the systemic blood flow and produced its systemic antioxidant effect, but the significant difference between parameters in groups 2 and 3 while studying of markers of OMP and LPO in systemic blood flow has been found out.

Conclusion. 1. Use of the reduced glutathione (hepaval) in the combined therapy of patients with GU and DU with the FMD is significantly more effective than the use of natural antioxidants (vitamin E) by a stable inhibition of processes activated by lipid peroxidation and oxidative modification of proteins.

2. Combined enteral and local use of glutathione reduced as applications on the oral mucosa showed higher efficiency compared to enteral use of glutathione as to restore of the balance of oxidant-antioxidant system both at the systemic and at the local levels, due to the elimination of negative prooxidant effect of fixed metal dentures in patients with gastric ulcer and duodenum ulcer.

Prospects for further research. Further studies may be devoted to evaluation of possible effect of reduced glutathione on parameters of antioxidant and detoxification systems of patients with gastric ulcer and duodenal ulcer that use fixed metal dentures.

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STRUCTURAL CHANGES IN OSSEOUS AND CARTILAGINOUS TISSUES OF THE MANDIBLE UNDER EFFECTS OF HEAVY METALS SALTS ON THE BODY

Abstract. *We have studied morphological changes in the osseous and cartilaginous tissue of the lower jaw of 72 experimental male albino rats whose bodies were influenced by the salts of heavy metals. We used anatomical, osteometric, spectrophotometric, histological, histochemical, morphometric, immunomorphological and statistical methods. It is shown that heavy metals salts cause the development of deep and persistent structural changes in the osseous and cartilaginous tissues of the mandible, which is accompanied by inhibition of growth, development of resorptive processes in the osseous tissue, reduced mineralization, depletion of inorganic matrix in macro- and microelements. Negative morphological changes develop in the process cartilage: zonal structure is impaired, proliferative activity of chondrocytes is inhibited.*
Key words: *osseous tissue, mandible, heavy metals salts.*

Introduction. Due to the intensification of chemical and mining industries, the environmental pollution increased and the antropogenic impact of chemical xenobiotics, including salts of heavy metals (SHM) on the human body and a damage of different organs and systems have grown [1,2]. Hygienic, Epidemiological and clinical studies have shown that heavy metals play a vital role in the occurrence of many diseases of the human body [7]. However, the features of the reaction of the dental system hard tissues under the effects of SHM on the body have not been studied sufficiently, even though it is shown that amalogenesis gets impaired under the influence of SHM as well as the incidence of dental caries in the areas, where the content of these chemical pollutants in the environment is increased, grow [5,6]. However, the literature does not give a detailed analysis of structural changes in the dentin under the influence of SHM [8].

Objective: to establish the features of structural changes in osseous and cartilaginous tissues of the mandible under the effects of heavy metals salts on the body.

Materials and methods. The study was conducted on 72 laboratory mature male albino rats in accordance with regulations adopted by the European Convention for the Protection of vertebrate animals used for scientific purposes (Strasbourg, 1986) and the Law of Ukraine "On protection of animals against cruelty» № 3477-IV of 21.02 .2006. The rats of the control and experimental groups were on a standard diet. The experimental animals received a combination of heavy metals found in excessive amounts in the northern Sumy region (increased amounts of zinc, copper, iron, manganese, lead, chromium). We used the anatomical, osteometric, spectrophotometric, histological, histochemical, morphometric, immunomorpho-

logical and statistical methods.

Results and discussion. In case of the SHM impact on the body, there is an inhibition of growth processes in the mandible and its formation. Lagging in osteometric indices of experimental animals compared to the intact rats remained at 5.02% - 8.58% (p <0.05).

At the microscopic level in the tissues of the lower jaw of experimental animals we observed changes of the structure, inhibiting proliferative activity of chondrocytes, which sometimes disappeared completely, atrophied or segregated in separate isogenic groups with very low proliferative activity. The number of cells reduced dramatically: they were irregularly shaped, the contours of chondrocytes were destroyed in some places, mitosis figures were hardly observed. The cells were mostly polygonal, there was a great number of layers of connective tissue and debris of damaged cells around them. The total width of the cartilage reduced by 4,89% (p <0,05).

There were signs of appositional growth inhibition in the compact substance as well as slower transformation of the membrane reticulated and osteoid osseous tissue into a splenial one. The ossification of intermediate osseous substance got impaired. The deformed and altered lines of adhesion, mosaic areas of calcification became clear. We marked disorders and inhibition of formation of secondary osteons, and, in contrast, an increased number of primary osteons, as evidenced by a decrease in their diameter and increased width of haversian spaces. The resorption cavities appeared.

In our view, the suppression of protein synthesis function by osteogenetic cells plays a significant role in the mechanism of development of deep regressive morphological changes in the osseous and cartilaginous tissues of the mandible influenced by SHM. It is confirmed by the low expression of group S100 proteins, since these proteins are actively involved in shaping the osseous tissue by mineralization of cartilage due to their ability to bind to calcium ions [3]. By forming both homo- and heterodimers, S100 proteins form complexes with proteins Ca2 + and Zn2 +. Entrapping these ions can change the spatial organization of S100 protein and provides connectivity to various proteins - targets and enhance their biological action [4]. Thus, by reducing the expression of calcium in the binding protein S100 in the osteogenetic cells the mineralization of the osseous tissue gets impaired and regressive morphological changes develop in it.

The results of chemical analysis of the osseous tissue showed that a reduction of calcium ions and basic osteotropic microelement zinc, which are forced out of hydroxyapatite crystals by cations of heavy metals due to their accumulation are observed in the crystal lattice of hydroxyapatite. It causes a depletion of bone inorganic substances. After a month of heavy metals impact on animals, we found deep disturbances in macro- and microelement composition of the lower jaw and the incisor. Changes in the chemical composition of the experimental organs reach significant values - 15,32% – 17,49% (p <0,01).

Thus, the intake of excessive SHM (chromium, manganese, iron, zinc, copper and lead) results in disturbances of the chemical composition of mineral component of the mandible and trace element metabolism in the experimental dentition organ, resulting in profound morphological changes in the crystal lattice. This, in turn, manifests itself by growth inhibition, deffects in the structure and shaping of the lower jaw and the incisor.

Conclusions. Organometric and histomorphometrical indicators, the chemical composition of the lower jaw under the effect of heavy metals on the body demonstrate the development of deep and persistent structural changes in the osseous and cartilaginous tissues. We observe inhibition of growth, development of resorptive processes in the tissue, reducing mineralization, depletion of inorganic matrix of macro- and microelements. Negative morphological changes develop in the process cartilage: zonal structure is impaired, proliferative activity of chondrocytes is inhibited, the width of the cartilage and some of its zones reduces by 4.80% -9.77% (p <0.05).

Prospects for further research. Considering

the proposed mechanism of the development of changes in the osseous tissue under the influence of SHM to study the ways of correction of changes in the bone tissue.

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SEX FEATURES OF INTERMITTENT HYPORBARIC HYPOXIA ACTION ON OXIDATIVE MODIFICATION CONTENT AND ON PRODUCTS OF LIPID PEROXIDATION IN THE GUM TISSUES OF RATS UNDER THE PHOTOPERIOD OF DIFFERENT DURATION

Abstract. *We have established in experiments on albino rats of different ages and sex that a systemic continued intermittent hypobaric hypoxia causes a slight decrease in the intensity of lipid and protein peroxidation and a significant reduction in enzyme activity in the gum tissues. Antioxidant-prooxidant index indicates the superiority of antioxidant activity in the tissues of the gums, and, respectively equal, to the strengthening of the resistance processes in these tissues.*
Key words: *age, rats, hypoxia, gums, photoperiod.*

The condition of epithelial tissues and mucous membranes covering the mouth largely depends on the systemic organismal factors, including the influence of hormonal factors, which have drawn an increasing attention. Effects of estrogen and progesterone of both endogenous origin and hormones of replacement therapy and contraceptives on the periodontal tissue of the female body in different ages including puberty period have been described in details [1]. When the body is affected by damaging factors, including bacteria, hypoxia and others, exept LP, an activation of oxidative modification of proteins (OMP) occurs. After the oxidative modification proteins become highly sensitive to proteolysis. Therefore, the determination of the total proteolytic activity (TPA) has been widely used in modern experimental dentistry [2, 3]. The systemic prolonged intermittent hypobaric hypoxia caused a slight decrease in the intensity of lipid and protein peroxidation and a significant decrease in activity of antioxidant enzymes in the gum tissues of immature male rats [4].

As the research of recent years has shown, hypoxia is seen not only as a damaging factor. Intermittent hypoxia becomes more practical as a factor for strengthening resistance under conditions of hypoxic hypoxia. It is based on the double function of ROS, which are formed under the influence of hypoxia. On the one hand, excess formation of ROS causes an oxidative stress, destroying structures of cells, including lipids and membranes, proteins and DNA. On the other hand, various processes, caused by low or moderate concentrations of ROS protect cells from oxidative stress caused by the same ROS and contribute to the restoration or maintenance of "redox balance" ("redox homeostasis") [5].

Objective: To determine functional properties of gum tissue of rats depending on age and gender and performance of the systemic intermittent hypobaric hypoxia and photoperiod of various duration.

Materials and methods. The study was conducted on 42 albino nonlinear sexually mature and immature male rats and sexually mature and immature females. The research was performed in compliance with the main provisions of GLP (1981), Rules of work using experimental animals (1977), Council of Europe Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes of 18.3.1986, EEC Directive №609 of 24.11.1986 and MHP of Ukraine №281of 01.11.2000.

We developed an original technique with simultaneous using the long-term intermittent hypoxic exposure to changes in photoperiod with different directions. We used a hypobaric hypoxia which was equivalent to an altitude of 4000 m above sea level, which was created in a transparent flow-type pressure cell by sucking air out with a vacuum compressor. The rate of "lifting" the animals to a given value was 24 km / h. Sessions of hypoxia lasting 2 h. were conducted every day from 9.00 to 11.00 for 14 days against the background of three modes of

lighting: natural light, typical for spring-summer period with duration of light period of day 15 hrs., continuous round clock lighting with intensity of 500 lux and permanent round clock darkness. The changed lighting mode was introduced a day before the first session of hypoxia, and the animals stayed 15 days under the changed photoperiod.

On the next day after the last session of hypoxia the animals were taken out of the experiment by decapitation under a light ether anesthesia. Euthanasia was performed in the morning from 9.00 to 12.00 for all groups of animals. After the exsanguination, the cold gum tissue samples were weighed on a torsion balance and homogenized in 1.2 ml of cooled TAE buffer and in 2 ml of chilled borate buffer. The homogenate was frozen and stored in a freezer at - 20˚C until it was used in a laboratory research.

Results and discussion. Under the normal lighting sex differences in the contents of OMP products were found in the gum tissues. For instance, the content of main oxidation-modified proteins in males was by 9.0% higher compared to females ($p < 0.05$), and the neutral ones were the same in both sexes.

Under the influence of intermittent hypobaric hypoxia such differences became even more pronounced. The immature female rats responded less actively on 14-day hypoxia than males. The females tended to have an increase in the main OMP products by 25.0% ($p > 0.05$), whereas the males experienced significant reduction in both neutral products ($p < 0.03$), and the main OMP ($p < 0.05$). The response of the animals to hypoxia is an important test (factor) for the ability of the gum tissue to withstand external hazards. Hypoxia is one of such natural factors. Reducing the partial pressure of oxygen leads to the production of reactive oxygen species (ROS), to formation of free radical compounds that have strong damaging effects on cells and tissues. At the same time, we know that the ROS have a dual function: their excessive production leads to oxidative stress and damage cellular structures; their low or moderate number is necessary for the formation of the defence, including the antioxidant one [5].

Considering the above, the results of the effects of hypoxia on the contents of OMP products in gum tissues of female rats can be regarded as a failure of the antioxidant system to resist the increasing content of OMP products, while the defence system in males turned out to be more effective. To some extent it is consistent with the existing idea that testosterone has a protective effect on the gums and periodontal tissues in general [1]. Since our results on the effects of hypoxia on the content of OMP products in gum tissues were obtained in immature animals, in which the testes do not produce testosterone yet, then, perhaps, this protective ability is a property of a male body that is genetically determined.

Changes in photoperiod of keeping animals duration have a significant impact on the content of oxidation-modified proteins in the tissues of the gums, provided the impact of intermittent hypobaric hypoxia on the animals. This is especially evident in a series of experiments, when hypoxia was used under 15 day darkness to which the animals were exposed (stimulation of pineal melatonin production). The females of this series of experiments had a significantly lower content of OMP products in the gum tissues (the main one by 22.9% and the neutral one by 16.5%) compared with animals that were only exposed to hypoxia ($P < 0.05$). Under the similar conditions of experiments the content of OMP products in the gum tissues of male rats remained the same. However, a combined effect of hypoxia and darkness reduced significantly the content of OMP products in the tissues of the gums (main OMP by 21.9% neutral one by 23.2% ($p < 0.05$)) in male rats compared to intact animals, kept in conditions of normal light and barometric pressure. In females OMP process in the gum tissues under conditions of combined effect of hypoxia and darkness was the same as in the intact animals. We have not found sexual features as to the content of OMP products in the gum tissues in the experiments while using the action of hypoxia against the background of 15-day lighting ("physiological" hypophys-ectomy).

The results led us to the need to introduce differentiation factors that cause activation of lipid peroxidation and oxidative modification of proteins into two groups in both sexes: 1) hormonal ones, caused by respective sex hormones available in the relevant sex; 2) non-

hormonal ones, which occur and act on the gum tissues in immature animals.

Conclusions. There are some gender features as to the defense system response in the gum tissue of sexually immature animals. The female animals respond with a significant reduction in products of lipid peroxidation and activity of antioxidant enzymes, deterioration of general condition of gum protection system and the lack of changes in content of oxidation-modified proteins in the gums to continued lighting. Males respond to light much less actively than females by a reduction of diene conjugates alone and the activity of antioxidant enzymes, with decreasing content of oxidation-modified proteins and proteolytic activity of the growth of collagen. Under long darkness sex differences in physiological state of the system to protect the gums appear even more clearly. Females respond to the darkness by reducing the content of diene conjugates in the gums, as well as malondialdehyde awhile the antioxidant enzyme activity and the content of oxidation-modified proteins remain unchanged and the integral indicator of the antioxidant-oxidative status is high. Males in the dark respond by high levels of lipid peroxidation products in the gums; the activity of antioxidant enzymes remains unchanged, and the levels of oxidation-modified protein and antioxidant-oxidant index reduce.

Prospects for further research. A study of gender and age features in the response of the gum tissues on hypoxia based on stomatological practice as to various lesions depending on sex and age.

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CONDITIONS OF EFFECTIVE DEPOSITION OF SUPERHARD MATERIALS IN MEDICINE

Abstract. *In order to obtain qualitative heterogeneous and superlattice structures we developed a method of formation of periodic structures by irradiating with a train of laser pulses. The theoretical and experimental study of the authors in the field of depositing films can be implemented in devices designed for superhard wear-resistant superlattice structures and be used in forming surfaces for implants.*

Key words: *superhard materials, irradiating, implants.*

Introduction. Obtaining superhard and high-strength material layers on the surfaces of implants, crowns and other structures is one of the major problems in Dentistry and Implantology. As a result of applying these layers, service properties of implants substantially improve, especially their wear resistance, fatigue strength and resistance to aggressive environments, which directly affect their operation life and use in tougher conditions.

To optimize the process of applying superhard layers of materials on implants and dental crowns we need information on basic parameters of vapor condensing on the substrate (base of the implant). However, those few previous papers [1,2], which had studied the properties of films obtained by sputtering the target with nanosecond laser pulses, had not practically studied the properties of the vapor phase At the same time, mass spectrometer and probe studies of the vapor phase allow identifying several important parameters of the condensation, optimizing the process of deposition and clarifying the understanding of the physics of evaporation in complicated targets.

Objective: To develop a method of forming periodic structures in irradiation with a train of laser pulses, which ensures qualitative heterogeneous and superlattice structures.

Materials and methods. Techniques for obtaining periodic thin-film structures based on liquid-phase, gas phase and molecular beam epitaxy occupy an important place in modern microelectronics. We have obtained periodic structures (mirrors of the soft X-ray band) in a multiphase laser deposition of films at a speed of 5-10/pulse. In this case the structures are formed over a long time, which imposes strict requirements to the vacuum in the working plants p≤10-9-10-10 torrs. To enhance the technological process of obtaining multilayer structures, to reduce requirements for vacuum conditions, we have proposed and implemented a way to create such periodic structures using a train of laser pulses [2].

Results and discussion. The most acceptable practical realization of the proposed method [1] is a deposition of the periodic structure with a train of pulses of nanosecond duration with the inter-pulse interval 10^{-5}-10^{-4} c. To achieve this goal we use both serial modified solid-state lasers (ruby, neodymium ones) operating in the self-oscillating mode and a CVL with average power P_{aver}=100 Вт.

In the condition when the descending part (slow of thecomponent) of the plasma bunch of the less remote target gets condenced on the substrate before the atoms from the rising edge (fast of the component) of the plasma bunch from a remoter target. This condition can be written down as

$$K = \frac{l_1}{l_2} \le \sqrt{\frac{m_2}{m_1}} \cdot \sqrt{\frac{E_{1\min}}{E_{2\max}}}, \qquad (1)$$

where l_1, l_2 are distances to the target, m_1 i m_2 – mass of the deposited material, $E_{1\min}$ and $E_{2\max}$ – minimum and maximum energy of laser plasma components.

This excludes interaction of plasma bunches with different targets during their transit to the substrate, providing an alternate deposition of layers with sharp borders. The total heads, emerging in this case, as well as energy activation of the substrate and, consequently, lower temperatures of oriented growth [2], reduce the diffusion process between the layers, which helps to ensure sharp boundaries between the layers.

For the proposed technique of deposition the requirements for vacuum conditions reduce dramatically. When the excess pressure in the vacuum chamber p≈10^{-5} torrs during the layers deposition Δt=10^{-5}-10^{-4} c on100 mm^2 of the surface will be deposited with residual gas molecules not more

$$\sigma = \frac{1}{4} nV\Delta t = \frac{p\Delta t}{\sqrt{2\pi mkT}} = 4\cdot 10^7 - 4\cdot 10^8$$ см$^{-2}$, (2)

where n – vapor density, V – speed of vapor components in a stream of plasma, m – molecular mass of the component, k – Boltzmann constant, T – plasma temperature.

This is 10^{-7}-10^{-6} of the monolayer, i.e. the requirements for vacuum conditions are only determined by the conditions of transit of plasma bunches through the target-substrate distance without collision with residual gas molecules. The condition (2) can be written down in an easy to use practical form $\gamma = P\Delta t \le 2{,}5\cdot 10^{-9}$ (torrs·s).

This method was implemented in the diposition of two-component periodic structure Si-SiC. We used a laser on neodymium glass, working in regular pulsations mode with τ=30 ns and an interpulse interval t=9-11μs (5-6 pulses in a train with energy of 0,5 J), and a laser LTY PT-7 with 12.5 Hz repetition rate. Targets (Si, SiC), and the substrate (cleavage KC1) were placed in a high-vacuum chamber, connected to transit time mass spectrometer (residual pressure in the system 5·10^{-6}-10^{-5} torrs). Working laser power density q=1,5·10^8 Вт/cm^2 $E_{1\min} = E_{2\min} = 10$ eB, $E_{1\max} = E_{2\max} = 200$ eB.

In our experiments (for the best mode) by (2) the distance substrate- target Si was L1 = 16 mm and the distance substrate –SiC- target - L1 = 50 mm. Radiation was divided by means of a light diffusing wedge so that 0.1 of total energy of the laser pulse fell on the Si-target, and 0.9 - on SiC-target. We also followed the criteria of periodic structure purity.

We have also carried out experiments to check the possibility of obtaining periodic structures both while holding the optimal conditions and violating them. The results are shown in Table.

Implementation of the discussed methods of laser deposition of films from synchronous torches can result into devices for heterogeneous [1] and superlattice [2] structures. The quality of a periodic structure was controlled by ion-photon spectroscopy. The resulting structures were bombed by Ar + ions, and their etching and purity of deposited layers was controlled by glow of the spectral line SiIλ288,2 and by lines of impurities in the

Table

Results of experimental deposition

Order №	$K = \frac{l_1}{l_2}$	p, torr	Δt, c	γ=p·Δt $\gamma_{опт}$=2,5·10^{-9}, torr·c	Positive effect	Note
1	0,32 (optimum)	10^{-5}	10^{-5}	10^{-10} γ<$\gamma_{опт}$	+	–
2	1	10^{-5}	10^{-5}	10^{-10}	–	overlapping plasma bunches

	$K > K_{onm}$			$\gamma<\gamma_{опт}$		
3	0,32 (optimum)	10^{-5}	$8\cdot10^{-2}$	10^{-6} $\gamma>\gamma_{опт}$	–	rooting impurities of residual gas in the spectrum of available lines N, C, O

spectrum. When the criteria (1) and (2) were fulfilled, as shown in the table. 1 № 1, we received a periodic structure of 6 Si layers and 6 SiC layers (optimal mode of deposition).

Failure to comply with condition (2), as shown in the table. 1 № 2, leads to overlapping plasma bunches and, consequently, the erosion of the periodic structure (mode of overlapping plasma bunches).

Conclusions. The results of theoretical and experimental studies indicate the possibility to obtain periodic superhard structures by irradiating laser pulses with a train in case of complying with elaborated conditions, the violation of which leads to no positive effect. Deposition of films from synchronous torches and development of devices designed to obtain superhard wear-resistant superlattice structures is one of the ways of embodiment of these theoretical and experimental studies. Such structures of previously modeled properties can be formed on the surfaces of the implants, dental crowns, prosthetic elements.

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HISTO-ULTRASTRUCTURAL ORGANIZATION OF NEUROMUSCULAR SYNAPSES OF THE MASSETER IN EXPERIMENTAL DIABETES MELLITUS

Abstract. *The paper deals with a study of the evolution of histological and ultrastructural changes in neuromuscular synapses (NMS) in streptozotocin-induced diabetes (SDM). We used histological and electron microscopic methods. It was established that on the 14th -28th days of SDM reactive changes dominated in the NMS, and they are characterized by a reduced length of the synaptic contact, perimeter of the axon terminal, the number and distance between the folds of the postsynaptic membrane. In the long term of SDM (42 days) the above changes become more intense, increasing dystrophic destructive processes in axon terminals and neurolemmocytes, which is confirmed by morphometry findings.*
Key words: *SDM, neuromuscular synapsis, muscle fiber.*

Introduction. The study of structural changes in organs and tissues in diabetes mellitus (DM) is one of the urgent problems of modern medicine [2]. Ukraine has officially registered more than 1 million diabetic patients, or 2% of the adult population [9]. The sharp rise in the prevalence of diabetes requires a deep study of the problem. The universal diabetic microangiopathy is one of the most frequent prognostically unfavorable manifestations of DM in which the vessels lesions of various organs are dominant [9]. In numerous scientific publications there are thorough data about the morphological manifestations of vascular disorders [8] in skeletal muscle in case of DM. However, almost no attention is paid to morphological changes in neuromuscular synapses (NMS) in the masticatory muscles (MM) in this disease [16]. There are no publications on the dynamics of morphometric changes of components of the NMS of MM in laboratories and in particular with streptozotocin-induced diabetes (SDM).

Objective: to study the dynamics of histo-ultrastructural changes in the NMS of MM during the development of streptozotocin-induced DM.

Materials and methods. The material for the study included the MM of twenty 12 month-old male Wistar rats, which were divided into 2 groups: the experimental and the intact ones. SDM in the experimental animals was simulated with a single intraperitoneal administration of streptozotocin (dissolved in 0.1 M citrate buffer with pH 4.5) at a dose of 6 mg per 100 g of weight [6]. The level of glucose in the experimental group of animals was measured daily in a drop of blood from the caudal vein using test strips for blood glucose meters by "Assu-Shec" (Germany). We selected the animals with the glucose rate higher than 13 mg / L for our study and sampled the material on the 14th, 28th, and the 42nd day of the experiment. We used histological and electron microscopic techniques. NMS was histologically found on tangential cuts of MM by impregnation with silver nitrate after Bilshovsky Gross. For the electron microscopic examination pieces of the material were fixed in 2% osmium tetroxide solution, and contrasted by a generally accepted method. Morphometry was performed on the electron-diffraction pattern using NIH USA "Image J" manually, taking into account the zooms. The computer data processing was performed using Statistical Package Stat.Soft.Inc; Tulsa, OK, USA; Statistica 6.

Results and discussion. As our study showed, the response of the NMS in SDM manifests itself on all structural components and has a strong

dynamic. After 14 days of SDM along the myelinated nerve fibers (MNF) on the histological specimens irregular narrowings and expansions appear. At the ultrastructural level in NMS, axon terminal perimeter is significantly reduced, so is the length of synaptic contacts, as well as the width and length of active zones of the presynaptic membrane (Table. 1). In the axoplasm the matrix of mitochondria is seen and their cristas get fragmented.

Table

Morphometric characteristics of axo-muscular synapses of the masseter at different times of SDM (M ± m, n = 20)

Structural elements and their parameters	intacts	duration		
		14th day	28th day	42nd day
Terminal perimeter ,microns	7,4± 0,02	7,1± 0,01#	6,7± 0,01#*	5,3± 0,01#*
Length of synaptic contact, microns	2,7± 0,01	2,4± 0,01#	2,1± 0,01#*	1,7± 0,02#*
Number of postsynaptic membrane folds	10,5± 1,23	9,9± 1,12#	8,8± 1,12#*	7,1± 1,01#*
The distance between the folds, microns	0,22± 0,004	0,26± 0,001#	0,31± 0,001#*	0,42± 0,002#*
The length of a fold, microns	2,7± 0,11	2,5± 0,08#	2,2± 0,08#*	1,9± 0,05#**
width of the active zone, microns	0,23± 0,001	0,21± 0,001	0,19± 0,001#*	0,14± 0,001#*
length of the active zone, microns	0,81± 0,003	0,78± 0,004	0,72± 0,004#*	0,54± 0,004#*
Number of vesicles throughout the active zone	165,3± 11,7	145,6± 6,52#	129,9± 6,35#*	102,1± 5,03#*
Number of vesicles in the active zone area	10,7± 0,33	9,8± 0,29#	9,1± 0,29#*	6,2± 0,22#*

Note: 1) #P <0,05 – reliability of the difference compared to the indices in the intact animals;
*2) *P <0,05; **P <0,01 – reliability of the values compared to the previous time of the experiment.*

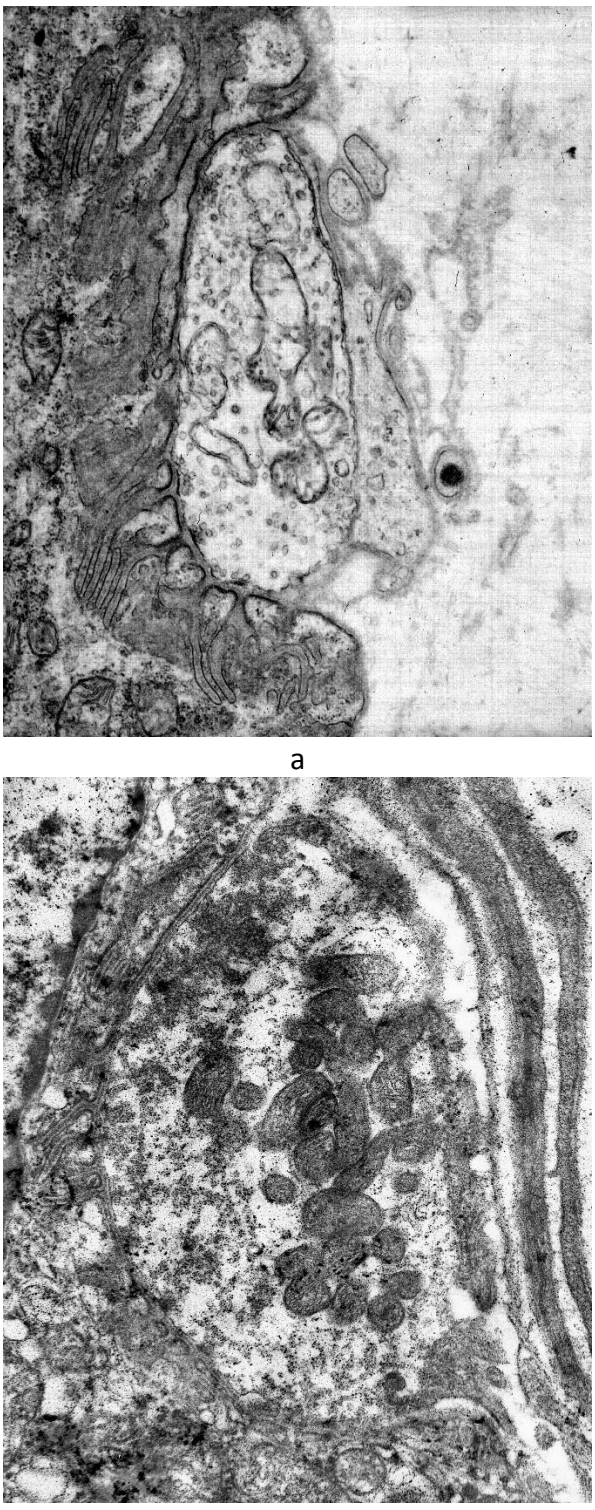
We found a swelling and separation of the myelin sheath (MS), which, according to many authors [3, 5], is a cause of its varicose. The chromatin in the neurolemmocyte nuclei condenses, a partial vacuolization of cytoplasm and the infiltration of mitochondrial matrix are observed. In the postsynaptic membrane the distance between synaptic folds increases due to their partial destruction. Changing the ultrastructural structure of the end neurolemmocytes in the experimental animals shows the development of stress reactions into a change in carbohydrate metabolism in SDM. An analysis of studies of a number of authors [1, 5] specifies that compensatory-adaptive response of neurolemmocytes results in hypertrophy of the morphological structures that are designed to ensure a sufficient level of synthetic processes in SDM.

On the 28th day from the start of simulating SDM the frequency and magnitude of MNF varicose on the histological specimens increases, particularly their preterminal departments, whereas the axon sprouting reduces. In electron microscopic examination the periaxonal space in the MNF is unevenly expanded. The degree of aggregation of filamentary-tubular structures in the axoplasm increases. This process is considered by some authors [1, 14] to be an outcome of axon transport disorders. O.S. Sotnikov and co-authors [10] indicate that neurofilaments aggregation and destructuration of microtubules occur under high acidity of the axoplasm, which, in turn, is the result of a release of oxidized products of protein

metabolism in the intercellular space, as a reflection of the distorted features of neurolemmocytes. Due to this stress reaction the cytoplasm of neurolemmocytes becomes oveloaded with lots of vesicles of different sizes [3], and the MS gets stratified [3, 14]. Degradation of MS is a nonspecific sign and a manifestation of a severe disturbance of phospholipid metabolism in the nervous system [4].

On the 28th day from the start of simulating SDM the total number of synaptic vesicles in the axoplasm of presynaptic membrane reduces. They usually have a different shape and small size. The synaptic fissure gets unevenly widened, the processes of the terminal neurolemmocytes grow into it, most of the postsynaptic membrane folds disintegrate. The number of mitochondria near the postsynaptic membrane decreases, they have small size and fragmented cristas (Fig. 1a). On the one hand it can serve as a morphological substrate of the disturbances in oxidative metabolism in SDM, on the other hand it explains the MM hypotrophy, resulting from impaired energy supply, axon transport delays, reduction of neurotransmitter and destructive changes in mitochondria [5, 11]. According to morphometry the terminal perimeter in this period decreases by 30.2%, and the length of synaptic contacts by 33.3%, resulting from a decrease in the number of subcellular components (see. Table. 1). According W.P. Hurlbrat [12] and H. Takekura et al. [15] the number of neurotransmitter vesicles and the number of mitochondria in the presynaptic axon terminal depends on synaptic neuronal activity and the axon transport speed [13].

Continuing the duration of SDM to 6 weeks leads to the destruction of certain MNF and their terminal branches, causing denervation of muscle fibers [1, 11]. Under these conditions the axoplasm is overloaded with synaptic vesicles of different diameters, and the length and number of active zones in the presynaptic membrane reduces. The length and width of synaptic contacts and of postsynaptic folds also decrease. The average area of NMS reduces compared with the control one by 25.6%, and compared to



a

b

Fig. 1. Ultrastructural changes in NMS on the 28th (a) and 42nd (b) days of SDM development: magn.: a, b) x 12000.

the last term of the experiment by 34.2% (see. Table. 1). It is noted that neurolemmocyte nuclei argyrophily increases in the area of NMS, and so does the number of these cells (satelitosis).

Conclusions. As a result of the study, histo-ultrastructural and morphometric changes in

the early stages of streptozotocin-induced diabetes were identified, and their pattern points to a close interaction between the neuromuscular endings and muscle fibers. It is shown that structural changes in neuromuscular endings depend on the duration of diabetes and include two stages: the first stage (14-28 days) reactive changes are observed, the second one (42 days) is dominated by degenerative processes.

Prospects for further research A comprehensive study of the patterns of changes in NMS and other constituents of MM in SDM.

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EXPERIMENTAL GROUNDS OF USING PLATELET-RICH PLASMA TO STIMULATE THE LIVER REGENERATION IN CASE OF CHRONIC HEPATITIS

Abstract. *The paper presents data on the possibilities of using platelet-rich plasma (PRP) in simulating CCl4- induced chronic hepatitis. It has been established that double PRP direct injection into the tissue of the organ leads to a rapid regeneration with normalization of biochemical parameters of the hepatic function, while the animals without PRP correction underwent active fibrosing and reduced protein synthesizing liver function. The experimental data give grounds to assert that the use of platelet-rich plasma is a promising method for stimulating liver regeneration in conditions of chronic hepatitis.*
Key words: *experiment; platelet-rich plasma; regeneration; liver.*

Introduction. A key problem of regeneration of an organ or tissue is forming an adequate blood supply through the formation of the vasculature. During the last decade, there have been many reports on applications of platelet-rich plasma (PRP) to correct the pathology of the musculoskeletal system components; for transplant engraftment and others. [1-3]. In this case there is an active process of neoangiogenesis that provides opportunities to recreate the morpho-functional state of the body not at the expense of the connective tissue, as happens the most frequently, but by reconstructing its parenchyma [4].

Objective: Considering the above, the purpose of our study was to ascertain the effectiveness of PRP in the liver regeneration in conditions of chronic hepatitis (CH).

Materials and methods. The study was conducted on mature male Wistar albino rats. The animals were divided into groups: Group I – rats, which were simulated CH by oral administration of oil solution of carbon tetrachloride (CCl4) at a concentration of 50% at a dose of 0.05 ml a day for 7 - 8 weeks [5]; The second group of rats with CH which were injected PRP into the right lobe of the liver (twice at intervals of 1 week). A separate group of animals served as an intact control.

Obtaining the platelet-rich plasma was carried out by its separation from the whole blood in a machine SmartPrep (manufacturer Harvester Corp, USA), certificate of state registration number 10179/2011 in accordance with the Order of State Inspection of Ukraine of 08 February 2011 r. Number 69.

The pathologic study of the liver was performed by a standard staining technique [6] using conventional and polarizing microscopy with a light microscope «Leica-DMLS». We measured the content of erythrocytes (E), leukocytes (L) and platelets (T) in all experimental animals; the content of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, total protein in the animals with simulated CH (the research was conducted at the CI "Center for Veterinary Medicine", Odessa).

The day after the termination of CCl4 effect or after the last injection of PRP was considered the first day of the experiment. Euthanasia of animals was carried out by shifting the cervical vertebrae under a light ether anesthesia on the 10th and 30th days of the experiment. Preparing the animals for the experiment, all invasive surgery, pain management and leaving the experiment were carried out in compliance with the relevant regulations (the Law of Ukraine "On protection of animals against cruel treatment» № 27 art.230 of 2006 as amended pursuant to Law number 1759- VI (1759-17) of 15.12.2009, VVR, 2010, № 9, art. 76 and general ethical

animal experimentation (National Congress on bioethics, 20.09.2001., Kyiv) and the ethic Code of scientists of Ukraine (NASU, 2009)).

Results and discussion. On the 10th day after cessation of SCl4 injection there was a sharp increase in the content of white blood cells and the concentration of cytolysis enzymes in the blood of the experimental animals (Table). At the same time there was a significant reduction in total protein and an increase of total bilirubin (Table).

Table

Hemogram values and liver function biochemical parameters in animals with chronic hepatitis and after correction with PRP

value (units)	Control	Group I (CH)		Group II (CH+PRP)	
		10th -day	30th day	10th day	30th day
E (10^{12}/l)	6,6±0,4	6,0±0,4*	5,3±0,2*“	6,7±0,8	6,9±1,3
L (10^9/л)	8,0±0,2	31,0±2,2*	27,4±1,8 * “	33,0±3,1*	24,4±2,0* “
Pl (10^9/l)	205±12,7	193,1±14,7	167,0±15,4 * “	218,0±11,4*	201,3±12,8 “
ALT (U / L)	57±3,5	78,0±6,3*	84,0±4,9 *	76,3±5,2*	61,0±4,9 “
AST (U / L)	184±4,9	245,0±7,2*	243,0±12,0*	237,0±14,2*	188,2±10,1 “
Total bilirubin (micromole /l)	2,4±0,1	4,6±1,1*	4,2±0,8*	4,3±0,7*	3,0±0,8 “
Total protein (g/l)	86,8±5,6	60,0±11,2	51,0±4,7* “	57,6±3,1*	77,3±5,2* “

*Note.: * - the difference is reliable relatively to the control (p<0,05);*
“ – the difference is reliable relatively to the previous term (p<0,05)

The pathological study revealed some foci of hepatocytes necrosis, areas of infiltration, vascular stasis, hepatocytes with signs of hydropic and fatty degeneration, perivenularly – an accumulation of the connective tissue, blood vessels hyalinosis (Figure 1).

On the 10th day after the last injection of PRP there was an increase of leukocytes in the blood of rats compared to the animals in the control group; the ALT content is reliably higher than in the control group and is not very different from this value in the first group, AST is reliably lower compared to the animals from the first group, but significantly higher than in the control group of animals; total bilirubin content is significantly higher than the control group; total protein content was not reliably different from the rate of animals from the first group and is significantly lower than that in the control group (Table 1).

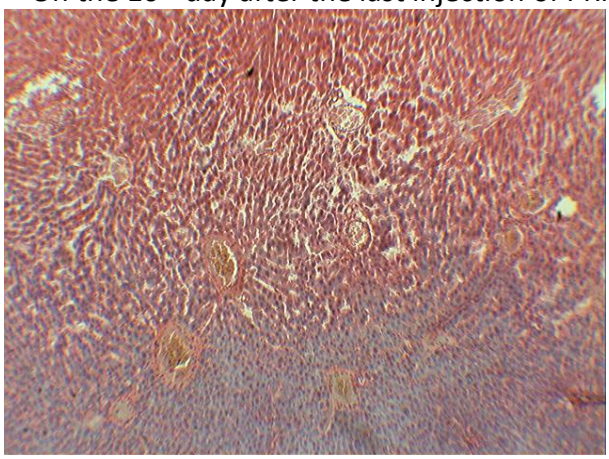

Figure 1. The structure of the liver of the animals in group I on the 10th day of the experiment (stained . hematoxylin-eosin, , magn. x200)

There are rare foci of necrosis in the hepatic tissue, blood vessels are dilated and filled, cells in a state of mitotic division are observed as well as a moderate perivascular fibrosis (Figure 2).

On the 30th day of the experiment the content of red blood cells and platelets in the blood of the first group animals continues to decrease, leukocytosis remains; the content of cytolysis enzymes remains significantly higher

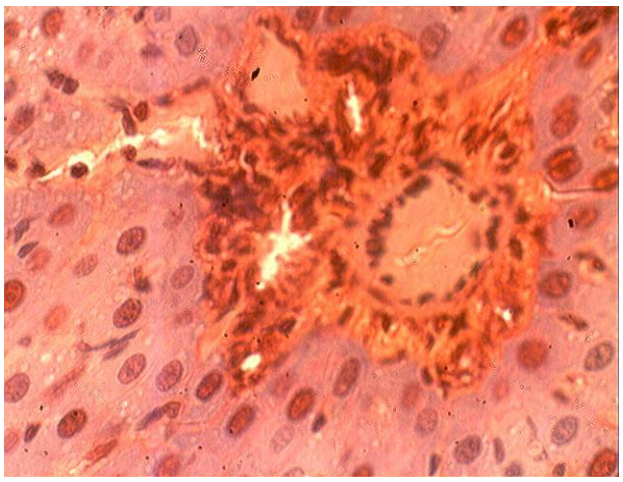

Figure 2. The structure of the liver of the animals in group II on the 10th day of the experiment (stained. hematoxylin-eosin, , magn. x400)

than that in the control group; total protein goes down both compared to the previous period, and to the control one, total bilirubin does not reliably change compared to the previous period, but remains significantly higher than the figure in the animals from the control group (Table 1).

There are disorders in the beam structure of the liver, necrosis foci, leukocyte infiltration, perivenular and perilobular fibrosis, fatty degeneration of hepatocytes (Figure 3).

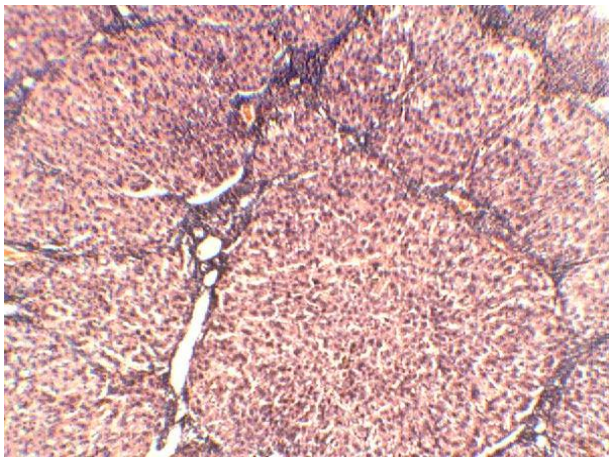

Figure 3. Hepatic tissue in the animals from the first group on the 30th day of the experiment (stained. hematoxylin-eosin, magn. x100)

In the second group of animals on the 30th day of the experiment, almost all hemogram values and cytolysis enzymes content were not reliably different from the control ones; leukocytosis remains, though with a noticeable tendency to decline; total bilirubin level is higher than in the control group, but this difference is not reliable; total protein is nearly identical to the control values (Table 1).

The beam structure in the liver is usual, the number of medium and small vessels increases, there are rare hepatocytes with signs of ballooning degeneration (Figure 4).

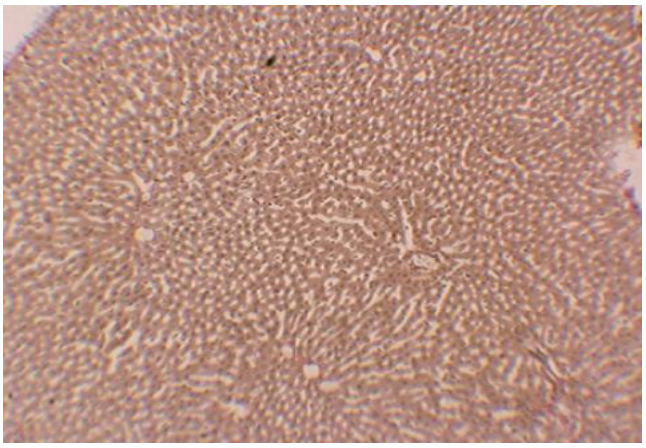

Figure 4. Hepatic tissue in the animals from the second group on the 30th day of the experiment (stained. hematoxylin-eosin, magn.x100).

The results show that in the group of animals with simulated pathological process distinct histological and functional changes, that are characteristic of active chronic hepatitis, occur. There were not any signs of regeneration in the liver up to the 30th day of observation after the cessation of toxic factor effect. The study that we had carried out earlier [7] showed a progressive proliferation of connective tissue, formation of false lobules, nodular transformation of the liver 6 weeks after the beginning of observation. However, there was a quite fast regeneration of the morpho-functional state of the liver in the group of animals which underwent correction of chronic hepatitis with platelet-rich plasma. This phenomenon can be explained by the influence of "cytokine cocktail" that comes as a part of PRP, namely: the vascular endothelial growth factor, platelet growth factor, endothelial growth factor and others. [8, 9]. The process of immune response to the damaging effect due to an activation of the immune system to minimize damage to cells for faster regeneration in a short term. [10] The powerful influence of high concentrations of biologically active substances that are involved in the processes of neoangiogenesis led to the growth of new blood

vessels that have created a unique framework for the diseased tissue, and progenitor cells came with the vessels. In addition, the progenitor cells in the PRP differentiate in the hepatic tissue into hepatocytes and stellate cells of the liver, preventing this way the formation of connective tissue components.

Conclusions. Considering the results of the experiment, it can be argued that the use of platelet-rich plasma is a promising method for stimulating liver regeneration in conditions of chronic hepatitis.

Prospects for further research. Further research should aim to clarify the duration of PRP effect, development of optimal doses and schemes of administration, identification of the basic biochemical processes occurring in the liver behind the correction with PRP. The question regarding the likelyhood of tumors at the areas where PRP is injected remains unanswered, we need a lnger experiment for that.

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ASSESSMENT OF ALUMINUM SALTS INFLUENCE ON INDEXES OF ION REGULATIVE RENAL FUNCTION IN MATURE AND IMMATURE RATS AGAINST THE BACKGROUND OF THE PINEAL GLAND HYPOFUNCTION

Abstract. *Deterioration of public health is due to anthropological environmental pollution, particularly by aluminum compounds. The question about the impact of aluminum salts on the ion regulatory function of the kidney has not been studied sufficiently. In order to assess the impact of nephrotoxic action of aluminum salts on the ion regulatory function of the kidney, and under the conditions of hypothyroidism of the pineal gland, we studied the effect of 14-day action of aluminum chloride compounds on the ion regulatory function in 24 white male rats. It was found that the environmental stress of aluminum salts is accompanied by a nephrotoxic effect, which is characterized by the development of the loss of sodium ions with urine due to a damage of the nephron tubular portion. Hypofunction of the pineal gland causes a nephrotoxic effect of aluminum salts in mature rats with a more significant manifestation of the syndrome of loss of sodium ions in the urine.*

Key words: *kidneys, aluminum chloride, pineal gland.*

Introduction. In recent years, public health in Ukraine has deteriorated significantly, due to anthropological environmental pollution, including compounds of aluminum, which influence everybody daily [1, 6, 8, 10]. Human aluminum requirement is 35-40 mg / day, exceeding this dose over 100 mg / day results in significant changes in the biological systems of the human body, including the kidney [2, 3, 4]. Despite the prevalence of aluminum compounds, the question about the impact of aluminum salts on the ion regulatory function of the kidney in mature and immature rats has not been studied sufficiently [5, 7, 9].

Objective: to study the influence of toxic effects of aluminum chloride on the ion regulatory renal function in mature and immature rats and under the conditions of hypothyroidism of the pineal gland.

Materials and methods. The experiments involved 24 mature and immature nonlinear male albino rats weighing 0,06-0,10 kg and 0,14-0,20 kg respectively. We studied a nephrotoxic effect of aluminum salts on ion regulatory renal function. Aluminum chloride in a dose of 200 mg / kg was administered intragastrically daily within 14 days of the experiment at 8.00 am and 8.00 pm with the 1% starch slurry. Hypothyroidism of the pineal gland was simulated by keeping the animals in conditions of constant illumination (24.00L: 00D) for 7 days. The ion regulatory function was assessed in terms of excretion of sodium ions and their concentrations in the urine, absolute and relative cation reabsorption, filtration charge and sodium ions clearance, sodium-potassium urine factor, concentration of sodium ions in the plasma concentration index of sodium ions, the values of the proximal and distal transport.

Results and discussion. The assessment of the ion regulatory renal function in intact immature rats which were administered aluminum salts (Table. 1) showed that the concentration of sodium ions in the urine increased. The excretion of sodium ions tended to increase. The filtration fraction of sodium ions in the conditions of administering aluminum salts in immature rats was characterized by a

downward trend compared to the control. The clearance of water free of sodium ions tended to reduce in the conditions of administering aluminum salts in immature rats.

Table 1

Values of the ion regulatory function of the kidney in intact immature rats under the influence of aluminum salts ($\bar{x} \pm S_{\bar{x}}$)

Values	Immature rats (Al) (n=6)	Control (n=6)
1	2	3
The concentration of sodium ions in the urine mmol / l	1,5±0,29	0,5±0,05 p<0,01
The excretion of sodium in urine mmol / 2h · 100 g	2,43±0,76	0,99±0,23
Filtration fraction of sodium ions, umol / min. · 100 g	17,5±5,44	25,51±5,32
The excretion of sodium, umol / min · 100 g	2,54±0,72	1,08±0,19
The excretion of sodium, mg / 100 ml Ccr	0,03±0,01	0,009±0,0004
Clearance of sodium-free water	1,62±0,16	2,2±0,27
Concentration index of sodium, stand. units.	0,01±0,002	0,003±0,0003 p<0,01

Note: 1. p – reliability of differences compared with the group of immature rats.
2. n – the number of observations.

The concentration index of sodium ions increased reliably. The distal reabsorption of sodium ions tended to reduce due to the administration of aluminum salts in immature rats.

An analysis of ion regulatory renal function values in mature intact rats after introducing aluminum salts (table 2) showed that the concentration of sodium in the urine increased. Filtration fraction of sodium ions in case of introducing aluminum salts in mature rats was. characterized by a downward trend compared to the control. The trend towards the growth

Table 2

Values of the ion regulatory function of the kidney in intact mature rats under the influence of aluminum salts ($\bar{x} \pm S_{\bar{x}}$)

Values	Mature rats (Al) (n=6)	Control (n=6)
1	2	3
The concentration of sodium ions in the urine mmol / l	1,9±0,15	0,7±0,03 p<0,001
The excretion of sodium in urine mmol / 2h 100 g	3,23±0,56	1,96±0,26
The excretion of sodium, umol / min 100 g	3,46±0,43	2,17±0,17 p<0,02
The excretion of sodium, mg / 100 ml Ccr	0,03±0,01	0,01±0,006
Clearance of sodium-free water, ml / 2 h 100 g	1,95±0,31	3,19±0,08 p<0,01
Relative reabsorption of sodium ions, %	98,7±0,95	99,5±0,38
Clearance of sodium ions ml /2 h · 100 g	0,03±0,004	0,01±0,0002 p<0,05
Concentration index of sodium, stand. units.	0,01±0,001	0,005±0,0002 p<0,001

Note: 1. p – reliability of differences compared with the group of mature rats.
2. n – the number of observations.

was recorded for the excretion of sodium ions, standardized by the glomerular filtrate speed The clearance of sodium-free water experienced a decrease in terms of administration of aluminum salts in mature rats. Relative reabsorption of sodium tended to be inhibited. The clearance of sodium was growing. The concentration index of sodium ions increased reliably.

An analysis of the values of ion regulatory renal function in mature and immature rats after introducing aluminum salts against the background of the pineal gland hypofunction (table 3) showed that the concentration of sodium in the urine was higher in mature rats. We established a similar pattern regarding the excretion of sodium ions. The trend towards growth was recorded for the excretion of sodium ions, standardized by the glomerular filtrate rate. The distal reabsorption of sodium ions after introducing aluminum salts in mature rats tended to decrease. The proximal reabsorption in the comparison group was higher in mature rats.

Table 3

Values of the ion regulatory function of the kidney in mature and immature rats under the influence of aluminum salts against the background of the pineal gland hypofunction ($\bar{x} \pm S_{\bar{x}}$)

Values	Mature rats (Al) (n=6)	Immature rats (Al) (n=6)
1	2	3
The concentration of sodium ions in the urine mmol / l	3,48±0,46	1,02±0,05 p<0,001
The excretion of sodium in urine mmol / 2h · 100 g	7,27±1,68	2,22±0,56 p<0,02
Filtration fraction of sodium ions, umol / min. · 100 g	33,02±10,13	22,77±5,05
The excretion of sodium, umol / min · 100 g	8,13±1,31	2,39±0,49 p<0,01
The excretion of sodium, mg / 100 ml Ccr	0,05±0,01	0,03±0,01
Distal reabsorption of sodium ions, micromoles/ 100 ml Ccr	0,87±0,26	1,96±0,14 p<0,01
Proximal reabsorption of sodium ions, micromoles/ 100 ml Ccr	23,31±9,21	24,87±8,06

Note: 1. p – reliability of differences compared with the group of mature rats.
2. n – the number of observations.

Conclusions. 1. The analysis of aluminum salts influence on the ion regulatory renal function in mature and immature rats showed that the studied environmental stress is accompanied by a nephrotoxic effect, which is characterized by the development of the loss of sodium through urine nephron tubular damage.

2. Hypofunction of the pineal gland causes nephrotoxic effect of aluminum salts in mature rats with a significant manifestation of the syndrome of loss of sodium in the urine.

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OXIDATIVE STRESS IN RATS GASTRIC TISSUES WHEN SIMULATING GASTROPATHY AT LACK AND EXCESS OF MELATONIN

Abstract. *The study has shown that different models of effects on the rats stomach, such as immobilization stress and chemical gastritis, against the background of a short lack and excess of melatonin cause different reactions of the prooxidant-antioxidant system. At the immobilization stress melatonin has gastroprotective effect, and at the simulated chemical gastritis it enhances the oxidative stress.*

Key words: *oxidative stress, prooxidant-antioxidant system, stomach, gastropathy, melatonin.*

Introduction. For the first time the term "oxidative stress" appeared in the 80s of the twentieth century when the German scientist Helmut Sies gave the following definition: "This is an imbalance of prooxidant-antioxidant system in favor of the prooxidants" [1]. The key concepts in this definition are the prooxidant and antioxidant.

The most active prooxidants in living systems are free radicals and ion-radicals, which contain unpaired electrons in atoms of oxygen, nitrogen, sulfur, chlorine: primarily they are superoxide anion ($O2^{\cdot -}$) that when interacting with other compounds turns into hydrogen peroxide (H2O2), which is not a radical, and very reactive hydroxyl radical ($HO^{\cdot}$), singlet oxygen (O21) and peroxynitrite ($ONOO^{-}$). All these forms of reduced oxygen ($O2^{\cdot -}$, H2O2, $NO^{\cdot}$) have a higher reactivity than oxygen molecule, so they were unifying called "reactive oxygen species" (ROS) [2,3].

The influence of oxygen and ROS leads to the formation of a number of organic radicals: R•, RO•, ROO•. A free radical actively react with neighboring molecules, thus forming a new radical, which causes a chain reaction. The stable level of free radical peroxidation (FRPO) in the body is maintained by the activity of the enzyme and non-enzymatic antioxidant systems (AOS).

AOS includes both non-enzymatic antioxidants: tocopherol, vitamin A, carotenoids, ubiquinone, ascorbic acid, thiol compounds, selenium derivatives, transferrin, lactoferrin, albumin, and enzymes: superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1), catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC (1.11.1.6), glutathione peroxidase (glutathione, hydrogen peroxide oxidoreductase, EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2), ceruloplasmin (iron II: oxygen oxidoreductase, EC 1.16.3.1) (Tsebrzhinskyi O.I., 2001, Baraboi V.A.,2006).

A number of factors leads to an imbalance of the prooxidant-antioxidant system. They are a stress, action of chemical and physical factors, the impact of xenobiotics, etc., leading to increased activity of free radical processes and consequently to increased concentrations of primary and secondary peroxidation products.

Hans Selye (1936) noted that the effect of numerous negative factors on the body, regardless of their nature and properties, have a standard response that is manifested as morphological triad: hypertrophy of the adrenal cortex; involution of thymus-lymphatic system (atrophy of the thymus and lymph nodes); formation of erosions and ulcers in the gastrointestinal tract.

At the stress the hypothalamic-pituitary-adrenal and sympathoadrenal systems activate. Their hormones influence the increased secretion of hydrochloric acid, pepsin, lead to

hypertonus of stomach, ischemia of gastric vessels. This causes intragastric proteolysis and damage to the mucosa [4,5,6]. Besides stress, including immobilization one, leads to increased FRPO, and as a result - to oxidative violation of tissue integrity, which causes gastroduodenal erosions and ulcers [7,8]. The imbalance in favor of FRPO with increasing concentrations of peroxidation products is the oxidative stress, which eventually leads to violation of tissues integrity. Therefore, there is a new deeper definition of "oxidative stress" in the scientific literature: "This is a temporary or permanent increase in level of ROS (compared to the stationary level), which violate the cellular integrity and metabolism, including processes with the participation of ROS, leading to oxidative modification of cellular components, which at a significant imbalance of PAS can lead to cell death by apoptosis or necrosis" (Lushchak, 2015).

In modern studies the following question should be focused on: "What can correct acute and chronic oxidative stress?" To prevent FRPO and correct pathologies caused by free radicals a range of antioxidants is used in modern medical practice.

Melatonin is such a stress protector, stimulator of the immune system, gero- and cancer protector, antioxidant (AO). It can positively affect the digestive tract and have anti ulcerogenic effect [9,10,11,12]. Its antioxidant action is due to its ability to neutralize free radicals and act as an indirect antioxidant activating antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPO) [13].

Objective: to determine changes of PAS when simulating gastropathy against the background of lack and excess of melatonin and to find out whether melatonin have the gastroprotective effect at various types of gastropathy.

Materials and methods. Study was performed on 90 Wistar rats. Keeping of animals and experiments were carried out due to the requirements of “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and “General Ethical Principles of Animals Experiments”, ratified on the First National Congress of bioethics (Kyiv, 2001).

Hypomelatoninemia (lack of melatonin) was simulated by the method described by V.M. Huraliuk (2008) [14] by non-stop lighting during 10 days. Since melatonin is synthesized in the epiphysis only in darkness, we supposed that the simulation partly reproduces hypomelatoninemia, considering that there is non-stop synthesis of the hormone by APUD system. It is known that power of light of 0.0005 mV/cm^2 is enough for white rats to reduce the amount of synthesized melatonin [15].

Maximum dose of melatonin single introduction, according to the literature [16, 17], is 5 mg/kg of body weight. In the experiment hypermelatoninemia was simulated by the introduction of melatonin at a dose of 1 mg/kg per day during 10 days.

Two types gastropathy were simulated in experiments: Selye stress ulcer and formation of erosions against the background of introduction of acetic acid. 24 hours before the simulation of gastropathy animals were not fed, but they had free access to water, because the starvation due to the activation of anaerobic glycolysis results in the reduction of protective factors in the gastric mucosa. The stress ulcer was simulated according to Selye by immobilization of the animals in tight boxes for 3 hours. Neuro-humoral factor plays a leading role in the mechanism of genesis of the simulated "stress ulcer", that is why this model is the most appropriate one among quick "acute" experimental ulcers (Zavodskaia I.S., Moreva E.V., 1981). The second type of gastropathy was simulated by the one-time introduction of 10% solution of acetic acid per os through a probe under light anesthesia. Gastropathy was simulated on the ninth day of the experiment, and on the tenth day the euthanasia was performed by bleeding with hexanal anesthesia due to the requirements of bioethics. The stomachs were used for further studies.

After macroscopic study the level of erosive and ulcerative damage to gastric mucosa was evaluated. Number of ulcers in gastric mucosa,

the average number of destructions per animal in the group, the percentage of animals with ulcers in the group (Hadzhai Ya.I. et al., 1962) were calculated, and the Pauls index (Pauls F., Wick A.M., Mac Key E.M. et al., 1947; Dobriakov Yu.I., 1978) [18, 19, 20] was calculated as follows:

$$\frac{n_{\_\text{the average number of destructio1 animal}}}{100} \times \%animals\_with\_ulcer$$

Also the antiulcer activity – AUA was calculated (the ratio of the Pauls index of control group to the one of experimental group).

Concentration of primary (diene conjugates (DC) by the method of Stalna I.D.) and secondary (thiobarbituric acid (TBA)-active products by the method of Stalna I.D., Haryshvyly T.G.) products of free peroxidation in the stomach homogenate was determined. Also activity of catalase by the method of Koroliuk M.A., Ivanova L.I., activity of glutathione peroxidase (GPO) by the method of Pakhomova V.A., activity of superoxide dismutase (SOD) by the method of Chevary S., I. Chaba I., and general proteolytic activity (GPA) by the method of Kunitz in stomach homogenate were determined.

The following methods of statistical analysis were used: checking of normality of distribution using the Shapiro-Wilk criterion, the reliability of the difference between the averages of each of biochemical parameters in the study groups was determined by Student t-test. Statistical analysis was carried out using a computer program Microsoft Office Excel 2007.

Results and discussions. As the study has shown, the content of primary lipid peroxidation products and TBA-active products when simulating immobilization stress against the background of the 10-day and normo-, hypo- and hypermelatoninemia changes, functioning of enzyme antioxidant protection changes too (Table 1).

There is an acute oxidative stress in animals in stressful conditions, which is characterized by

Table 1.

Changes of some parameters of PAS when simulating immobilization stress against the background of lack and excess of melatonin, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without stress (Intact) (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)
DC mmol/kg	6.438± 0.365	11.01± 0.513***	6.75± 0.571	9.26± 0.371*** ▪	4.431± 0.589 *	8.699± 0.498** ▪▪
TBA-active products, µmol/kg	7.821± 0.26	19.81± 2.68**	8.014± 0.34	13.51± 1.66**	9.23± 1.54	13.4± 2.35*
Activity of SOD, st.units/g	0.285± 0.05	0.214± 0.05	0.143± 0.033*	0.182± 0.016	0.219± 0.027	0.168± 0.031
Activity of catalase, mkat/kg	2.559± 0.074	2.09± 0.09**	2.113± 0.106 **	2.116± 0.076***	2.895± 0.131*	2.195± 0.042***
Activity of GPO mkat/kg	5.46± 0.308	5.07± 0.293	4.64± 0.312	4.93± 0.442	4.11± 0.302**	4.41± 0.276*

*Note: *, **, *** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group; ▪, ▪▪, ▪▪▪ the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the control stress.*

sharp changes in parameters of PAS: the concentration of diene conjugates in the gastric tissues homogenates increases in 1.7 times compared to the intact group (by 71,02% (ρ = 0.001)); the concentration of TBA-active products in rat gastric tissues homogenate increases in 2.5 times compared to the intact group (ρ = 0.001); there is a decrease in enzyme antioxidant system activity - SOD activity decreases slightly (by 24.9%), catalase activity decreases slightly (by 18.3% (ρ≤0.01)), GPO activity decreases slightly (by 7%).

Also there is an acute oxidative stress at immobilization stress against the background of lack of melatonin: DC concentration in the gastric tissues homogenates increases compared to the intact group (by 43.8% (ρ = 0.001), but is lower than at the stress control (by 15.9% (ρ≤0.05)); the concentration of TBA-active products in rat gastric tissues homogenate increases in 1.7 times compared to the intact group (ρ≤0.01) but is slightly lower than at the stress control; also there is a decrease in enzyme antioxidant system activity - SOD activity decreases slightly (by 36.1%), catalase activity decreases slightly (by 17.3% (ρ=0.001)), GPO activity decreases slightly (by 9.7%) compared to the intact group. The acute oxidative stress also occurs at the immobilization stress against the background of introduction of melatonin per os at a dose of 1 mg/kg: DC level at hypermelatoninemia increases compared to the intact group (by 31.2% (ρ≤0.01)), but is lower by 21% compared to the stress control group (ρ = 0.01) and slightly lower than at stress with hypomelatoninemia; the concentration of TBA-active products in gastric tissues homogenate increases in 1.7 times (71% (ρ≤0.05)) but is slightly lower than at the stress control (32.4%); SOD activity decreases slightly compared to the intact group (41.1%) and almost does not differ from the activity at the stress and at the stress with hypomelatoninemia; catalase activity decreases (by 14.2% (ρ = 0.001)), but also does not differ from the activity at the stress and at the stress with hypomelatoninemia; GPO activity also decreases (by 19.2% (ρ≤0.05)) compared to the intact group and is lower than at the stress control (13%).

The study shows the increase in the severity of ulcerative process in the rats stomach at simulated immobilization stress: the frequency of damages increases, the average number of destructions per animal increases, the average degree of ulcer in the group increases, the Pauls

Table 2.

Morphological changes in gastric mucosa of white rats at simulated immobilization stress on the 10th day of the normo-, hypo- and hypermelatoninemia, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without stress (Intact) (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)	Without stress (n=8)	At stress (n=8)
Frequency of damages, %	12.5	87.5***	0	100***	25**	62.5**
Average number of destructions per rat	0.25	9.63***	0	13.25***	0.5**	3.38**
Average level of ulcer (LU) in the group	0.125	1.5***	0	2.125***	0.375**	1***
The Pauls index (PI)	0.031	8.42***	0	13.25***	0.125***	2.11***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group*

index increases (Table 2), and there is a significant worsening in all the parameters at immobilization stress against the background of hypomelatoninemia.

Our calculations of antiulcer activity (the ratio of the Pauls index of control group to the one of experimental group with simulated immobilization stress against the background of hypermelatoninemia) indicate the gastroprotective activity of melatonin.

Analyzing the data, it can be noted that prophylactic introduction of melatonin have not prevented the emergence of acute oxidative stress in this model of gastropathy but immobilization stress caused less damage to the gastric mucosa (GM).

But as it is shown by the study, at another model of gastropathy, namely the chemical gastritis, the parameters of prooxidant system and antioxidant enzymes changes in different way (Table 3).

At the simulated chemical gastritis there is no

Table 3.

Changes of some parameters of PAS when simulating chemical gastritis against the background of lack and excess of melatonin, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)
DC mmol/kg	6,305 ± 0,102	4,617 ± 0,206**	7,812 ± 0,314***	14,42 ± 1,311***■■■	7,759 ± 0,177***■■■	10,33 ± 0,363***■■■
TBA-active products, µmol/kg	5,769 ± 0,228	7,359 ± 0,422**	19,5 ± 1,269***■■■	12,82 ± 0,759***■■■	95,88 ± 0,824***■■■	100,6 ± 1,063***■■■
Activity of SOD, st.units/g	0,644 ± 0,032	0,373 ± 0,096*	0,56 ± 0,065■■	0,264 ± 0,048***	1,161 ± 0,026***■■■	1,147 ± 0,037***■■■
Activity of catalase, mkat/kg	2,1 ± 0,088	2,166 ± 0,084	1,542 ± 0,205*■■	1,424 ± 0,164**■■■	1,928 ± 0,016	1,788 ± 0,094*■■
Activity of GPO mkat/kg	5,69 ± 0,172	7,88 ± 0,251***	7,32 ± 0,263***	8,14 ± 0,09***	7,31 ± 0,088***	7,98 ± 0,145***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group; ■■, ■■■ the difference between the samples means is statistically significant with ρ≤0.01, ρ≤0.001 respectively compared to the group with chemical gastritis*

acute oxidative stress in animals: the concentration of diene conjugates in the gastric tissues homogenates decreases slightly compared to the intact group (by 26.8% (ρ = 0.01)); the concentration of TBA-active products in the gastric tissues homogenates of rats increases compared to the intact group (by 27.6% (ρ = 0.01)); SOD activity decreases in 1.7 times (ρ≤0.05); catalase activity does not change; and GPO activity increases (by 38.5% (ρ = 0.001)).

There is an acute oxidative stress at simulated chemical gastritis against the background of 10-day hypomelatoninemia: the

concentration of diene conjugates in the gastric tissues homogenates increases in 2.3 times (ρ = 0.001); the concentration of TBA-active products in the gastric tissues homogenates of rats increases in 2.2 times compared to the intact group (ρ = 0.001); there is a decrease in enzyme antioxidant system activity - SOD activity decreases compared to the intact group in 2.4 times (ρ = 0.001) and is lower than in the group with chemical gastritis (by 29.2%); catalase activity also decreases compared to the intact group (by 32.2% (ρ = 0.01)) and is also lower than in the group with chemical gastritis (by 34.3% (ρ = 0.001)); and only GPO activity increases compared with the intact group (by 43.1% (ρ = 0.001)), and almost does not differ from the group with the simulated chemical gastritis.

At the simulated chemical gastritis against the background of 10-day hypermelatoninemia contents of primary and secondary products are also high: thus DC concentration increases compared to the intact group (by 63.8% (ρ = 0.001)), but decreases compared to the group with the simulated chemical gastritis at the hypomelatoninemia (by 28.4% (ρ≤0.05)); the concentration of TBA-active products in the gastric tissues homogenates of rats increases in 17.4 times (ρ = 0.001) and increases in 7.9 times (ρ = 0.001) compared to the group with the simulated chemical gastritis at the hypomelatoninemia. As for the antioxidant system, there is an increased activity of SOD compared to the intact group in 1.8 times (ρ = 0.001), compared to the group with the chemical gastritis - in 3 times (ρ = 0.001), compared to the group with the chemical gastritis at the hypomelatoninemia - in 4.4 times (ρ = 0.001). The activity of catalase is lower than in the intact group (by 14.9% (ρ≤0.05)) and is slightly higher than in the group with the chemical gastritis at the hypomelatoninemia (by 16%). GPO activity is higher than in the intact group (by 40.3% (ρ = 0.001)), and does not differ from the activity of the group with the chemical gastritis and of the group with the chemical gastritis at the hypomelatoninemia.

As it can be seen from Table 4 at the simulated chemical gastritis there is an increase in the severity of ulcerative process in the stomach of rats compared to the intact group: the frequency of damages increases, the average amount of destructions per animal increases, the average level of destructions in

Table 4.

Morphological changes in gastric mucosa of white rats at simulated chemical gastritis on the 10th day of the normo-, hypo- and hypermelatoninemia, (M±m)

Parameters	Experimental conditions					
	Natural lighting Normomelatoninemia		Permanent lighting Hypomelatoninemia		Natural lighting+ melatonin introduction Hypermelatoninemia	
	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)	Without effect on GM (n=7)	At the simulated chemical gastritis (n=7)
Frequency of damages, %	0	100***	100***	100***	57.14***	100***
Average number of destructions per rat	0	3.43***	5.43***	7***	3***	13***
Average level of ulcers (LU) in group	0	1.57***	1.71***	2.29***	0.86***	2.57***
The Pauls index (PI)	0	3.43***	5.43***	7***	1.71***	13***

*Note: *, **,*** - the difference between the samples means is statistically significant with ρ≤0.05, ρ≤0.01, ρ≤0.001 respectively compared to the intact group*

the group increases, the Pauls index increases. At the lack of melatonin and simulated chemical gastritis all the parameters worse, but at the hypermelatoninemia and chemical gastritis all the parameters worse even more compared to the group with the chemical gastritis: the average amount of destructions increases in 3.8 times, the average degree of ulcer increases in 1.6 times, the Pauls index rises in 3.8 times.

Also the antiulcer activity of melatonin AUA was calculated (the ratio of the Pauls index of the group with the chemical gastritis to the one of the group with the chemical gastritis at the hypermelatoninemia): in this model of gastropathy melatonin does not reveal his activity, as (due to Pauls, 1947) AUA value should be higher than 2 units.

Conclusions: 1. There is an acute oxidative stress in the rat gastric tissues at the simulation of gastropathy by the immobilization stress.

2. There are no significant changes of parameters of PAS at the simulated chemical gastritis, which indicates the absence of the acute oxidative stress.

3. In the group with the simulated immobilization stress the introduction of melatonin at a dose of 1 mg/kg per day during 10 days has gastroprotective effect.

4. In the group with the simulated chemical gastritis the introduction of melatonin at a dose of 1 mg/kg per day during 10 days does not have gastroprotective effect, and the concentration of free radical peroxidation products is higher than in all other experimental groups, which indicates the presence of the acute oxidative stress.

5. Reactions of prooxidant-antioxidant system of rat stomach at various types of are different, which indicates there are different mechanisms of adaptation of the system to the action of negative factors.

Prospects for further research. It is reasonable to perform further study of the reactions of prooxidant-antioxidant system in the stomach at the oxidative stress against the background of the prolonged lack and excess of melatonin.

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ABOUT THE DISEASE, THE CAUSE OF DEATH AND THE METHOD OF EMBALMING THE BODY OF M.I. PYROHOV (to the 205th anniversary of birth) (literature review)

Abstract. *literature review deals whith biography, life, death and the method of embalming the body of M.I. Pyrohov.*
Key words*: M.I. Pyrohov, embalming.*

In December last year the global surgical community celebrated 205th birth anniversary of the great surgeon, pioneer of military-field surgery, thinker, pedagogue, public figure Nikolai Pyrohov. He lived in Ukraine more than 20 years and here still found his eternal rest.

Ukraine remembers and piously saves everything connected with the name of N. Pyrohov. Of course, over time, each of an outstanding figure is contemplated differently and an inevitable revaluation is made. For the historian everything that touches the lifetime of such prominent figures has an extraordinary value.

Nikolai Pyrohov was born on 13 (25) November 1810, in Moscow to a major in the commissary service of I. Pyrohov. He was the youngest among his 14 brothers and sisters.

In September 1877, during a business trip to the theatre of the Russo-Turkish war to Bulgaria, where Nikolai Ivanovich carried out an inspection of the military hospitals, he met Dr. David Vivodcev, who proudly showed him his invention – apparatus for embalming corpses.

It should be noted that embalming during the military conflict had a particular importance at that time. As it is known from history, Russia has always been a proponent of grabbing foreign territories, that also takes place in our time on the example of the Crimea and Eastern Ukraine.

During these conflicts a lot of high rank military officers and members of the Royal family died and were sent for burial to Russia. Considering the lack of rapid transport, in order to save the remains of the victims, they had to be embalmed. The same time, during the war in the Balkans, for this purpose the military doctor D. Vivodcev was serving.

In 1880, between the media went around the news what the community has decided to celebrate the 50-year anniversary of the scientific activities of the N. Pyrohov. It was decided to celebrate the holiday in May in Moscow where he was born. In order to invite the hero of the day on the celebration Professor M. Sklifosovsky arrived. On arrival to hometown N. Pyrohov discovered a surprise – he received the title of honorary citizen of Moscow. All European universities gave him honorary doctorate degrees. But the final day of the Jubilee celebration was sorrowful through the information about the disease of Pyrohov. Having lost the last upper jaw the year before, he refused to insert artificial teeth. In the place of the removed tooth a small ulcer appeared. He didn't give it a special importance. During the consultations of leading Moscow surgeons, it was recognized that the ulcer was malignant and the patient was offered to remove it immediately. The surgeons had a confidence in the successful completion of the operation. Nikolai's wife Alexandra refused surgery and

decided to look for advice of the famous Vienna surgeon Theodore Bil'rot. She took her husband to Vienna directly from the celebration. After examining the patient Bil'rot quietly and confidently convinced the patient that the disease is not serious and does not require surgical intervention. After the death of N. Pyrohov Bil'rot stated in the media that during the consultation the patient had evident signs of cancer with metastases, so surgical treatment had no meaning and that patient having cataract and other concomitant diseases just wouldn't transfer operation. Until the death of their father the sons did not know about the intention of the stepmother to embalm the body of Pyrohov. This is evidenced by the fact that his eldest son Nicholas appealed to the Holy Synod with the request to give permission for the burial of his father on the territory of the farmstead. Synod didn't grant the request.

It is also known that well in advance before the illness Nikolai expressed a wish to be buried on the territory of the family estate and 21 days before his death he reminded his relatives. about it. The idea of embalming the body of her husband arised under the influence of an article in the magazine «Niva» in which was told about that in France, namely in Nantes, in recent years it became trendy to bury noble people in family crypts with mandatory embalming. With this request a month before the death she turned to capital doctor D. Vivodcev.

The letter has been preserved: "Dear Sir David, excuse me if I will upset you with my letter. Nikolai lies on the bed of death. You sent him your book about embalming to the anniversary. I hope that you will take work of embalming his body that I would have wanted to keep in the original form. If you agree, then notify me on your own terms, and when the God stops his cruel suffering, I'll inform you. If you acknowledge to come earlier telegraph then. Yours sincerely A. Pyrohova. October 25, 1881."

The second letter Alexandra wrote on November, 5, 1881, 20 days before the death of her husband. In it she again makes D. Vivodcev a request to carry out embalming and reports that did not know about a necessity to have a license on this occasion from corresponding instances.

As later wrote M. Dahl on the fourth day after death, November 26, 1881 D. Vivodcev came to the family estate and on his own methodology embalmed the body of Pyrohov. For this he used injection liquid, consisting of thymol – 5.0, alcohol – 45.0, glycerine and distilled water 2160.0 – 1080.0.

Fistly, in two stages 9 liters of solution is injected under pressure to 3 atm. in the carotid and femoral arteries in the direction opposite to the normal flow of blood in order to remove the maximum of blood from vessels, and in the second phase 6 liters of solution is injected to maximize the removal of embalming liquid dissolved in 9 liters of water.

This fluid not only preserved, but also ceased decomposition of the corpse, which already had signs. His original method of embalming the corpses of people and animals D. Vivodcev described in his monography: "Embalming and ways to preserve anatomical specimens and animal corpses", which was published in one of the Petersburg publications in 1881. Originally the method was intended to anatomical dissection and preservation of animals organs and in particular used by D. Vivodcev in his famous description of the ways of lung lymph flow of dogs. Later the method was tested for embalming corpses and especially – the eminent persons, as for example the Chinese ambassador who suddenly died while being to St. Petersburg, whose embalmed body D. Vivodcev preserved for a long time traveling to Beijing. All the necessary tools for the procedure were also designed by D. Vivodcev, including specially made folding table.

For his method on January 19, 1876 Dr. Vivodcev was awarded the first prize at the Philadelphia International Exhibition. Embalming process lasted about 4 hours. During the process of embalming the priest, two doctors and two paramedics were present. By the method of D. Vivodcev autopsy was not performed, preservative was administered under pressure by the syringes.

As later the doctor S. Shklyarevskyy, who was present at this procedure mentioned, the effect was spectacular, deceased began to look like a sleeper.

Immediately after the scientist's death his family appealed to the relevant authorities of St. Petersburg for permission to carry out the burial of her husband in the family estate. The answer came quickly, but it was reported that Pyrohov's desire can be satisfied only if his heirs give written consent that eventually in case of transition to the new owners of the estate Nikolai's ashes will be reburied in a family estate at another location. Pirigov's family members didn't agree with this proposal.

Even before the death of her husband wife Alexandra ordered a special design of sarcophagus in Austria and at the village cemetery Sheremeta she bought a land where a temporary crypt and chapel began to build.

It was a cold winter, construction work progressed slowly. Due to the fact that neither the crypt-tomb or sarcophagus has not yet been prepared, the embalmed body of Nikolai in November 29, 1881 was temporarily moved to a small, century-old, wooden church in the village Sheremetka that was able to bear several dozen people.

Finally, it was officially announced that the funeral will be held on January 24, 1882 at 12 o'clock. The arrival of deputies began, wreaths were sent. The open coffin with the body of Pyrohov was raised and transferred to the vault, where it was installed on a pedestal by the scientist's sons, doctors and officers.

And only later, in 1885, designed by Kiev architect above the crypt the church-shrine was built, consecrated in honor of St. Nicholas where the sarcophagus was established and in which the body of Pyrohov still remains.

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STRUCTURAL-GEOMETRIC REMODELING OF THE LEFT VENTRICULAR AND ENDOTHELIAL VASCULAR FUNCTION IN CASE OF ARTHERIAL HYPERTENSION AND DIFFERENT BODY WEIGHT OF PATIENTS

Abstract. *This investigation deals with changes of structural-morphologic, geometric, systolic-diastolic indices of remodeling of the left ventricular and endothelial vascular function parameters in patients with arterial hypertension with normal body weight, obesity and low body weight.*
Key words: *arterial hypertension, obesity, deficiency of body weight, structural-geometric remodeling of left ventricular, systolic-diastolic parameters, endothelial dysfunction.*

Introduction. When precautionary methods are applied to prevent the impact on development of cardiovascular complications the main attention is drawn to obesity that more frequently is estimated by means of Quetelet index, that is, the body weight (kg) to body height (m) ratio. Among the mechanisms through which pathological impact of obesity is implemented, arterial hypertension (AH), hypercholesterolemia and glucose intolerance play a critical role, increasing prevalence among overweight individuals.

However, as numerous epidemiological research studies state, among other 32-year observations in Framingham, the increased mortality from IHD and other non-infectious diseases is observed among patients with incomplete body-weight index in comparison to average body-weight index patients. It is essential, that this interdependence has self-sufficient effect and is not influenced by smoking and hidden diseases. In the studies of native scientists the range of minimal mortality is observed when Quetelet index is 24-27 kg/m².

Whereas Quetelet index decrease is accompanied by decrease of systolic and diastolic arterial pressure, total cholesterol and low density lipoprotein and by increase of high density lipoprotein cholesterol, glucose intolerance, individuals with low weight of body probably should have favorable cardiovascular disease risk profile. Increase of mortality caused by cardiovascular system disease among them is seemed more paradoxically in comparison with average Quetelet index individuals.

In fact, when AH and abdominal obesity (AO), specific target lesions occur which afterwards can appear as independent risk factors of cardiovascular sequelae [2,5,6]. Characteristics of myocardial and endothelial dysfunction of patients with AH and deficiency of body weight remain incompletely studied. We did not find convincing data pertaining to this issue in the native literature.

Determination of special aspects of structural-geometric, systolic-diastolic remodeling of left ventricular (LV) and endothelial dysfunction of vessels as early markers of atherosclerosis among patients with AH and different body weight was the target of our research.

Materials and methods. 69 male patients with hypertensive disease (HD) stage II, in accordance to Ukrainian Association of Cardiology (2008), at the ages from 60 to 85, in an average – 75±5,9 years old were enrolled in the study. 25 patients with AH had concomitant AO, 15 – deficiency of body weight, 29 patients – normal body weight.

Duration of the disease was less than 10 years. Abdominal obesity type was determined when waist circumference to thighs circumference ratio was ≥ 0,95 and body mass index (BMI)>25 kg/m², deficiency weight of body – when BMI<18,5 kg/m².

Echocardiography was performed with the use of M – modal, two-dimensional regime, pulsed wave and permanent wave Doppler regime with a help of ”LOGIQ 500” (General Electric, USA) 2,5 – 3,5 MHz sensor machine with phased array. The thickness of cardiac walls and the size of cardiac cavity were determined by means of through Penn convention method and by formula of L. Teichholtz. Myocardium mass of the left ventricule of the heart (MMLVH) was calculated by formula of Devereux R.B. with subsequent indexation to

body surface square. MMLVH criteria that exceeded 134g/m2 for males, was taken as LV hypertrophy (LVH) level. Doppler sonography of the carotid and brachial arteries (BA) was conducted using supersonic diagnostic scanner "LOGIQ 500" (General Electric, USA). The endothelial function was studied using samples with reactive hyperemia (endothelium-dependent vasodilation, EDVD) and nitroglycerin (endothelium-independent vasodilation, EIVD).

Dilation of the brachial artery against the background of reactive hyperemia that exceeds more than 10% of intact diameter is conditionally commonly believed as normal reaction, lower indicator or vasoconstriction is considered to be pathological.

Statistical data processing, provided in M+m form, was conducted by means of variation statistics methods with the use of Student t-test on PC on the basis of Microsoft Excel program of statistical analysis. Difference was considered as adequate when $p<0,05$.

Results and discussion. Characteristics of myocardial function in investigated patients are presented in table 1.

In our investigation patients with HD and AO had adequately higher SWT and PWT, ESD and EDD, and also LVMMI in comparison with the group of patients with HD but without AO that was indicative of liability to predominantly concentric LVH progression. These data matche with the results of majority of investigations [1,3,4,7]. In fact, eccentric LVH typical for gynoid (gluteofemoral) obesity type is connected with left ventricular afterload and dilation increase. In other words, preload by volume causes compensatory myocardium hypertrophy. Concentric LVH, as the most unfavorable remodeling type, is more often diagnosed in patients with AH and android (abdominal) obesity type, in pathogenesis of which neurohumoral component plays a leading role [8]. Hyperinsulinemia directly or via activation of mediators of sympathetic activity and hormones of renin–angiotensin–aldosterone system causes to cellular growth increase and rebuilding of collagen matrix inside myocardium. In some investigations there was revealed the adequate LVMM association with levels of immunoreactive insulin, C-reactive protein and renin activation; it was found that this coherence did not depend on the degree of obesity, duration of AH.

Alterations detected when analyzing transmitral flow have the same trend in all the groups of patients and reflect the formation of hypertrophic type of diastolic function abnormality.

However, intergroup analysis of changes in diastolic function of patients with normal body weight and with AO revealed that abnormality of LV diastolic filling was more often detected in patients with associated AO. Only 1,8 % of patients with AO (14 % without AO) did not have abnormality of LV diastolic filling, and detection frequency of hypertrophic type was 86 % (76 % in patients without AO). Pseudonormal type of diastolic dysfunction also more often was detected in patients with associated AO (12,2 and 10 % respectively), predominantly when eccentric LVH that could be explained by pressure increase in the left atrium (LA) and, thus, increase of blood flow inside early diastole. Restrictive type of diastolic function abnormality was not observed in any patient. When analyzing echocardiographic indices of patients with deficiency of body weight there was detected the tendency to formation of concentric LVH and diastolic function abnormality stage I. In the group of patients with deficiency of body weight when comparison to the group of patients with adequate nutritional status the firm LVMM decrease was observed, that was indicative of mildly less LVH grade and DT decrease associated with myocardium stiffness that negatively affected the heart contractility.

Characteristic of endothelial function of vessels in the examined groups of patients is presented in table 2. To compare the results of BA Doppler sonography and to avoid any doubt we compared foregoing groups of patients with one more group, - control group, which consisted of 20 virtually healthy individuals with an adequate nutritional status.

Outlet diameter of the brachial artery in examined groups did not vary adequately. However in patients with hypertensive disease stage II according to data of investigation there were detected firm pathological EDVD BA decrease and decrease of BA sensation to shear stress, regardless body weight, however it was well-marked in patients with deficiency weight of body and in patients with associated AO. Wherein, EIVD was retained in response to nitrates.

Interaction between LVH stage and endothelial dysfunction was also determined by a variety of experimental and clinical investigations.

Coherence between myocardium remodeling and endothelial dysfunction may be explained by NO synthesis decrease, as a result of induction of proliferative processes inside the heart and vessels and formation of predominantly

Table 1.

Indices of echocardiogram in patients affected by HD stage II and with different body weight

Indices	Indice values (M±m) in groups of patients					
	HD with stand. body weight,29 patients (1 group)	HD with AO 25 patients (2 group)	p1-2	HD with deficiency of body weight, 15 patients (3 group)	p1-3	p2-3
Size of LA, см	3,67 ± 0,1	3,8 ± 0,1		3,59 ± 0,1		
EDD, cm	4,87 ± 0,1	5,76 ± 0,1	<0,001	4,84 ± 0,1		<0,001
ESD, cm	3,15 ± 0,1	3,76 ± 0,1	<0,001	3,10 ± 0,1		<0,001
SWTd ,cm	1,19 ± 0,01	1,27±0,01	<0,001	1,18 ± 0,1		<0,001
PWTd ,cm	1,24 ± 0,01	1,38±0,01	<0,001	1,22 ± 0,1		<0,001
RWT	0,46± 0,02	0,49±0,02		0,42±0,02		<0,05
EF , %	63,4 ± 1,1	55,8 ± 2,2	<0,001	64,4 ± 2,1		<0,001
LVED , ml	118,26±4,8	144,8 ± 4,9	<0,05	116,18±4,1		<0,05
LVES , ml	43,18 ± 2,2	49,9 ± 2,4	<0,05	42,16 ± 2,1		<0,05
SD , ml	76,4 ± 1,8	84,4 ± 2,1	<0,001	74,4 ± 2,1		<0,001
MCO, l/min.	5,4 ± 0,2	6,28 ± 0,4		5,2 ± 0,4		
LVMM , g.	258,4 ± 7,4	300,8 ± 5,1	<0,001	230,8 ± 6,1	<0,001	<0,001
LVMMI ,g/m²	136,7 ± 4,1	148,8 ± 4,4	<0,05	135,8 ± 4,1		<0,05
PVR ,dyn.s.cm-5	1642,4±60,0	1704±58,2		1582±59,1		
E/A $_{TF}$	0,95 ± 0,06	0,82 ± 0,05		0,99 ± 0,05		<0,05
E_{TF}, m/s	0,52 ± 0,02	0,54±0,027		0,50±0,024		
A_{TF}, m/s	0,65 ± 0,02	0,56±0,028	<0,05	0,63±0,026		<0,05
IVRT, s	0,12 ± 0,02	0,13 ± 0,02		0,11± 0,02		
DT, s	0,238±0,002	0,230±0,002	<0,05	0,220±0,002	<0,01	<0,001

Notes (used in Table 1): LA – left atrium, EDD – end-diastolic dimension, ESD – end-systolic dimension, SWTd – septum wall thickness, diastole, PWTd – Posterior wall thickness, diastole, RWT – relative wall thickness, EF – ejection fraction, LVED – left ventricular end diastolic, LVES – left ventricular end systolic, SD – systolic discharge, CO – cardiac output, LVMM – Left Ventricular Myocardium Mass, LVMMI – Left Ventricular Myocardium Mass Index, PVR – peripheral vascular resistance, ETMK – peak early filling velocity of transmitral flow, ATMK –peak atrial systole velocity, IVRT – Isovolumetric (Isovolemic) relaxation time, DT – Deceleration time (DT) of the early filling velocity.

Table 2

Alterations of endothelial function of vessels in patients with HD and different body weight.

Indices	Indices value (M±m) in groups of patients			
	Control n = 20	HD n = 29	HD + AO n = 25	HD with deficiency of body weight n = 15
outlet diameter of BA, mm	3,9 ± 0,2	4,1 ± 0,2	4,4 ± 0,2	3,9 ± 0,3
EDVD , %	10,3 ± 0,6	8,4 ± 0,2*	4,6 ± 0,8**	4,0 ± 0,4**
EIVD , %	17,6 ± 1,3	14,0 ± 1,5	13,5 ± 2,1	12,8 ± 2,1

Notes: * - p <0,01; ** - p <0,001 (in comparison with control group)

concentric LVH in response to peripheral vascular resistance and SD appears. If NO synthesis remains high, the absence of LV walls thickening becomes the consequence of antiproliferative NO parameters, that jointly with dilation of the heart chambers acts as prerequisite of formation of eccentric LVH.

Presence of endothelial dysfunction in patients with HD and AO may be a marker of atherosclerosis. ”Mute” behavior of atherosclerosis in individuals with AH granting insulin resistance makes obvious the necessity of early detection of the abnormality of vasodilation in actual patients category.

Endothelial dysfunction in individuals with HD and deficiency of body weight is pathogenetically related with progression of atherosclerosis in them. It should be admited, that when analyzing lipid metabolism in forgoing category the levels of total cholesterol and triglycerids were in reference range. Collected results indicate the necessity of further investigation of pathophysiological characteristics of AH and their impact on target organs in patients with deficiency of body weight. Perhaps, when conducting large population studies and acknowledgment of cardiovascular risk there would appear the necessity in remodeling of optimal parameters of lipid profile for actual category of patients in order to enhance their medical treatment and preventive measures of development of cardiovascular complications.

Determination of endothelial function of vessels in patients with HD and deficiency of body weight may be also an actual cardiovascular risk marker in current category of patients. Unfortunately, deficiency of body weight issue is neglected in modern preventive cardiology, however continuous increase of cardiovascular mortality requires redouble attention to all the patients, which may be in potential high-risk group. That is why, further investigation of endothelial function of vessels is absolutely necessary for development of clear criteria of preclinical disease diagnostics, determination of process intensity and development of pathogenetically sound tactics of medical treatment.

Conclusions: 1. In group of patients with HD and associated AO there the tendency to development of predominantly concentric LVH was detected.

2. In patients with associated AO abnormality of diastolic LV filling jointly with formation of hypertrophic type of diastolic dysfunction was frequently detected.

3. Abnormality of vasodilation as response to compression is typical for all the patients with HD regardless of body weight, however it was well-marked in patients with deficiency weight of body and in patients with associated AO.

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