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Inhaber: Marina Kisiliuk

Tel.: + 49 51519191533

Fax.: + 49 5151 919 2560

Email: info@dwherold.de

Internet: www.dwherold.de

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Prof. Zamiatin P.M.

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Zayats O.V.,*Assistant, Department of Physiology SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine
o.v.zaiats@gmail.com***Voronych-Semchenko N.M.***Chief of the Physiology Department, Professor, SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine*

STATE OF L-ARGININE/ARGINASE SYSTEM AND DIHYDROGEN SULFIDE OF ORAL FLUID IN CHILDREN WITH PRECLINICAL IMBALANCE OF IRON AND THYROID HOMEOSTASIS

Abstract. *The parameters of L-arginine/arginase system and dihydrogen sulfide of oral fluid in children with mild deficiency of iodine, latent iron deficiency, combined deficiency of iodine and iron and their effect on oral cavity were analyzed in the study. The increase in the content of NO₂, NO₂+NO₃, peroxynitrite on the background of the enzymatic activity of arginase decreasing, especially in children with combined iodine and iron deficiency, were established. More marked changes were observed in boys.*

Key words: *L-arginine, arginase, dihydrogen sulfide, oral fluid, iodine deficiency, latent iron deficiency.*

Introduction. Western Ukraine belongs to the endemic region with iodine deficiency in the biosphere, characterized by a high level of thyroid gland diseases. In recent years, residents of this region have had a tendency to worsen the dental status of the children's population. This dynamics is associated with a number of objective factors, such as: a decrease in the socio-economic status of the population, unbalanced nutrition, changes in environmental processes that have a negative impact on the balance of micro and macro elements, primarily essential ones (iodine, iron, selenium) [1]. Therefore, an in-depth study of the origin of dysfunction of thyroid gland and comorbid pathology remains relevant both for the physiology of the endocrine system and dentistry. The main cause of thyroid homeostasis disorders is an inadequate supply of iodine to the body, iodide organification disorders and the deficiency of other essential elements involved in the secretion of thyroid hormones, including iron, which is a cofactor of enzymes of thyreogenesis. These factors can affect the condition of the oral cavity, in particular, the permeability of the vascular wall of the mucous membrane. At the same time, it is known that nitric oxide (NO) plays an important role in regulating physiological and biochemical processes in the body as a whole and in the dentofacial area. NO detects, simultaneously activates and inhibits various metabolic processes in the human body, though it

is active only for a few seconds. Under its influence, the regulation of signaling pathways, which trigger a series of adaptive-compensatory reactions of the organism, is carried out. Changes in the NO metabolism system can lead to hypoxic necrobiosis in the cells.

A biomarker for the study of dental status in the human body is an oral fluid, the characteristics of which are specific for prediction and detection of periodontal diseases, as well as qualitative changes in the oral cavity [2]. In recent years, salivary glands (SG) have been studied as a special organ for the control of the formation of NO entering the oral cavity with saliva, and contoured by the mechanism of autoregulation. At the same time, NO affects hemodynamics and proliferation of SG cells, neurotransmission and saliva secretion. L-arginine participates in such physiological processes as regulation of the immune response, maintenance of nitrogen balance, antiproliferative actions, and is a substrate for the formation of NO. In the human body there is a nitrite reductase system that provides the formation of nitrate (NO₃) and nitrite (NO₂), which do not exhibit vasoconstrictor action [3], L-arginine → NO → NO₂ → NO₃ – the cycle of NO metabolism [4]. Nitrite and nitrate reductase system is the largest exporter, providing the intake of NO [5]. Nitrite reductase reactions of oral bacterial microflora have a significant influence on NO production [6, 7]. It is known that

almost 25% of plasma nitrates are excreted with saliva into the oral cavity, leading to an increase in nitrate concentration in the oral fluid ten times more, concerning their content in plasma [8]. NO excess leads to the formation of peroxynitrite, which has a signal effect and pathogenic influence on a cell, causing nitroso-active and oxidative stress of cells.

The purpose of the study was to study the parameters of the nitric oxide system in the oral fluid of children of the endemic region with a mild hypothyroid dysfunction, latent iron deficiency and combined microelementosis (iodine and iron deficiency).

Materials and methods of research. 78 children aged 6-11 years old who were randomized for age-sexual characteristics and clinical diagnosis were examined. All schoolchildren were divided into four groups: with adequate iodine and iron supplementation (gr. I, n=24), with mild iodine deficiency (gr. II, n=18), with latent iron deficiency (gr. III, n=17) and with combined deficiency of bioelements (gr. IV, n=19). Differentiation of diagnoses was carried out on the basis of complaints, anamnesis, clinical and laboratory methods of research. Functional state of thyroid gland was determined by the content of thyroid hormones in serum: thyroid stimulating hormone (TSH), free T₄ and T₃ hormones. Median ioduria was determined for establishing a level of iodine supplementation in children. To characterize the iron base, the content of serum iron and ferritin, the total iron binding capacity of the blood serum and the coefficient of transferrin saturation by iron were determined. The study of the NO system in the oral fluid was carried out by the content of NO⁻, NO₂⁻, NO₂⁻+NO₃⁻, H₂S, arginase and L-arginine activity [8]. Statistical analysis was performed using computer programs (Statistic Soft 7.0). Comparison of the samples was carried out according to the Student t-criterion. The error p<0.05 was considered statistically significant.

Results of the research and their discussion. As a result of the study, there were significant differences between the investigated parameters depending on the changes of the micronutrient panel. Thus, in the case of a mild iodine deficiency in boys, an increase in NO₂ content (by 15 times, p₁₋₂<0.05), NO₂+NO₃ (almost by four times, p₁₋₂<0.05) in the oral fluid was observed regarding the control values. In girls of the same group, the increase of peroxynitrite (by two times, p₁₋₂<0.01)

relative to healthy peers, was found. Boys with latent iron deficiency had increased NO₂ (by 3.6 times, p₁₋₃<0.01) in the oral fluid, as compared to control. More significant parameters changes were observed in girls of this group, in particular, reduction of NO₂ (by 45%, p₂₋₃<0.05) against the background of peroxynitrite content increase in oral fluid (almost by three times, p₁₋₃<0.05). Changes in the balance in the L-arginine/arginase system were the most significant in combined iodine and iron deficiency. Boys of group IV had an increase in NO metabolism products in oral fluid: NO₂ – by 8.3 times, p₁₋₄<0.001), NO₂+NO₃ (by 3.3 times, p₁₋₄<0.05), peroxynitrite (by 2.8 times, p₁₋₄<0.05) against the background of arginase activity decreasing (by 33%, p₁₋₄<0.05) as compared to the data of control group of children. It should be mentioned that girls of the same age had only a significant increase in the content of peroxynitrite in oral fluid by 2.5 times (p₁₋₄<0.01) relative to the source data. Attention is drawn to the significant discrepancies between the indicators of children in study groups III and IV. Thus, girls with combined iodine and iron deficiency had an increase in the content of NO₂ in the oral fluid (by 3 times, p₃₋₄<0.05), NO₂+NO₃ (by 47%, p₃₋₄<0.05) in relation to data in group III. Accumulation of nitrates and nitrites in oral fluid with decreasing activity of arginase is a dangerous sign of inflammatory processes in the mucous membrane of the oral cavity and gums. It has been established that NO affects the aetiology and pathogenesis of periodontal diseases as a result of reduction of antimicrobial activity of oral fluid [9, 10]. There is a metabolic acidosis in the tissues, which contributes to the development of inflammatory and dystrophic processes, accompanied by gum hyperemia [11]. This effect is due to increased vascular wall permeability, an inhibitory effect on platelet aggregation and osteoclast activation.

Conclusion. Changes in the NO system in the oral fluid of school-age children indicate a disturbance in the balance of the L-arginine/arginase system in preclinical stages of thyroid homeostasis and the development of iron deficiency anemia. Particular attention is paid to the change in the rates in children with combined microelementosis and the gender difference in the indicators in the studied groups. Such differences can be the result of poor hygiene in boys against the background of microelement

Table 1

State of L-arginine/arginase system and dihydrogen sulfide of oral fluid in children with iodine deficiency (ID), latent iron deficiency (LID), combined deficiency of bioelements (ID+LID), adequate iodine and iron supplementation children aged 6-11 years old (M±m)

Indexes	Group I (control)		Group II (ID)		Group III (LID)		Group IV (ID+LID)	
	Boys (n=8)	Girls (n=8)	Boys (n=9)	Girls (n=9)	Boys (n=8)	Girls (n=8)	Boys (n=10)	Girls (n=8)
H ₂ S, (μmol / l)	67.22±11.33	68.18±8.23	70.18±3.93	78.25±3.74	72.91±6.89	63.3±0.85	71.74±5.89	75.5±11.53
NO ₂ , (μmol / l)	0.27±0.09	0.77±0.62	4.17±1.18 p ₁₋₂ <0.05	1.09±0.24	0.98±0.18 p ₁₋₃ <0.01	0.42±0.05 p ₂₋₃ <0.05	2.23±0.31 p ₁₋₄ <0.001	1.22±0.15 P ₃₋₄ <0.001
NO ₂ +NO ₃ , (μmol / l)	1.44±0.32	2.97±1.54	5.39±1.70 p ₁₋₂ <0.05	2.60±1.25	2.79±1.03	2.20±0.14	4.81±1.37 p ₁₋₄ <0.05	3.24±0.36 P ₃₋₄ <0.05
Peroxynitrite, (μmol / l)	5.30±0.91	3.69±1.08	12.23±3.98	11.98±1.65 p ₁₋₂ <0.01	10.96±3.15	10.57±2.72 p ₁₋₃ <0.05	14.63±3.58 p ₁₋₄ <0.01	9.09±0.79 P ₁₋₄ <0.01
L-arginine, (μg / ml)	37.03±5.06	28.25±7.67	42.28±6.13	42.12±7.88	37.67±9.01	40.13±0.42	38.52±9.73	37.3±3.11
Arginase, (μmol / min • mg)	0.27±0.04	0.23±0.09	0.28±0.06	0.24±0.06	0.205±0.02	0.25±0.03	0.18±0.01 p ₁₋₄ <0.05	0.33±0.07

Note. P with Arabic numerals is a significant difference between the indicators in the respective research groups imbalance, which potentially negatively affects the stomatological status of children. The revealed changes can affect the oral cavity, mineralizing and anti-inflammatory functions of the oral fluid, with subsequent effects on hard tissue of the teeth, periodontal and mucous membranes.

Prospects for further research. Investigation of dental status in children – residents of endemic regions and schoolchildren with iron deficiency anaemia. Implementation of modern preventive measures for pupils with microelement imbalance, who form the risk group of endocrine, hematologic and dental pathology.

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Hrygorieva O.A.,*MD, PhD, DSc, Professor, Chief, Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine***Matvieishyna T.M.,***MD, PhD, Associate Professor, Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine, matvieishyna_tn@meta.ua***Topolenko T.A.***MD, PhD, Associate Professor, Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine*

DYNAMICS AND MORPHOLOGY OF DENDRITIC CELLS OF THE NASAL SUBMUCOSA OF RATS' PHARYNX AFTER ANTENATAL ANTIGEN INFLUENCE

Abstract. *Researching determining mechanisms of structural and immunological homeostasis under physiological conditions, alteration of neurohumoral and metabolic control, consider to affirm that DCs are the most important modulators of skin and mucosal structure. Purpose of the study is to describe the dynamics and morphology of nasopharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus. DCs were detected on the cryostat sections of the pharynx tissue using the Vahstein-Meisel method. DCs have high activity of ATPase. Results. It is settled that in all groups DCs number increases by 14 days of life, simultaneously with increasing of antigenic load on the body. In antigen-primed animals DCs activation take place earlier than in control, namely at the 7th day of life. In antigen-primed animals, the DCs number does not change, as compared to control, but the number of their dendrites is greater compared to control. DCs activation at 14th day of life is probably explained by an increased antigenic load on the body due to changing of the type of food. DCs high activity of ATPase is explained by activation of proteases in the endocytosis process of antigens. In conclusion, antenatal antigen influence does not cause changes in the ATP⁺ DCs number in nasopharyngeal submucosa, but in antigen-primed animals, the intensity of deposition of ATP⁺-material in the cytoplasm of the cells is higher and the number of dendrites in the cells is greater than that of the control and intact groups. In animals of all observed groups DCs activation passes at the first day after birth. In antigen-primed animals, the second wave of activation ATP⁺ DCs takes place at the 7th day of life, namely a week earlier than in animals of the intact group.*

Keywords: *ATP, antenatal antigen action, dendritic cells, nasopharynx.*

Introduction. Dendritic cells (DCs) are the one of fundamental representations of the immune system, as they are the most efficient APCs for the activation of naive T cells. This process leads to the induction of primary immune responses. DCs reside in unflappable tissues in an immature form where they are very capable of taking up antigens but weak at stimulating T cells. After the influence of a variety of danger signals, including pathogens, proinflammatory cytokines, and dying cells, DCs undergo a process of differentiation or maturation and migrate to the T cell areas of secondary lymphoid organs [2, 5].

Researching determining mechanisms of structural and immunological homeostasis under physiological conditions, alteration of neurohumoral and metabolic control, consider to affirm that DCs are the most important modulators of skin and mucosal structure. This point is based on current data according to effects

of their participation in skin and mucosal structural homeostasis maintaining. DCs participate in processes of innate and adaptive immunity, as well as in switching between immunity and tolerance depending on their activation and maturation states [1].

Purpose of the study is to describe the dynamics and morphology of nasopharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus.

Material and methods. The object of the study is pharynx of 124 white laboratory rats. Withdrawal of animals from experiment was carried out on 1, 3, 7, 14, 21, 45 days of postnatal life with decapitation. Animals are divided into 4 groups: I – intact, II – animals, which were exposed to antenatal antigen influence on the 18th day of prenatal development with the method of Voloshyn M.A. (2010) [8], III – animals, which were exposed to amniotic fluid antigen influence on the

18th day of prenatal development with the method of Voloshyn M.A. (2011) [9], IV – control animals, which were exposed to antenatal intrafetal injection of saline solution on the 18th day of prenatal development. The split virus inactivated Influenza vaccine Vaxigrip, have been used as antigen. DCs were detected on the cryostat sections of the pharynx tissue using the Vahstein-Meisel method. DCs have high activity of ATPase, because of the activity of the ATP-dependent proton pump depends on the gradual decrease of pH in the endosomes and lysosomes, activation of proteases in the endocytosis of antigens. Other pharyngeal cells exhibit moderate to low activity of ATPase. Control of the reaction was carried out with histological preparations rich in ATPase. Preparations were contained in glycerol-gelatin. The number DCs dendrites was counted in a nasopharyngeal submucosa on a unit area of $15000 \mu\text{m}^2$ under a microscope with oil immersion technique (ocular lens 8, objective lens 90). The variation statistics methods via program STATISTICA 6.1 was used to compare differences in number of DCs and DCs dendrites. The $p \leq 0,05$ were considered significant.

Results of study. ATP-positive DCs are found in the nasopharyngeal submucosa. At the day 1st of life in intact animals, the body of the cells has the form of an elongated triangle with spatially orientated dendrites, preferably with the button-shaped ends. DCs is present $1 \pm 0,2$ at $5000 \mu\text{m}^2$ in nasopharyngeal submucosa (Table).

The number of DCs dendrites in nasopharyngeal submucosa is $2,7 \pm 0,05$ at $15000 \mu\text{m}^2$ (Table). The absolute number of DCs ATP⁺ in the submucosa is $1,2 \pm 0,15$ at $5000 \mu\text{m}^2$ and $1,4 \pm 0,35$ at $5000 \mu\text{m}^2$ in animals of the second and the third groups (Tabl. 1).

At day 7th of life in intact animals, the number of DCs ATP⁺ increases in comparison with the previous observation period. The tendency of DCs number increasing in antigen-primed animals remains up to the 7th day of life. The number of DCs dendrites in the nasopharyngeal submucosa in experimental animals is statistically significantly higher compared to intact animals.

At the 14th day of life, the accumulation of ATP-positive material in the cytoplasm of DCs increases, it manifests itself by darker DCs color, also the number of dendrites increases, simultaneously their length decreases, the form changes into the fan-shaped.

In experimental animals, DCs are more vividly

Table

ATP⁺-DCs' Dynamics in Rats' Nasopharyngeal Submucosa on the Unit Area ($5000 \mu\text{m}^2$, Vashhtein-Meisel reaction), ($M \pm m$)

Day of Life	Group of Observation	Number of DC	Number of DC Dendrites
1	I	$1 \pm 0,2$	$2,7 \pm 0,05$
	II	$1,2 \pm 0,15$	$4 \pm 0,13^*$
	III	$1,4 \pm 0,35$	$3,9 \pm 0,08^*$
	IV	$1 \pm 0,2$	$2,7 \pm 0,05$
7	I	$1,4 \pm 0,3$	$2,8 \pm 0,08$
	II	$1,7 \pm 0,35$	$3,9 \pm 0,1^*$
	III	$1,9 \pm 0,15$	$4 \pm 0,05^*$
	IV	$1,4 \pm 0,3$	$2,8 \pm 0,08$
14	I	$3 \pm 0,3\#$	$3,4 \pm 0,08\#$
	II	$3,2 \pm 0,5$	$4,7 \pm 0,23^*$
	III	$3,4 \pm 0,35$	$4,6 \pm 0,08^*$
	IV	$3 \pm 0,3\#$	$3,4 \pm 0,08\#$
21	I	$3,2 \pm 0,15$	$3 \pm 0,25$
	II	$3,5 \pm 0,5$	$4 \pm 0,05$
	III	$3,7 \pm 0,65$	$3,8 \pm 0,03$
	IV	$3,2 \pm 0,15$	$3 \pm 0,25$
45	I	$3 \pm 0,3$	$2,6 \pm 0,18$
	II	$3,4 \pm 0,35$	$3,5 \pm 0,1$
	III	$3,5 \pm 0,5$	$3,5 \pm 0,05$
	IV	$3 \pm 0,3$	$2,6 \pm 0,18$

Notes: I – intact, II – animals, which were exposed to antenatal antigen influence, III – animals, which were exposed to amniotic fluid antigen influence, IV – control animals; the symbol * means that the result is statistically probable with respect to the intact group, the symbol # means that the result is statistically probable in relation to the previous observation period.

colored throughout all observation periods than in control. Most often there are cells with enormous number of dendrites than in common DCs, having a predominantly fan-shaped form. In experiment the DCs dendrites are visually thicker and more intense coloring than in control. The content of DCs in the submucosa throat in experimental rats tends to increase compared to the intact group.

During the period from the 21 to the 45 day of life in intact animals, intense deposition of ATP-positive material in the DCs cytoplasm doesn't change, the shape of the dendrites is predominantly fan-like. DCs number is at the level of the previous observation period. In antigen-primed animals at the 21st day of life, there the DCs number is higher compared to control, there are cells with a greater number of short dendrites (Tabl. 1). The shape of dendrites is different: fan-shaped endings prevail, except them button-

shaped dendrites are also present.

Discussion. DCs number increases throughout first two weeks, simultaneously with increasing of antigenic load on the body. In antigen-primed animals, regardless of the input method of antigen, DCs activation take place earlier than in control, namely at the 7th day of life. In antigen-primed animals, the DCs number does not change, as compared to control, but the number of their dendrites is greater compared to control. DCs activation at 14th day of life is probably explained by an increased antigenic load on the body due to changing the type of food.

DCs high activity of ATPase is explained by activation of proteases in the endocytosis process of antigens. Adenosine is a well-studied neurotransmitter, but as a part of ATP it also exerts profound immune regulatory functions. Extracellular ATP acts as a “danger” signal and stimulates immune responses, i.e. by inflammasome activation [6]. Its degradation product adenosine also acts rather anti-inflammatory, as it down regulates functions of DCs and dampens T cell activation and cytokine secretion [3]. DC derived adenosine can also act back onto the DCs in an autocrine manner. This leads to suppression of DCs functions that are normally involved in stimulating immune responses. Moreover, ATP and adenosine production thereof acts as “find me” signal that guides cellular interactions of leukocytes during immune responses [4, 7].

The more ATP is accrued in DCs, the more credibly DCs will derivate adenosine. It is consequents to suppresses activation of T cells. Also derivation of adenosine can preventing DCs maturation and development of effectors' functions. This probably leads to decrease in the reactions of local immunity.

Conclusions. Antenatal antigen influence does not cause changes in the ATP⁺ DCs number in nasopharyngeal submucosa, but in antigen-primed animals, the intensity of deposition of ATP⁺-material in the cytoplasm of the cells is higher and the number of dendrites in the cells is greater in control. In animals of all observed groups DCs activation passes at the first day after birth. In antigen-primed animals, the second wave of activation of ATP⁺ DCs takes place at the 7th day of life, namely a week earlier than in animals of the control and intact groups.

Prospects for further research. It is planned to

describe the dynamics and morphology of oropharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus.

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Popko S.S.,*PhD, High teacher of the Department of Histology, Cytology and Embryology Mayakovsky Avenue 24a, Department of Histology, Cytology and Embryology, Zaporozhye, 69035, Ukraine***Yevtushenko V.M.,***MD, Professor of the Department of Histology, Cytology and Embryology ZSMU, Zaporozhye, Ukraine***Syrtsov V.K.***MD, Professor, Head of the Department of Histology, Cytology and Embryology ZSMU, Zaporozhye, Ukraine*

FEATURES OF BALT IN RATS WITH EXPERIMENTAL CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ADMINISTRATION OF THIOMETRISOL

Abstract. *Background. Cigarette smoke is a major etiological factor associated with the development of chronic obstructive pulmonary disease (COPD). Despite the importance and growing prevalence of COPD, little progress has been made in developing effective drug therapy. The development and progression of COPD is characterized by chronic progressive inflammation. An objective of the research: to establish the morphological parameters of immunocompetent cells in the composition of BALT rats with experimental chronic obstructive pulmonary disease and the administration of thiometrisol.*

An object of this study: 60 adult rats of Wistar line, using immunohistochemical method by determining the CD4, CD8, CD20, CD67 (Thermo Scientific, USA) and administration of thiometrisol. As a result of our research, it was revealed that BALT consists of lymphoepithelial lymphoid nodules. Lymphoid nodules were a highly organized structure of lymphocytes and antigen-presenting cells. CD20+ cells formed lymphoid nodules, sometimes with a germinal center, which are surrounded by a perifollicular area consisting of T cells of predominantly CD4+ origin. CD67+ cells are located between the T cells for transport and the presentation of antigens from the lumen of the bronchi to the T cells. Lymphocytes enter the BALT through high endothelial venules (HEV), which are present in the lymphoid nodules. Sometimes there were other lymphoid accumulations, mainly in the lung parenchyma, which did not meet all the criteria for BALT. These lymphoid structures were not in contact with the bronchial epithelium and were more in contact with the lumen of the alveoli than with the bronchial or bronchiolar lumen. They were predominantly CD20+ cells with a weakly pronounced germinal center, surrounded mainly by CD4+ T cells, but also diffusely located CD8+ T cells. These lymphoid accumulations were more often observed in animals after exposure to tobacco smoke, which was the trigger factor for their development. Thus, the study showed that in experimental animals there are violations of the cellular, humoral and nonspecific parts of the immune system. An increase in the content of B-lymphocytes (CD20 + cells), the main lymphocyte phenotypes (CD4 + and CD8 + cells) provide an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines. Conclusion. The results confirm the need for adequate treatment of patients with COPD for the elimination of chronic inflammation; they justify the use of thiometrisol in complex therapy. Its use can successfully supplement standard therapy and improve the state of the local immune system of the lungs.

Keywords: COPD, rats, BALT, immunohistochemical method, thiometrisol.

Introduction. In recent years, there has been an increase in the number of chronic respiratory diseases, a special place among which is chronic obstructive pulmonary disease (COPD), which is a significant medical and social problem. According to the World Health Organization, about 220 million people in the world today suffer from this disease, which in 2020 will take the third place in the world in terms of mortality [1]. In Ukraine, the

current epidemiological situation is characterized by the incidence of COPD at the level of 7% of the population of Ukraine, or about 3 million people.

The development and progression of COPD is characterized by an incompletely reversible airway obstruction, accompanied by chronic progressive inflammation, mucociliary dysfunction, structural changes with airflow restriction, as well as a systemic component. One of the insufficiently

studied phenomena in the study of morphological changes in chronic nonspecific lung diseases is the reaction of connective tissue elements, such as microvessels and immunocompetent cells [2, 3, 4].

Cigarette smoke is a major etiological factor associated with the development of chronic obstructive pulmonary disease (COPD). Despite the importance and growing prevalence of COPD, little progress has been made in developing effective drug therapy [5]. For the treatment strategy to work, it is necessary to slow down and inhibit the inflammatory and destructive processes underlying this disease. A morpholine-based chemical compound 2(5-(4-pyridyl)-4(2-methoxyphenyl)-1,2,4-triazol-3-ylthio)acetate (hereinafter abbreviated as thiometrisol) is an original active substance, with cytoprotective, antioxidant, anti-inflammatory properties. On the basis of deep scientific research, its effectiveness has been proven due to the presence of anti-inflammatory, antioxidant, anti-hypoxic, and immunocorrective action in it [6].

Obviously, the morphofunctional characteristics of the influence of thiometrisol on the local immune system of the lungs remain relevant and not fully studied.

An objective of the research: to establish the morphological parameters of immunocompetent cells in the composition of BALF rats with experimental chronic obstructive pulmonary disease and the administration of thiometrisol.

Material and methods. The work was performed on 60 adult rats of Wistar line, divided into 3 groups: 1 - 20 intact animals; 2 - experimental group, 20 animals with experimental chronic obstructive pulmonary disease; 3 - 20 animals to whom, on the background of a modeled chronic nonspecific lung disease, thiometrisol was administered. To simulate an experimental chronic obstructive pulmonary disease, the world-famous model (Geraghty P. et al., 2014) will be applied, according to which experimental animals are exposed to tobacco smoke in a specially designed chamber for 4:00 a day, 5 days a week for 2 months with a total solids concentration of 80 mg / m³. For the correction of chronic non-specific lung disease, thiometrisol was used - a chemical compound based on morpholinium 2 (5- (4-pyridyl) -4 (2-methoxyphenyl) -1,2,4-triazol-3-ylthio) acetate (intraperitoneally in the amount of 25 mg per kg of

animal body weight 2 times a day for 5 days).

The animals were taken out of the experiment according to the prescribed time limit. For an immunohistochemical study, material from the bronchi and lungs was first fixed in a 10% solution of neutral buffered formalin for 10–12 hours and compacted into paraffin. Slices made 4-6 µm thick were mounted on adhesives SuperFrost Plus slides (Menzel Glaser, Germany). In our study, CD4, CD8, CD20, CD67 (Thermo Scientific, USA) were used as primary antibodies. The next stage of immunohistochemistry was performed using the latest generation Quanto imaging system (Thermo Scientific, USA). Secondary antibodies with a high content of horseradish peroxidase molecules were applied in sections and incubated in humid chambers for 10 minutes with washing in TRIS-buffer solution between each stage for 5 minutes. The reaction was identified by applying a DAB chromogen under microscope control. pas for 20 seconds to 1 minute, with specific structures showing dark brown color. Morphological study of the obtained sections was performed using a Primo Star light microscope (Zeiss, Germany) with a documentation system Using computer morphometric analysis among elements of lymphoid tissue associated with the trachea and bronchi, the average number of immunocompetent cells was noted: CD4+, CD8+, CD20+, CD67+ cells.

A quantitative analysis of the results of a morphometric study and statistical processing of morphometric data will be carried out according to generally accepted statistical methods and using Microsoft Office Excel and Statistica 6.1. The significance of differences between the values of independent micrometric values is determined by Student's criterion

Results. Protection of the mucous membrane of the respiratory tract is carried out by the mucous barrier with the epithelium, which provides mucociliary clearance, intraepithelial lymphocytes, and lymphoid tissue associated with the mucous membrane of the bronchi. As a result of our research, it was revealed that BALF consists of lymphoepithelial lymphoid nodules. Lymphoid nodules were a highly organized structure of lymphocytes and antigen-presenting cells. CD20+ cells formed lymphoid nodules, sometimes with a germinal center, which are surrounded by a

perifollicular area consisting of T cells of predominantly CD4⁺ origin. CD67⁺ cells are located between the T cells for transport and the presentation of antigens from the lumen of the bronchi to the T cells. Lymphocytes enter the BALT through high endothelial venules (HEV), which are present in the lymphoid nodules. Sometimes there were other lymphoid accumulations, mainly in the lung parenchyma, which did not meet all the criteria for BALT. These lymphoid structures were

not in contact with the bronchial epithelium and were more in contact with the lumen of the alveoli than with the bronchial or bronchiolar lumen. They were predominantly CD20⁺ cells with a weakly pronounced germinal center, surrounded mainly by CD4⁺ T cells, but also diffusely located CD8⁺ T cells. These lymphoid accumulations were more often observed in animals after exposure to tobacco smoke, which was the trigger factor for their development (tab. 1).

Table

Dynamics of the quantitative composition of immunocompetent cells in the composition of BALT of rats

Group	Type of immunocompetent cells			
	CD4 ⁺	CD8 ⁺	CD20 ⁺	CD67 ⁺
I	35,48±0,95	25,90±0,50	19,15±0,16	11,22±0,33
II	45,60±0,71*	36,24±1,24***	38,25±2,91***	25,37±2,13***
III	37,86±2,51	27,19±1,96**	20,56±0,61	14,94±1,98***

I - intact group; II - animals with experimental chronic obstructive pulmonary disease; III - animals with experimental chronic obstructive pulmonary disease and administration of thiometrisol.

* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$ (in comparison with intact animals).

Discussion. Thus, the study showed that in experimental animals there are violations of the cellular, humoral and nonspecific parts of the immune system. An increase in the content of B-lymphocytes (CD20⁺ cells), the main lymphocyte phenotypes (CD4⁺ and CD8⁺ cells) provokes an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines. All this creates conditions for the formation of chronic immune deficiency, which is a marker of an adverse course of COPD [2, 5].

Conclusion. The results confirm the need for adequate treatment of patients with COPD for the elimination of chronic inflammation; they justify the use of thiometrisol in complex therapy. Its can use successfully supplement standard therapy and improve the state of the local immune system of the lungs.

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Todoriv T.V.,*Assistant, Department of Physiology SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine, taniastrokosh@gmail.com***Bagrii M.M.,***Associate Professor, Department of Pathomorphology and Forensic Medicine, SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine***Voronych-Semchenko N.M.***Chief of the Physiology Department, Professor, SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine*

STRUCTURAL PECULIARITIES OF MYOCARDIUM IN RATS UNDER CONDITIONS OF IODINE DEFICIENCY AND OBESITY

Abstract. *The article represents the results of the study of the structural peculiarities of myocardium in rats under conditions of iodine deficiency and obesity in the experiment. Under the conditions of iodine deficiency there were observed: an increase of interstitium in the myocardium due to its edema, in the arterioles – swelling and vacuolation of the endothelial cell cytoplasm, in medial membrane of small arteries – small vacuoles. More significant changes in the structural organization of the myocardium were found in the rats that were on a high-calorie diet. Fragmentation of parenchymal cells of the heart is determined, where there could be observed small amount of vacuoles which are small, of round form and transparent. In the tiny arteries, the solitary focal lucent areas of the cytoplasm of the innercellular membrane are visualized due to the presence of small transparent vacuoles, as well as around solitary tiny arteries there is a slight expansion of connective tissue fibers.*

Key words: *iodine deficiency, obesity, cardiovascular system, myocardium*

Introduction. Lack of iodine in the environment for many countries of the world creates an significant medical and social problem. According to WHO experts, one third of the Earth's population is at risk of iodine deficiency (ID). Approximately one billion inhabitants of the planet have clinical manifestations of ID [1]. Deficiency of hormones of the thyroid gland in the body leads to a breakdown of carbohydrate, water-electrolyte, lipid, protein metabolism, causing morphofunctional and biochemical changes in various organs and systems. In particular, ID are associated with an increase in cardiovascular morbidity [2-3]. In spite of the successes in the prevention of cardiovascular events, cardiovascular diseases occupy a leading place among the causes of mortality in the industrialized world. An important factor in the development of cardiovascular disease is obesity, which is one of the most common metabolic diseases in the world [4]. In most countries, between 30% and 60% of adults have excess body weight. It is known that excess body weight reduces the standard of living, human capacity, complicates physical and intellectual activity, leads to premature aging, early disability, reduction in life expectancy [5].

The purpose of the study. Investigate the structural features of myocardium in rats under iodine deficiency and obesity in the experiment.

Material and methods. The study was conducted on 60 white adult mature rats weighing 150-180 g that were divided into two experimental groups: animals with iodine deficiency (1st experimental group, n = 20) and obese animals (2nd experimental group, n = 20). For comparison, similar studies were performed on intact animals (control group, n = 20). The condition of ID was reproduced by a two-month iodine-deficient diet [6]. To simulate obesity animals were kept on a high-calorie diet [7]. To assess the thyroid status of animals, the content of thyroid hormones in blood serum and urinary iodine concentration were determined. Control over the reconstruction of alimentary obesity was performed by calculating the body mass index (BMI). Histologic cuts of the heart were stained with hematoxylin and eosin, according to Shabadash (identification of glycogen), alcian blue according to Stidmen (determination of nonsulfated glycosaminoglycans), and also PAS-staining (verification of glycoproteins) was performed. Histological studies were carried out on the Leica DME light-optical microscope. In

order to objectivize the quantitative studies, computer morphometry of objects in histological preparations was performed. Morphometric analysis of the myocardium was performed taking into account the following parameters: the thickness of the cardiomyocyte (TC), the average perimeter of the cardiomyocyte nucleus (APCN), the average area of the cardiomyocyte nucleus (AACN), the parenchyma-Interstitial index (PII) was calculated. Euthanasia of animals was carried out by decapitation under ketamine quenching (100 mg / kg body weight). The keeping, feeding and euthanasia of animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986). The statistical analysis of the results was performed using Microsoft Excel and Statistica 5.5 computer programs.

Results of research and discussion. As a result of the study in animals with iodine deficiency there is a tendency to increase TC, APCN with a clear visualization of the borders of most muscular cells of the heart. The nuclei are mainly localized in the central part of the cells, somewhere in the paracentral. The shape of the nuclei is ovoid, somewhere round and spindle-shaped. The nuclei are mostly with a clear karyolemma, chromatin of nuclei is finely dispersed, moderately basophilic, uniformly distributed in the nucleoplasm. Some cells contain central and paracentral tiny, rounded nucleoli. Focal muscular cells are unevenly enlarged in diameter, have a wavy appearance, plasmolemma is veiled, the cytoplasm is unevenly stained with eosin, with moderate lucent areas. The nuclei of such parenchymal cells of the myocardium are slightly lumenized, some of them – are reduced in size, with a wavy karyolemma, and somewhere nuclei are not visualized due to karyolysis. There is an increase in interstitium due to its edema, which is evidenced by a sharp decrease in PII at 76% ($p < 0.001$) in relation to control (Table 1). At the same time, the loose connective tissue of the interstitium is visualized by an lumenized, somewhere vacuolated, there are some macrophages and lymphocytes in a small number. When staining of histological sections with alcian blue according to Stidman in the extended interstitial zones the accumulation of nonsulfated glycosaminoglycans of greenish color is visualized, which is a reflection of deficiency of thyroid hormones on the basis of

iodine deficiency with the development of mucinous edema (Fig. 1). An increase in the interstitial layers is observed, which is accompanied by a violation of the ordered structure of the muscular cells of the heart, which appear to be flaky in this case. Somewhere there is a fragmentation of cardiomyocytes into separate areas. In the arterioles of the myocardium, insignificant condensation of the chromatin of the endothelial cells nuclei is observed, in some of them – there are swelling and vacuolation of the endothelial cells cytoplasm, single macrophages and lymphocytes are periarteriolar. In the lumen of arterioles, in most cases, there are red cells located compact.

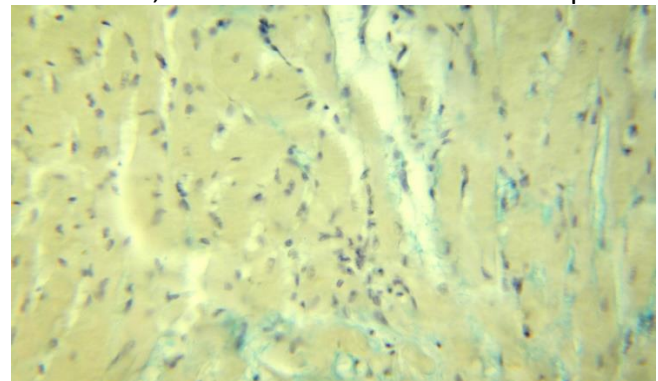


Fig. 1. Mucosal myocardial edema of a rat under the conditions of iodine deficiency. Staining: Alcian blue according to Stidman. Magnification: $\times 400$

Tiny arteries with swelling and vacuolation of the endothelial cell cytoplasm, which bulges slightly into the lumen of the vessels. The nuclei are slightly hyperchromic, irregularly rounded. Other endothelial cells that do not undergo edematous phenomena, spindle-shaped, elongated along the wall of the nucleus. In the middle membrane of small arteries in the cytoplasm of smooth myocytes, there are small vacuoles in the form of small rounded lumenized areas (Fig. 2). The nuclei of such smooth myocytes are slightly hyperchromic, of irregular, ovoid form. Around the individual tiny arteries of the myocardium there are cellular infiltrates in a small amount, mainly represented by macrophages and lymphocytes. In the lumen of such small arteries, moderate amount of red blood cells is found. Small veins of the myocardium in the lumen contain somewhere compactly located erythrocytes. Endothelial cells are with clear spindle-shaped homogeneous nuclei elongated along the walls of vessels, insignificant amount of cytoplasm without signs of vacuolation, clasmotosis. The presence of focal vacuolization of

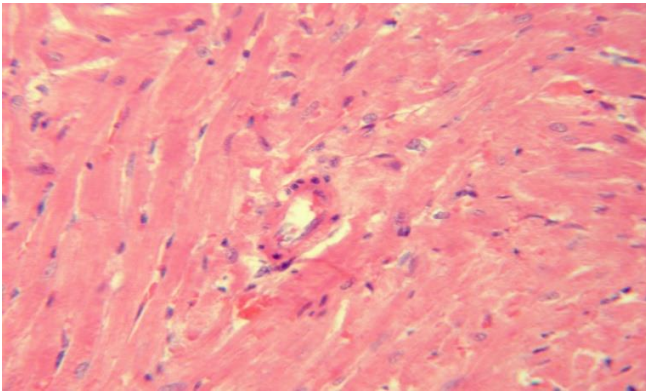


Fig. 2. Artery of the rat's myocardium under the conditions of iodine deficiency. Staining: hematoxylin and eosin. Magnification: $\times 400$

these cells is determined in the middle membrane in the thickness of smooth myocytes.

More significant changes in the structural organization of the myocardium were found in rats that were on a high-calorie diet. In particular, a significant decrease in the thickness of the nucleus was observed (22%, $p < 0.001$), somewhere uneven staining of cellular cytoplasm through enlightenment in some of them (see Table 1). In such cells, the cross-striation is veiled and slightly blurred in their contours. In some areas there is the fragmentation of parenchymal cells of the heart. Cardiomyocytes in this case are represented fragmented, the borders of cells are veiled. During staining according to Shabadash, it was determined that a small part of the cardiomyocytes in the cytoplasm contains a reduced amount of glycogen (Fig. 3). Focally are traced small, of round form, transparent, in a small number of vacuoles in the cytoplasm of parenchymal cells of the heart that do not displace the nuclei of cardiomyocytes and do not violate the integrity of cytomembrane. The nuclei of cardiomyocytes are of the ovoid form, located centrally with fine-grained chromatin. Their

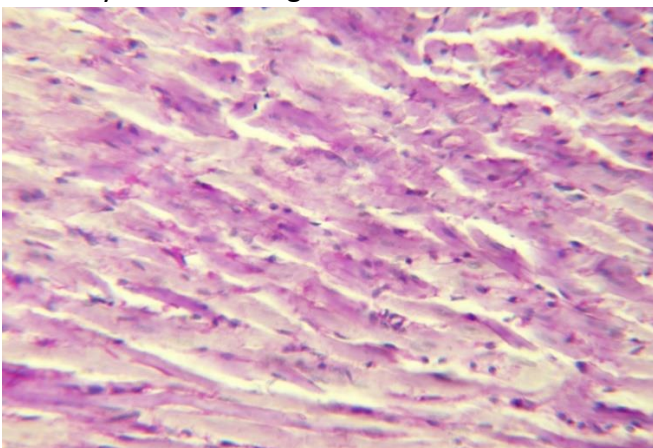


Fig. 3. Glycogen in cytoplasm of rat's cardiomyocytes under obesity conditions. Staining: according to Shabadash. Magnification: $\times 400$.

perimeter and area are smaller than in intact animals at 25 and 36%, respectively ($p < 0.05$). The thickness of the parenchymal cells of the heart under the conditions of obesity is less at 22% ($p < 0.05$) relative to the control data. These morphological changes may reflect biochemical changes in the organism in obesity, in particular, be due to the reduced allocation of adiponectin and leptin increased excretion. Under such conditions the loose connective tissue between the cardiomyocytes is expanded (parenchymatous-interstitial index is lower than that of intact animals at 74%, $p < 0.001$), sometimes it contains a small amount of macrophages, lymphocytes, fibroblasts (see Table 1). The extended and full-blood hemocapillaries are visualized in the interstitium, around individual ones – there are single freely located leukocytes. A small amount of leukocytes is observed in the lumen of individual capillaries along with erythrocytes. Endothelial nuclei are elongated, homogeneous. In the small arteries the solitary focal lumenized areas of the cytoplasm of cells of the inner lining are visualized due to the presence of small transparent vacuoles, accompanied by a slight protrusion of the cytoplasm to the capillary lumen (Fig. 4). The nuclei of such endothelial cells retain an elongated character. Around the solitary small arteries there is a slight growth of connective tissue fibers (Fig. 5).

As a result of the comparative analysis, in obese animals, the decrease of the thickness of cardiomyocytes (34%, $p < 0.05$), perimeter and area of nuclei (at 29 and 39%, respectively, $p < 0.001$, $p < 0.05$) regarding the analogous indices in rats that were on the iodine-deficient diet. Such data emphasize the important role of obesity in

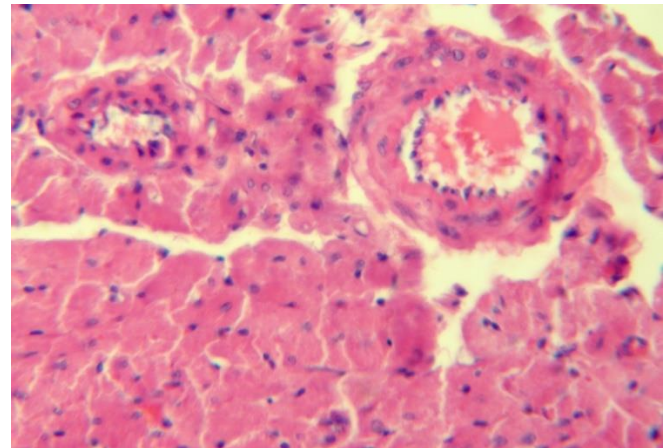


Fig. 4. Arteries of the rat's myocardium in obese conditions. Staining: hematoxylin and eosin. Magnification: $\times 400$

the development of cardiac pathology, which can potentiate in the regions of goiter endemic areas.

Conclusions. Iodine deficiency and high calorie diet accompanied by the

development of structural changes of the myocardium, which significantly increases cardiovascular risk under such conditions.

Prospects for further studies. Relevant topics for further study can be metabolic processes in the myocardium, the dynamics and reversibility of the revealed structural changes.

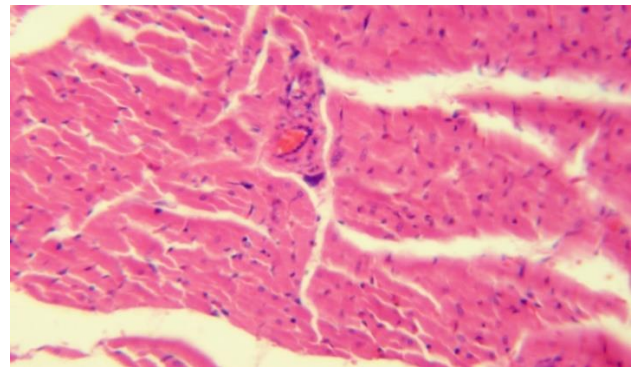


Fig. 5. Myocardium of the rat in obese conditions. Staining: hematoxylin and eosin. Magnification: $\times 400$

Table 1

Changes in the morphometric indices of rats' myocardium against the background of iodine deficiency and obesity (M \pm m)

Animal groups	Thickness of cardiomyocyte, μm	Perimeter of the nucleus, μm	Area of the nucleus, μm^2	Parenchyma-interstitial index, relative units
Control group (n=20)	14.96 \pm 1.19	40.55 \pm 3.31	88.93 \pm 11.24	45.15 \pm 2.11
1 st research group (Iodine deficiency, n=20)	17.57 \pm 2.47	42.61 \pm 1.97	93.69 \pm 11.12	10.74 \pm 1.03 [#] P _{K-1} <0.001
2 nd research group (Obesity, n=20)	11.65 \pm 1.05* p ₁₋₂ <0.05	30.15 \pm 3.50* p ₁₋₂ <0.01	57.05 \pm 10.11* p ₁₋₂ <0.05	11.90 \pm 1.15 [#]

Note. Reliable difference regarding control * - $p < 0.05$, # - $p_{1-2} < 0.001$; p with Arabic numerals – significant changes between indices in experimental groups.

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Sapunkov O.D.,
Kosakovskiy A.L.,
Sapunkova S.S.,
Bogdanova L.O.

Department of Pediatric Surgery and Otolaryngology, of the Higher State Educational Institution of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine

EVOLUTION OF THE LOWER WALL OF TYMPANIC CAVITY IN THE EARLY PERIOD OF ONTOGENESIS

Abstract. *The structure and syntopy of the inferior wall of the tympanic cavity have been studied in 58 fetuses and 11 newborns. It is established that it begins to be formed at the eighth month of intrauterine development. In newborns, the thickness of the inferior wall of the tympanum is 1.06 ± 0.03 mm, the length of the jugular fossa is 12.5 ± 0.29 mm, the width of the jugular fossa is 8.51 ± 0.27 mm.*

Key words: *anatomy, fetus, jugular fossa, ontogenesis, tympanic cavity.*

Introduction. At this time, the problem of the pathology of the middle ear in newborns and infants is very topical due to the high frequency of their detection of otitis media [1]. It connected with certain anatomical characteristics of the structure of the nasal cavity and the middle ear, and the presence of amniotic fluid in the middle ear [5]. Most authors are engaged in the study of the pathology of the middle ear of term infants and children of the first year of life [3]. Some researchers consider the disease of the middle ear in premature infants [5, 6]. In the analysis of the pathology of the middle ear, they pay more attention to exudative otitis media [2, 8].

There are few works on the pathology of the middle ear in premature infants, although in the first months of life many of them receive continuous therapy (Continuous Positive Airway Pressure) and are on enteral feeding, which can lead to inflammation changes in the cavity of the nose, nasopharynx, auditory tube and provoke the development of exudative otitis media [4].

Therefore, the nursery of children with low and extremely low body weight and an increase of the number of cases of such children surviving to 7% [1], the study of the problem of the pathology of the middle ear is very actual today.

These traumatic lesions of soft tissues of the nose and nasopharynx in premature infants may lead to pathological processes in the middle ear

what can reach 42% [4]. At the same time in 10% of premature infants the otitis media goes quite difficult and in the future it is necessary to conduct surgical intervention - miringoplasty or shunting of the tympanic cavity [6]. That's why the study of embryological aspects of the development of the middle ear, its anatomy and topographic anatomy in the fetuses, remains very important. Modern scientific achievements have considerably expanded the practical possibilities of endoscopic operations with diseases of the ear. The introduction of them contributed to the development of the priority direction - endoscopic operations, which have several advantages over traditional otomicrosurgical operations [7].

The aim of the study. To study the peculiarities of the development of the inferior wall of the tympanic cavity in the early period of ontogenesis.

Materials and methods. The study was carried out on corpses of 58 fetuses of 271,0-375,0 mm of parietal-coccygeal length (PCL) and 11 newborns by methods of ordinary and fine preparation under the control of binocular enlarging lens, macromicroscope and morphometry, photomicrography of the "OLIMPUS μ 1000 All-weather 10.0 Mpix" digital camera.

Result. We have found out that the inferior wall of the tympanic cavity limits the jugular fossa and begins to form on the 8th month of intrauterine development in the fetuses of 271.0 -

310.0 mm PCL as an processus of the petrosal part of the temporal bone. The development of internal jugular vein contributes to the formation of this wall. Within process of increasing of its diameter the lower edge of the annulus tympanicus begins to depart from the promontory and the tilt angle of the upper edge increases. As

a result the annulus tympanicus moves from a horizontal position in a more inclined. The thickness of the inferior wall of the tympanic cavity during this period is $0,63 \pm 0,03$ mm. The width of the jugular fossa is $6,25 \pm 0,27$ mm, and its length reaches $8,50 \pm 0,32$ mm (Fig. 1, 2).

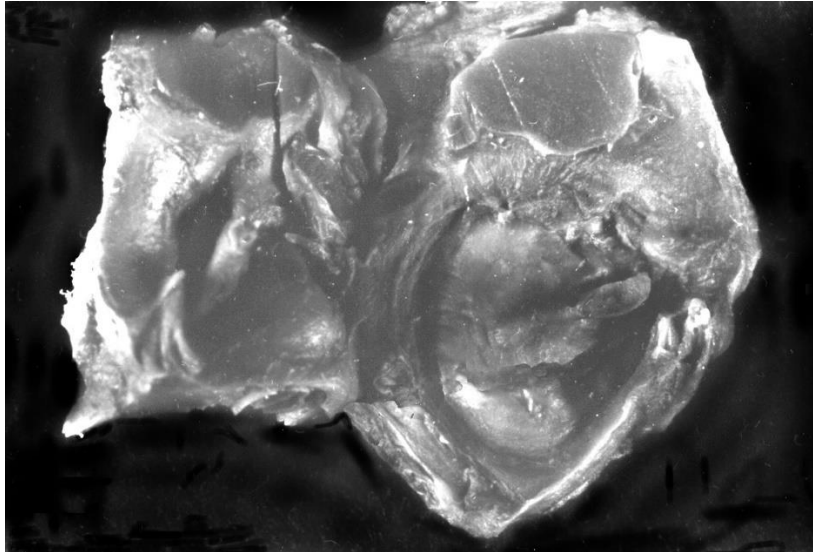


Fig. 1. Surgical incision the tympanic cavity of the fetus 275,0 mm PCL. Increase 1: 3,8. 1 – medial wall of the tympanic cavity, 2 – eardrum.

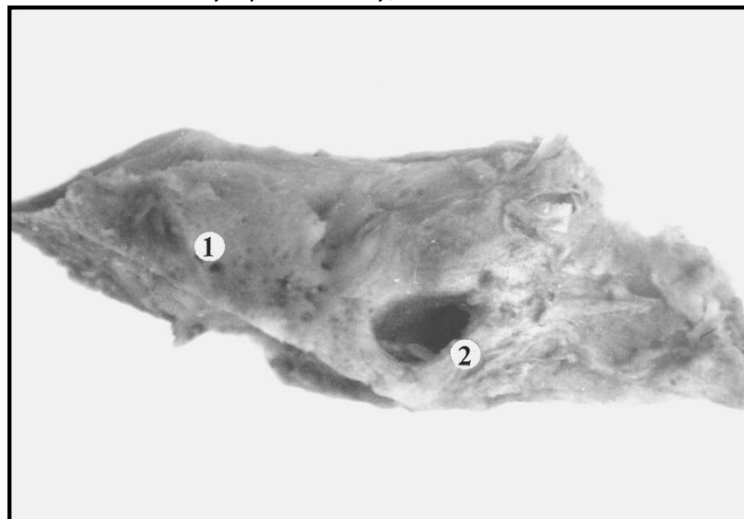


Fig. 2. The temporal bone of the fetus 290,0 mm PCL (bottom view). Increase. 1:3,2. 1 – the jugular fossa, 2 – canal of the internal carotid artery.

During the ninth month of intrauterine development that is in the fetuses 311.0 - 345.0 mm PCL the tympanic cavity becomes more irregular in shape, numerous cells appear on its walls. The inferior wall of the tympanic cavity increases its size and the lower edge of the annulus tympanicus departs from the promontory even more, and the angle of inclination of the upper edge increases. As a result the tympanic cavity from the almost horizontal position moves in the inclined. The thickness of

the inferior wall of it is $0,72 \pm 0,02$ mm. The width of the jugular fossa is $7,25 \pm 0,18$ mm, its length is $9,50 \pm 0,24$ mm (Fig. 3).

During the tenth month of intrauterine development that is from the fetuses of 346.0 - 375.0 mm PCL the formation of the inferior wall of the tympanic cavity continues as a result of the increase of the diameter of the internal jugular vein. Its thickness is $0,87 \pm 0,04$ mm. The width of the jugular fossa is $7,75 \pm 0,26$ mm, its length is $10,15 \pm 0,27$ mm (Fig. 4).

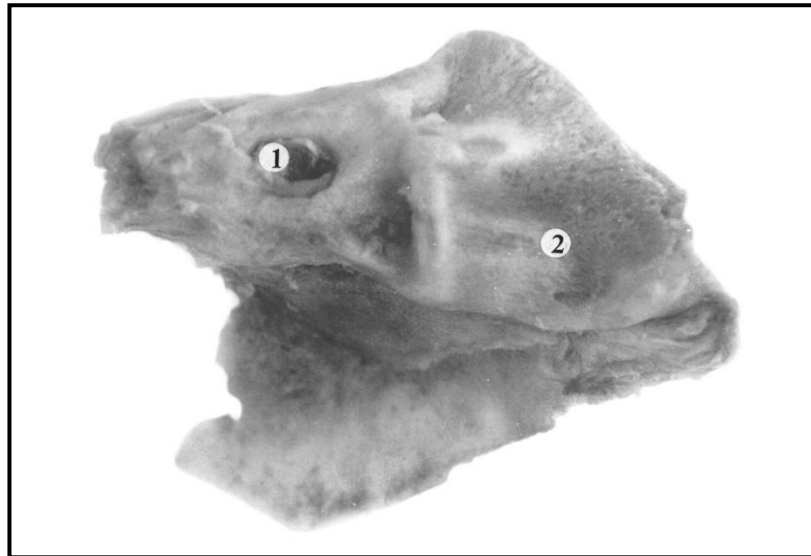


Fig. 3. The temporal bone of the fetus 336,0 mm PCL (bottom view). Increase 1: 3,2. 1 – canal of the internal carotid artery, 2 – the jugular fossa.

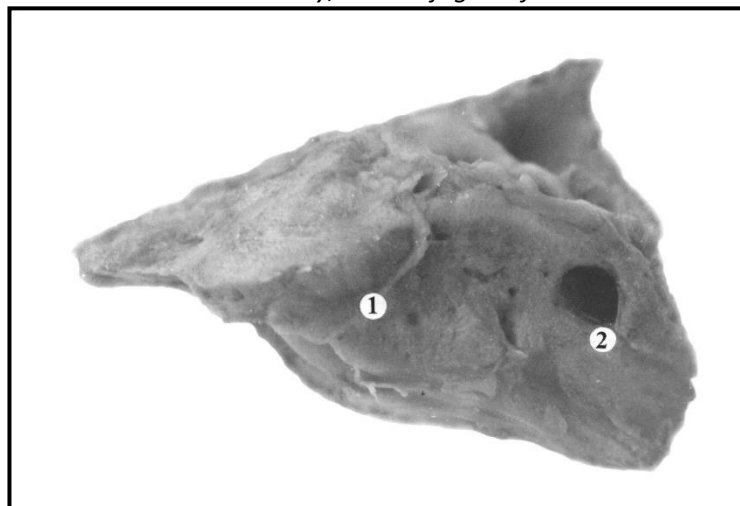


Fig. 4. The temporal bone of the fetus 350,0 mm PCL (bottom view). Increase 1: 3,2. 1 – canal of the internal carotid artery, 2 – the jugular fossa.

The tympanic cavity of the newborns has the form an irregular cube with rounded edges that is filled with embryonic tissue. The inferior wall of the tympanic cavity is a bone plate under which the bulb of the jugular vein is located. Its thickness is small and is $1,06 \pm 0,03$ mm. The width of the jugular fossa is $8,51 \pm 0,27$ mm, its length is $12,50 \pm 0,29$ mm (Fig. 5).

Discussion. From the eighth month of prenatal life to the period of newborn's birth, the thickness of the inferior wall of the tympanic cavity

is increased by 1.68 times. The dimensions of the jugular fossa which is limited by this wall increase the length for 1,47 times and the width for 1,36 times.

Beginning from the 8th month of the fetal period of development and till the period of newbornnes,

the movement of the annulus tympanicus with the tympanic membrane is in a more vertical position as a result of the increase

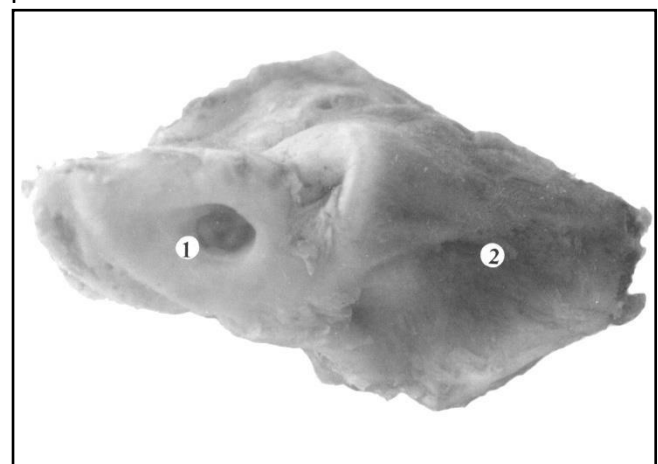


Fig. 5. The temporal bone of the newborn (bottom view). Increase 1: 3,2. 1 – canal of the internal carotid artery, 2 – the jugular fossa.

of the width of the jugular fossa.

The results of this study will contribute to the rational choice of methods for surgical interventions in the middle ear of newborns and babies in the first months of life.

Conclusions. 1. The thickness of the inferior wall of the tympanic cavity is 1.06 ± 0.03 mm of newborns, which should be taken into account when performing manipulations and surgical interventions on the middle ear.

2. The thickness of this wall of newborns does not differ from the thickness of it of children of 1-3 years.

3. The thickness of this wall of the fetuses of the 8th - 10th months is thinner than of newborns.

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Vepruk Y.M.,*Candidate of Medical Sciences, Associated Professor, Department of medical biology and genetics of the Higher State Educational Institution of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine***Zakharchuk O.I.***Doctor of Medical Sciences (MD), Head, Department of Pharmaceutical Botany and Pharmacognosy, of the Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi, Ukraine***Tovkach Y.V.***Candidate of Medical Sciences, Associated Professor, Department of anatomy, topographical anatomy and operative surgery of the Higher State Educational Institution of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine, tovkach11@rambler.ru***Rykhlo I.S.***The 3d year student of the Higher State Educational Institution of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine*

INDICATORS OF RENAL FUNCTIONS UNDER THE CONDITIONS OF ALUMINUM CHLORIDE INTRODUCTION IN DIFFERENT AGE GROUPS OF ANIMALS

Abstract. *The experiments on 36 immature and mature nonlinear male albino rats, weighing 0,60-0,10 kg respectively, under the condition of aluminium salts introduction, have shown more significant nephrotoxic effect of studied xenobiotic in immature rats on excretory and acid regulating function, according to the increase of concentration and excretion of protein in urine. However, in mature rats, it was detected a trend towards an increase of ammonium excretion and ammonia ratio under the condition of aluminum salts introduction.*

Key words: *kidneys, aluminium chloride, immature rats, mature rats.*

Introduction. Chemical contaminants of the environment can get into the body via respiratory, digestive, dermal and mucous layers and as a result, cause pathological changes of different organs. Also, increasing pollution by industrial waste plays an important role in ecological disasters [1, 3, 4, 6]. Given problematics is also associated with a high level of urbanization, industrialization and high psycho-emotional stress. The question about the impact of aluminum salts on the excretory and acid regulating functions of kidneys in mature and immature rats is still remained insufficiently studied [2, 5, 8].

The aim of the study was to investigate the nephrotoxic effect of aluminum chloride on the excretory and acid regulating renal function in different age groups of animals [7, 9].

Materials and methods. We performed experiments on 36 immature and mature nonlinear albino male rats weighing respectively 0,16-0,21 kg; studied the impact of aluminum chloride on the excretory and acid regulating functions of kidneys in group comparison, during 14 days.

Results and discussions. Analysis of the evaluation of excretory and acid regulating renal function in intact immature rats, under the

condition of aluminum salts (table 1) showed, that the level of diuresis in immature rats against the background of aluminum salts introduction was characterized by a downward trend. Indicators of concentration and excretion of potassium ions with urine did not undergo changes. The concentration of protein in the urine and its excretion against the background of aluminum salts introduction in immature rats, while the excretion of titrated acids was inhibited. It was manifested a tendency towards an increase in ammonia excretion and probable increase of ammonia ratio upon the introduction of aluminum salts in immature rats.

Characteristics of indicators of excretory and acid regulating renal functions in intact mature rats under the impact of aluminum salts (table 2) showed, that the level of diuresis and the creatinine excretion in mature rats against the background of aluminum salts introduction decreased in group comparison. The concentration of protein in the urine and its excretion increased when introducing aluminum salts in mature rats. It was detected the probable increase in ammonia ratio upon the introduction of aluminum salts in mature rats, as to the control.

Table 1

Indicators of renal function in intact immature rats under the condition of aluminum salts

introduction ($\bar{x} \pm S_x$)

Indices	Immature rats (Al) (n=6)	Control (n=6)
The diuresis, ml / 2 h · 100 g	1,62±0,164	2,21±0,27
The concentration of protein in urine, g / l	0,22±0,017	0,065±0,001 p<0,001
The excretion of protein, mg / 2 h · 100g	0,39±0,039	0,14±0,019 p<0,001
The excretion of titrated acids, kmol / min 100 r	4,65±0,831	14,2±3,12 p<0,02
The ammonio ratio, conventional units.	5,2±0,324	2,64±0,311 p<0,001

Table 2

Indicators of renal function in intact mature rats under the condition of aluminum salts introduction

($\bar{x} \pm S_x$)

Indices	Mature rats (Al) (n=6)	Control (n=6)
The diuresis, ml / 2 h · 100 g	1,97±0,314	3,21±0,088 p<0,05
The excretion of kreatinine, mkmol/ 2 h · 100 g	1,22±0,217	2,27±0,213 p<0,05
The concentration of protein in urine, g / l	0,24±0,019	0,07±0,008 p<0,001
The excretion of protein, mg/ 2h · 100 g	0,45±0,049	0,22±0,006 p<0,001
The ammonio ratio, conventional units.	1,46±0,188	1,04±0,048 p<0,001

The usage of forest plot of meta-analysis of the comparative evaluation of the nephrotoxic effect of aluminium salts introduction in mature and immature rats, on the assumption of low sodium diet at water induced diuresis in the volume of 5% of the body weight, provided the opportunity to demonstrate more significant nephrotoxic effect of the investigated environmental factor on immature animals (figure)

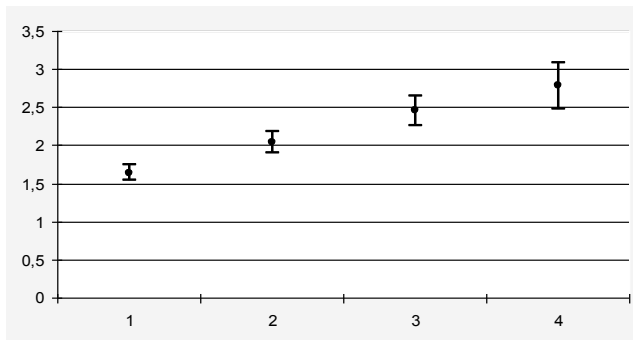


Figure.1 Forest plot of the meta-analysis of the comparative evaluation of the nephrotoxic effect of aluminum salts in mature and immature rats under low sodium diet at water induced diuresis in the volume of 5% of body weight. 1 – the excretion of potassium ions (mmol / l) in mature rats; 2 – the excretion of protein (mg / 2 h · 100 g) in mature rats; 3 – the excretion of potassium ions (mmol / l) in immature rats; 4 – the excretion of protein (mg / 2 h · 100 g) in immature rats. Control for all studies is presented in the form of a horizontal line and accepted for 1.

Conclusion. Thus, impact analysis of aluminum

chloride on the excretory and acid regulating function of kidneys in mature and immature rats showed, that the investigated environmental load is accompanied by a nephrotoxic effect characterized by proteinuria due to the damage of tubular part of the nephron.

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Khitrik A.,*Assistant of the Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhia, Ukraine, Mayakovsky Avenue 24A, 69035, Orhidea_72@ukr.net, ORCID 0000-0003-0111-6016***Yevtushenko V.,***MD-PhD, Professor, of The Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhia, Ukraine***Nemyrskyi V.,***Student, Zaporizhzhia State Medical University, Zaporizhia, Ukraine***Bushman V.,***Assistant of The Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhia, Ukraine, ORCID 0000-0002-5833-0155***Necheporenko A.,***Assistant of The Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhia, Ukraine, ORCID 0000-0003-0981-3641***Aksamiteva M.***Assistant of The Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhia, Ukraine, ORCID 0000-0002-8168-1869*

MORPHOGENESIS OF URINARY BLADDER OF RATS IN EARLY POSTNATAL PERIOD

Abstract. *The actual problem of practical medicine is the treatment of diseases of the lower part of the urinary system and about 20% of this pathology accounts for the bladder pathology. The purpose of this research was to study the morphological characteristics of the bladder of rats in the period of early postnatal ontogenesis. A histological survey of the bladders of 40 rats was conducted. Quantitative analysis of the results of the morphometric study was carried out using methods of variation statistics using Excel and STATISTICA. The aim of the study was to determine the average thickness of the lamina propria, the average number of microvessels in the unit of area, the number of lymphocytic cells. During the early stage of postnatal ontogenesis (up to 90th day of life of rats), the following changes in the structure of the bladder were observed: an increase in the thickness of the bladder mucous membrane; an increase of cells of the immunomorphological complex (lymphocytes, macrophages, and lymphocytic cell clusters), changes in their quantitative aspect and in the diversity of the cellular composition; an increase in the number of blood vessels in the microcirculatory bed. These changes occurred progressively, with maximum expressiveness on the 30th day of life and subsequent stabilization of indicators. This may be caused by a change in the diet of rats, since from 14th to 21st day there was a transition from dairy to natural nutrition. It is planned to conduct an investigation of the effect of antigenic stimulation on the structure of the wall of the bladder in the future.*

Key words: *postnatal ontogenesis, bladder, lymphocytes, microcirculatory channel.*

Introduction. Currently, the actual problem of practical medicine is the treatment of diseases of the lower urinary system, and about 20%^[1] of this pathology accounts for the bladder. Our studies are devoted to the normal morphology of the rat bladder and the morphofunctional changes of this organ in the period of early postnatal ontogenesis.

Objective: to study the morphological features of the bladder of rats in the period of early postnatal ontogenesis.

Materials and methods. The material for the study was the bladder of "Vistar" rats aged 1, 14, 30, 60, 90 days. In each age group, 8 animals were

studied. After euthanasia the bladders were fixed in 10% neutral formalin and embedded in paraffin according to the standard technique. A number of microsections were made with a thickness of 5-6 microns. Coloring was performed with hematoxylin and eosin, alcian blue^[2]. Morphometric studies and photomicrography were carried out using a ZEISS microscope with an Axiocam 105 color digital microplotter^[3]. For the analysis, the methods of variation statistics were used using Excel and STATISTICA^[4,5]. The average thickness of the lamina propria of the mucosa, the average number of microvascular vessels, lymphocyte cells and lymphocyte accumulations

per 5000 μm^2 were determined.

Results of the study. Obtained the data is presented in Table 1 below.

Discussion. At the age of one day, the three-layer structure of the bladder wall is noticeable: mucous membrane, muscle membrane and serous (adventitial) membrane. The average

thickness of the mucosa is 3.78 microns. It is represented by areolar tissue. The microcirculatory bed of the mucous membrane is represented by arterioles in the amount of 1.19 (per 5000 μm^2 , as well as all cell quantities given below), venules in the amount of 2.29 and capillaries in the amount of 9.71.

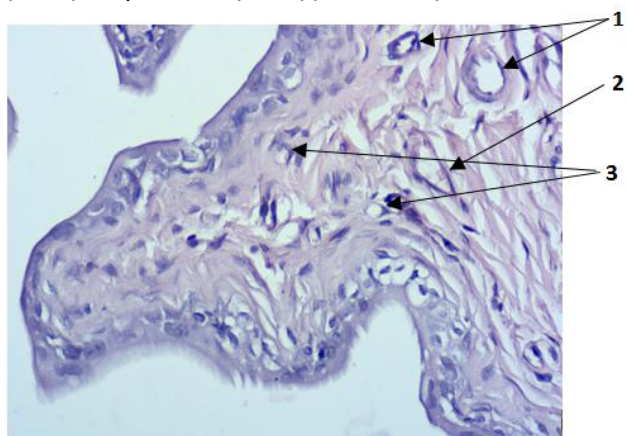
Table 1.

Morphometric indicators of the structure of the bladder wall of rats in the early prenatal period

Age, days	Height of the areolar tissue, μm	Arterioles in mucosa	Venules in mucosa	Capillaries in mucosa	Lymphocytes	Lympho-cytic cell clusters
1	3,78 \pm 0,64	1,19 \pm 0,26	2,29 \pm 0,52	9,71 \pm 0,70	1,25 \pm 0,28	
14	18,1 \pm 1,00	2,20 \pm 0,71	1,92 \pm 0,46	10,62 \pm 4,98	1,45 \pm 0,31	
30	29,52 \pm 1,19	7,29 \pm 1,59	7,24 \pm 2,40	24,87 \pm 6,95	8,08 \pm 0,83	0,50 \pm 0,47
60	34,80 \pm 2,87	4,96 \pm 3,73	6,46 \pm 4,12	27,92 \pm 2,99	8,62 \pm 1,83	0,08 \pm 0,15
90	25,35 \pm 1,91	1,82 \pm 1,00	2,36 \pm 1,23	15,20 \pm 7,69	4,26 \pm 0,51	0,10 \pm 0,16

In the mucosa lymphoid cells are present. The diameter of these cells is 2-4 microns, these are small lymphocytes (in this work, we took lymphocytic cells as small with a diameter of 2-4 microns, as medium – 5-6 microns, as large – 7-8 microns). They are located mainly in perivascular region, rarely – in the subepithelial region. The average number of cells is 1.25.

During the period of ontogenesis, after 14 days, the rats begin to switch to a mixed diet and, during this period, the following structural changes in the bladder are observed. The loose fibrous connective tissue of the lamina propria of the mucous membrane has a thickness of 18.10 microns. A clear separation of the lamina propria and submucosa is not observed, however, there are isolated smooth myocytes in the lamina propria. The number of arterioles (2.33), venules (1.58), capillaries (8.04)(Picture 1).



Picture 1. Mucosa of the 14 day old rat. Hematoxylin and eosin stain, $\times 40$. 1 – Arterioles; 2 – Venules; 3 – Capillaries

Lymphocytic cells are located diffusely and perivascular, represented by small lymphocytes in the amount of 1.46. There are single macrophages in an amount of 0.25 and neutrophils 0.12, located perivascular.

When studying the structure of the urinary bladders of the 30 day old rats, mucosa is without a distinct muscle layer and is made of loose fibrous connective tissue. The thickness of its own submucosa plate is 29.92 microns. The mucous plate of its own contains arterioles (7.71), venules (5.83) and a large number of capillaries (27.39). There are diffuse and perivascular lymphoid cells and clusters in the mucosa^[6,7]. The average number of cells was: lymphocytes – 8.08, macrophages – 1.07, neutrophils – 0.70, plasma cells – 0.50, lymphoid clusters – 0.50, all located mainly perivascular. When studying the structure of the urinary bladders of the 60 day old rats, the lamina propria of the mucosa was without a clearly defined muscle layer and is made of loose fibrous connective tissue. The thickness is 34.80 μm . The mucosa contains arterioles (4.96), venules (6.46) and a large number of capillaries (27.92). There are diffuse and perivascular lymphoid cells and their clusters in the mucosa. The number of lymphocytes is 8.63, macrophages 0.83, neutrophils 0.12, plasma cells 0.08, lymphoid clusters 0.08, all located perivascular.

The loose fibrous connective tissue of the mucous membrane of 90 days old rats has a thickness of 25.35 microns. A clear separation of the lamina propria and tela submucosa was not

observed, however, there are isolated smooth myocytes in the lamina propria. The number of arterioles – 1.82, venules – 2.36, capillaries – 15.20. Lymphocytic cells are located diffusely and perivascular, represented by lymphocytes in an amount of 4.27, macrophages in an amount of 1.37. There are rare single lymphoid accumulations (0.1).

Conclusions. After studying the morphological changes that occur in the bladder of the rat in the early stage of postnatal ontogenesis, we came to the following conclusions:

1. Along with a pronounced thickening of the mucous membrane of the bladder, the number of microvasculature vessels increases, with a maximum increase observed on 30th day of life and subsequent stabilization of indicators. Moreover, a pronounced increase in performance is more typical for the capillary link;

2. An increase in numbers of the immunomorphological cells (lymphocytes, macrophages, as well as lymphocyte cell clusters), changes in quantitative terms and in the diversity of the cellular composition. All changes occur faster by the end of the first month of life.

Prospects of further studies. Considering the results of the data obtained, it is planned to conduct a study on the effect of antigenic stimulation on the structure of the bladder wall in the future. An unknown effect of dietary changes (namely changing dairy nutrition to natural one) that occurred in this study in 14th-21st day period also may warrant further investigations.

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Voitenko S.G.,
Chycherska M.V.,
Martenchuk A.J.,
Tovkach Yu.V.,
Vepriuk Yu.M.

Higher State Educational Institution of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine

LASER COAGULATION OF VASCULAR PLACENTAL ANASTOMOSES AT FETAL FATAL TRANSFUSION SYNDROME

Abstract. *The fetal-fetal transfusion syndrome criteria are the difference in body weight of more than 20% and different concentrations of hemoglobin in the fruits. As a result, one fetus from which the blood is pumped becomes a donor of blood, the other - the recipient, as a result of the fetus papiraceus and, conversely, overload of the recipient's circulation (fetus acardiacus). The survival of one and two fruits was 77 and 57% as a result of laser therapy (results of the study Eurofetus). Children who survived newborns after FFTS treatment may have a brain injury in the form of periventricular leukomalacia. The perspective of the development of technologies of laser fetoscopic interventions is, of course, an optimal solution to the problem of FFTS provided early and adequate diagnosis. It makes it possible not only to improve the incidence of the disease among the fruits with monochorial multi-fertility, but also is safe for the mother's health.*

Key words: *fetal-fatal transfusion syndrome, multiple pregnancy, monochorous double, fetus-donor, recipient fetus.*

Introduction. The fetal-fetal transfusion syndrome (FFTS) is one of the leading places among the most significant complications of multiple pregnancy. The FFTS was first described by Schatz in 1982. Its morphological substrate is an anasthetic vessel between two fetal circulatory systems. Monocular multiproliferation refers to high-risk pregnancy due to the presence of specific complications, based on the presence of vascular placental anastomosis. With monochoric double as a result of uneven distribution of blood between the fruits through arterio-venous anastomosis there is a fetal-fetal transfusion syndrome (FFTS). That is, this is a specific complication that is observed with a monozygotic double with a monochorous type of placentation. Fetal-fetal transfusion syndrome (FFTS) occurs in 5-20% of monochorous pregnancies and leads to significant perinatal mortality and morbidity.

The FFTS criteria are the difference in body weight of more than 20% and different concentrations of hemoglobin in the fruits. One fetus from which the blood is pumped becomes a donor of blood, the other – the recipient, of the fetus papiraceus and, conversely, overload of the recipient's circulation (fetus acardiacus) (Fig. 1).

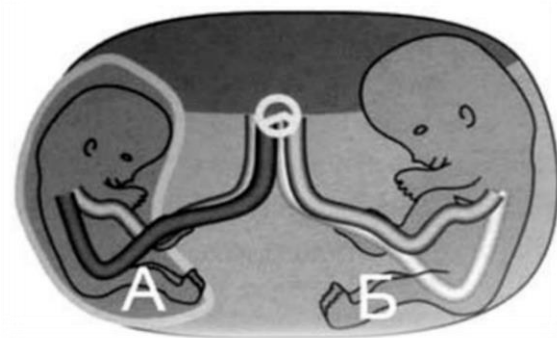


Fig. 1 Fetal Syndrome - Fetal Transfusion
A the fetus is a donor; B fetus is the recipient

The mechanisms of the SFFT disinfection are to lower the blood pressure of the fetus donor due to the uneven distribution of the joint placenta between the twins, and the placental insufficiency that occurs in the fetus that has gotten a smaller part of it; and in the emergence of a number of symptoms, as for the fetus - the donor, delayed fetal growth, general underdevelopment, anemia; and for the fetus-recipient - an increase in blood pressure, cardiomegaly, hydramnion, hypervolemia, polycythemia.

In the absence of adequate treatment and the detection of signs of this pathology up to 25 weeks in 80% of cases FFTS causes the death of the fruit.

Survival thanks to the method of laser coagulation of vascular anastomoses (FLKAA) is about 53-56% (Sichinova L.G.).

Aim. Whether to determine whether laser coagulation of anastomoses is a "gold standard" of FFTS treatment in the European experiment Eurofetus, as it is a highly effective operation for antenatal treatment of FFTS. The survival of one and two fruits was 77 and 57% as a result of laser therapy (results of the study Eurofetus). Children who survived newborns after FFTS treatment may have a brain injury in the form of periventricular leukomalacia.

Materials and methods. Patients with monochorous twins, complicated by FFTS, with a pregnancy less than 26 weeks. Before conducting FLC, an ultrasound examination was carried out (Pic. 2, 3) to evaluate the fetometric parameters, to exclude fetal abnormalities, placenta localization, and the number of amniotic fluid, and a dopplerometric study to evaluate hemodynamics in the arteries of the umbilical cord, middle cerebral artery and venous duct of both fruits. Also, the dopplerometric evaluation of hemodynamics of both fruits was carried out before intervention, after 1 hour and the next day.

FLK of the surface anastomoses of the placenta was performed under conditions of long-term epidural anesthesia. An amniotic cavity of the recipient fetus through the anterior abdominal wall under ultrasonic control was performed on an operating tubule with two separate channels for a 1 mm fetoscope and 0.6 mm Nd: YAG diode laser (A.V. Mikhailov, A.N. Romanovsky).

When localization of the placenta on the anterior wall of the uterus, a curved operating



Pic.2 Monochrome Double



Pic. 3 Interstitial vascular Anastomosis

tube and a flexible fetoscope of the same diameter were used. In some cases, in order to improve the visualization, an amniofusion of warm physiological solution was performed. Surface arterio-venous anastomosis was detected at visual inspection and coagulated. After FLC, an amniocentesis was performed under ultrasound control through an operating tubule until normal indexes of the amniotic index were reached. In the case of a successful outcome of the FLC, the patient was observed outpatient with an interval of 1-2 weeks with the obligatory conduction of ultrasound and dopplerometric study.

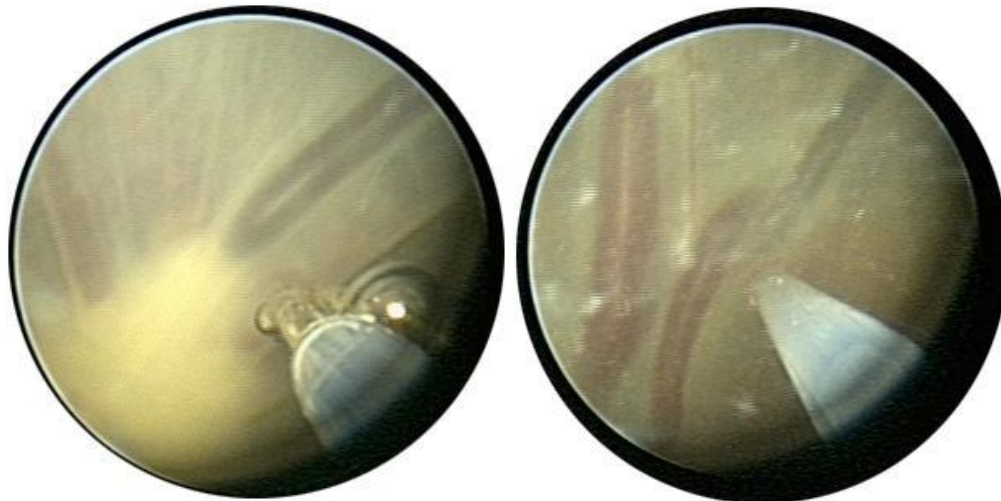
Results of the research and their discussion.

After laser coagulation of placental anastomoses in the case of FFTS, there is a significant decrease in mean blood pressure, increased cardiac output and systolic volume in the mother. Also, after laser coagulation, possible complications, such as the development of the syndrome TAPS (anemia-polycythemia of twins) to 13%. In such cases, the former recipients after the operation are anemic, and twin twins are polycythemic.

A particularly dangerous situation in the prognostic plan under FFTS is the intrauterine death of one of the fruits observed in monochoric pregnancy in 25% of cases. The main factors influencing the prognosis are the term of pregnancy, which indicates the death of the fetus. If the death of a twin is diagnosed in the first trimester, then more than 90% of the fruits will have a favorable prognosis. If the death of the fetus occurred in the second or third trimesters, then the favorable prognosis for the living fetus will be 50-60%. (Guseva OV) In the case of the

death of one of the fetus, the presence of the circle of blood circulation significantly impairs the livelihoods of another twin. Live fetus begins to shed blood from the dead through vascular anastomosis, which leads to the development of hypovolemia, severe damage to parenchymal organs due to hypoperfusion and death from hypovolemic shock. Studies show that the risk of

death of the second twin in the antenatal or early neonatal period reaches 38%. In the fetus, which was born after the fetal death of its twin, there are often pathologies of the central nervous system, urinary system, liver, and gastrointestinal tract. In 20% of these newborns, there is multicystosencephalomalacia (N. Harkevich, V. L. Semenchuk, S. K. Kletsky.)



A: to coagulation

B: after coagulation of anastomosis

Fig.4 Arterio-venous anastomosis and tip of a laser conductor

The twin death causes a dilemma regarding the benefits of early delivery over expectancy tactics, which in turn threatens the death of a second fetus in 25% of cases and increases the risk of its disability.

Conclusions. The perspective of the development of technologies of laser fetoscopic interventions is, of course, an optimal solution to the problem of FFTS provided early and adequate diagnosis. It makes it possible not only to improve the incidence of the disease among the fruits with monochorial multi-fertility, but also is safe for the mother's health. When solving the problem of conducting phthoscopic laser coagulation, careful ultrasound scans should be carried out to find possible combinations of anomalies, taking into account the prognosis of the child's life and health, depending on the defects found and the possibilities for their correction.

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Horodynskyi S.I.,
Andriyets M.M.

Higher State Educational Establishment of Ukraine, "Bukovinian State Medical University", Chernivtsi, Ukraine

INFLUENCE OF THE REGULAR YOGA PRACTICE ON THE HEART RATE VARIABILITY OF THE OVERWEIGHT INDIVIDUALS

Abstract. Purpose of the work was to investigate the influence of the regular Yoga practice to the heart rate variability of the persons with overweight. Materials and methods: 32 overweight previously non-yoga practitioners were investigated during 24 weeks since beginning of regular Yoga practice. Main anthropometric, hemodynamic and heart rate variability parameters were analyzed trice (Week 0, 12 and 24), and received data were compared with parameters of 16 normal weight persons. Heart rate variability investigation was provided on the cardiac monitor "Diacard" AO "Solveig" (Ukraine). Results: We found out the significant reduction of the time and spectrum heart rate variability parameters of parasympathetic heart function regulation in overweight subjects before beginning of the regular Yoga practice. Dynamic control discovered progressive positive influence of Yoga to autonomic cardiac regulation with significant growth of parasympathetic activity and normalization of sympathetic/parasympathetic balance with strong tendency to decreasing of resting heart rate, systolic and diastolic blood pressure, body weight, body mass index and waist circumference. Correlation analyze detected negative dependence between waist circumference and reduction of parasympathetic influence to the heart activity. Conclusions: The regular Yoga practice prominently improves the balance of autonomic regulation because of significant growth of parasympathetic influences and reduction of sympathetic stimulations of the heart function of overweight persons.

Key words: Yoga practice, obesity, overweight, heart rate variability.

Introduction. The overweight and obesity was accepted by WHO as the major public health problem and the global pandemic in 1997 [17]. In 2015, a total of 107.7 million children and 603.7 million adults were obese. Since 1980, the prevalence of obesity has doubled in more than 70 countries and has continuously increased in most other countries. High body mass index (BMI) accounted for 4.0 million deaths globally, nearly 40% of which occurred in persons who were not obese. More than two thirds of deaths related to BMI were due to cardiovascular disease [5, 10, 16].

Many factors play a role in the relationship between overweight and cardio-vascular diseases (CVD), such as insulin resistance, hypertension, and reduced high-density lipoprotein. However, an imbalance in autonomic regulation of the cardiac function might be the mechanism for the increased prevalence of CVD in obesity [9]. Because the autonomic nervous system controls a significant part of the internal functions of the body, fat disequilibrium in obesity is an important negative factor [14, 18].

Heart rate variability (HRV) is a simple non-invasive instrumental method for the detection and investigation of cardiac autonomic

dysfunction in different medical conditions, including obesity [3]. Low HRV is an established predictor of CV morbidity and mortality [11, 13].

Major health organizations, such as the International Association for the Study of Obesity (IASO) and the American College of Sports Medicine (ACSM), consistently support the need for more than 150–250 min/week of moderate-intensity physical activity to prevent weight gain. However, there is currently a lack of guidance for obese individuals on feasible strategies for weight loss and prevention of weight regain [12]. Yoga is an ancient system of practices based on the scientific principles of exercise, breathing and meditation, and philosophical beliefs concerning life and thinking. The origin of yoga has been ascribed to the Indus Valley Civilization (2600–1900 BCE) although some researchers suggest more ancient origins. Participation in yoga has increased dramatically throughout the world in recent decades. Many styles of yoga have been tested in the clinical setting and most involve the performance of physical postures (*asanas*), breathing exercises (*pranayama*) and meditation [4, 6].

A growing number of research studies have shown that the practice of Yoga can improve

strength and flexibility, and may help control such physiological variables as blood pressure, respiration and heart rate, and metabolic rate to improve overall exercise capacity [7, 8, 15]. Investigations suggest that Yoga practice is effective for decrease of the general stress, awareness on satiety, positive influence for over eating and weight reduction [2].

However, the chronic effects of Yoga training on HRV in obese persons and its correlation with anthropometric characteristics improvement remain inconclusive and need to be investigated.

The purpose of the work was to investigate the influence of the regular Yoga practice to the heart rate variability of the persons with overweight.

Materials and methods. 32 overweight previously non-yoga practitioners were investigated during 24 weeks since beginning of regular Yoga practice. There were 18 female and 14 male persons with BMI >25 kg/m² and from 25 to 47 years of age (40,3±2,4 y.o.). All investigated persons have agreed to participate in an experiment. Comparative analyses of HRV parameters was conducted with data of 16 non-obese subjects with the same age/gender characteristics. Informed written consent was taken from all the subjects and they were screened for any history of drugs/alcohol intake, familial history of hypertension and cardiac diseases, or presence of any medical illness likely to affect the HRV parameters based on clinical history and physical examination. Yoga training was conducted three times in a week; every class lasted 90 min and consisted of asanas, pranayama and meditation under the supervision of the experienced Yoga master. HRV investigation was provided trice (beginning of Yoga practice and after 12 and 24 weeks) in comfortable conditions. The blood pressure of each subject was measured

in supine position. Analysis of HRV was performed based on 5 min ECG recorded at rest in the supine position. Recordings were taken during 08:00 am – 11:00 am to avoid any hemodynamic effect on HRV on the cardiac monitor "Diacard" AO "Solveig" (Ukraine). HRV analyses and results estimation were based on the classic approach for analysis of variability of heart rate that is recommended by the European society of cardiology and North American society of pacing and electrophysiology [1]. The parameters of autonomic regulation of heart rate and results of spectral analysis were registered; and received data were calculated via support of statistical program «Kubios HRV». Statistical analysis was performed with Statistical Package for the Social Sciences software for Windows (version 21). Differences in variables were tested using Mann–Whitney U test for the data having nonparametric distribution and Student's t-test for the data with parametric distribution. The results were presented as mean ± standard deviation (SD). Pearson's correlation was used to correlate the HRV measures and the obesity indices. Statistical significance was considered to be $p < 0.05$.

Results. The anthropometric characteristics of the investigated subjects present in the Table 1. It shows comparable age of overweight and non-overweight subjects with prominent differences in the body weight (BW), BMI and waist circumference (WC) between groups.

The time domain, frequency domain variables of HRV of both the groups were calculated and are presented comparatively in Table 2. Among the time domain measures, SDNN, RMSSD, and pNN50% were calculated. All these time domain variables were significantly less ($p < 0.05$) in the overweight group as compared to the normal weight group. Spectral parameters – high-

Table 1

Baseline characteristics of overweight and non-overweight subjects

Characteristic	Control group (n=16)	Total cohort (n = 32)	Male (n = 14)	Female (n = 18)
Age (y)	41,2±3,5	40,3±2,4	38,2±10,1	42,3±11,4
Body weight (kg)	76,8±6,3	95,5±16,3	89,4±12,2	97,3±14,2
BMI (kg/m ²)	23,4±2,1	30,5±4,2	29,9±5,1	30,9±3,3
Obese (BMI 30+) (n; %)	0; 0%	11; 34%	5; 36%	6; 33%
Waist circumference (cm)	81,3±8,4	94,7±14,7	93,9±14,5	95,3±15,1

frequency component of the spectrum, HF (ms²), low frequency component of the spectrum – LF (ms²) also were significantly reduced in overweight subjects. However, the LF/HF ratio as an indicator of the balance of sympathetic and parasympathetic autonomic regulation and one of

the indirect sympathetic marker in overweight subjects exceeded the same one from the non-obese persons.

Next HRV investigations on the Week 12 and Week 24 of the regular Yoga practice we conducted to detect possible influence of that

exercise complex to the autonomic regulation of the cardiac functions. Received data are presented in the Table 3 and suggest significant ($p < 0.05$) growth of the main time and spectrum characteristics of HRV in overweight persons during continuous Yoga practice.

Dynamic investigation of the resting heart rate (RHR), systolic (SBP) and diastolic blood pressure

(DBP), BW and BMI (Table 4) revealed tendency of the positive influence of the regular Yoga training to investigated characteristics of overweight persons. We concluded that all investigated parameters progressively decreased partially coming into normal ranges in some subjects. BW and BMI normalized in 4 obese (36%) and 6 overweight persons (29%).

Table 2

Comparison of HRV measures between overweight and normal weight groups

Variables	Overweight subjects (n=32)	Control group (n=16)
SDNN (ms)	32,4±8,7	44,5±7,6 *
RMSSD (ms)	26,5±4,5	42,7±9,8 *
PNN50%	5,8±1,5	21,6±6,4 *
LF ms ²	252,9 ±28.3	456,7±56,9 *
HF ms ²	165,5±38,7	530,5±112,7 *
LF/HF	1.2±0.95	0.67±0,8 *

Note: * - $p < 0,05$, comparing with the control group

Table 3

Dynamics of HRV characteristics of the overweight subjects during investigation

Variables	Week 0	Week 12	Week 24
SDNN, ms	32,4±2,7	37,5±3,4	41,4±4,7 *
RMSSD, ms	26,5±4,5	32,6±6,3	38,7±5,9 *
pNN50, %	5,8±1,5	12,4±3,5	15,8±5,2 *
LF, ms ²	252,9 ±28.3	354,4±46,8	398,5± 56,8 *
HF, ms ²	195,5±38,7	365,7± 82,7	464,3±91,7 *
LF/HF ratio	1,2±0.95	0.97 ± 0.95	0.86 ± 0.95 *

Note: * - $p < 0,05$, comparing between week 0 and week 24

Table 4

Changes of hemodynamic and anthropometric characteristics during investigation (n=32)

Parameters	Week 0	Week 12	Week 24
Resting heart rate (bpm)	78,3±8,5	73,9±6,3	67,4±9,1
Systolic blood pressure (mmHg)	134,3±5,6	130,5±6,3	126,8±8,1
Diastolic blood pressure (mmHg)	85,7±5,9	82,5±6,7	77,1±5,4
Body weight (kg)	95,5±16,3	93,7±11,5	89,8±9,9
Body mass index (BMI) (kg/m ²)	30,5±4,2	28,8±5,3	26,4±3,7

Correlation indexes were calculated to reveal possible dependence between HRV data and anthropometric and hemodynamic parameters of the investigated subjects. Moderate negative correlation was detected between WC and HF ($r = -0,412$), WC and RMSSD ($r = -0,324$), WC and SDNN ($r = -0,432$), BMI and DBP ($r = -0,375$), RHR and SDNN ($r = -0,356$), RHR and HF ($r = -0,432$). However, positive correlation was revealed between LF and WC ($r = 0,441$), LF/HF and WC ($r = 0,318$). Other HRV parameters were not significantly correlated with anthropometric and hemodynamic characteristics of the observed individuals.

Discussion. In present work, we investigated influence of the 24-weeks Yoga practice to HRV variables of 32 overweight person. Comparative analyses was conducted with normal-weight

subjects comparable in terms of their age and general health characteristics. However, obese persons had significantly higher BW, BMI, and WC than normal weight controls. The resting heart rate was significantly higher in the overweight subjects in comparing to the normal-weight group, which corresponds to several studies about finding of tachycardia in obese people as risk factor of the CVD [5].

The HRV variables SDNN, RMSSD, pNN50, HF indices that reflect the cardiac parasympathetic nerve activity [13] were significantly lower in overweight than in normal weight persons. Besides that, the sympathetic marker LF/HF ratio significantly exceeded in obese subjects in comparison to normal weight controls. According to studies, obese and overweight persons suffer

from an increased mortality risk supposedly due to CVD related to either continuously lowered parasympathetic or heightened sympathetic activation [11, 14].

Moreover, correlation analysis showed that the HRV parasympathetic variables, like SDNN, RMSSD, HF ms², were negatively dependent from WC, while RHR had moderate positive relation with LF and LF/HF ratio. Based on absence of any significant dependence between HRV parameters and BMI, we can suggest that WC is the more important predictor of CVD in overweight subjects, which should be carefully controlled and corrected by physical exertions.

Taking for attention, that Yoga practice tends to be very popular all over the world, it was important to analyze it's positive effects for reduction of the CVD risks including dysregulation of the sympathetic-parasympathetic balance. Although the mechanism by which yoga influences autonomic activity is not well investigated, regular Yoga practice appears to directly stimulate the vagal nerve and enhance parasympathetic output leading to parasympathetic dominance and normalization of the cardiac function, mood, and energy states, as well as stimulation of neuroendocrine, metabolic, cognitive, and immune responses.

Our work shows not only progressive positive anthropometric (reduce of BMI, WC, BW) effects from the regular Yoga training of the overweight persons, but also sufficient hemodynamic (decrease RHR, SBP, DBP) results and significant positive influence to HRV parameters with tendency to normalization of the main time and spectrum variables (SDNN, RMSSD, pNN50, LF, HF, LF/HF ratio). 24 weeks` Yoga practice realized adequate stimulation of the parasympathetic regulation of heart function in overweight subjects leading to reduction of cardio-vascular risks. During the period of supervision, BMI, WC, BW and HRV variables had strong tendency to normalization, but did not fully came into normal ranges. According to that, continuation of regular Yoga training is strongly recommended for this category of persons.

Conclusions. Time (SDNN, RMSSD, pNN50) and spectrum (HF, LF/HF ratio) HRV parameters of parasympathetic part of cardiac function regulation in overweight persons were significantly ($p < 0,05$) reduced in comparing with normal-weight subjects, suggesting about complex negative influence of obesity as a predictor of cardiac problems. Negative moderate

correlation between parasympathetic variables of HRV and WC, also positive moderate correlation between sympathetic spectrum variables (LF, LF/HF) and WC without strong relationship between HRV parameters and BMI recognize the abdominal type of overweight as more important negative factor of vegetative dysregulation.

The regular Yoga practice helps in balancing of autonomic regulation because of significant growth of parasympathetic influences and reduction of sympathetic stimulations of the heart function, which was confirmed by HRV monitoring and dynamic analyzes of hemodynamic parameters (resting heart rate, systolic and diastolic blood pressure) of overweight persons.

Heart rate variability test is a modern, non-invasive adequate method of estimation of the heart function's autonomic regulation in overweight subjects and may be used not only for evaluation of the predictors of cardiac problems but also as an objective reflection of positive changes of health during physical training.

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Kovalchuk K.S.

PhD-student, Department of Human Anatomy, Operative Surgery and Topographic Anatomy, Zaporizhzhia State Medical University, Maiakovskiy avenue 26, Zaporizhzhia, Ukraine, 69035, kovalchuk.ks@zsmu.zp.ua

DYNAMICS OF OVARIES' FOLLICLES OF RATS' OFFSPRING DURING THE FIRST THREE MONTHS OF POSTNATAL LIFE IN NORM AND AFTER INTRODUCTION OF PROGESTERONE DURING PREGNANCY

Abstract. *The objective of the work is to examine distribution and correlation of ovarian follicle classes of the rats' offspring during first three month after birth in norm and after introduction progesterone during pregnancy. Serial sections of ovaries' tissue were stained with hematoxylin and eosin and with alcian blue with a 0,2M as a critical concentration of MgCl₂. To achieve the aim a light microscopy and methods of variation statistics for examination of dynamics of relative areas occupied by the follicles of different classes, connective tissue, blood vessels and corpus luteum were used. The introduction of progesterone from the 15th to 18th day of pregnancy of rats resulted in decreasing of the relative area occupied by primordial follicles from the 9th day up to the end of the third month of life. The changes in the ratio of follicles' classes were also observed - the earlier appearance of vesicular follicles in the experimental group of animals. During the period of observation, the relative area of connective tissue in the experimental offspring significantly increased.*

Key words: ovaries, rat, progesterone, pregnancy, follicles, connective tissue, blood vessels

An important process of development and valuable functioning of ovaries is forming of primordial follicles which form ovarian reserve or follicle pool. In rats during the first three days after birth there is an assembly of primordial follicles appeared by destruction of the clusters, which develop on the base of oocytes surrounded by the layer of flat somatic cells – the squamous pregranulosa cells [1]. The cells in clusters are bound by intercellular bridges that appear as a result of incomplete cytokinesis of cells. The formation of clusters provides the accumulation of nutrients for the further development of oocytes [2, 3].

After the formation of primordial follicles the majority of them remains at constant for a long time, and only a small part fills up the pool of growing follicles. It distinguishes the initial and cyclic recruitment of follicles. The process of the initial recruitment of follicles occurs throughout life, since the formation of primordial follicles. A cyclic recruitment coincides with beginning of the puberty. Also the differences are present in regulation of follicles' growth: before puberty, growth is controlled by local growth factors, and then it depends on the level of the follicle

stimulating hormone and expression of its receptors on the follicles [4]. The initiation of the growth of "nonactive" follicles is an obligatory process for the providing the necessary number of mature oocytes at the moment of ovulation and preserve the pool of follicles and its premature exhaustion [5]. At formation of ovarian reserve, the excessive number of germ cells is reduced by apoptosis in order to save the most quality selected oocytes for fertilization in future [6, 7].

The processes of distribution, correlation and maturation of follicles have to be studied perfectly, because fetal ovaries undergo the influence of different factors during intranatal period of development. Progesterone is usually administrated to pregnant women in order to prevent the abortion.

However, the use of hormones during pregnancy can cause disturbances in the "mother-placenta-fetus" system and result in impairment of morphogenesis and further functioning of the reproductive system's organs. Since the destruction of clusters and the subsequent collection of primordial follicles, the correlation and interaction of hormones of estrogen and progesterone is also crucial. It is known that 17β-

estradiol (E2) is the most active form of into the case of endogenous estrogen, and addition of E2 to the neonatal culture of ovaries of mice the process of disintegration of clusters and assembly of primordial follicles is repressed [8]. Also, a high level of maternal estrogen in the blood prevents the destruction of clusters after birth in rodents [9]. But except influence of high levels of maternal estrogen, there are data on the synthesis of own sex hormones by newborn to control the development of primordial follicles assembly [10]. The cultivation of the neonatal ovaries of rodents in the presence of progesterone also results in inhibition of the process of breakdown of clusters and subsequent delay the assembly of primordial follicles [8, 11]. So, the prenatal effects of progesterone in the "mother-placenta-fetus" system can lead to violation of the formation of the ovarian pool and in future to its premature exhaustion.

The purpose of the study - to determine the dynamics and correlation of ovarian follicle classes, relative areas occupied by connective tissue, blood vessels and corpus luteum in the offspring of rats from the 9th up to the 90th day of the postnatal life.

Materials and methods: the object of the study - the ovaries of white laboratory rats at the 9th, 14th, 21st, 30th, 45th, 60th and 90th days of postnatal life, got from females with the dated term of pregnancy. The animals were retained in the conditions of vivarium, with free access to water and meal. During work with animals followed requirements and recommendations of the European Union Directive 2010/10/63 EU on experiments on laboratory animals, the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1961), that is used with a scientific aim, and also "General ethic principles of experiments, on animals" that is accepted by First National Congress on Bioethics.

The offspring of rats included in a study were divided into 3 groups: I - intact, II - experimental group of rats, who were injected by 0,33 ml of progesterone oil solution at the 15th to 18th day of pregnancy and III - control group of rats which were injected by 0.9% Sodium Chloride solution in equivalent dosage. Starting from the 90th day of life, the phase of the estral cycle was determined by vaginal smear method. Ovaries were fixed in 10

% neutral formalin solution, conducted in increasing concentrations of alcohol and poured into mixture of wax and paraffin-rubber. Serial sections were stained with hematoxylin and eosin and with alcian blue with a 0,2M as a critical concentration of MgCl₂. Using a light microscope studied a dynamics, a correlation and a relative area, that occupy the follicles of different classes (primordial, primary, secondary, vesicular and tertiary (antral), connective tissue, blood vessels and corpus luteum. The data were processed by methods of variation statistics. The certainty difference of data of experimental and control groups was assessed by Student's test. The compared results considered such, that for certain differ at $p < 0,05$.

Results. Since the data on the distribution of the investigated structures of the intact and control groups did not differ, the results of the experimental and control groups are compared in the future.

At the 9th day of life in the ovaries of offspring of control animals, most of the area is cortical substance represented by primordial, primary and secondary follicles, the relative area of the connective tissue is $13,2 \pm 0,08$ %. The blood vessels take $5,8 \pm 0,04$ % of the ovaries' area. Primordial follicles form groups, of several follicles, predominantly under the tunica albuginea in the peripheral part of the ovaries. The relative area occupied by primordial follicles is $36,7 \pm 0,18$ %. A slightly smaller area is represented by primary follicles – $32,1 \pm 0,18$ %, respectively. Secondary follicles occupy the central part of the ovary, they take $3,2 \pm 0,08$ % of the area. In the offspring of animals got after intrauterine introduction of progesterone solution, the area occupied by primordial follicles is statistically significantly lower compared to control ($36,7 \pm 0,18$ % in the control and $30,3 \pm 0,16$ % in the experimental group, respectively). In relation to primary and secondary follicles, the relative area they occupy is also lower than that of control animals. However, in the offspring of experimental animals, in contrast to the control, $4,4 \pm 0,13$ % of the area of the organ is occupied by vesicular follicles. The relative area of connective tissue of rats' ovarian in the experimental group is almost twice exceeds an analogical index in control animals ($26,1 \pm 0,09$ %

in experimental animals versus $13,2 \pm 0,08$ % in control animals). The relative area occupied by blood vessels in experimental rats is lower than in control - $2,4 \pm 0,3$ % accordingly.

At the end of the second week of life at comparison of indexes of relative area, occupied by primordial and primary follicles it is settled that in experimental group this index continues to lag behind against control ($27 \pm 0,10$ % and $25,8 \pm 0,04$ % in experimental animals and $33,2 \pm 0,17$ % and $32,2 \pm 0,11$ % respectively). The relative area occupied by the secondary follicles in both of the studied groups increases in comparison with the previous observation period, but in animals of the experimental group, towards the animals of control group this index is a little bit lower ($12,5 \pm 0,05$ % and $14,1 \pm 0,19$ % accordingly). As well as at the 9th day of observation, in animals of the control group the vesicular follicles are not revealed. Thus in offspring of animals after the introduction of progesterone, the relative area occupied by vesicular follicles remains at the level of the previous day of observation - $4,9 \pm 0,06$ %. A similar tendency of increasing of relative area occupied by connective tissue is observed, but in animals of the experimental group this index is much higher than in control - $27,3 \pm 0,08$ % and $14,7 \pm 0,21$ % respectively. The relative area of blood vessels also increases, but in animals after intranatal exposure to progesterone, it lags behind the control indexes - $2,5 \pm 0,06$ % and $5,8 \pm 0,07$ % accordingly.

At the 21st day of life, the relative area occupied by primordial follicles in animals of the control group increases in comparison with the 14th day. In the offspring of animals in the experimental group, this index is significantly lower ($21,3 \pm 0,21$ % in experimental animals and $24,1 \pm 0,04$ % in control respectively). In relation to the area occupied by primary follicles in both investigated groups there is no considerable difference ($20,5 \pm 0,05$ % for the animals of experimental group and $21,6 \pm 0,05$ % in a control group). At the end of the third week of life, vesicular follicles appear in the ovaries of control animals, they occupy $5,4 \pm 0,05$ % of the area of the ovary. A similar index in animals of the experimental group is significantly different - $12,8 \pm 0,05$ %, respectively. The relative area of connective tissue increases in both studied groups of animals, but in the offspring of animals

after fetal progesterone introduction, this index is significantly higher than in control ($33,4 \pm 0,10$ % and $28 \pm 0,04$ % respectively). The relative area occupied by the blood vessels in the control animals remains at the level of the previous observation period, while in animals of the experimental group this index is increased, but is less than in control - $4,5 \pm 0,07$ %, respectively.

At the end of the first month of life in both groups of the investigated animals the pool of primordial follicles is substantially exhausted, however more expressed changes are found in the experimental group of animals ($9,4 \pm 0,08$ %) than in control ($11,8 \pm 0,11$ %). Similar changes are also common for primary follicles ($9,2 \pm 0,03$ % in experimental animals and $13,1 \pm 0,08$ % in control, respectively). A relative area of secondary follicles increases considerably in both groups, but in offspring of animals after progesterone exposure it is slightly smaller ($22,3 \pm 0,08$ %) than in control - $26,8 \pm 0,12$ %. At comparison with the previous observation period the area occupied by vesicular follicles increases in the control group - from $5,4 \pm 0,05$ % up to $8 \pm 0,11$ %. But more expressed increase of relative area of these follicles is common for the animals of experimental group - $14,5 \pm 0,07$ %. There is also a trend of an increase in the index of relative area occupied by connective tissue and vessels in animals of both groups ($38,5 \pm 0,10$ % and $5,7 \pm 0,08$ % in experimental offspring and $33,5 \pm 0,08$ % and $7,2 \pm 0,05$ % in control animals).

At the 45th day of observation, the relative area occupied by primordial and primary follicles continues to decrease in control animals - $6,5 \pm 0,08$ % and $7 \pm 0,03$ % respectively. More expressed changes in comparison with control are observed in the pool of primordial follicles of experimental offspring - $5,3 \pm 0,11$ % and $4,9 \pm 0,07$ % respectively. The most of the area is occupied by secondary follicles, however in posterities of experimental animals it is for certain less than in control - $22,7 \pm 0,10$ % against $28,9 \pm 0,04$ %. Increase of relative area of vesicular follicles compared to the 30th day of life is common for both groups of animals, but in animals after fetal effect of progesterone, this index is significantly higher ($18,8 \pm 0,07$ % in the experimental group and $11,8 \pm 0,12$ % in control). The dynamics of the relative area of the

connective tissue and the blood vessels during the period of observation from the 45th to the 90th day of life coincides with the earlier obtained data [12].

At the 60th day, the relative area occupied by secondary follicles in both studied groups of animals changes in comparison with the previous observation period. This index diminishes in both group (from $22,7 \pm 0,10$ % to $19,4 \pm 0,10$ % in experimental and from $28,9 \pm 0,04$ % to $25,2 \pm 0,07$ % for control animals accordingly). The relative area occupied by vesicular follicles increases in the control group in comparison with the 45th day of life, however, in the experimental animal group, this index is statistically significantly higher – $17,8 \pm 0,08$ % and $21,3 \pm 0,14$ % respectively. The percentage of the area occupied by primordial and primary follicles ($3,4 \pm 0,09$ % and $3,7 \pm 0,12$ % in experimental and $5,1 \pm 0,08$ % and $4,5 \pm 0,11$ % in control animals, respectively) continues to diminish.

Starting from the 90th day of life, the estrous cycle begins to be established in the rats' offspring. Accordingly, the correlation of types of follicles varied, as tertiary (antral) follicles appear. Also after ovulation the certain percent of area is occupied by corpus luteum. At the end of the third month of life relative area occupied by primordial follicles continues to decrease, but in animals of the experimental group, these changes are more expressed – $2,9 \pm 0,09$ % and $4 \pm 0,07$ % in control animals, respectively. A similar tendency is common for primary follicles, at comparison of indexes of both groups no significant differences were found – $2,8 \pm 0,10$ % in the control and $3,2 \pm 0,11$ % in the experimental offspring, respectively. Regarding the previous observation period in control animals, the relative area of the secondary follicles decreases. However, in offspring of animals after prenatal exposure to progesterone, the reduction of this index is more expressed – $11,2 \pm 0,14$ % versus $13,1 \pm 0,15$ % in the control. In both groups of animals, at the moment of observation, the relative area occupied by vesicular follicles is significantly reduced, but in experimental animals, these changes are more expressed in comparison with the control group ($4,1 \pm 0,12$ % in experimental animals and $5,8 \pm 0,16$ % in controls, respectively). In animals after the prenatal effect of progesterone the index of

relative area of tertiary follicles presents $9,3 \pm 0,10$ % accordingly. In animals of the control group, the similar indicator is somewhat higher – $11,9 \pm 0,07$ %, respectively. A significantly higher share of ovarian area is occupied by corpus luteum in experimental animals – $14,3 \pm 0,34$ % and $11,4 \pm 0,23$ % in control [12].

Discussion. Thus, during the first three months of life a gradual reduction to the pool of primordial follicles takes place in ovaries of rats, since form an initial point for a continuous process of folliculogenesis. After the assembly of primordial follicles, majority of them perish by apoptosis at different stages of folliculogenesis. Only a small part of follicles passes all stages of ripening and takes part in the process of ovulation. In the ovaries of offspring of rats after intrauterine exposure to progesterone, in comparison with control, there is a significant decrease in the relative area occupied by primordial follicles during all period of observation – from $30,3 \pm 0,16$ % up to $2,9 \pm 0,09$ %, respectively. Statistically reliable lag of index of relative area of primordial follicles for experimental animals is may be related to violation of process of assembly of primordial follicles during the first 3 days after birth and its potential lesser number [11, 13]. Analyzing the results, it is possible to assume that exhaustion of follicular pool after the prenatal effects of progesterone can lead to premature ovarian failure and early onset of a menopause in future.

In offspring of animals after introduction of progesterone at 9th day of life vesicular follicles are present. This is not common for the control group of animals at the analogical period of observation, since vesicular follicles appear only at the 21st day of life. A significant increase of the relative area occupied by vesicular follicles is related to the fact that the offspring of animals in the experimental group while estimating the area of separate vesicular follicles these indexes exceeded control values considerably [14]. Reduction of this index in both groups of animals from the 60th up to the 90th time of observation is explained by beginning of cyclic changes in ovary and by appearance of tertiary follicles.

An area that is occupied by secondary follicles in both groups gradually grows from the 9th day of life. Starting from the 30th day and till the end of

the observation period, secondary follicles become the predominant type of follicles in both groups, occupying a significantly smaller area in experimental animals – $22,3 \pm 0,08$ % in comparison with control $26,8 \pm 0,12$ %. The above-described tendency may be associated with a smaller number of primordial and primary ovarian follicles in the offspring of rats on the background of progesterone's fetal activity.

At comparing the relative area of tertiary follicles in both studied groups of animals, no significant differences were found – $11,9 \pm 0,07$ % in control and $9,3 \pm 0,10$ % in experimental animals, respectively.

In the offspring of animals of both groups, the ratio of the follicular to connective tissue component varies as follows: the relative area of connective tissue grows up with the increase of the observation period, reaching the maximum data by the 90th day of life. At the same time, the relative area occupied by the follicles of all types decreases accordingly. The got results in relation to the reliable increase of the content of connective tissue in the ovaries of experimental animals correlate with data in relation to the reliable increase of content of fibrocytes and gradual decline of fibroblasts.

The relative area occupied by blood vessels in both groups gradually increases up to the end of observation period. However, in offspring of animals after prenatal effect of progesterone, there is a probable lag of this index towards the control one up to the 90th days. The data obtained regarding the reduction of the area of blood vessels is also confirmed by the data obtained by T.A. Topolenko towards negative effect of progesterone influence on the vascular component of rats' testicles [15].

Conclusions.

1. In the offspring of rats after administration of progesterone in the fetal period the relative area occupied by primordial follicles decreases from the 9th day ($30,3 \pm 0,16$ % in experimental animals and $36,7 \pm 0,18$ % in control) to the end of the third month of life ($2,9 \pm 0,09$ % in the experimental and $4 \pm 0,07$ % in the control animals, respectively).

2. In the animals of the experimental group from the 9th day and in the control group from the 21st day of life, the relative area of the vesicular

follicles increases up to the 60th day inclusive, and starting from the 90th day starts to diminish.

3. The relative area of the secondary follicles is gradually increased in both investigated groups, and starting from the 30th day up and to the end of the observation period, however in offspring of animals of experimental group these changes are more expressed ($11,2 \pm 0,13$ % at experimental posterity and $13,1 \pm 0,15$ % for control animals accordingly).

Prospects of researches. In further it is planned to investigate correlation and dynamics of follicles in newborn rats after prenatal influence of progesterone.

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Sarafinyuk L.A.,*Head of the Department of Physical Education and Medical Physical Culture of the National Pirogov Memorial Medical University, Doctor of Biological Science, Professor 73A/28, st. Dovzhenka, Vinnitsia, Ukraine, 21001, e-mail: lsarafinyuk@gmail.com***Khavtur V.O.***assistant of the Medical Biology Department SHEI "I. Ya. Horbachevsky Ternopil State Medical University of the Ministry of Health of Ukraine", Ternopil, Ukraine; e-mail: khavtur@tdmu.edu.ua***Fedoniuk L.Ya.***Ph.D., MD, Professor, Chief of the Medical Biology Department SHEI "I. Ya. Horbachevsky Ternopil State Medical University of the Ministry of Health of Ukraine", Ternopil, Ukraine; e-mail: Fedonyuk22Larisa@gmail.com***Khapitska O.P.***Ph.D., Assistant Professor of Internal Medicine Department of the National Pirogov Memorial Medical University, Vinnitsia, Ukraine; e-mail: olga.hapitska@ukr.net*

EVALUATION OF THE BLOOD VENOUS LEAVE IN VOLLEYBALL PLAYERS OF THE MESOMORPHIC SOMATOTYPE

Abstract. *The peculiarities of the amplitude of the diastolic wave, the dicrotic and diastolic indices of the thigh and shin in the youth volleyball players of the mesomorphic constitutional type are presented in the paper. The lower values of the diastolic wave amplitude and hemodynamic indices in mesomorphic volleyball players were established in compare with non-athletes, that means and improve good venous outflow of blood. The lower values of the diastolic wave amplitude and hemodynamic indices in mesomorphic volleyball players and non-athletes were established, results indicated good venous outflow of blood in volleyball players.*

Key words: *reovasogram of thigh and shin, venous outflow, youth volleyball players, mesomorphic somatotype.*

Sports is a special kind of activity, which is combined with regular high (often extreme) physical and emotional stresses, high standards of health of an athlete. Assessment of the functional state of the body during sports is important for optimal construction of the training process, a kind of "outlet" of athletes to the highest level of functional readiness, which, together with other factors, ensures the achievement of the maximum possible sports results [1, 2]. Adaptation mechanisms of muscles blood supply are very important for the growth of sports achievements. It is known that the weight of the muscles is about 36% for women, 42% for men. Athletes - 50% or more. The largest range of changes in blood supply is a characteristic feature of the skeletal muscle's blood supply. In a state of rest, skeletal muscle receives 18-20 % of the total volume of oxygen consumed, which is 50-60 ml min⁻¹. During the physical trainings the blood supply of skeletal muscles can increase by 10-20 times in comparison with the state of rest, and consequently, the proportion of oxygen, which enters to the active muscles is increased to 2.8-3.3 l min⁻¹ [3, 4]. In the process of intensive work of the

muscles are appear adaptive changes in the activity of the cardiovascular system, the nature and tension of which depend not only on the power and duration of loading, but also to a lesser extent on the action that was used [5]. A sufficiently large factual material has been accumulated concerning the physiological processes associated with the exercise of physical activity and, above all, dynamic loads. But a number of issues of muscular work adaptation remains inadequate. Considering the above facts, it is obvious that the system of peripheral vessels, in particular the state of venous outflow of blood, is of great importance for the proper performance of training and competitive loads.

The purpose of the study was to determine the state of venous blood outflow in athletes of the mesomorphic somatotype, who are engaged in volleyball, by determining the reovasographic indexes of the thigh and shin.

Materials and methods. We conducted a comprehensive survey of youth volleyball players (boys from 17 to 21 years old and girls from 16 to 20 years) with high level of athletic skill (from the first adult level to sports master). They conducted an

anthropometric study using Bunak's technique [6], and somatotypological investigation according to the estimated modification of the Heath-Carter method [7]. It was found that 32 male volleyball players and 29 female volleyball players belonged to the mesomorphic constitutional type. The control group was consist of individuals of the same somatotype (23 boys and 33 girls) who were not engaged in sports, and were practically healthy at the time of the survey. A statement on their health was made after a clinical and laboratory study, which included echocardiography, sonography of the thyroid gland, kidney, urinary bladder, liver, gall bladder and spleen, chest X-rays; spirometry, tetrapolar rheography. The research was conducted on the base of the Scientific Laboratory of Functional Morphology and Genetics of the Scientific-Research Center of the E. Pirogov Vinnitsa National Medical University. The reovasographic parameters of the thigh and shin were determined using tetrapolar rheography on a computer diagnostic complex. The estimation of quantitative parameters was carried out according to time, amplitude and other parameters according to Ronkin and Ivanov's method [8]. Reliability of difference values between independent quantitative variables were determined using the U-Mann-Whitney criterion and programme STATISTICA 5.5 (license number AXXR910A374605FA)

Results. Firstly, the state of venous blood outflow is characterized by such indices to reovazograms, as amplitude of diastolic wave, dicrotic and diastolic indices. We have found that the amplitude of the diastolic wave on the thigh in the volleyball players of the mesomorphic somatotype (0.006 ± 0.002) Ohm, compared to those of the same constitutional type who were not engaged in sports (0.007 ± 0.002) Ohm, was slightly lower ($p > 0.05$). At the shin of the volleyball players, this indicator had a value (0.019 ± 0.005) Ohm, in the control group - (0.021 ± 0.007) Ohm, the difference in comparison of these values is not reliable.

The value of the dicrotic index in the thigh of the mesomorphic somatotype volleyball players was (58.76 ± 13.34) %, in the control group - (59.66 ± 16.97); at the shin of the athletes, the average values of this indicator were within (30.28 ± 12.67) %, in non-athletes - (36.21 ± 11.79)%. Thus, it was found that on the thigh the magnitude of this indicator did not reveal statistically significant

differences between volleyball players and non-sportspersons, and the dicrotic index was less ($p < 0.05$) than in the control group.

The diastolic index, determined by the ratio of the amplitude and parameters of time of the thigh rheogram, in the mesomorphs volleyball players (50.56 ± 12.76) % were significantly lower ($p < 0.05$) than in the control group (57.88 ± 13.32) %. The value of this index on the shin did not differ statistically significantly between athletes (37.50 ± 8.73) % and those not engaged in sports (40.32 ± 8.894) %, but although it is necessary to note the lower values of the diastolic index for volleyball players.

Discussion. Under the influence of sports training, the functional capabilities of athletes are increased, the parameters of aerobic performance of the organism increase, the efficiency of the functioning of the cardiovascular and respiratory system increases [9], longer load increases the significance of the aerobic system [10].

The amplitude of the diastolic wave, which reflects the ratio of arterial and venous blood flow, at a young age, under conditions of high elasticity of the arterial bed, it is mainly a wave of reflection from the smallest arteries and arterioles [11]. Because the degree of this reaction is functionally associated thought the venular-arteriolar reflex with a state of venous organ perfusion, it indirectly depends on the state of venous outflow. With the loss of the elastic properties of the arterial bed, which may be due to age or different pathological states, the amplitude of the diastolic wave increases due to overlapping reflection waves in the more central artery regions [12].

The dicrotic index characterizes the state of tone of contractive elements of small arteries and arterioles and allows to assess the state of microcirculation and depends on the state of peripheral vascular resistance. Significant changes in the dicrotic index (in the direction of increase) indicate a violation of the correlation between arterial flow and venous outflow in small vessels, the growth of peripheral resistance and the appearance of signs of venous congestion in the vessels of the precapillary bed [13].

The diastolic index reflects, basically, the state of blood outflow from the arteries to the veins [11]. Diastolic index - is the ratio of the magnitude of the amplitude at the level of the dicrotic to the

maximum amplitude of the rheographic wave [12]. It is known that this parameter reflects the state of the vessels of the postcapillary bed, the venules and venous tone, venous blood flow (the ratio of outflow and blood flow) and is an indirect parameter of the elasticity of the vascular wall [13, 14].

The smaller values of the diastolic wave amplitude, the dicrotic and diastolic indexes that we observed in the volleyball players of the mesomorphic somatotype, indicate the best venous outflow, which formed under the influence of systematic physical activity. An improved venous outflow of volleyball players of this constitutional type can be considered as a compensatory adaptation of peripheral hemodynamics to the needs of this kind of sport. In previous studies, we revealed the negative changes in regional circulation in volleyball players of the general group (without division into somatotypes). They showed the most pronounced slowing of the blood flow on the shin and thigh, reducing the elasticity of the vascular walls of the arteries of medium and small diameters, high peripheral resistance, and increasing the tone of arteries of different diameters [15, 16].

Conclusion: in volleyball players of the mesomorphic somatotype compared to non-athletes, improved venous outflow of blood was established, as evidenced by lower values of the amplitude of the diastolic wave, of the dicrotic ($p < 0.05$ on the tibia) and of the diastolic ($p < 0.05$ on the hip) indices.

Prospects for further research. The results of this study can be used in predicting the parameters of the reovasograms of the thigh and shin in volleyball players to achieve their high results.

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Kononova O.V.

Senior Research Assistant, Ph.D., O.M.Marseev Institute of Public Health of the NAMS of Ukraine, Kyiv, Ukraine,
vladoks2010@gmail.com

IMMUNOLOGICAL INDICATORS IN PATIENTS WITH GENERALIZED PARODONTITIS AND PSYCHOEMOTIONAL STRESS

Abstract. Psychological stress of patients promotes development of periodontal disease. It should be considered in a comprehensive treatment of patients with periodontal disease. Objective: to determine possible relations between the level of psychological stress in the organism of a patient with generalized periodontitis, and evaluate the parameters in the immune system cellular part. Material and methods. The study was conducted on the group of 40 young patients. They were divided into the following 4 subgroups, 10 people each. The first group: clinically healthy individuals with no manifestations of stress with clinically healthy periodontal tissues. The second group: patients with generalized periodontitis without manifestations of stress. The third group: persons with manifestations of psychological stress without visible lesions of the periodontal tissues. The fourth one: patients with generalized periodontitis and manifestations of psychological stress. The level of psychological stress was assessed using questionnaires. All patients with generalized periodontitis were subjected to a thorough clinical examination of the oral cavity. Cortisol content was determined in the oral fluid samples using the RP Elecsys kit (Roche Diagnostics, USA). Immunophenotyping of lymphocytes was performed in venous blood of patients. Results. The lowest level of cortisol in saliva was found in patients without clinical signs of the disease - 14.67 ± 2.71 nmol/l. The presence of periodontal disease slightly increases the level of cortisol to 24.33 ± 5.47 nmol/l. In case of psychoemotional stress, the level of cortisol increases to 29.33 ± 2.55 nmol/l. When psychoemotional stress is combined with generalized periodontitis, the level of cortisol increases to 41.67 ± 5.67 nmol/l. Determination of the parameters of subpopulation composition of lymphocytes: CD3 +, CD4 +, CD8 + in this category of patients showed a decrease in their absolute number in patients with periodontal disease (generalized periodontitis - 2nd subgroup), psychoemotional stress (3rd subgroup) and in case of combination of periodontal disease and psychoemotional stress (4th subgroup). Most of these changes are pronounced in patients of the 4th subgroup in case of combination of periodontal disease and psychoemotional stress. Determination of the parameters of subpopulation composition of lymphocytes: CD3 +, CD4 +, CD8 + in this category of patients showed a decrease in their absolute number in patients with periodontal disease (generalized periodontitis - 2nd subgroup), psychoemotional stress (3rd subgroup) and in case of combination of periodontal disease and psychoemotional stress (4th subgroup). Most of these changes are expressed in patients of the 4th subgroup in case of combination of periodontal disease and psychoemotional stress. Conclusions. The conducted research enables to confirm a certain relationship between the manifestations of psychoemotional stress, periodontal disease (generalized periodontitis) and cellular immunity. Such a condition requires appropriate medical treatment of this group of patients with generalized periodontitis and manifestations of psychoemotional stress.

Key words: generalized periodontitis, psychoemotional stress, cortisol, cellular immunity.

Interrelations between opportunistic microorganisms of the biofilm and patient's (host's) organism are important in pathogenesis of generalized periodontitis [13]. Immune response of the host's organism is essential in these interrelations [5]. Many various factors influence on them, namely, general somatic diseases, social-economic condition of the patient, smoking and psychological stress. Psychological stress, in particular, is an

important factor of development of many pathological conditions including periodontal diseases [9, 14, 17]. Available stress results in decrease of immune resistance and development of clinical important disorders of immune response [3, 8].

One of objective parameters determining availability of psychological stress and reflects the level of immune protection is cortisol content in the

oral fluid [2, 11]. Detection of cortisol level in the oral fluid enables to find certain relations between the level of stress and periodontal diseases.

Changes occurring in the parameters of the functional activity of the immune system cellular part are an important factor of psycho-emotional stressors [1, 5]. They are affected by stress parameters, state of the anti-stress system, determining peculiarities of immunologic response, which is valuable for the selection of therapeutic tactics of a patient with generalized periodontitis.

Objective: to determine possible relations between the level of psychological stress in the organism of a patient with generalized periodontitis, and evaluate the parameters in the immune system cellular part.

Materials and methods. The study was conducted on the group of 40 patients of a young age. They were distributed into the following 4 subgroups. The first subgroup included 10 clinically healthy individuals without stress signs and healthy periodontal tissues. The second subgroup included 10 patients with generalized periodontitis and without stress manifestation. The third subgroup included 10 individuals with psychological stress signs without vivid lesions of the periodontal tissue. And the fourth group included 10 patients suffering from generalized periodontitis and psychological stress signs.

The level of psychological stress was assessed using questionnaires (DASS-21; Spielberg-Hanin's scale). [12, 16]. The examined patients filled in Spielberg-Hanin's questionnaire. It helps to determine personal and situational anxiety. The answers were estimated according to the keys, the total score was calculated considering all the scales separately (situational anxiety scale and individual anxiety scale).

During examination of the oral cavity the color and consistency of the mucous membrane of the vestibule, its depth, condition and height of frenula attachment were evaluated. The state of the mucous membrane of the cheeks, soft and hard palates, tongue and oral fundus was determined as well. The gums were examined from the vestibular and oral sides. The color of the gums, availability of lack of swelling, consistency, and relief of the gingival margin were determined. Availability, localization and intensity of inflammatory process were determined by means of Pisarev-Schiller's test

[6, 7]. Special attention was paid to dental deposits: their condition, consistency, amount and localization. To find dental deposits (dental plaque) diagnostic dyes were used. Oral hygiene was evaluated by means of Green-Vermillion index (1964). PMA index was used to determine the degree of gingival inflammation [6, 7].

Cortisol content was determined in the oral fluid samples. The patients were asked to abstain from taking meals and drinks to hours before taking saliva for test. The oral fluid was collected in the morning on an empty stomach in special Eppendorf tubes. After the samples were transported to the laboratory, they were centrifuged at 3000 rotations per minute during 5 minutes. In case of necessity saliva samples could be kept at the temperature of -20 ° C. 20 mcl were taken from saliva and put into the sample's cup. Cortisol content was determined in nmol/L by means of RP Elecsys set (Roche Diagnostics, USA) in the analyzer Cobas e 411 [4, 15].

Venous blood was used as the material to examine immunologic parameters. The blood was taken in the morning on an empty stomach in the amount of 5 ml in Vacuum Tubes EDTA.K3 (whole blood). The blood was examined during 2 hours after it had been taken. Lymphocytes were immunophenotyped by means of immunofluorescence analysis using flow laser cytometer BD FACS Canto II (Becton Dickinson, USA) after an automatized preparation of the whole blood samples by BD FACS Sample Prep Assistant II (Becton Dickinson, USA). An absolute (10^9 cells/L) and relative (%) content of certain parameters of the lymphocyte subpopulation content were determined: CD3+, CD4+, CD8+. The ratio CD4+/CD8+ (T-helpers/T-suppressors) was further determined.

The results obtained were statistically processed in the packet "STATISTICA 6.1" applying parametric and non-parametric methods. Correct distribution of signs according to every variation series, mean values according to every signs and their standard errors and deviations were estimated [10].

Results. The patients from the first (control) subgroup did not present stress signs, common somatic diseases, and had clinically healthy periodontal tissues. Their level of oral hygiene was close to excellent, and hygiene index was on an average 0,15. Inflammation of gums was practically lacking. Yellowish color was found in certain parts of

the gums only. PMA index was on an average 4,7%. Cortisol content in the oral fluid was $14,67 \pm 2,71$ nmol/L. This level was accepted as a norm in case of comparison with parameters of patients from other groups.

The second subgroup included patients suffering from I degree generalized chronic periodontitis. The patients from the second subgroup did not present psycho-emotional stress signs either. Their level of oral hygiene was close to good, and hygiene index was on an average 1,33. Cortisol content in the oral fluid was $24,33 \pm 5,47$ nmol/L.

The third subgroup included patients with psycho-emotional stress signs and practically unnoticeable lesions of the periodontal tissues. Inflammation of gums was practically absent, only yellowish color was found in certain parts of the gums. Cortisol content in the oral fluid was $29,33 \pm 2,55$ nmol/L.

The fourth subgroup included patients suffering

from I degree generalized chronic periodontitis and with psycho-emotional stress signs. Their level of oral hygiene was close to good, and hygiene index was on an average 1,57. Inflammation of gums was not considerable. OMA index was on an average 38,6%. Cortisol content in the oral fluid was $41,67 \pm 5,67$ nmol/L.

Therefore, association of psycho-emotional stress with periodontal diseases, namely, generalized periodontitis, results in the most considerable increase of cortisol level in the oral fluid – up to $41,67 \pm 5,67$ nmol/L. Its content differs statistically reliably ($p < 0,05$) from cortisol level of patients in other subgroups.

The parameters of the lymphocyte subpopulation content CD3+, CD4+, CD8+ in patients from different groups are presented in Table 1. The parameters of the first group were used as control values for the parameters obtained in patients from other groups.

Table 1

Content of lymphocyte subpopulations in the examined patients ($M \pm m$)

Parameters		I subgroup	II subgroup	III subgroup	IV subgroup
CD3+	%	$37,4 \pm 3,7$	$37,9 \pm 3,8$	$40,3 \pm 4,1$	$41,2 \pm 4,2$
	10^9 kl/L	$0,65 \pm 0,21$	$0,62 \pm 0,23$	$0,59 \pm 0,23$	$0,52 \pm 0,23$
CD4+	%	$12,4 \pm 1,3$	$10,9 \pm 3,3$	$9,17 \pm 3,21$	$8,67 \pm 3,21$
	10^9 kl/L	$0,17 \pm 0,07$	$0,15 \pm 0,06$	$0,13 \pm 0,06$	$0,12 \pm 0,05$
CD8+	%	$5,3 \pm 1,7$	$5,2 \pm 1,7$	$2,52 \pm 2,12$	$1,30 \pm 1,15$
	10^9 kl/L	$0,08 \pm 0,07$	$0,08 \pm 0,07$	$0,07 \pm 0,07$	$0,07 \pm 0,07$
CD4+/CD8+		$1,95 \pm 0,17$	$1,86 \pm 0,17$	$1,86 \pm 0,17$	$1,71 \pm 0,17$

Detection of the parameters of the lymphocyte subpopulation content: CD3+, CD4+, CD8+ in patients from the second subgroup (suffering from I degree generalized chronic periodontitis) showed decrease of their absolute amount (Table 1). An absolute amount of CD3+ lymphocytes, for example, statistically 4,62% decreased from $0,65 \pm 0,21$ (10^9 kl/L) to $0,62 \pm 0,23$ (10^9 kl/L). An absolute amount of CD4+ lymphocytes, for example, statistically 11,76% decreased from $0,17 \pm 0,07$ (10^9 kl/L) to $0,15 \pm 0,06$ (10^9 kl/L). An absolute amount of CD8+ lymphocytes remained on the level similar to that of the control group - $0,08 \pm 0,07$ (10^9 kl/L). Parameter CD4+/CD8+ (T-helper/T-suppressor) unreliably 3,08% decreased from $1,95 \pm 0,17$ to $1,86 \pm 0,17$.

The fourth group included patients suffering from I degree generalized chronic periodontitis and

with psycho-emotional stress signs. Detection of the parameters of the lymphocyte subpopulation content: CD3+, CD4+, CD8+ in this category of patients, demonstrated decrease of their absolute amount (Table 1). An absolute amount of CD3+ lymphocytes, for example, statistically unreliably 20,00% decreased from $0,65 \pm 0,21$ (10^9 kl/L) to $0,52 \pm 0,23$ (10^9 kl/L). An absolute amount of CD4+ lymphocytes statistically unreliably 29,41% decreased from $0,17 \pm 0,07$ (10^9 kl/L) to $0,12 \pm 0,05$ (10^9 kl/L). An absolute amount of CD8+ 12,5% decreased from $0,08 \pm 0,07$ (10^9 kl/L) to $0,07 \pm 0,07$ (10^9 kl/L). The parameter CD4+/CD8+ (T-suppressors) 12,31% unreliably decreased from $1,95 \pm 0,17$ to $1,71 \pm 0,17$.

Conclusions. The conducted investigation enables to confirm certain interrelations between psycho-emotional stress signs and periodontal

diseases – generalized periodontitis. It should be noted that cortisol level in the oral fluid is more affected by psychological stress available than pathological process in the periodontal tissue. Certain interrelations between psycho-emotional stress signs, periodontal diseases (generalized periodontitis) and cellular links of immunity are confirmed.

Therefore, the conducted investigation enables to suggest a negative effect of psycho-emotional stress on the periodontal tissue. This condition requires an appropriate pharmacological treatment of this group of patients suffering from generalized periodontitis with psycho-emotional stress signs.

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Borysenko A. V.*Doctor of Medical Sciences, Professor, Head of the Department of Therapeutic Dentistry, O.O.Bogomolets National Medical University, Kyiv, Ukraine, anatoliyborysenko.nmu@gmail.com***Vatankha T.V.***Ass. Professors, Department of Therapeutic Dentistry, O.O.Bogomolets National Medical University, Kyiv, str. Zoologicheskaya, 1, Kyiv, Ukraine, Vatankha2@gmail.com*

THE NEAREST RESULTS OF TREATMENT OF PERIODONTAL DISEASES IN WOMEN TAKING ORAL CONTRACEPTIVES

Abstract. Nowadays the use of hormonal contraceptives is a popular means of contraception. However, these agents also contain estrogens, which can provoke periodontal inflammation. Therefore, the development of methods of treatment of periodontal diseases in this category of women is an urgent issue. **Objectives:** to determine clinical efficacy of the suggested method of treatment of inflammatory periodontal disease in women taking hormonal contraceptives for the purpose of planning pregnancy. **Materials and methods.** Clinical investigation was conducted on the groups of 90 women taking oral contraceptives for the purpose of planning pregnancy. Pharmacological treatment of these patients was performed using the suggested medical composition. A comprehensive examination of patients with periodontal tissue was conducted before and after treatment. To evaluate a clinical efficacy of treatment Schiller-Pisarev test (1962), the index of PMA by C. Parma (1961), hygienic index OHI-S (1964), PBI by H.R.Muhlemann and others were used. **Results.** As a result of investigation it was found that the usage of the suggested medical composition in a comprehensive therapy of inflammatory periodontal diseases in women taking oral contraceptives produces a positive effect. It is confirmed by decline in the index OHI-S, PMA, PBI, improving oral hygiene. **Conclusions.** The use of the suggested medical composition increases the effectiveness of treatment of inflammatory periodontal diseases in women taking oral contraceptives.

Key words: women taking oral contraceptives, inflammatory periodontal diseases, medical composition.

According to the clinical evidence available periodontal lesions in pregnant women are associated with hyperestrogenemia [5, 6, 9]. Nowadays administration of hormonal contraceptives is a popular means of contraception. Though, these agents contain estrogens, and they can provoke occurrence of inflammatory processes in the periodontal tissue. Meanwhile, instructions to hormonal contraceptives do not contain indications concerning possible complications from the side of periodontal tissue [7].

Our previous experimental work [1] indicated that a long administration of the hormonal contraceptive "Yaryna" causes development of gingivitis in the gums. It is evidenced by biochemical examinations, namely, a considerable increase of elastase level, a marker of inflammation, in the blood of experimental animals. Mineralizing activity of the periodontal osseous tissue decreased in rats taking this contraceptive. It resulted in an increased degree of bone atrophy of the mandibular alveolar process.

To prevent occurrence of gum inflammation under experimental conditions in rats taking a hormonal contraceptive the bioflavonoid angioprotector "Normoven" was used. It contains diosmin and hesperidin in the dose of bioflavonoids 84 mg/kg, stimulates mineralizing activity of the periodontal osseous tissue, and reduces degree of alveolar process atrophy. Though, it does not eliminate inflammatory signs in the periodontal tissue completely [15].

Considering the obtained experimental data for medical treatment of periodontal inflammation in women taking hormonal contraceptives, with the purpose of family planning, a method of treatment of the periodontal tissue in women taking oral hormonal contraceptives was suggested.

Objective: to determine clinical efficacy of the suggested method of treatment of periodontal inflammation in women taking hormonal contraceptives with the purpose of family planning.

Materials and methods. To achieve the goal clinical-radiological examination of 120 women was

conducted. The main group included 90 (72,73%) women taking oral contraceptives with the purpose of family planning. The control group included 40 (27,27%) women, who were consulted at the Stomatological Center at O.O.Bogomolets National Medical University, and the University students. Exclusive criteria for the main group were: smoking and other bad habits; pregnancy; abortions; comorbid common somatic diseases. Entry criteria were: patients of a reproductive age (from 19 to 35 years) taking low doses of oral contraceptives containing 0,03 mg of ethinyl estradiol and 3 mg of drospirenone no less than for a year.

Distribution of patients into groups according to the degree of disease and age was practically similar (Table 1).

Inflammation in the periodontal tissues was determined by means of Schiller-Pisarev test [8]. Greene-Vermillion index and simplified oral hygiene index OHI-S [4], gingival index [11] were used to assess hygienic state of the oral cavity. To determine intensity of inflammation in the periodontal tissue papillary-marginal-alveolar (PMA) index by C.Parma [14] was applied. Papillary bleeding index (PBI) by H.R.Muhlemann [13], alveolar plaque index (API) to determine dental plaque by Lange [10] was used as well. Condition of the osseous tissue was determined by means of orthopantomogram. The diagnosis of periodontal lesion was made according to M.F.Danylevsky's classification [2, 3]. The obtained experimental and clinical data were statistically processed according to the standard processing programs [12].

To treat inflammatory lesions of the periodontal tissue in women taking hormonal contraceptives with the purpose of family planning, a method of treatment was suggested (Declaration Patent of Ukraine № 131972 of 11.02.2019). The treatment includes elimination of local irritating factors and topical pharmaceutical treatment of symptomatic gingivitis using the bioflavonoid angioprotector "Normoven" produced by Ltd "Kyiv Vitamin Plant" (Ukraine) against the ground of general treatment with the same agent "Normoven". For the general treatment the drug "Normoven" was indicated per 1 tablet twice a day (in the morning and in the evening) during meals. The duration of the course depended on pronunciation of symptomatic gingivitis and was on an average 10 days. For the local treatment "Normoven" 0,5 in tablets was used.

It was grinded in a mortar and medical composition was prepared *ex tempore*:

Normoven 0,5 g

Metrogyl-denta gel 0,5 g

The agents were mixed and applied on the afflicted area of the gums. Application lasted 20-30 minutes. This medial composition was applied twice a day by a patient herself. The period of treatment was 7-10 days.

All the patients used a manual toothbrush, dental floss, toothpaste "Lacalut" and lotion "Lacalut" for professional oral hygiene. All the irritants of the periodontal tissue were removed completely (dental plaque, tartar etc.). Subgingival dental deposits were removed with further treatment of surfaces of dental roots (so-called SRP – Scaling and Root planning).

Results and discussion. A comprehensive examination of the periodontal tissue before treatment showed that in 80 women of the main group and 30 women of the control group inflammatory and dystrophic-inflammatory diseases of the periodontal tissues were found. Chronic catarrhal gingivitis of a mild and moderate degree was diagnosed more often among inflammatory diseases. It was found in 68 (85,0%) women of the main group and 24 (80,0%) women from the control group. The main complaints included unpleasant sensations in the gums and edema of the gums. They were found in 67(83,75%) women from the main group. Complaints on bleeding were found in 66 (82,5%) women. Edema of the gums was found in 24 (80,0%) women, bleeding from the gums - in 23 (76,67%) women from the control group. Dystrophic-inflammatory diseases of the periodontal tissue were found in 12 (15,0%) women from the main group including 7 (8,75%) women with diagnosed chronic generalized periodontitis of I degree, and 1 (1,25%) woman with generalized periodontitis of an initial degree of chronic course. Dystrophic-inflammatory diseases were found in 6 (20,0%) women from the control group including 5 (16,67%) of them with detected generalized periodontitis of an initial degree of chronic course and 1 (3,33%) woman – generalized chronic periodontitis of I degree. The data of assessment of the oral hygiene in women from the main group (Table 2) ranged within 1,2-3,0 of OHI-S, on an averagey $2,1 \pm 0,1$ API ranged within 14%-100% – on an average $37,7 \pm 0,3\%$. In the control group the values of these indices were $2,4 \pm 0,1$ (OHI-S) and

31,3±0,3% - API respectively. It is indicative of a relatively satisfactory condition of the oral hygiene in both groups. PMA index in the main group was on an average 34,8±0,3%, and in the control one – 29,2±0,3%. Bleeding of the gums determined by PBI in women from the main group was within 1,25±0,1 points and 1,28±0,1 points in women from the control group respectively. Therefore, a

comparative analysis of the periodontal tissue condition among the women from the examined groups is indicative of relatively satisfactory condition of the marginal gums due to unsatisfactory condition of oral hygiene.

After the treatment with application of the suggested methods certain changes in the oral hygiene were determined (Table 2)

Table 1

Distribution of patients with periodontal diseases of the main and control groups

Groups	Periodontal diseases				Age (years)	General number of patients	
	Chronic catarrhal gingivitis		I degree of generalized periodontitis			abs.	%
	abs.	%	abs.	%			
Main group	68	75,56	22	24,44	23,67	90	100,0
Control group	24	60,0	16	40,0	24,33	40	100,0
Total	92	70,77	38	29,23		130	100

Table 2

Dynamics of the hygienic index OHI-S in the nearest terms of observations (points)

Groups of patients	Before treatment	Terms of observation after treatment		
		2 weeks	1 month	3 months
Main group	1,94±0,17	0,78±0,07	0,52±0,05	0,58±0,05
Control group	1,86±0,17	0,74±0,07	0,68±0,06	0,8±0,08
p	>0,05	>0,05	<0,05	<0,05

Primary examination of the oral cavity of all the patients from both groups determined that hygienic state was practically similar and was within the limits "satisfactory hygiene". The hygienic index OHI-S was 1,94±0,17 and 1,86±0,17 points respectively. 2 weeks after the treatment this index in patients from the main group approximately 2,5 times improved. A month later hygienic index was 0,52±0,05 points among the patients from the main group and 0,68±0,06 points among the patients from the control group. 3 months later OHI-S in patients from the main group remained on a former level of good hygiene. It remained on a previous level of good hygiene in the main group, and in patients from the control group it increased to 0,8±0,08 points. In spite of the fact that both indices are within the range equal to the assessment "good hygiene", in patients from the main group who underwent the suggested treatment this index was 30% better. Beginning with the term of observation of 1 month the value of OHI-S in patients from the main group and control group differed reliably ($p<0,05$). The obtained results enabled to predict more stable clinical effect and longer remission of periodontal pathological process.

After the course of treatment the degree of inflammation in the periodontal tissue decreased as it can be evaluated according to the values of PMA index (Table 3).

A month after treatment the value of PMA index approximately 4,63 times decreased in patients from the main group: from 39,52±2,1% to 8,53±1,2%, and in patients from the control group - 2,72 times: from 40,34±2,1% to 14,84±1,3%,. 3 months later in patients from the main group the value of PMA index remained on the same level: 8,23±1,1%. In patients from the control group the index and tendency to exacerbation increase, since PMA index is close to the upper level of "mild" gingivitis: 15,32±1,1%. The difference between the values of PMA index in patients from the groups of observation is statistically reliable ($p<0,05$).

Therefore, the dynamics of anti-inflammatory action correlated directly with the oral hygiene state. PMA index corresponded to the assessment "moderate gingivitis".

The suggested method of treatment produces certain capillary-protective action, which was evidenced by the changes of PBI (Table 4).

Table 3

Dynamics of PMA index in the nearest terms of observation (%)

Groups of patients	Before treatment	Terms of observation after treatment		
		2 weeks	1 month	3 months
Main group	39,52±2,1	8,53±1,2	8,52±1,2	8,23±1,1
Control group	40,34±2,1	14,84±1,3	14,36±1,3	15,32±1,1
p	>0,05	>0,05	<0,05	<0,05

Table 4

PBI dynamics in the nearest terms of observation (points)

Groups of patients	Before treatment	Terms of observation after treatment		
		2 weeks	1 month	3 months
Main group	1,46±0,1	0,85±0,07	0,49±0,03	0,51±0,04
Control group	1,51±0,1	0,93±0,07	0,68±0,05	0,75±0,06
p	>0,05	>0,05	<0,05	<0,05

Therefore, the conducted clinical studies and examination of patients in the nearest terms of observation showed that the suggested treatment produces a certain capillary-protective action.

Conclusions. Results of treatment improved gradually during one and three months after the conducted treatment. They were maximum 3 months later in patients from the main group. In patients from the control group maximum was determined a month later, and 3 months later these results deteriorated. The difference between the patients from the main and control groups within the terms of three months was statistically reliable ($p<0,05$).

Therefore, examination of patients in the nearest terms of observation showed the efficacy of the suggested therapeutic-hygienic complexes. According to the index assessment of the periodontal tissue state the results of treatment of patients from the main group differed reliably ($p<0,05$) from the data of patients from the control group.

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Stetsevyat V.B.*Assistant, Department of Physiology SHEE „Ivano-Frankivsk National Medical University”, f10bff@gmail.com***Bagrii M.M.***Ass. Professors, Department of Therapeutic Dentistry, O.O.Bogomolets National Medical University, Kyiv, str. Zoologicheskaya, 1, Kyiv, Ukraine, Vatankha2@gmail.com***Voronych-Semchenko N.M.***Chief of the Physiology Department, Professor, SHEE „Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine*

SEXUAL DIMORPHISM OF THE STRUCTURAL ORGANIZATION OF THE LIVER OF THE INSULIN-RESISTANT RATS

Abstract. *In animals, under the conditions of insulin resistance (IR), a violation of lamellar organization of the liver due to dystrophic changes in hepatocytes in all its lobes, but mainly in their peripheral departments, has been revealed. Liver cells are enlarged in size. In females there is a more pronounced zonal character of degenerative processes of hepatocytes compared with males, whose dystrophy is predominantly diffuse in nature. A distinctive peculiarity in females with IR is also the presence of foci of fatty degeneration of hepatocytes.*

Key words: *insulin resistance, structural organization of the liver, sexual dimorphism.*

Topicality of the study. Diabetes mellitus (DM) is a relatively common disease of the endocrine system in the world, that has a tendency to increase. The total number of patients with DM can reach 440 million up to 2030 [1]. The risk of DM is in its multiple-organ damage, as well as in the high risk of development of comorbidity. In recent years, attention has been drawn to the diseases of the liver, which from year to year remain the leaders of the digestive tract pathologies. The liver plays a leading role in the metabolic processes of the organism, and the violation of its functional state is associated with the changes in the metabolic processes of various genesis [2, 3]. Therefore, it is relevant to study the structural organization of an organ under the conditions of DM.

The objective of the research. To study the structural peculiarities of the liver in rats with insulin resistance taking into account sexual dimorphism, in the experiment. **Materials and methods.** The study was performed using 40 nonlinear, mature rats (males and females) weighing 150-180 g. Rats of the experimental group (20 males and 20 females) were modeled IR by adding the 10% fructose solution to drinking water during 8 weeks [4]. Control group animals (20 males and 20 females) followed the standard diet. Care, feeding and euthanasia (decapitation using ketamine anesthesia, 100 mg/kg of the body weight) were in line with current international requirements regarding the humane

treatment of animals. Histological preparations were stained with hematoxylin and eosin according to Shabadash for evaluation of structural changes in the liver, and PAS-reaction was performed [5]. When analyzing indices, gender peculiarities were taken into account. Histological studies were performed on the Leica DME light-optical microscope. In order to objectivize quantitative studies, computer morphometry of objects in histological preparations was performed. During the first stage, the digital copies of the optical image of microscopic preparations were obtained using a Nikon Coolpix 4500 digital camera, followed by an analysis of digital copies of the images using the computer software Image Tool 3.0 for Windows. The morphometric analysis of the liver was performed taking into account the following parameters: the average perimeter and area of the hepatocyte, the average perimeter and nucleus area of the hepatocyte, the average perimeter and nucleolus area of the hepatocyte, and the average thickness of the vascular wall. The nuclear-cytoplasmic index and the index of the ratio of the nucleolus area to the area of the nucleus of the liver cell were also calculated. Statistical analysis of the results was performed using Microsoft Excel and Statistica 5.5 (Multiple Regression) computer software.

Results of the research and their discussion. The structural construction of the liver in animals of the control group, both males and females, is characterized

by a trabecular organization (Fig. 1). Hepatocytes of rectangular shape, with a granular eosinophilic cytoplasm, contain small granules of glycogen with an average area of $3.23 \pm 0.65 \mu\text{m}^2$ (Fig. 2).

Granules are round-shaped, rich in purple tint, are diffusely located in the cytoplasm of cells. The liver cells have a centrally located round nucleus with heterogeneous chromatin. The nucleus is characterized by a central and paracentral rounded form of the nucleoli. Between the trabeculae of hepatocytes there are sinusoidal hemocapillaries that contain erythrocytes. In peri-sinusoidal spaces, solitary macrophages are visualized. In the peripheral parts of the lobes, the portal tracts are visualized, in the central departments – there are central veins, which are mainly filled with blood. The examined metric indices of the hepatocytes are represented in Table 1, and indicate a lack of a significant difference between animals of different genders. In males with IR, the lamellar organization of the liver is violated due to the dystrophic changes in hepatocytes in all the lobes, but mainly in their peripheral areas. Hepatocytes are of polygonal form, the borders of cells are slightly veiled. Liver cells have light cytoplasm, in which eosinophilic granularity is noted. The analysis of the staining according to Shabadash indicates that the cytoplasmic lucent areas are due to the loss of liver cells of glycogen (Fig. 3).

The liver cells are enlarged in size (average perimeter and area of hepatocytes at 29% and 65% exceed the control data, $p < 0.05$). An increase in metric parameters of hepatocytes is accompanied by their compact location. The nuclei of the liver cells are mostly round regular-shaped, with a fine-particle uniformly dispersed chromatin in the karyoplasm, with a central and paracentral rigorous visualization of the rounded form of the nucleoli. The morphometric parameters of liver hepatocytes in rats with IR are represented in Table 1. In some cases, hepatocytes with more than one nucleolus in the nucleus, accompanied by an increase of the metric parameters of the nuclei, are observed. Somewhere there are marked single binuclear hepatocytes. A significant increase of the nuclear-cytoplasmic index (at 19% in males and at 25% in females, $p < 0.05$), and the presence of binuclear hepatocytes suggest the development of regenerative processes in the cell, in particular, in the nucleus, but the tension of the regenerative

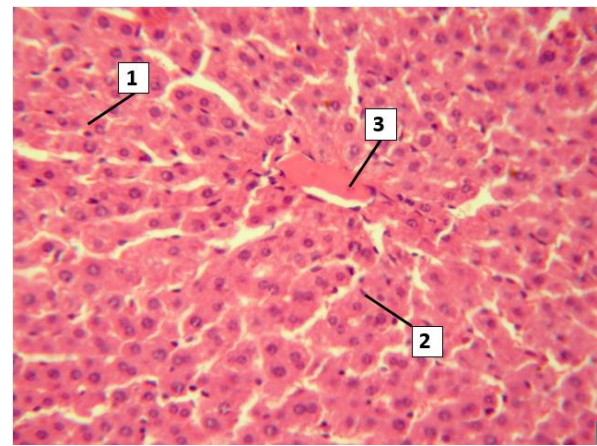


Fig. 1. Structural organization of the liver in the control group animals. Staining: hematoxylin and eosin. Magnification: $\times 400$. 1 – trabeculae, 2 – sinusoidal hemocapillaries, 3 – central vein.

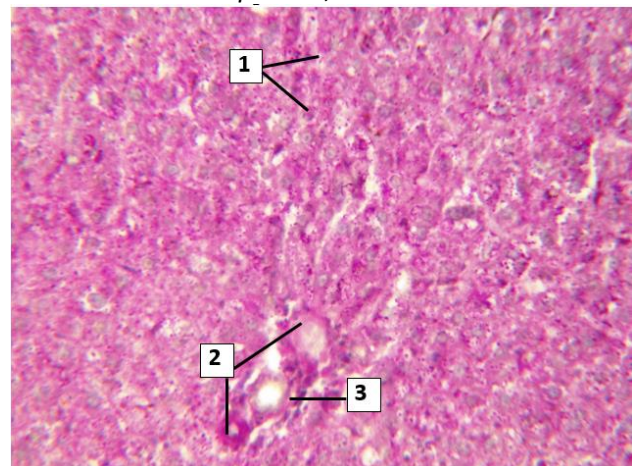


Fig. 2. Glycogen granules in the liver in the control group animals. Staining: according to Shabadash. Magnification: $\times 400$. 1 – granules of glycogen, 2 – vessels of the portal tract, 3 – interlobular bile duct.

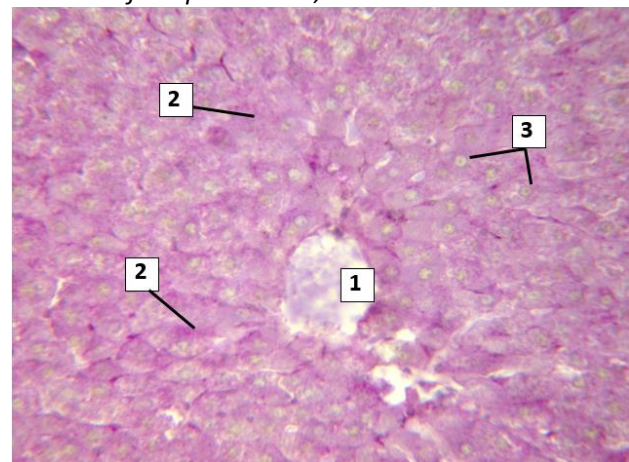


Fig. 3. Liver of rat-male with insulin resistance. Staining: according to Shabadash. Magnification: $\times 400$. 1 – central vein, 2 – solitary glycogen granules in the cytoplasm of the liver cells, 3 – nuclei of hepatocytes. process is low, an index of which is the reduction of the ratio of the nucleolus area to the area of the hepatocyte nucleus. In some hepatic cells, the nuclei are compact, somewhere fragmented, deformed,

Table 1

Morphometric parameters of liver hepatocytes of control (intact animals) and experimental (animals with insulin resistance) groups of animales (M+m)

Parameter	Control group		Experimental group	
	Male	Female	Male	Female
Average perimeter of the hepatocyte, mkm	71.82±5.81	73.45±3.15	92.89±8.26*	82.64±8.75
Average area of the hepatocyte, mkm ²	348.12±2.59	362.04±10.40	574.63±37.61*	446.99±12.45**
Average perimeter of the hepatocyte nucleus, mkm	30.64±2.92	31.97±1.15	40.18±3.49*	35.86±2.16
Average area of the hepatocyte nucleus, mkm ²	56.41±3.77	57.16±3.01	111.75±13.58*	87.63±11.37**
Nuclear-cytoplasmic index	0.16±0.02	0.16±0.02	0.19±0.01	0.20±0.02
Average perimeter of the hepatocyte nucleolus, mkm	9.45±1.05	9.81±1.23	12.22±2.24	10.58±1.13
Average area of the hepatocyte nucleolus, mkm ²	7.87±0.93	7.55±1.04	9.74±1.52	6.98±1.45
Ratio of the nucleolus area to the area of the nucleus of the hepatocyte	0.14±0.01	0.13±0.1	0.09±0.01*	0.08±0.01*

Note: * - reliable difference to control ($p<0.05$); # - reliable difference to data in males within the group ($p<0.05$)

and sometimes they have the form of bent sticks. A cellular stromal reaction is not observed around such degenerative altered hepatocytes (Fig. 4). The compact location of the hepatocytes enlarged in size, is accompanied by the compression of sinusoidal hemocapillaries, their deformation

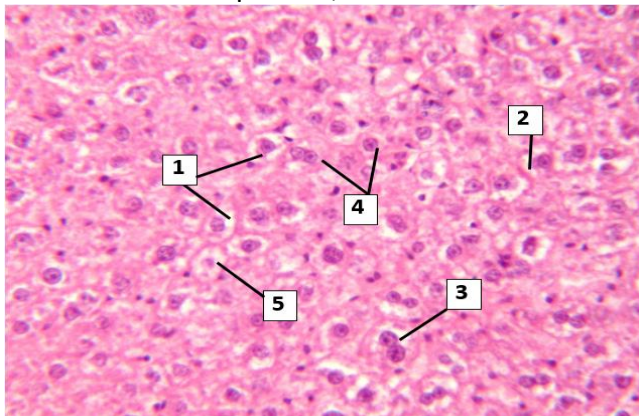


Fig. 4. Liver of rat-male with insulin resistance
Staining: hematoxylin and eosin. Magnification: $\times 400$. 1 – increased in size hepatocytes with light granular cytoplasm, 2 – compressed sinusoidal hemocapillaries, 3 – binuclear hepatocytes, 4 – centrally located nucleoli of rounded shape, 5 – apoptotic altered hepatocytes.

(see Fig. 4). In the lumen of the capillaries there are compactly located erythrocytes, in the single fields of view – with solitary leukocytes. The central veins and vessels of the portal tract are filled with compactly located erythrocytes with preserved tinctorial properties. In portal tracts there are solitary macrophages and lymphocytes, in some cases – insignificantly pronounced infiltration with

non-parenchymal cellular elements with the presence of lymphocytes and macrophages, which do not extend beyond the boundary plate and are not accompanied by the degenerative changes of surrounding liver cells. The wall of the part of the vessels is insignificantly and segmentary thickened and substantially (2.4-fold, $p<0.05$) exceeds the control group data. In these zones, the wall is represented in the form of a homogeneous saturated eosinophilic nuclear-free mass, on the inner side of which there are localized elongated endothelial cells along the perimeter, outwards – solitary lymphocytes, macrophages. Staining using the PAS method indicates the accumulation of glycoproteins in the form of saturated pink nuclear-free masses in the wall. The wall of individual vessels is segmentary unevenly thickened; in the PAS reaction the accumulation of glycoproteins of light-purple color with focus foam is observed, which is a reflection of plasma oozing (Fig. 5).

Co-directed structural changes are also determined in females with IR. At the same time, in females under the conditions of IR, there is a more pronounced zonal character of hepatocyte degenerative processes compared to males, whose dystrophy has a more diffuse nature. A distinctive peculiarity is the presence of cells of fatty dystrophy of hepatocytes. As in males, an increase of the nuclear-cytoplasmic index indicates the intensity of the regenerative process in the cell (Fig. 6).

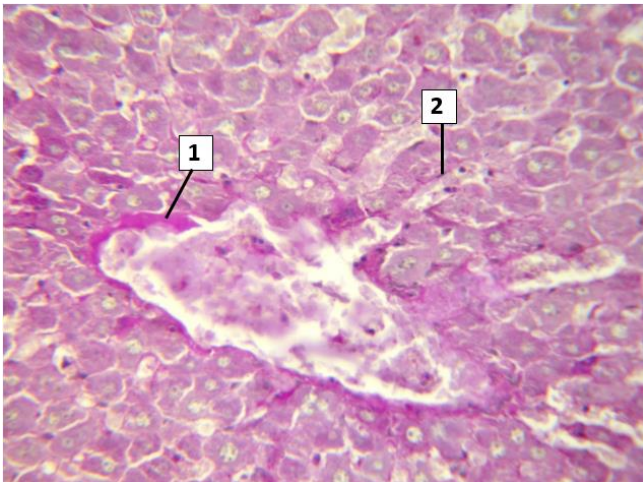


Fig. 5. Liver of rat-male with insulin resistance
Staining: PAS. Magnification: $\times 400$. 1 – plasma
oozing of the central vein wall, 2 – basal membrane of
sinusoidal hemocapillaries.

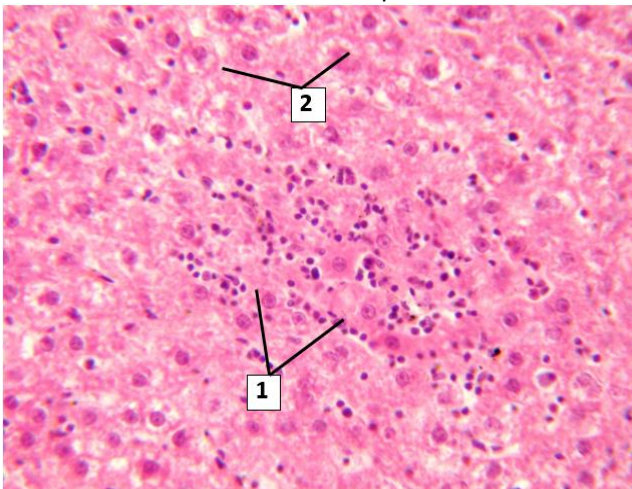


Fig. 6. Liver of rat-female with insulin resistance
Staining: hematoxylin and eosin. Magnification: $\times 400$. 1
– leukocytes in the lumen of sinusoidal hemocapillaries,
2 – dystrophically altered hepatocytes with enlightened
cytoplasm.

Conclusion. In rats with IR, there was revealed
the development of regenerative processes in the

cell, in particular, in the nucleus, which is indicated
by an increase of the nuclear-cytoplasmic index and
the presence of binuclear hepatocytes, but the
intensity of the regenerative process is low
(decrease of the ratio of the nucleolus area to the
area of the hepatocyte nucleus). Sexual dimorphism
is in the presence of foci of the fatty dystrophy of
hepatocytes and the pronounced zonal character of
hepatocyte dystrophic processes in females
compared with males, whose dystrophy is more
diffuse in nature.

Prospects for further research. Study of the
dynamics of the structural changes in hepatocytes
as the IR formation and their reversibility.

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Azadaliyeva S.F.

Odlar Yurdu University, Baku, Azerbaijan khuda1949@mail.ru

THE INFLUENCE OF INITIAL ACIDITY (pH) ON THE PRODUCTION OF SILVER NANOPARTICLES BY *SACCHAROMYCES ELLIPSOIDEUS* BDU – XR1

Abstract. The aim at the presented article was to investigate the influence of initial acidity (pH) on the production of silver nanoparticles by *Saccharomyces ellipsoideus* BDU - XR1 obtained from culture collection of Microbiology Department of Baku State University. The reaction mixture contained 10 g wet biomass of yeast and 10^{-3} mol AgNO_3 solution was separately incubated in the acidity of pH 4.0; pH 5.0; pH 6.0; pH 7.0 and pH 8.0 at 30°C for 7 days. Spectrophotometric analyses of the samples showed that the incubated samples at pH 6.0, 7.0 and 8.0 have a absorption (peak), in the 408 to 412 nm wavelengths characteristic for silver nanoparticles. So the synthesis of silver nanoparticles by this strain occurs in the range of pH 6.0 – 8.0 and the optimum initial acidity was pH 7.0. Synthesis of silver nanoparticles was not observed when pH was 4.0 and 5.0.

Key words: yeast, *Saccharomyces ellipsoideus* BDU – XR1, initial acidity, silver nanoparticles, UV spectrum.

Introduction. Synthesis of nanoparticles is one of the fastest developing areas of nanotechnology. Unlike large-size materials, these particles have specific biological properties that are characterized by physical, chemical, magnetic, thermal, optical, and quantum sizes. Nanoparticles are widely used in medical diagnostics and treatment, as carriers of pharmaceuticals, cosmetics, dyestuffs, food products, packaging products, transportation of products, oil production, agriculture and, finally the environment. In the most developed countries of the world, large-scale synthesis of metal nanoparticles is carried out using physical, chemical and biological methods [4, 8, 10].

Recently, special attention is paid to the use of biological methods for the production of nanoparticles. Based on the biological synthesis process of nanoparticles, three basic steps are to use medium-sized solvents for synthesis, select ecologically harmless agents and select non-toxic materials for stabilizing nanoparticles. Many synthetic methods depend on organic solvents, which also result in the hydrophobicity of the used agents. So biological methods (using microorganisms and plants) for the synthesis of nanoparticles have some advantage compared with physicochemical methods. gives an opportunity to increase the biomass and size of the formed nanoparticles. Particular attention is paid to the application of yeast and mould fungi and bacteria in this process [6, 9, 11].

It was possible to synthesize metal nanoparticles, such as silver, gold, zinc, selenium, titanium and platinum, using the yeast [1, 2, 8,

13]. Silver nanoparticles attract more attention due to their large surface area, unique physico-chemical and biological properties. As a result of large-scale researches carried by scientists, it was possible to obtain various metal nanoparticles from yeast of *Saccharomyces* [5, 7, 12].

In our previous studies, the property of *Saccharomyces ellipsoideus* BSU – XR1 to form silver nanoparticles have been determined. The influence of incubation time, temperature and biomass quantity on the formation of silver nanoparticles have been revealed [3].

Objective: The main aim at the presented work was to investigate the influence of initial acidity (pH) on the formation of silver nanoparticles by *Saccharomyces ellipsoideus* BDU – XR1.

Materials and Methods. As a research object, was used *Saccharomyces ellipsoideus* BDU - XR1 obtained from the culture collection of Microbiology Department of Baku State University.

Saccharomyces ellipsoideus BDU – XR1 was cultivated in nutrient medium having the following content: yeast extract – 10 g, sucrose – 20 g, pepton - 20 g, distilled water – 1 liter. The culture was cultivated for 48 hours at 30°C in thermostat. The obtained yeast biomass was separated by filtration from yeast culture and washed 3 times with 100 ml sterile distilled water. Adding 10 gram wet biomass into 90 ml sterile distilled water was prepared the suspension, then added 10 ml 10^{-3} molar AgNO_3 solution and was incubated in dark medium for 7 days at a temperature of 30°C in the acidity of pH 4.0; 5.0; 6.0 and 7.0.

The formation of silver nanoparticles was primarily observed by the change of the reaction mixture color from light yellow to dark brown. Under the same conditions, no color change was observed in the incubated control tube (no AgNO_3 solution added).

Then the biomass was separated by filtration and spectrophotometric analysis of these samples was performed on the spectrophotometry "UV - Vis SPECORD 250 plus" (Germany).

Results. The formation of silver nanoparticles by *Saccharomyces ellipsoideus* BDU – XR1 initial acidity were studied depending on the (pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0). It was determined that when silver nanoparticles assembled in the medium it darkened the reaction mixture. This phenomenon is considered to be the primary indication of the existence of silver nanoparticles. From the 3rd day of the incubation, noticeable variation in the reaction mixture has begun (Fig.1). Then spectrophotometric analyses were



Fig.1. The color darkening of the reaction mixture - of *Saccharomyces ellipsoideus* BDU - XR1. A – control; B – experiment

carried out taking samples from these reaction mixtures. The absorption spectra were observed in the 408-412 nm wavelengths on the UV-VIS spectrophotometry of samples taken from the reaction mixtures pH 6.0; 7.0 and 8.0. Based on spectrophotometric analyses of the samples taken from the variants of the poor color change pH 4.0 and 5.0 the absorption has not been observed (Fig. 2).

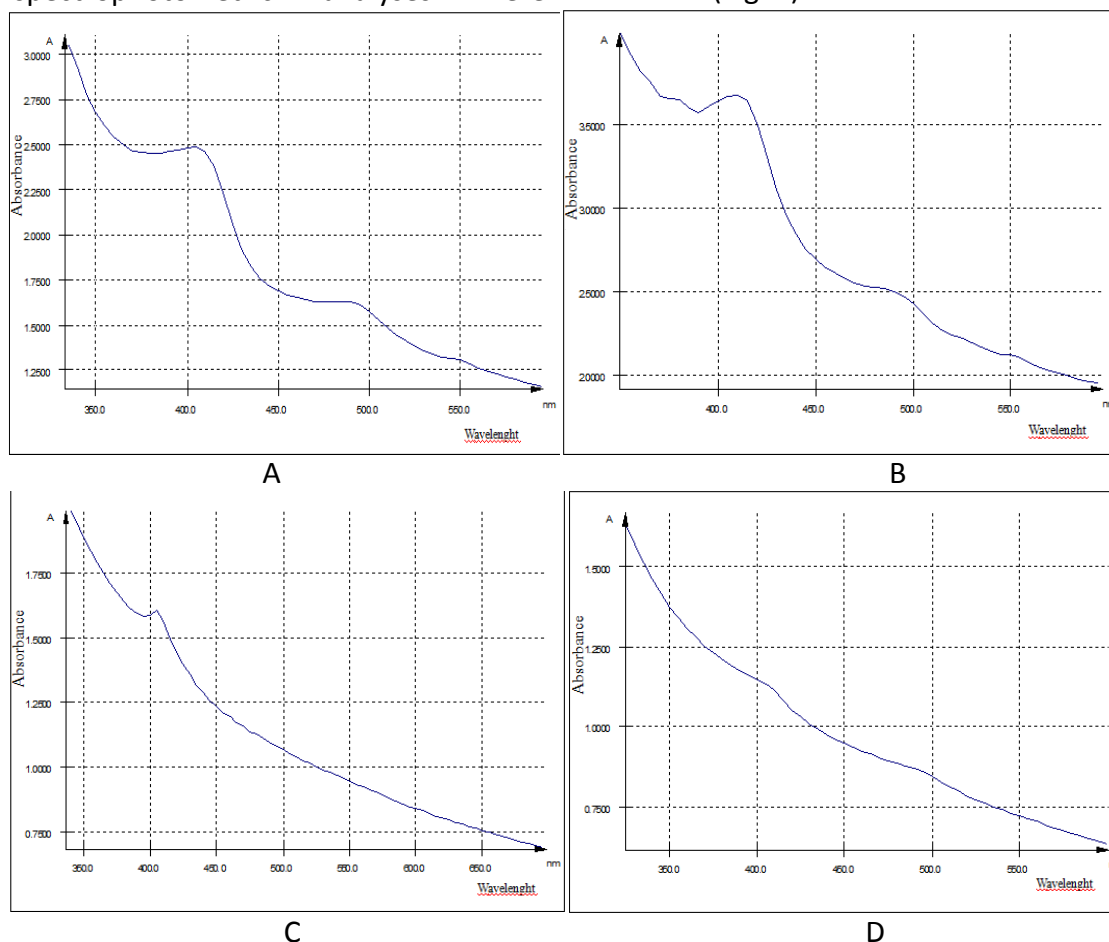


Fig.2. The UV – spectrum of silver nanoparticles depending on various ambient acidity of the *Saccharomyces ellipsoideus* BDU – XR1. pH 6,0 (A); pH 7,0 (B); pH 8,0 (C); pH 5,0 (D);

Discussion. The presence of the above data shows that the synthesis of silver nanoparticles by *Saccharomyces ellipsoideus* BDU – XR1 occurs in the range of acidity pH 6.0 – 8.0 and optimum pH is 7.0. At the same time maximum absorbance of UV-VIS

spectra was 412nm. These data are the same with results in *Saccharomyces boulardii* [14]. However, UV-VIS spectra absorbance of silver nanoparticles, produced by other strains of *Saccharomyces* were different. Silver nanoparticles produced by

Saccharomyces cerevisiae shown absorbance in 430 nm [15] by *Sacch. Cerevisiae* – in 450 nm [16] by commercial bakers yeast - in 413 – 455 nm [17]. These differences perhaps related with formation of clusters of silver nanoparticles.

Conclusion. Thus, the influence of the acidity on the formation of silver nanoparticles by *Saccharomyces ellipsoideus* BDU – XR1 were investigated. It has been discovered that the synthesis of silver nanoparticles occurs at pH 6.0 – 8.0. The optimum initial acidity (pH) was pH 7.0. Synthesis of silver nanoparticles was not observed when the pH was 4.0 and 5.0.

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