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THE ROLE OF AUTOPHAGY PROCESSES IN MAINTAINING VITAL ACTIVITY OF TUMOUR CELLS UNDER CONDITIONS OF STRESS ON THE PATTERN OF MELANOMA CELL LINE

Abstract. Due to autophagy cells are able to supply the lack of nutrients and energy under stressful conditions and return to normal vital activity. Autophagy does not only promote enhancing of vital activity of cells but their death as well. A transition moment between accumulation of the reserve abilities of cells with the purpose to preserve vital functioning and the onset of autophagic type of cellular death still remains unknown. The knowledge of autophagy processes is especially important to understand pathogenesis of oncological diseases. The study was conducted on 793 pattern of melanoma cell line cultivated under stressful conditions simulated by changing culture environment into decreased level of nutrients in different terms of time. Autophagy processes were investigated in our study with addition of rapamycin (sirolimus) and chloroquine, and the role of EGFR in maintaining vital activity of cells. A therapeutic effect of target EGFR on 793 modeled cell line of melanoma was proved.

Key words: autophagy, apoptosis, EGFR, rapamycin (sirolimus), chloroquine, melanoma.

Introduction. Autophagy is a process when internal components of the cell are transmitted inside of its lysosomes or vacuoles and experience degradation in them [1]. The role of autophagy in maintaining vital activity of cells and activation of their death under stressful conditions have been unknown for a long time, and only in 2016 the Japanese researcher Yoshinori Ohsumi was awarded the Nobel Prize in physiology and medicine due to discovery of the main regularities of this process.

The main stimuli promoting occurrence of autophagy processes in cells can be the following: lack of nutrients, availability of damaged organelles, partially denatured proteins or their aggregates in the cytoplasm. Autophagy can be also induced by oxidative or toxic stress. In general, due to autophagy cells are able to supply the lack of nutrients and energy under stressful conditions and return to normal vital functioning [2].

There are three types of autophagy: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy and chaperone-mediated

autophagy are activated only under stressful conditions. Therefore, they attract the most attention for further detailed investigations. In case of macroautophagy the cytoplasm containing any organelles is surrounded by the membrane compartment similar to the cistern of the endoplasmic network. As a result, this part is separated from the rest of the cytoplasm by two membranes. Such structures with double layer membranes are called autophagosomes. Autophagosomes are combined with lysosomes forming autophagolysosomes, in which organelles and the rest of the content of autophagosomes are digested [3].

Chaperone-mediated autophagy is characterized by a directed transport of partially denatured proteins from the cytoplasm through the lysosome membrane into its cavity where they are digested. This process occurs with participation of cytoplasmic proteins-chaperones of hsc-70 family, auxiliary proteins and LAMP-2, serving as a membranous receptor of the chaperones-protein complex awaiting their transport into the lysosome.

Autophagy does not only promote enhancing

of vital activity of cells but their death as well. In case of autophagic type of cellular death all the organelles in the cell are digested, and remaining cellular debris is completely absorbed by macrophages [4]. A transition moment between accumulation of the reserve abilities of cells with the purpose to preserve vital functioning and the onset of autophagic type of cellular death still remains unknown.

The knowledge of autophagy processes is especially important to understand pathogenesis of oncological diseases, as from one side it is the method of survival of cells, and from another side the method of activation of cellular death, which in a number of cases give a perspective of sensitization of tumour cells [5].

With this purpose many scientists deal with investigations of activators and inhibitors of autophagy. The mechanism of autophagy action (rapamycin, tamoxifen, perifosine, erlotinib, etc.) is mainly based on inhibition of mTOR-signal way or increased expression of *Atg* autophagy genes, while inhibitors (bafilomycin A, chloroquine) prevent fusion of autophagosomes with lysosome [6, 7]. EGFR blocking is used in practical medicine with the aim to treat and prevent relapses of oncological diseases, although the signs of autophagy processes in the course of treatment of oncological patients still remain unknown [8].

Objective. To study the role of autophagy in maintaining vital activity of cells under stressful conditions and with the use of antibodies to EGF on the pattern of melanoma cell line.

Materials and methods. The experiment was conducted on 793 melanoma cell line obtained from ATCC (Great Britain). The cells were kept at the temperature -80°C . The cells were reproduced on the water bath at the temperature $+37^{\circ}\text{C}$ followed by centrifugation during 5 minutes at $1000 \times g$ (Heraeus Biofuge). The received supernatant was isolated and added to 1 ml of cellular suspension in 4 ml of culture medium RPMI 1640 in the flask for cultivation (JET BIOFIL TCF-012-050). The work with cells was performed in safety cabinet (Steril-VBH). The cells were calculated by means of the microscope Leica (within the norm no less than 3×10^5 cells / ml). Unfrozen cells were incubated in CO_2 incubator at the temperature of $+37^{\circ}\text{C}$ (5 % CO_2 , Heraeus).

72 hours later RPMI 1640 was changed for isolation of cryopreservative. 7 days after changing the culture medium the cells were distributed into 4 groups. At the beginning of the

experiment every group of 793 melanoma cell line grew in the culture medium RPMI 1640 with addition of 10 % FBS (fetal bovine serum), 2 mM L-glutamine and 1% Pen-Strep (penicillin-streptocid) (Sigma-Aldrich, USA). In the first group of cell stressful conditions were simulated on the 4, 8, 12 and 24 hour, reproducing starvation conditions by means of changing culture medium into the medium with low content of nutrients (RPMI 1640 with addition of 0,1 % FBS, 2 mM L-glutamine and 1 % Pen-Strep). The second group of cells was cultivated in saturated culture medium RPMI 1640 with addition of 10 % FBS, 2 mM L-glutamine, 1 % Pen-Strep, which was changed on the 4th hour with addition of 10 nM rapamycin, on the 8th hour – with addition of 50 nM rapamycin, on the 12th hour – with addition of 75 nM rapamycin, and on the 24th hour – with addition of 100 nM rapamycin (Sigma-Aldrich, USA). In the third group of cells stressful conditions were simulated on 4, 8, 12 and 24 hour, reproducing starvation conditions by means of changing culture medium into the medium with reduced content of nutrients and 10 nM chloroquine (Sigma-Aldrich, USA). In the fourth group of cells stressful conditions were simulated on 4, 8, 12 and 24 hour, reproducing starvation conditions by means of changing culture medium into the medium with reduced content of nutrients and antibodies to EGF in the concentration $0,2 \mu\text{g}/\text{ml}$ (Sigma-Aldrich, USA). All the groups of cells were incubated in CO_2 incubator at the temperature $+37^{\circ}\text{C}$ (5 % CO_2 , Heraeus).

To assess the vital functioning of the examined groups of cells colorimetric test was made with tetrazolium staining agent 3-(4,5-dimethylthiazole-2-il)-2,5-diphenyl-tetrazolium bromide (MTT-test) according to the standard procedure (Sigma-Aldrich, Saint Louis, MO, USA) [9]. Colour intensity (optic density) was measured at 530 nm by means of microplanchette (Infinite F50, TECAN). The experiment was conducted three times.

The results were statistically processed by means of dispersive and correlation analysis using the standard functions of the package MS Excell 2010 (Microsoft Inc., USA). Criterion values and the main calculations were made by means of the specialized software ANOVA (Stat View 4.0 software, Abacus Concepts, Berkeley, CA, USA). As the criterion of difference credibility of the indices was significance level $P < 0,05$.

Results and discussion. Proliferation of cells of 793 melanoma cell line was determined by means of MTT-test by the colour intensity level. At the beginning of the experiment the vital activity of cells was similar in every group of 793 melanoma cell line and was accepted as 100 % (Table 1, Figure 1).

In the control group under stressful conditions reproduced by means of changing culture medium with decreased level of nutrients in different period of time, the vital activity of cells decreases gradually, although at the end of the experiment

it was higher as compared to other groups ($P < 0,05$). Thus, on the 4th hour of starvation the vital activity of cells decreased by 10 %, on the 8th hour – by 20 %, on the 12th hour – 25 %, and on the 24th hour – by 28 % in comparison with the beginning of the experiment ($P < 0,05$). Although cellular proliferation rate in the control group began to decrease on the 12th hour of starvation, and it was the lowest on the 24th hour. At this term the vital activity of cells became only 3% lower in comparison with the same value on the 12th hour. The highest decrease of cellular proliferation rate

Table 1.

Assessment of the vital activity of melanoma cell line against the ground of induction and inhibition of autophagy processes under stressful conditions in 4, 8, 12 and 24 hours since the beginning of cultivation ($M \pm m$, %).

	Groups	Beginning of the experiment	Stressful conditions (starvation)			
			4 hours	8 hours	12 hours	24 hours
1.	Control	100±0,23	90,99±0,27	79,78±0,11	75,97±0,16	72,87±0,21
2.	Cells with rapamycin	100±0,33	66±0,19	62±0,17	56±0,1	54±0,22
		(0 nM)	(10 nM)	(50 nM)	(75 nM)	(100nM)
3.	Cells with chloroquine	100±0,12	64,29±0,22	57,89±0,25	40,81±0,32	54±0,21
4.	Cells with anti-EGF	94,47±0,15	81,12±0,14	78,38±0,23	75,96±0,27	64,88±0,31

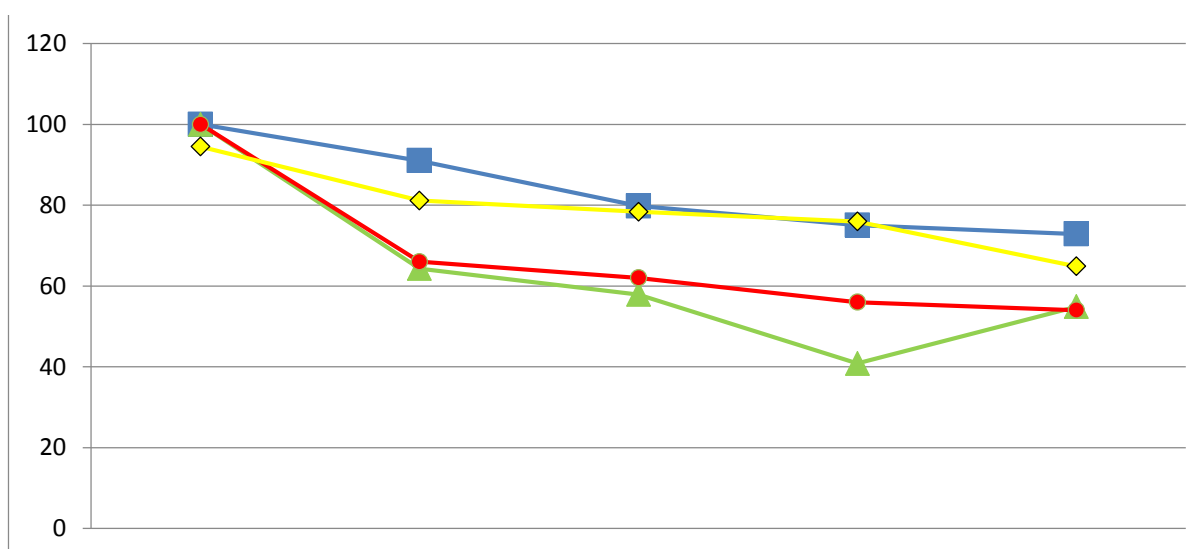


Fig.1. Vital activity of melanoma cell line against the ground of activation and inhibition of autophagy processes under conditions of stress in 4, 8, 12 and 24 hours since the beginning of cultivation ($P < 0, 05$). Signs: ■ control; ● cells with rapamycin (10, 50, 75, 100 nM); ▲ cells with chloroquine; ◆ cells with antibodies anti-EGF

occurred in the period from 4 to 8 hour of starvation, while the vital activity of cells became 10% lower. Therefore, against the ground of starvation beginning with the 12th hour cells begin to adapt to stressful conditions. Dephosphorilation of mTOR-signal way probably occurs exactly at this period inducing increased

vital activity of cells by means of autophagy activation. Although, at the beginning of occurring stressful conditions intensive destruction of cells is observed which is indicative of activation of autophagic death of cells or apoptosis, preceding the increase of general vital activity of cells. Nevertheless, molecular mechanisms of this

transmission remain unknown and require further investigations.

Against the ground of additional administration of rapamycin as an autophagy activator in different concentrations (10, 50, 75 and 100 nM) to culture medium without simulating stressful conditions the vital activity of cells reduced considerably as well as compared to the experiment. Thus, addition of rapamycin in the concentration of 10 nM promoted decreased cell proliferation as much as 34%, in the concentration of 50 nM –38 % less, in the concentration of 75 nM – 44 % less, and in the concentration of 100 nM –46 % less as compared to the beginning of the experiment ($P < 0,05$). Therefore, the lowest vital activity of cells was found with the concentration of rapamycin in the dose of 10 nM, where cell proliferation was in 1,5 times less than in the group of cells without administration of rapamycin ($P < 0,05$). The highest decrease of the vital activity of cells was found with the concentration of rapamycin of 100 nM, where cell proliferation was by 1,9 times lower than in the group of cells without rapamycin ($P < 0,05$). Therefore, a conclusion can be drawn that the more intensive dephosphorilation of mTOR-signal way occurs, the more intensive activation of autophagic type of cell death is. Although in case the block of this signal way is not considerable, the rate of proliferative properties decreases less. Thus, the degree of activation of autophagy depends on the intensity and time of block of mTOR-signal way. Probably at the beginning of starvation death of cells occurs, since dephosphorilation of mTOR-signal way is not sufficient and autophagy processes fail to activate reserve abilities of cells to maintain their vital activity. In case dephosphorilation of mTOR-signal way is appropriate, autophagy is activated promoting maintenance of the vital activity of cells. Although, the more intensive dephosphorilation of mTOR-signal way occurs, the more probable autophagic death of cells is. Probably at the beginning of stressful conditions the processes of cell apoptosis are activated followed by autophagic inhibition directed to the increase of the vital activity of cells. And as far as all possible resources are exhausted autophagic death of cells occurs. Therefore, activation of apoptosis with the purpose to treat oncological diseases can promote increased cell resistance, and then this suggestion requires more detailed investigation.

When chloroquine, as autophagy inhibitor, is added with simulation of stressful conditions on the 4, 8, 12 and 24th hour a considerable decrease of the vital activity of cells occurs as compared to the cells from the control group cultivated under the similar stressful conditions ($P < 0,05$). On the 12th hour of starvation in the group of cells with chloroquine the lowest index of cell proliferation is found (40,81 %), which was in 1,86 times less than in the control group with simulation of stressful conditions at the same time ($P < 0,05$). Thus, undoubtedly autophagy increases the vital activity of cells, and the highest activity of this process occurs on the 12th hour of starvation. It is also evidenced by decreased intensity of vital activity loss exactly on the 12th hour of starvation in the control group.

The use of antibodies to EGF, which in the norm should activate EGFR and, therefore, stimulate phosphorylation of mTOR-signal way causing intensive growth of cells, and considerable inhibition effect on cell proliferation in comparison with the control group was not provoked ($P < 0,05$). In case of dephosphorilation of mTOR-signal way by means of antibodies to EGF considerable decrease of the vital activity of cells was found on the 4th hour in 1,12 times in comparison with the control group under the similar stressful conditions ($P < 0,05$). Thus, when EGFR stimulation is absent proliferation of melanoma cells decreases, and stressful conditions on the 4th hour do not cause activation of autophagy. Although on the 8th and 12th hour of cell starvation there was no difference in cell proliferation in both groups found. On the 24th hour of simulation of stressful conditions the vital activity of cells was lower than in the control group by 1,12 times ($P < 0,05$). Thus, with EGFR block against the ground of stress on the 8th hour other signal ways are involved which are directed to autophagy activation and maintenance of the vital activity of cells. Review of literary sources gives the ground to predict that a central role in this process belongs to JNK-signal way (c-jun N-terminal kinase). Different stress factors are able to activate JNK by means of conformation disorders of sensitive phosphatases which in the norm inhibit this signal way and proteins activating it. In its turn, JNK activation promotes phosphorylation of Beclin 1 protein which is a trigger of autophagy. JNK can work as promoters of cell apoptosis. And JNK role in activation of apoptosis depends on the type of cells and kind of

stimulus. The influence of JNK activation on apoptosis was suggested to depend on the activity of other signal ways, for example, ERK or NFkB-mediated, which enables to suggest that JNK activation facilitate but not initiate apoptosis process [10, 11, 12]. Although, decrease of the vital activity of cells with the use of antibodies to EGF on the 24th hour of starvation of cells gives the evidence of therapeutic effect of the target EGFR therapy for the patients with melanoma, and transmission of JNK from autophagy to the maintenance of apoptosis in case of more prolonged effect of stressful conditions.

Conclusions: Increase of the vital activity of cells of 793 melanoma cell line under stressful conditions occurs on the 12th hour of starvation.

1. With the concentration of 10 nM of rapamycin proliferation of 793 melanoma cell line decreases in 1,5 times as compared to the cells without administration of rapamycin ($P < 0,05$), which is the lowest index of decreased cell proliferation when rapamycin is administered. With 100 nM of rapamycin proliferation of 793 melanoma cell line decreases in 1,9 times in comparison with cells without administration of rapamycin ($P < 0,05$), which is the highest index of decreased cell proliferation when rapamycin is used.

2. On the 12th hour of starvation in the group of 793 melanoma cell line with the concentration of 10 nM of chloroquine the highest decrease of cell proliferation occurs (40,81 % less), which is in 1,86 times less than in the group of cells with simulation of stressful conditions at the same time ($P < 0,05$). The highest inhibition of autophagy processes on the pattern of 793 melanoma cell line occurs on the 12th hour of simulation of stressful conditions.

3. On the 24th hour of simulation of stressful conditions with the use of antibodies to EGF the vital activity of 793 melanoma cell line is lower than that in the control group in 1,12 times ($P < 0,05$). Therapeutic effect of target EGFR therapy of 793 melanoma cell line under stressful conditions is found on the 24th hour.

Perspectives of further studies. To investigate the expression of autophagy markers in comparison with expression of proteins of EGFR – signal way under stressful conditions with the purpose to find objects for target therapy of melanoma directed to inhibition of autophagy processes is rather perspective. Examination of

trigger mechanisms of autophagy activation and transition to autophagic cell death could supplement the knowledge concerning pathogenesis of oncological diseases and promote the development of new approaches of personalized medicine to the treatment of oncological patients.

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SURGERY OF CORDIAL DAMAGES

Abstract. *The experience of the clinic staff of the Institute of General and urgent surgery. T. V. Zaitsev NAMS of Ukraine concerning surgical treatment of various heart damage, including mine-explosive gunshot and bullet wounds. The analysis of the highly-specialized surgical care with the use of modern technology. Experience of providing surgical care to victims with injuries of the heart. The data relating to the substantive provisions about the anatomical background of various wounds and dependence clinical manifestations, diagnosis and treatment of patients. Discussed generally accepted statements, the classification of these injuries, the optimal algorithms of diagnostic and therapeutic measures. Summarizes the main provisions of the surgical doctrines in the provision of medical care in surgical hospitals General profile and the possibility of its improvement in the treatment of patients in specialized clinics. Special attention is given to surgical tactics aimed at timely and effective damage detection of intracardiac structures, restoring the integrity of the vital vessels and tissue, including the use of technologies of artificial blood circulation. Described surgical technique depending on the localization and extent of the damage. Recommendations for intensive care and anesthesia support at all stages of treatment of victims with injuries of the heart. The article discussed data on the use endocardial assisted shunting and circulation in typical damage to intracardiac structures; videoarticles and angiographic minimally invasive surgical procedures.*

Key words: *mine-explosive and gunshot wounds ball heart, diagnosis, surgical tactics.*

Introduction. Nowadays, one of the greatest dangers of human life are mine-explosive and bullet heart damage. Among other damages the heart damage is characterized by high risk of the deaths (8,3-40,0 %) but , at the same time, there is a high rehabilitation opportunity and patients preservation of efficiency who survived due to the qualified medical aid. The success of the surgical treatment of those who have heart wounds depends on the chosen tactic which remains the subject of discussions.

Materials and methods. In the clinic of the department " Institute of General and Emergency surgery named after V.T. Zaitseva NAMS of Ukraine over 410 victims with heart injuries have been treated, among which 307 were diagnosed with penetrating injures, 74 - mine-explosive and bullet injuries, 34 – inner heart structures damage. We have reviewed clinics perennial experience in surgical treatment of patients with different heart lesions. The main group was 276 victims per period of 2001-2015 with application of modern innovations and an observation group – 134 injured who treated at the institution's clinic in 1969-2000.

The diagnoses were established objectively using radiological, electrocardiographic,

ultrasound, echocardiographic and phonoscopic methods of computer tomography. The additional diagnostic information was obtained in the research of acid-base parameters, coagulation, serum electrolytes, hemoglobin and hematocrit. The statistical processing of researching results was carried out of using a standard application package such as R, Microsoft Excel 2007 and STATISTICA 6.0. All data were processed by the method of statistics variation using student's criterion. There were used correlation, many factoring system and regression analysis for choosing the most informative indicators.

Results and discussion. It should be distinguished three main mechanisms of hemodynamic disorders with heart damage: acute blood loss, hypovolemia, tamponade of the heart and disturbance of the heart's pumping function which are caused by the damage of the myocardium, coronary arteries, valves, interstices and leading paths. Attached to the heart wounds the typical for cardiograms is the teeth reduction in the voltage, displacement of the interval S-T above the isolation, signs of myocardial infarction.(table 1)

Clinically cardiac injuries are diagnosed and based on Beck's triad:

Table 1

Frequency of electrocardiographic changes registration in patients suffering from mine-explosive heart damage (%)

Indicator	Comparison group	Main group
Change of the T teeth	56 (75,7)	40 (74,0)
Heart rhythm disturbance	51 (68,9)	33 (61,1)
Deflection of the electric heart axis	34 (45,9)	16 (29,6)*
Change of the P teeth	26 (35,1)	9 (16,7)**
Change of the segment	26 (35,1)	8 (14,8)**
Conduction disturbance	7 (9,5)	3 (5,6)

Notes: * - $P < 0,05$, ** - $P < 0,01$ compared with those who were not heart injured

- an abrupt drop of blood pressure
- rapid and significant increase of central venous pressure
- an abrupt relaxation of heart tones and – absence of heart pulsation during the X-ray examination

A valuable diagnostic and, in some cases, therapeutic event for the detection and evacuation of blood from the pericardium is its puncture. In recent years, we use the needle Veresha as a less traumatic and thoracic port. Also, endovideotoracoscopy is performed and during which the localization of heart wounds is determined, pericardium integrity, the presence of blood in the chest cavity and a warm shirt, hematomas of the heart and pericardium; pericardioscopy as a diagnostic and therapeutic measure and subxyphoid pericardiotomy. In the clinic, an algorithm for diagnostic manipulations was created in case of wounded heart suspicion.

According to the clinic, the informative of heart damage symptoms was: deafness of the heart tones – 97,0%; the pain in the area of the heart – 25,0%; systolic noise at the top – 8,3%; pericardial friction noise – 5,0%; tachycardia ($HS > 120 \text{ min}^{-1}$) – 15,0%; reduction of systolic pressure ($< 60 \text{ mm hg.art.}$) – 46,0%; lowering pulse pressure ($< 15 \text{ mm hg.art.}$) – 43,0%. In case of the damage suspicion between the atrial and interventricular membrane it is possible to use a simplified verification of blood leakage through the traumatic defect. To do this, you should perform a blood sample from the right atrium (the using of the central vein) and make a puncture of the pulmonary artery (when there is no arterial access) and an aorta with subsequent blood transfusion and determination of the blood saturation with oxygen (the using of the gas analyzer).

$Q_p:Q_s = (\text{Sat(aorta)} - \text{Sat(SVC)}) / (\text{Sat (vena pulmonalis)} - \text{Sat (arteria pulmonalis)})$, where Q_p – pulmonary circulation; Q_s – systemic circulation;

Sat aorta – arterial saturation (necessary puncture of the aorta or using of the blood collection from the peripheral arterial line); Sat (SVC) – mixed venous carbonation (the using of the blood transfusion from the central venous catheter or right anesthetic puncture); Sat (vena pulmonalis) – lung vein saturation (usually taken in 100%); Sat (arteria pulmonalis) – saturation in the lung artery (necessary execution of the pulmonary artery puncture with subsequent blood transfusion).

This equation can be used to determine the relative flow of the blood between the body and the lungs. In patients without damage between the ventricular and periaapinal membranes this ratio will be equal to 1 (that means that the blood flow in the lungs and in the body is the same). In patients who have inter-ventricular or periaapinal membrane damage the blood flow in the lungs will be higher than in the aorta and this ratio will be higher than 1. This is a reliable definition of the shunt presence in the membranes of the heart.

The realization of this research allows surgeon perform a diagnosis after sewing of the heart wound, in case of the interventricular or periaapinal membrane suspicion of the damage and determine the degree of the membrane damage severity already on the operating table. When the ratio $Q_p: Q_s$ is in range from 1 to 2 there are moderate defects of the membrane that require cardio surgeon consultation in the next day. When this number is 2 or more these are major defects and the definition of further treatment tactics should be carried out immediately.

After analyzing the obtained data it is possible to suspect and sometimes completely diagnose intracardiac damage, which allows to solve the issue of further surgical-organizational tactics intraoperatively and urgently. During the surgical intervention, all victims with heart wound was advised to use transtreatment ultrasound

examination of the heart chambers. In some cases, angiographic examination of heart cells by known techniques helps to verify the diagnosis.

Modern technologies are widely used in the clinic and the active surgical tactic allows to avoid diagnostic errors and choose the most correct type of surgical intervention. The most important component of surgical care when there are heart injuries is reinfusion of blood that significantly reduces the volume of the hemotransfusion. In clinics there are many reliable and easy in exploitation different systems for blood reinfusion, the question of the device choice is determined mainly by the cost and availability of consumables, as well as the clinics priorities.

In the clinic, an access to the damaged interventricular membrane is proposed through the recanalized entrance channel in the myocardium of the ventricle under artificial blood circulation.

A portable system of extracorporeal circulation Cardiohelp (Maquet, USA) which, firstly, was designed for carrying out of an emergency room during transportation and in extraordinary situations deserves particular attention. The benefits of this system for treating heart injuries

are: the possibility of rapid cannulation of the femoral vessels by Seldinger which gives an opportunity to use system even on the reception or by the brigades of an ambulance; ease of work, lack of special infrastructure, low risk of air embolism; a variety of regimes depending on the clinical situation with the possibility of circulatory support up to 7 l/min; continued circulatory support up to 14 days at one oxygenator.

To take into account achieved results, the use of an emergency room in urgent cardiac surgery of heart injuries has a great prospects. Modern systems for extracorporeal circulation are safe, reliable, easy to use and must become an integral part of the technological arsenal of general surgical clinics dealing with the provision of an emergency care for injuries. Using an emergency room while there are heart injuries allows to rapid stabilization of hemodynamics, complete full correction of intracardiac lesions and, if it's necessary, transporting a patient to a specialized center.

In the table 2 below was not taken into account the experience of treating 74 troops and civilians who came from the ATO zone because the conditions for their delivery to medical

Table 2

Characteristic of victim groups

Indicator	Comparison group (n=134)	Main group (n=276)
Time from heart injury, hour	2 ± 0,28	2,78 ± 0,32
Time before operation, hour	0,42 ± 0,02	0,31 ± 0,01*
The size of the heart wound, centimeters	1,42 ± 0,18	1,48 ± 0,23
Arterial pressure on the upper limbs, mm.hr.st.		79,73 ± 4,35
Systolic	82,20 ± 3,37	41,02 ± 3,52
diastolic	42,60 ± 3,00	
Heart rate, min ⁻¹	102,18 ± 2,23	116,12 ± 3,2*
Central venous pressure, mm.hr.st.	126,31 ± 8,51	121,24 ± 9,57
Blood in the pleural cavity, ml	845,44 ± 55,32	753,68 ± 70,02
Blood in the pericardium, ml	251 ± 15,86	240,72 ± 18,56
Hemoglobin, g/l	105,14 ± 2,73	107,15 ± 3,29
Bed day, days	18,09 ± 1,23	14,34 ± 1,32*

Notes: *— $P < 0,05$ differences with the comparison group

institutions and the distribution to specific hospitals did not coincide with the average sample of patients in Kharkiv and Kharkiv region.

The significant differences in central venous pressure between the main group (121,24 ± 9,57) mm.hr.st. and comparison group (126,31 ± 8,51) mm.hr.st. also, the amount of blood in the pleural and pericardial cavities, respectively (753,68 ± 70,02)ml, (240,72 ± 18,56) ml in the main and (845,44 ± 55,32) ml, (251 ± 15,86) ml in the comparison group was not noticed. The heart rate

was reliably higher in the main group (116,12 ± 3,2) min⁻¹ than in the comparison group (102,18 ± 2,23) min⁻¹ that can indicate more severe traumatic injury in the wounded of a main group. The concentration of hemoglobin in the blood with which the injured came was: in the main (107,15 ± 3,29) g/l and in the comparison group (105,14 ± 2,73) g/l, the sizes of heart injuries in the main group (1,48 ± 0,23) centimeters and in the comparison group (1,42 ± 0,18) centimeters did not differ.

A significant decrease in the time that passed from the hospitalization of the victim to the operation in the main group ($0,31 \pm 0,01$ hour) showed the effectiveness of the developed scheme for the organization of diagnostic and therapeutic tactics in the injured with heart injuries. Besides, a weighty decrease in the number of bed days to ($14,34 \pm 1,32$) days attracts attention what indicates an improvement in the quality of both diagnostic and therapeutic measures.

The most important causes of the fatal consequences are late delivery of victim to medical center, tamponade of heart, untimely surgical intervention with intense blood loss, as well as hard thoraco-abdominal damage with heart wounds and organs of the belly cavity. The analysis of fatality gives the clearest idea about the effectiveness in using developed approaches in the treatment of injured with heart injuries. Patients death was observed at different times of stay in a hospital. The statistical importance of the differences was estimated with a help χ^2 (table 3).

With the number of freedom degrees for this table which is equal to one ($v=1$), the probability of differences between the main and control group more than 5%, it can be argued that reduction of mortality in the main group is statistically reliable.

Posttraumatic pericarditis and septic complications were the most frequently observed postoperative complications. Only 3 patients with intracardiac injuries were registered, 20 surgical interventions were performed on the damage of patients intracardiac structure after heart injury (table 4).

Table 3

Statistical importance of the differences by deaths

Indicator	Comparison group		Main group		χ^2
	Abs.	%	Abs.	%	
Surviving	97	72,39	225	81,52	4,15
Death	37	27,61	51	18,48	

Notes: * - $v = 1$, $\alpha = 5\%$

Mortality in this group was 15% (3 patients) which was the most often associated with severe purulent-septic and infectious complications.

Conclusions. Timely delivery of the victim to a surgical clinic; conducting resuscitation measures fully, aimed at eliminating shock manifestations and heart tamponade; urgent surgical intervention for vital signs and effective resuscitation and anesthetic support; sewing

Table 4

Posttraumatic pericarditis

Disturbance	The number of injured	
	Abs.	%
Traumatic defect of atrial fibrillation	1	5
Traumatic defect of interventricular membrane	13	65
Left ventricular aneurysm	1	5
Traumatic valve insufficiency	3	15
Traumatic defect of sinus Valsavi	1	5
Intraventricular conduction disturbance	1	5
In all:	20	100

heart wounds, reliably bleeding stop, relief of the tamponade phenomena and blood loss; intraoperative diagnostics of intracardiac injuries; surgical correction of intracardiac injuries using artificial blood circulation technology; adequate postoperative integrative therapy should be considered as basic principles of surgical care in case of heart damage on the basis of given data.

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PECULIARITIES OF MYOCARDIAL ULTRASTRUCTURE OF RATS AT THE LATE TERMS OF OPIOID INTOXICATION

Abstract. *In recent decades drug addiction in Ukraine has spread very quickly. In 2015 the WHO estimated approximately 400 000 injection drug users in Ukraine. Every year the number of drug addicts in Ukraine becomes 8-9% more. A negative tendency is the fact that 70-75% of drug addicts are young people under 25 years of age. A considerable use of narcotic substances, their increased circulation and spread of drug addiction stipulate the necessity of detailed studies of opioid effect on the human organism, and morphological structure of the internal organs in particular. The majority of conclusions are based on clinical administration of drugs without adequate experimental studies concerning morphological changes, which is intolerable. Therefore, the objective of our study is investigation of opioid effect on the human organism and morphological structure of the internal organs in particular, determine and describe morphological changes in the myocardium of rats and its blood microcirculation on the ultra-structural level under Nalbuphine effect on the 28th and 42nd days of experimental opioid intoxication. The study was conducted on 48 laboratory mature albino male rats with the body weight of 130-200g, aged from 3,5 to 4,5 months. The animals were divided into two groups – experimental and control. The experimental animals were every day intramuscularly injected with the opioid Nalbuphine (nalbuphine hydrochloride) produced by Rusan Pharma with increasing the dose from 25 mg/kg to 35 mg/kg according to the following plan: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg, V week – 30 mg/kg, VI week – 35 mg/kg. The rats from the control group were intramuscularly injected with sodium chloride. Heart samples were used as the material for investigation. The material for morphological examination was taken under control of biochemical blood indices (LPO, superoxide dismutase, catalase, malonic dialdehyde, glutathione peroxidase, glutathione transferase). The method of electron microscopy of samples was applied. The material was examined and photos taken by means of the microscope YEMB-100K (Ukraine) with accelerating potential 75kW and 4000 – 24000 x magnification on the microscope screen. The results are indicative of the fact that against the ground of injection of the opioid analgesic Nalbuphine on the 28th day of the experimental opioid intoxication deep destructive changes of cardiomyocytes similar to those of myocardial infarction are found: destruction of cardiomyocytes, mitochondrial crystallolysis, intercellular swelling, sludge syndrome and cellular detritus available in the vascular lumen. On the 42nd day of the experimental opioid intoxication the signs of decompensation and destruction of cardiomyocytes increase, the perivascular space dilates due to swelling and cellular detritus blocking the vascular lumen, changed erythrocytes and formation of clots. Villous damage of the cardiomyocyte sarcolemma is characteristic for this term of the experiment.*

Key words: *opioid, Nalbuphine, myocardium, blood microcirculation, rat.*

Introduction. In recent decades drug addiction in Ukraine has spread very quickly. In 2015 the WHO estimated approximately 400 000 injection drug users in Ukraine. Every year the number of drug addicts in Ukraine becomes 8-9% more. A negative tendency is the fact that 70-75% of drug addicts are young people under 25 years of age. Gender analysis is indicative of the fact that boys use drugs more often than girls. 73% of drug users are urban residents, although a part of the rural

youth increases gradually. 97% drug addicts tried drugs for the first time at the age of 12-19, and every fifth drug addict is a female [10]. Therefore, a considerable use of narcotic substances, their increased circulation and spread of drug addiction stipulate the necessity of detailed studies of opioid effect on the human organism, and morphological structure of the internal organs in particular.

The organs affected by narcotic substances

microscopically resemble clinical signs peculiar for chronic inflammatory diseases. At the same time internal organs are filled with blood irregularly, the majority of the blood vessels are spasmodic, and blood in them is mostly watery, dark, with single loose clots [7].

Acute poisoning with narcotic substances is characterized by hyperemia of the cerebral tunics and hemorrhages into its matter [4]. Deep destructive changes of nerve cells, their organelles, cytoplasm blooming, formation of vacuoles, and development of microangiopathy are found [1].

In addition to swelling of the drainage glia manifested by perivascular and pericellular swelling microcirculatory disorders in the form of erythrocyte stasis in the capillaries, general venous plethora, and erythrocyte sludge are found. Sometimes hemorrhages involve pia mater [3].

Microscopic examination of the adrenal glands detects areas with lost structure of the glomerular zone, absence of the borders between the cortical zones, and focal delipoidization. Hemodynamic disorders are characterized by plethora, signs of erythrocyte stasis, formation of sludge and clots. In addition, pyknotic dense nuclei of chromaffin and supporting cells, and cytoplasm vacuolization are found [2]. Morphological examination of the kidneys after opioid intoxication detects vascular reactions of the glomeruli in the sub-capsular and juxtacellular areas of the renal cortex in the form of hypercellularity, dilation and overfilling of the capillary loops with erythrocytes, dilation of the mesangial matrix of the renal glomeruli. In addition, thickening of the collagen fibers of the external layer of the glomerular capsule and lymphohistiocytic infiltration of the periglomerular stroma are found [5]. Massive swelling in the lungs is detected by macroscopic examination. Vascular plethora with the signs of stasis and swelling are found on histological specimens. Emphysema areas are found in the lung parenchyma interchanging with atelectasis areas. The groups of swollen alveoli with admixtures of neutrophil leukocytes are found rather frequently. A number of alveolocytes and macrophages were found in these areas which was indicative of availability of small pneumonic foci in the lungs [6].

The liver is characterized by the signs of fatty degeneration and stasis plethora of the portal tracts. Periportal cellular inflammatory infiltration

and dystrophic changes of the liver cells up to focal necrosis were found on histological examinations [7]. The liver cells have dystrophic changes manifested by moderate swelling of the liver cells with granulation in their cytoplasm which is indicative of granular dystrophy of the liver cells. The nuclei of the cells are of different sizes. In many cases small and moderate fatty drops were found located in the center of the lobes which is indicative of fatty degeneration [6]. Nalbuphine intoxication of the liver is characterized by changes of the blood microcirculation in the form of dilation or narrowing of sinusoids, hemostasis with formation of clots and hemorrhages, and lymphohistiocytic infiltration, focal necrosis and increased amount of apoptotic changed cells prevail [8].

Many researchers describe morphological changes of the heart of drug addicts both on the micro- and ultra-structural levels, although pathogenesis of opioid effect on the myocardium and its blood microcirculation, and what morphological changes are caused by opioids still remain unknown. The majority of conclusions are based on clinical administration of drugs without adequate experimental studies concerning morphological changes, which is intolerable.

Objective: to determine and describe morphological changes in the myocardium of rats and its blood microcirculation on the ultra-structural level under Nalbuphine effect on the 28th and 42nd days of experimental opioid intoxication.

Materials and methods. The study was conducted on 48 laboratory mature albino male rats with the body weight of 130-200g, aged from 3,5 to 4,5 months. The animals were carefully selected for the study. Every rat was examined, weighed, and marked. The selected group of animals was kept in a separate cage on standard food in the vivarium. A careful examination prevented to involve the animals with signs of intra-vivarium infection into the groups of control and experiment. The animals were divided into two groups – experimental and control. The experimental animals were every day intramuscularly injected with the opioid Nalbuphine (nalbuphine hydrochloride) produced by Rusan Pharma with increasing the dose from 25 mg/kg to 35 mg/kg according to the following plan: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg, V week –

30 mg/kg, VI week – 35 mg/kg [9]. The rats from the control group were intramuscularly injected with sodium chloride. Heart samples were used as the material for investigation. The material for morphological examination was taken under control of biochemical blood indices (LPO, superoxide dismutase, catalase, malonic dialdehyde, glutathione peroxidase, glutathione transferase). The material for the study was taken every week by means of exsanguination of animals after intraperitoneal injection of sodium thiopental. All the investigations were carried out according to the regulations of the Directive of the European Commission dated November 24, 1986, and were approved by the Bioethical Committee at Danylo Halytskyi Lviv National Medical University, the minutes №3 dated March 16, 2015.

To obtain ultra-thin sections the fragments of the rats' hearts were cut by means of a blade, and placed immediately into a large drop of 2% osmium tetroxide solution on 0,1 M phosphate buffer (pH 7,36) with sucrose. After that the strips of the cardiac tissue 0,8×0,1×0,1cm in size were cut by means of a blade degreased in acetone, and they were quickly placed into another drop of a fixing solution of the same content located on the plate of dental wax lying on an ice slab. Small pieces (tissue blocks) of the cardiac tissue, cubic in shape and 1mm³ in volume were cut from the strips. The tissue blocks were fixed in 2% osmium tetroxide solution on 0,1 M phosphate buffer (pH 7,36) with sucrose addition during 2 hours. After that they were washed by buffer solution of the same content (4 fresh portions 15 minutes in each). The material was conducted according to the standard scheme [11]. Ultra-thin sections were prepared on the ultramicrotome YMTП-3M by means of glass knives prepared on the device CCH-1. The sections of a silver or delicately lemon colour were selected for the study. First the sections were contrasted in 2% uranyl acetate solution, followed by lead citrate [12]. The material was examined and photos taken by means of the microscope YEMB-100K (Ukraine) with accelerating potential 75kW and 4000 – 24000 x magnification on the microscope screen.

Results and discussion. Ultramicroscopic examination of rats intramuscularly injected with the opioid Nalbuphine during 4 weeks of the experiment on the 28th day detected that sarcolemma of the cardiomyocytes was thickened, and destructed in some areas.

Mitochondria are broken, glycogen granules are absent. The sarcolemma of cardiomyocytes is destroyed with invagination areas. Mitochondria destroyed and changed by their shape and size are located marginally. Myofibrils are broken. Intracellular swelling is found (Fig.1) and myofibrils, Z-disks and M-lines are ruined. T-system is dilated, destructed, with visible lysosomes.

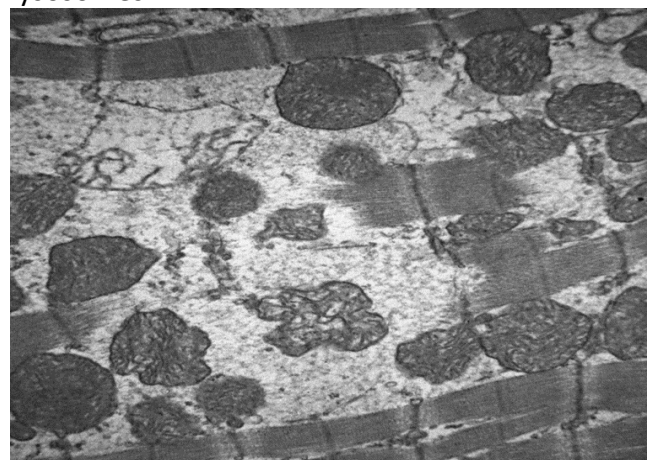


Fig.1. Intracellular swelling in the myocardium in 28 days of the experiment. Electronic micrograph. Magnification : ×10000

Many broken mitochondria are found. Preserved mitochondria are changed by their size and shape, and their mitochondrial matrix is compact. Sarcoplasmic reticulum is dilated and partially lysed. In some areas myofibrils are destructed, Z-lines, M-lines are ruptured, and mitochondria are changed by their shape and size. A considerable damage of the sarcoplasmic reticulum is found between myofibrils. Glycogen blobs are absent which is indicative of decompensation processes in the myocardial cells (Fig.2).

Although at this term of the experiment Z-disks and M-lines are preserved in certain areas of cardiomyocytes (Fig.3).

Marginal changes of cardiomyocyte sarcolemma, destruction of myofibrils, thickening of the contacts between cardiomyocytes were found in the experimental animals. Chromatin is marginally located in the fibroblast nucleus. The folds of the cytoplasmic membrane are found (Fig.4).

In the area of histohaematin barrier blood capillaries adhere closely to the surrounding cardiomyocytes. Invaginations and expansions are found on the luminal surface of the endothelial cells. Erythrocytes are changed in the lumen of blood capillaries by their shape and size, platelets

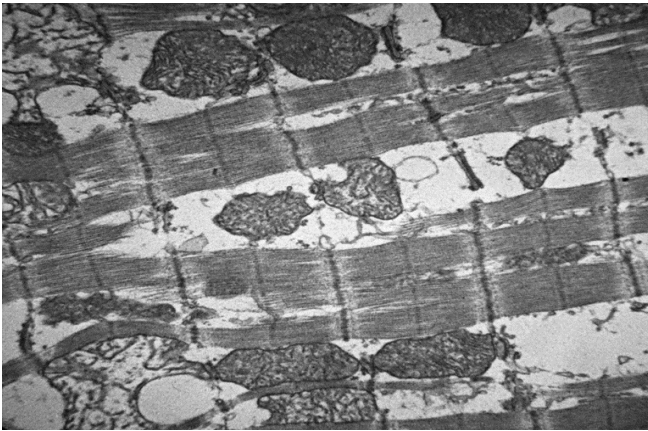


Fig. 2. Central part of cardiomyocyte of a rat 28 days after the experiment. Electronic micrograph. Magnification: x 10 000.

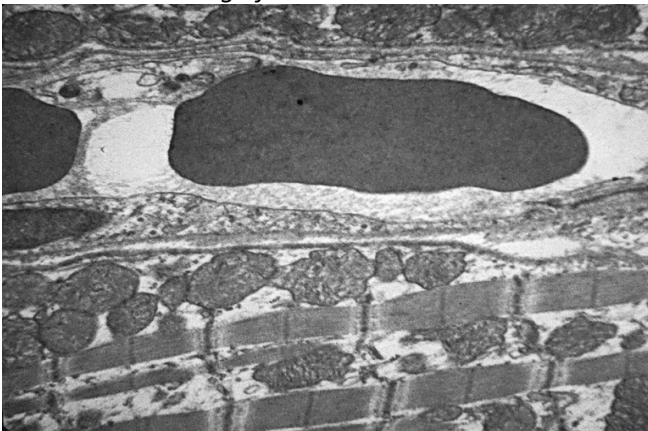


Fig.3. Marginal fold of the luminal surface of the endotheliocyte. Electronic micrograph. Magnification : x 5000

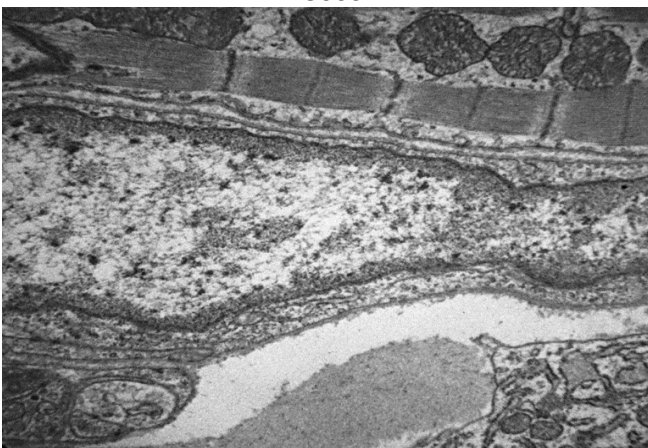


Fig. 4. Fibroblast in the atrial myocardium of a rat 28 days after the experiment. Electronic micrograph. Magnification : x 10 000.

adhesive to the luminal membrane of the endotheliocytes are found between them. The basal membrane of the majority of capillaries is laminated and destructed in some areas. The number of pinocytic vesicles in endotheliocytes at this term is not changed.

At this term of the experiment the lumen of microvessels is filled with cellular detritus (Fig.5). The luminal surface of the endotheliocytes is changed which is presented by numerous folds

and invaginations. Numerous pinocytic vesicles and destruction of mitochondrial apparatus are available in the cytoplasm of endotheliocytes. Cellular detritus is found in the lumen of blood capillaries. Pathological folds are found on the luminal surface of endotheliocytes which are 3-4 times as much as marginal ones. Pinocytic vesicles and lysosomes are found in the cytoplasm of endotheliocytes.

On the 42nd day of the experiment a dilated intercellular space is found due to swelling, deformation of sarcolemma, swelling with destruction of sarcoplasmic reticulum is found between myofibril fibers, the majority of mitochondria are destructed, although some of them are preserved (Fig.6).

Vacuolization and mitochondrial crystallolysis are detected between myofibrils and under sarcolemma (Fig.7).

Mosaic damage of the surrounding cardiomyocytes is found in the areas of the central parts of cardiomyocytes where practically undamaged cardiomyocyte is located close to the damaged

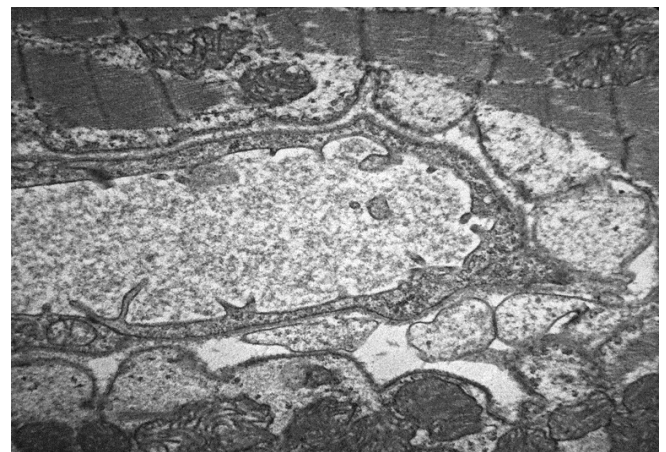


Fig. 5. Venule 28 days after the experiment. Vascular lumen is filled with cellular detritus. Electronic micrograph. Magnification : x 8000.

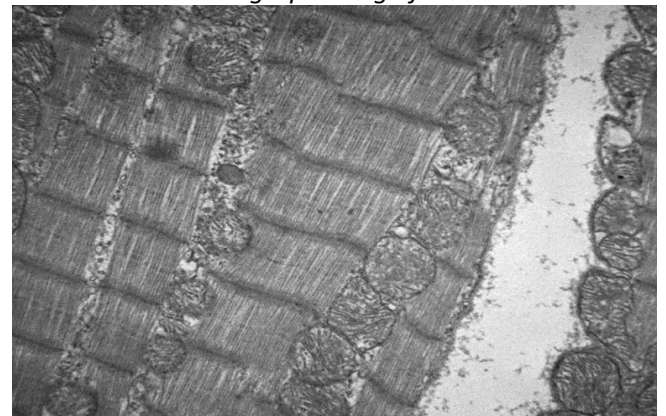


Fig. 6. Intercellular space in the rat's myocardium is dilated at the expense of swelling 42 days after the experiment. Electronic micrograph. Magnification : x 5000.

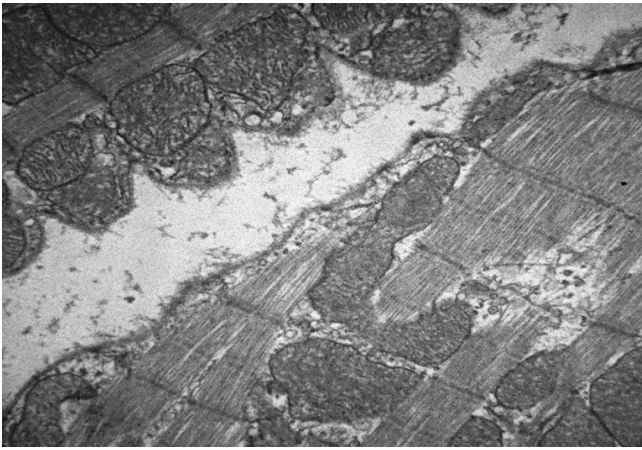


Fig. 7. The area of two neighbouring cardiomyocytes 42 days after the experiment. Vacuolization and crystallolysis of mitochondria are seen between myofibrils and under sarcolemma. Electronic micrograph. Magnification : x 5000.

one. Exfoliation of myofibrils, destruction of Z-disks and M-lines with simultaneous preservation of these structures in the neighbouring myofibrils, destruction of mitochondrial crests, and dilation of T-system canals are found (Fig. 8).

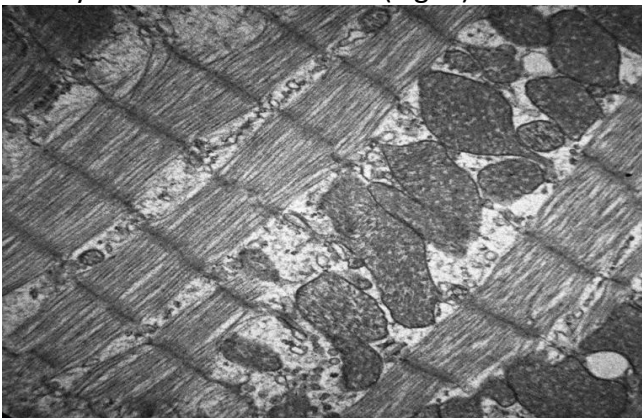


Fig.8. Area of the central part of the cardiomyocyte of a rat 42 days after the experiment Electronic micrograph. Magnification : x 5000.

A considerable perivascular swelling in the area of histohaematic barrier, ruined sarcolemma of cardiomyocytes, ruined Z-disks between myofibrils are determined. Sarcolemma of cardiomyocytes has folds with the signs of swelling, glycogen blobs are absent, mitochondrial crests are ruined. Myofibrilolysis of cardiomyocytes and dilation of T-system tubes are detected (Fig.9).

Villous damage of the cardiomyocyte sarcolemma is specific for this term of the experiment (Fig.10).

Blood capillaries with thickened fold membrane and cellular detritus in their lumen are found (Fig.11).

The vascular wall is swollen and exfoliated, the internal layer of membranes has folds and finger-

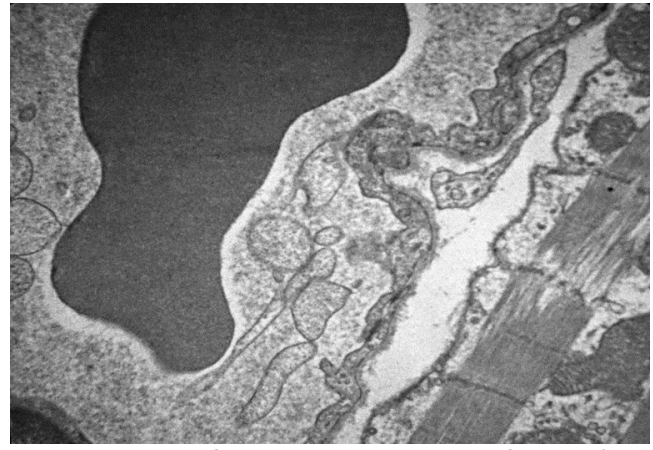


Fig. 9. Area of histohaematic barrier of the rat's myocardium 42 days after the experiment Electronic micrograph. Magnification : x 5000.

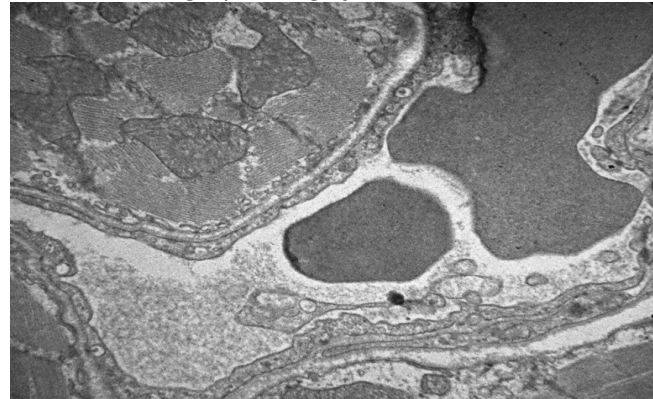


Fig. 10. Villous damage of the cardiomyocyte sarcolemma of a rat 42 days after the experiment. Fragments of platelets, erythrocytes changed by their shape and size are found in the vascular lumen. Electronic micrograph. Magnification : x 5000.

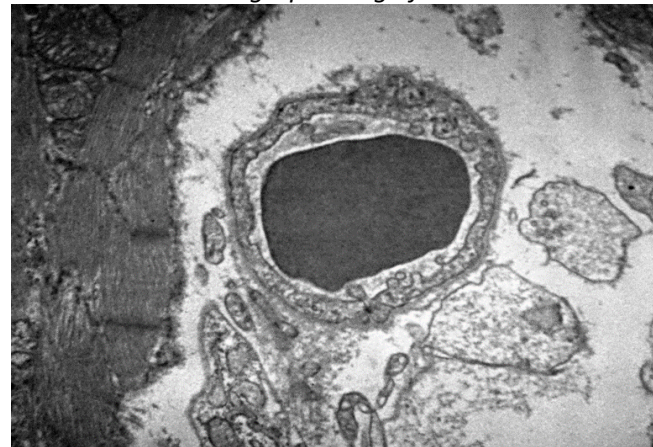


Fig. 11. Area of histohaematic barrier of the rat's myocardium 42 days after the experiment Electronic micrograph. Magnification : x 5000.

like expansions. Interendothelial contacts are thickened. Smooth muscular contacts are damaged. Cellular detritus of formed blood elements is found in the vascular lumen (Fig.12).

In the area of histohaematic barrier a considerable dilation of the interstitial space, swelling and partial destruction of the internal layer of the membrane are detected. The vascular

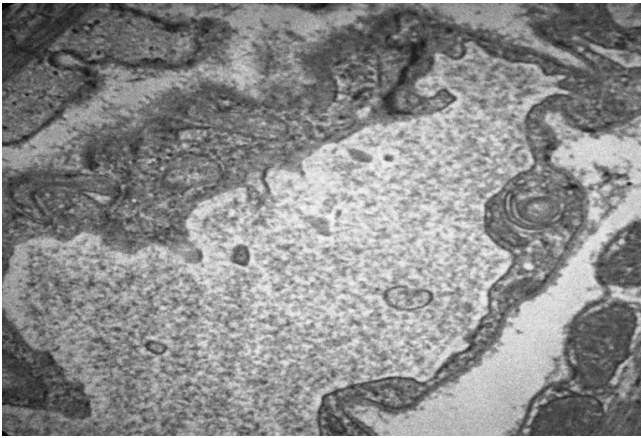


Fig. 12. Area of histohaematic barrier of the rat's myocardium 42 days after opioid intoxication. Electronic micrograph. Magnification : x 5000.

lumen is filled with changed erythrocytes and remains of the formed blood elements and cellular detritus.

Conclusion. On the 28th day of the experimental opioid intoxication deep destructive changes of cardiomyocytes similar to those of myocardial infarction are found: destruction of cardiomyocytes, mitochondrial crystallolysis, intercellular swelling, sludge syndrome and cellular detritus available in the vascular lumen.

On the 42nd day of the experimental opioid intoxication the signs of decompensation and destruction of cardiomyocytes increase, the perivascular space dilates due to swelling and cellular detritus blocking the vascular lumen, changed erythrocytes and formation of clots. Villous damage of the cardiomyocyte sarcolemma is specific for this term of the experiment.

Prospects of further studies. The obtained results enable to extend the notion and solve the issue concerning opioid effect on the heart structure and its blood microcirculation forming morphological basis for better understanding of pathogenesis and further search of optimal methods of treatment of cardiologic diseases among patients who have to take opioids for a long period as well as drug addicts.

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WUNDHEILENDE EIGENSCHAFTEN UND STABILITÄT DER NANOHALTIGEN BIODEGRADIERENDEN POLYMERMATERIALIEN IN VERSCHIEDENEN AUFBEWAHRUNGSZEITEN (EXPERIMENTELLE UNTERSUCHUNG)

Abstract. Zusammenfassung: die Behandlung der Kranken mit eitrig-entzündlichen Erkrankungen bleibt weiterhin ein aktuelles ungelöstes Problem. Eine der modernen Methoden der lokalen Wundbehandlung unterschiedlicher Genese ist der Einsatz der Polymerwundüberzüge. Einen wichtigen Platz besitzen die Entwicklung, die Forschung und die Einführung eines solchen Mittels in die Klinik. Es wurden von uns Polymermaterialien in Form der biodegradierenden Polymerbasis «Biodep» und des gesättigten mit nanoskaligen Zinkoxid-Partikeln (ZnO) Folien «Biodep-nano» synthetisiert. Ziel: wundheilende Eigenschaften und die Stabilität der biodegradierenden Polymermaterialien in unterschiedlichen Fristen ihrer Aufbewahrung zu forschen. Forschungsmaterialien und Methoden: Es wurde die Forschung der dampfdurchlässigen Fähigkeit, Ausscheidungseigenschaften, des Schwellungsgrads im Labor «Des Zentrums für Bioelementologie» und des Lehrstuhls für Allgemeine Chirurgie von der SHBE «Die nationale medizinische Universität Iwano-Frankiwsk» durchgeführt, wundheilende Eigenschaften im Experiment in vivo wurden in der klinisch-biologischen Abteilung (Vivarium) von der SHBE «Die nationale medizinische Universität Iwano-Frankiwsk» untersucht. Die Forschung wurde an 24 Meerschweinchen mit dem Gewicht von 300-400 g (die Art *Cavia porcellus*) getestet. Ergebnisse: die Untersuchung der Stabilität zeigte eine allmähliche Verringerung der dampfdurchlässigen Fähigkeit als Basisfolie und Folie vom gesättigten nanoskaligen Zinkoxid bis zu 6%, der Schwellungsgrad der Folie verringert auf 6,5%, die Ausscheidungsfähigkeit zeigte auch die Tendenz zu einem leichten Senkung der Indikatoren. Die Untersuchung der Suffizienz der Schnittwunde zeigte eine leichte Senkung der Materialwirkung auf Narbe, was praktisch keinen Einfluss auf seine Bildung und Reißfestigkeit hatte. Schlussfolgerungen: Die Polymermaterialien haben gute wundheilende Eigenschaften und gute Stabilität unabhängig von der Aufbewahrungsfrist. Die Polymermaterialien sind eine vielversprechende Richtung der Wundchirurgie und erfordern weitere Forschung.

Stichworte: Wunden, Behandlung, Folien, Stabilität

Die Einführung. Das Problem der Behandlung der Patienten mit eitrig-entzündlichen Erkrankungen bleibt weiterhin aktuell. Die Prävalenz der chirurgischen Infektion, neue Stämme von Mikroorganismen und deren Resistenz gegen Antibiotika-Therapie, die ungünstigen Ergebnisse der Behandlung von eitrig Wunden, die mit langen Heilzeiten verbundenen und septischen Komplikationen diktieren die Notwendigkeit, neue Methoden und

Materialien der chirurgischen Wundbehandlung zu suchen [1].

In der postoperativen Periode ist eine der wichtigsten Voraussetzungen für Verlaufsversorgung des Wundprozesses ohne Komplikationen die Vorbeugung der Sekundärinfizierung der Wunden und die Schaffung der günstigen Bedingungen für die Epithelisierung. Eine wichtige Rolle dabei spielen die Verbände [2, 3].

In einer Reihe mit bereits bekannten Verbandsmitteln ist eine der modernen Methoden der lokalen Wundbehandlung verschiedener Genese die Verwendung der polymeren Wundüberzüge, die einen Einfluss in den verschiedenen Phasen des Wundprozesses haben [4, 5, 6].

Solche Materialien müssen bioverträglich, nicht toxisch sein und allmählich mit der Ausscheidungsmöglichkeit des Arzneimittels degradieren [7].

Einen wichtigen Platz besitzen die Entwicklung, die Forschung und die Einführung eines solchen Mittels in die Klinik. Die wichtigste Aufgabe der Entwicklung des neuen fertigen Arzneimittels ist die Forschung seiner Stabilität während der Aufbewahrung, wobei die Eignung des Arzneimittels durch organoleptische und physisch-chemische Parameter, mikrobiologische Stabilität und spezifische pharmakologische Aktivität gesteuert wird [8].

Es wurden von uns die Polymermaterialien in Form der biodegradierenden Polymerbasis «Biodep» [9] und der gesättigten mit nanoskaligen Zinkoxid-Partikeln (ZnO) Folie «Biodep-nano» synthetisiert [10].

Das Ziel: wundheilende Eigenschaften und die Stabilität der biodegradierenden Polymermaterialien in unterschiedlichen Fristen ihrer Aufbewahrung zu forschen.

Die Materialien und Methoden: in der Forschung wurden von uns entwickelten biodegradierenden Polymermaterialien in Form der Folien verwendet: 1. biodegradierende Polymerbasis «Biodep»; 2. Biodegradierende Polymerfolie "Biodep-nano". Die Folien wurden unter aseptischen Bedingungen hergestellt und nach der Sterilisation mit UV-Licht innerhalb von 24 Monaten in einem verschlossenen Plastikbeutel luftdicht bei Raumtemperatur und Normaldruck aufbewahrt. Die Forschungen wurden in einer Laufzeit von 12 und 24 Monate entsprechend durchgeführt.

Experimentelle Untersuchung in vivo wurde in der klinisch-biologischen Abteilung (Vivarium) der SHBE « Die nationale medizinische Universität Iwano-Frankiwsk» an 24 Meerschweinchen mit einem Gewicht von 300-400 g (Art *Cavia porcellus*). Die Tiere wurden gehalten und aus dem Experiment gemäß der sanitär-hygienischen

Normen (wissenschaftlich-praktische Empfehlungen zu der Haltung der Labortieren und die Arbeit mit ihnen, 2002), der Anforderungen der «Allgemeinen ethischen Prinzipien der Tierversuchen» erlassen vom I. Nationalkongress für Bioethik (20.09.2004, Kiew, Ukraine), genehmigt mit den «Regeln für die Ausführung der Arbeiten der Verwendung der Versuchstiere», bestätigt mit dem Befehl des Ministeriums für Gesundheitswesen der Ukraine und des Gesetzes der Ukraine «Über den Tierschutz gegen Misshandlung» (Nr. 1759-VI vom 15.12.2009) und den Regeln der Europäischen Konvention über den Schutz der Wirbeltieren (European convention for the protection of vertebrate animals used for experimental and other scientific purposes. — Council of Europe. — Strasburg, 1986) beseitigt [11]. Unter Vollnarkose wurde das Operationsfeld vorbereitet und eine Schnittwunde im interskapulären Bereich gemacht, eine Polymerfolie in die Wunde in der Größe von 3 mm zu 20 mm gelegt und in den bestimmten Forschungsfristen (3, 7, 12 Tag) die Einschätzung des Aussehens der Wunde und ihrer Heilung durch die Untersuchung der Angemessenheit der Narbe auf die von uns entwickelten Wundtensiometer durchgeführt [12].

Die Stabilität der Polymermaterialien wurde im Labor «Des Zentrums für Bioelementologie» und des Lehrstuhls für Allgemeine Chirurgie von der SHBE «Die nationale medizinische Universität Iwano-Frankiwsk» geforscht.

Von uns wurde die Untersuchung der Veränderungen des Schwellungsgrades, der durchlässigen und ausscheidenden Eigenschaften der Proben von Polymermaterialien durchgeführt, jede Untersuchung wurde dreimal gemacht.

Für die Untersuchung des Schwellungsgrades wurden dir vorher gewogenen Proben in einem Glas mit Wasser platziert, die in bestimmten Zeitfristen herausgenommen wurden, das Wasser wurde mit Filterpapier abgetrocknet und wurden wiederholte Wägungen des Gewichts mit Wagen AXIS AD200 mit einer Genauigkeit von 0,001 G) in 1 Stunde und 12 Stunden durchgeführt [13].

Die durchlässige Fähigkeit wurde mit der Platzierung der Folienproben auf den Kolben mit destilliertem Wasser und weiterer Wägung des Kolbens in 1 Stunde und 24 Stunden geforscht [14].

Die Ausscheidungseigenschaften wurden

mittels kolometrischen Testsystems auf Zink Aquaquant® mit einer Empfindlichkeit von 0,1-5 mg/l der Firma MerckKGaA (Deutschland) geforscht [15].

Alle Untersuchungen wurden dreimal mit der Bestimmung der Mittelwerte und der statistischen Bearbeitung der Daten mit der Eintragung der Ergebnisse in die entsprechenden Tabellen durchgeführt. Es wurde die parametrische beschreibende Statistik angewendet und wurden die Lizenzpaketen der statistischen Analyse Microsoft Excel[16] benutzt.

Die Einschätzung der Forschungsergebnisse der dampfdurchlässigen, ausscheidenden Fähigkeit und des Schwellungsgrades wurde durch die Berechnung der Verhältnisse der erhaltenen Mittelwerten der durchgeführten Untersuchungen in 12 und 24 Monate zu den erhaltenen Werten der durchgeführten Untersuchungen, die gleich nach der Herstellung der Proben von Polymermaterialien erhalten wurden, nach der folgenden Formel durchgeführt:

$$a = \frac{a_2 - a_1}{a_1} \cdot 100\%$$

wo, α – Forschungsgrad;

α_2 –die Werte der Untersuchungen, die in verschiedenen Aufbewahrungszeiten erhalten wurden;

α_1 – die Werte der Untersuchungen, die gleich nach der Herstellung erhalten wurden.

Ergebnisse. Bereitgestellte Muster haben im Laufe der Untersuchung ihre Farbe und organoleptischen Eigenschaften nicht verändert. Flexibilität und Elastizität bleiben unverändert. Die Untersuchung von dampfdurchlässigen Eigenschaften hat eine leichte Verringerung der *Wasserdampfdurchlässigkeit* der Polymerfolien gezeigt, um 1,8% pro eine Stunde bei Raumtemperatur im Laufe von 12 Monaten und um 4,7% im Laufe von 24 Monaten und in einer anderen Untersuchung beträgt diese Verringerung schon 24 Stunden später etwa 2% in 12 Monaten und 4,1% in 24 Monaten. Die Untersuchungsergebnisse bei der Temperatur 37°C zeigten auch eine Tendenz zur Verringerung der *Wasserdampfdurchlässigkeit* maximal bis 6,5%. Die erhobenen Untersuchungsergebnisse sind in Tabelle 1 dargestellt.

Tabelle 1

Verringerung der Dampfdurchlässigkeit von Polymermaterialien in 12 und 24 Monaten.

Muster	12 Monate		24 Monate	
	1 Std.	24 Std.	1 Std.	24 Std.
Basisstoff	1,87%	1,9%	4,7%	5,2
Basisstoff +ZnO5%	2%	1,95%	4,1%	5,8%
Basisstoff bei 37°C	2,1%	2,5%	5,1%	5,4%
Basisstoff +ZnO 5% bei 37°C	2,4%	2,9%	5.7%	6,5%

Eine weitere Untersuchung von Polymerfolien zeigte Tendenz zur Verringerung ihres Quellwerts um 6,2% in 24 Monaten im Laufe erster Untersuchungsstunde und um 7,3% in 24 Untersuchungsstunden. Die Folie, die mit nanoskaligem Zinkoxid gesättigt wurde, zeigte ebenfalls eine Verringerung der *Quelleigenschaften* um 6,9% in 24 Monaten während der ersten Untersuchungsstunde und dementsprechend um 7,7% in 24 Untersuchungsstunden (Tabelle 2).

Die Forschung der Ausscheidungsfähigkeit zeigte ebenfalls eine Tendenz zur Verminderung in 12 Monaten und 24 Monaten dementsprechend, aber schon in 48 Stunden war die Abscheidung von ZnO fast gleichwertig im

Verringerung des Quellwerts

	Zeitspanne, Monat	Verminderung des Quellwerts, %	
		in 1 Std.	in 24 Std.
Basisstoff	12	5,3%	6,3%
	24	6,2%	7,3%
Basisstoff + Zink 5%	12	5,7%	5,9%
	24	6,9%	7,7%

Tabelle 2

Vergleich zu Primäresultaten gleich nach der Herstellung (Tbl. 3).

Tierexperimentelle Untersuchungen zeigten auch Tendenz zu einer geringen Verringerung von der Wirkung der Folien innerhalb von 6%. So

Tabelle 3.

Die Ausscheidungsfähigkeit von Polymerfolie mit 5% nanoskaligem Zinkoxid (ZnO).

Aufbewahrungszeit, Monate	Verringerung der Ausscheidung Zn ²⁺ , %		
	1 Std.	24 Std.	48 Std.
12	4,8%	5,4%	1,3%
24	5,7%	5,1%	1,8%

zeigten die durchgeführten Untersuchungen in 12 Monaten eine Verkleinerung der Narbe nach einer Schnittwunde bei der Anwendung des Basispolymerfolien am dritten Forschungstag um 4,6% , am siebten Tag um 5,8,0% und am zwölften Tag um 4% im Vergleich zu ähnlichen Forschungen gleich nach der Herstellung der Folie. Ähnlich waren die Ergebnisse in 24 Monaten. So ergeben Folien mit nanoskaligen *Zinkoxid*-Partikeln unwesentlich bessere Ergebnisse in 12 und 24 Monaten: am dritten Tag betrug die Verringerung 4,3%, am siebten Tag - 6,2% , am zwölften Tag - 2.4 %.

Diskussion: die von uns ausgewählte und durchgeführte experimentelle Tierforschung wurde heute sehr häufig eingesetzt und erlaubt uns, das untersuchte Objekt objektiv zu bewerten [17].

Die von uns erzielte Ergebnisse bestätigen große Bedeutung und Potenzial der von uns entwickelter moderner bioabbaubarer Systeme mit der möglichen berechneten Dosis von Arzneimitteln an dem *Verletzungsbereich* bei der Wundbehandlung, was eigentlich auch weltweit bekannte wissenschaftliche Studien beweisen [18].

Es muss darauf hingewiesen werden, dass das Vorhandensein von aktiven Elementen in den Folien, in dem Fall von Nanopartikeln des Zinkoxids, keinen wesentlichen Einfluss auf die Größe der Verringerung der *Wasserdampfdurchlässigkeit* und anderer Eigenschaften der Folien hatte.

Schlussfolgerungen 1. Die von uns untersuchten bioabbaubaren Polymerfolien verlieren ihre wundheilenden Eigenschaften während langer Aufbewahrungszeit nicht.

2. Während der Aufbewahrungszeit verlieren Polymermaterialien etwas an Stabilität, was wiederum ihre Eigenschaften nicht beeinflusst.

3. Bioabbaubare Polymerfolien benötigen

weitere experimentelle Untersuchungen.

Perspektiven für weitere Forschung:

Die Untersuchungen von neuen chirurgischen Folien zum Zweck der Behandlung von Wunden unterschiedlicher Herkunft und Vorbeugung der Wundkomplikationen ist eine vielversprechende Richtung in der *chirurgischen* Wundversorgung. Die von uns entwickelten bioabbaubaren Polymermaterialien sind flexibel, so dass sie an verschiedene anatomische Strukturen und Wunden gelegt werden können, haben gute dampfdurchlässige Eigenschaften, dies gewährleistet ziemlich feuchte Umgebung in der Wunde, haben die Fähigkeit, den Wirkstoff abzuscheiden und allmählich zu verfallen, besitzen stabile Eigenschaften zu unterschiedlichen Aufbewahrungszeiten, was in der Gesamtheit ihre hohe Wirksamkeit bei der Wundbehandlung beweist. Die durchgeführte Forschung der Stabilität und wundheilenden Eigenschaften von entwickelten Polymermaterialien lässt sich sicher über ihre Qualität, Relevanz und Zukunftsaussichten der weiteren experimentellen Forschungen sowie bei der Wundbehandlung und als auch bei der Vorbeugung von Komplikationen behaupten.

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FORMATION OF SILVER NANOPARTICLES USING BIOMASS OF BACTERIUM *ACTINOMYCES SPP. NSX-333*

Abstract. In this article, we studied biosynthesis of silver nanoparticles using biomass (cells) of *Actinomyces spp. NSX-333*, isolated from the Azerbaijan soil. AgNO_3 solution was added to the cell culture and reaction mixture was incubated at 28 °C for 24 hours in the dark condition. Derived silver nanoparticles were characterized using UV-visible spectroscopy, Scanning Electron Microscopy (SEM) micrographs and Energy Dispersive Analysis (EDS) methods.

Keywords: *Actinomyces spp.*, biomass, biosynthesis, silver nanoparticles.

Introduction. Currently, nanoparticles find a wide application in medicine (synthesis, delivery and disposal of drugs, cancer treatment), biology (immune research, use of nanoparticles as biomarkers in the study of intracellular processes) and technology (electronics, information technology, obtaining new materials with improved properties) [4, 6]. There are a lot of publications about the biological synthesis of gold, silver, selenium, platinum, quartz and other compounds with bacteria, actinomycetes, fungi and yeasts, as an active search of effective bioobjects is conducted for obtaining nanoparticles of different chemical nature [1, 2, 5, 7]. However, despite the stability, biologically produced nanoparticles are not homogeneous and the synthesis is rather slow. To overcome these problems, a comprehensive study of all the factors affecting this process is necessary [6, 8].

A lot of physico-chemical methods have been developed for the synthesis of nanoparticles. However, despite their successful application, these methods often remain expensive and require the use of hazardous chemical compounds. Therefore, for the synthesis of various nanoparticles using microorganisms, there

is a need to develop effective methods safe for the environment and human [9-11].

We have previously shown silver nanoparticles formation using the cell-free culture fluid of *Actinomyces spp. NSX-333* [3]. The purpose of this work was to obtain silver nanoparticles using the biomass of *Actinomyces spp. NSX-333*.

Materials and methods. The strain *Actinomyces spp. NSX-333*, isolated from the soil, was used as an object. The pure culture of actinomycete was stored in a microbial culture collection of Baku State University.

To study the ability of the actinomycete to form silver nanoparticles, the culture was grown on the Gauze liquid mineral medium with the following composition (g /l): soluble starch -20; K_2HPO_4 -0.05; KNO_3 -0.1; FeSO_4 -0.005; MgSO_4 -0.05; agar-20. The liquid media was inoculated with the actinomycete culture and incubated at 28 °C for 7-14 days. The biomass was separated from the culture medium by centrifugation and to remove traces of nutrients, it was washed three times with sterile distilled water. After washing, the biomass was resuspended in 100 ml of sterile distilled water and incubated at 28 °C for 24 hours. Then, the suspension was filtered and obtained biomass

was subsequently used for the synthesis of nanoparticles.

To synthesize silver nanoparticles, 10 g of wet biomass was mixed with 100 ml of an aqueous solution of 1 mM silver nitrate (AgNO_3) and incubated in a thermostat at 28 °C under dark conditions for 7, 14, and 28 days. The control without AgNO_3 was kept under the same conditions as described above.

At the end of the incubation, the biomass was separated by filtration and liquid was analyzed on a SPECORD 250 plus spectrophotometer (Germany). The size and morphological characteristics of silver nanoparticles were examined on a scanning electron microscope JEOLJSM-7600F (Japan). EDS analysis of the silver nanoparticles was also carried out.

Results. The formation of silver nanoparticles in the reaction mixture can first be visually determined from the color change of the solution, inoculated with biomass. Color change was observed 15-20 days later. The color of the suspension, which does not contain silver ions, was remained pale yellow. The reaction mixture changes its color to dark brown in the presence of silver ions (Fig. 1). The color change during the reaction from pale yellow to dark brown confirms the cellular (biomass) formation of nanoparticles.

Spectrophotometric analysis of the biomass filtrate of *Actinomyces spp.* NSX-333 showed weak absorption at a wavelength of 420 nm, for a culture incubated 7, 14 and 28 days (Fig. 2).

Analysis of the sample on a scanning electron microscope showed that silver nanoparticles have a spherical shape with a size of 22.4 nm. Agglomeration of nanoparticles is observed (Fig.3).



Fig.1. Color change of reaction mixture during formation of silver nanoparticles with the biomass of *Actinomyces spp.* NSX-333: a-experiment; b-control

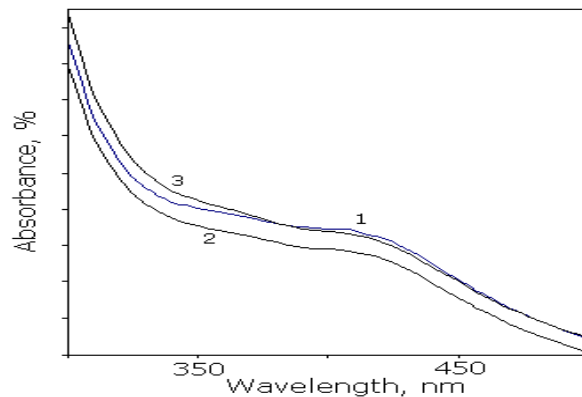


Fig. 2. UV-spectr of silver nanoparticles synthesized with the biomass of *Actinomyces spp.* NSX-333. 1- 28 days; 2- 14 days; 3- 7 days

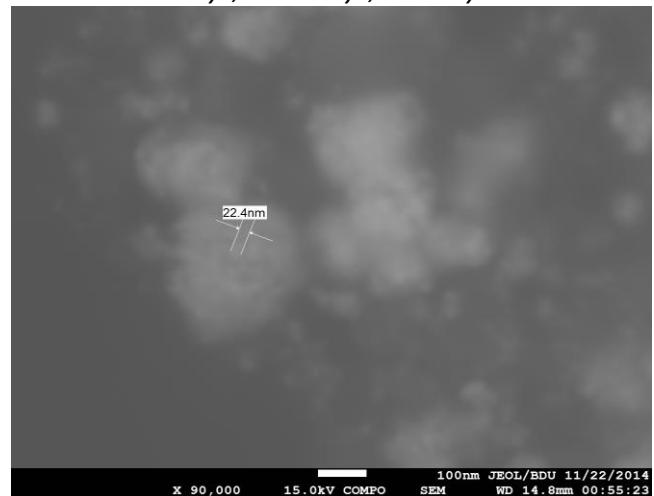


Fig.3. SEM images of silver nanopartilcs, synthesized with the biomass of *Actinomyces spp.* NSX-333

Energy-dispersive X-ray spectroscopy of the sample showed a characteristic peak (AgLa1), indicating the presence of silver nanoparticles (Fig.4).

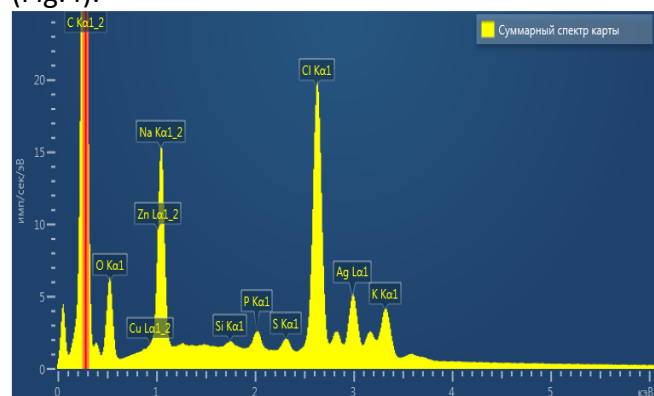


Fig.4. EDS-spectr of nanoparticles, synthesized with the biomass of *Actinomyces spp.* NSX-333

Discussion. It should be noted that this culture is able to form silver nanoparticles by extracellular (culture liquid). The silver nanoparticles formed by the biomass of the culture differ from the extracellularly formed silver nanoparticles by the spherical shape and stable size. So that,

extracellularly formed silver nanoparticles are irregular in shape and their sizes vary from 20 to 130 nm [5].

Conclusion. Thus, spherical silver nanoparticles with a size of 22.4 nm. are formed in the presence of biomass (cells) *Actinomyces spp.* NSX-333, whereas nanoparticles of an irregular shape and with the size of 20-130 nm. are formed in the presence of a cell-free culture liquid. Further research on the role of temperature, acidity (pH), concentration of AgNO₃ and other parameters will be carried out.

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NON-SURGICAL TACTICS OF LIGATURE CORRECTION OF FACIAL SOFT TISSUE AGE CHANGES AND SUBSTANTIATION OF THEIR USE IN COSMETIC DERMATOLOGY ON THE BASIS OF PATHOMORPHOLOGICAL INVESTIGATIONS CONDUCTED

Abstract. *To determine reasonability of ligature correction use by means of introduction of resorptive and permanent grafts on the basis of investigating pathomorphological changes in the skin. The study was conducted on 30 mature albino male rats of “Wistar” line with the body weight of 100-130 grams. Experimental animals were divided into 2 groups depending on the type of threads grafted. Rats of all the groups (14, 30 and 90 day) were divided into 3 subgroups 10 animals each. All the 10 rats in every subgroup were implanted with suture material of the same type. Threads produced by Aptos, namely, Excellence Visage (EV) and Light Lift Thread (LLT) were used for the study. Examination of morphological changes in the peri-implant area determined that collagen is formed in all the groups of experimental rats at different chronological stages with different term of biological degradation and in different amounts. Dependence between the type of grafting thread (structure) and stimulation of collagenosis has been determined. The experiment demonstrated that collagen is formed in the peri-implant areas in both groups of experimental animals at different chronological stage with different term of biological degradation and in different amounts. At the expense of the structure of threads (springs, thread with incisions) and polylactic acid available collagenosis was found to be stimulated considerably. The information provided by the producer concerning the time of disintegration in 365 days correspond to the real facts, since on the 90th day of the experiment resorptive thread was not disintegrated completely; it confirms reasonability of use of these cosmetic threads in esthetic dermatology with the purpose of a long lifting correction of the skin. Polylactic acid available in the content of threads is not an indicator factor of collagenosis efficacy. The structure of the ligature itself must be of greater effect.*

Key words: *collagen, collagenogenesis, permanent graft, resorptive graft, ligature correction.*

Introduction. In recent years the esthetic issues have gained more topicality. Today beauty industry has a number of methods and means of correction and rejuvenation of the face and body, and lifting in particular [9, 10].

The procedure is directed to stimulation of formation of additional collagen in the skin ensuring improvement of turgor and elasticity at the expense of strengthening the frame.

Collagen (Greek kolla — glue + genos — genus) is one of the extracellular, closely related proteins that are the main component of the connective tissue and provides its strength and elasticity. It is fibrillar protein of glycoprotein structure consisting of macromolecules possessing a unique three-spiral structure. It constitutes approximately $\frac{1}{3}$ of all the proteins of the mammalian organisms and 70% of protein mass in the skin. Collagen in the human body fulfills an

important “regulatory role” of the connective tissue functioning (qualitative content of the tissue structure, ensuring flexibility and elasticity of the tissue, prevention of its dehydration, providing moistening of deeper skin layers and inhibition of its aging, improvement of the hair and nails condition) [6, 8].

In the body collagen is synthesized from protocollagen in fibroblasts and chondrocytes, is contained in the connective tissue, fills intercellular space and together with proteoglycans participates in the intercellular interaction, influences upon the mobility of cells, morphogenesis of the organs and tissues during development of the organism [6-8].

The number of transverse connections in a collagen molecule increases in the body with age, resulting in the reduction of its resistance and elasticity, decrease of strength in the cartilages

and ligaments, increase of bone fragility. The structure of collagen fibers depends on the type of tissue and its "specialization". The most widespread types of collagen are: 1) the main component of the skin, tendons, ligaments and bones; 2) over 50% of protein in the cartilage tissue; 3) restorative component of the walls of the blood vessel and intestines; 4) restorative component of the basal epithelial plate in the filters of the blood capillaries and nephron glomeruli [3, 6].

Fibrillar collagen in the skin of an adult (1, 3 and 5 types) is the biggest collagen fraction: the 1st type collagen constitutes approximately 80-90% and the 3rd type collagen constitutes 8-12% [7].

The use of threads in esthetic medicine is based on their ability to stimulate collagenogenesis, which is stated by producers, although this information does not have any grounds, since none of the studies could confirm this hypothesis [2-5].

Therefore, we have decided to check collagen formation with the use of one of the most aggressive methods of cosmetic dermatology – resorptive grafting threads.

Objective: to determine reasonability of ligature correction use by means of introduction of resorptive and permanent grafts on the basis of investigating pathomorphological changes in the skin.

Materials and methods. The study was conducted on 30 mature albino male rats of "Wistar" line with the body weight of 100-130 grams. Experimental animals were divided into 2 groups depending on the type of threads grafted.

Rats of all the groups (14, 30 and 90 day) were divided into 3 subgroups 10 animals each. All the 10 rats in every subgroup were implanted with suture material of the same type. Threads produced by Aptos were used: Excellence Visage (EV – thread with folds, 30 % caprolactone + 70% polylactic acid, time of biological degradation \geq 365 days) and Light Lift Thread (LLT- thread with incisions welded with needles, both contain 50 % of caprolactone + 50 % of polylactic acid, time of biological degradation \geq 365 days).

All the animals were kept under conditions of vivarium. The animals were taken out from the experiment by means of overdosage of ether narcosis (exposition for 5-7 minutes) on the 14th,

30th and 90th day of the experiment. The fragments of the skin with subcutaneous adipose tissue from the back were used for morphological examination [1]. Paraffin blocks were made from every fragment, histological staining was made according to the common method and Mallory's histochemical staining.

Results. The cosmetic thread Excellence Visage was used in the first group of experimental animals. On the 14th day since the beginning of the experiment lymphoplasmocytic infiltration was detected in all the ten experimental specimens.

In all the necrotic materials of the skin angiogenesis of the similar intensity was found. The areas of angiomatosis were located more distally from the places of implantation. The number of fibroblasts in the peri-implant areas was high, and they form a "sleeve" of the connective tissue around implants.

At the 2nd stage of the study – on the 30th day – intensity of infiltration in the peri-implant areas decreased. Degree of angiogenesis remained on the previous level and was characterized by the capillaries, single arterioles and venules available. The number of fibroblasts around the areas of implantation remained on the level of the first stage of the experiment.

On the 90th day since the beginning of the experiment any infiltration in the areas around implants was practically absent. Intensity of angiogenesis was similar to that of the previous stages, although the vessels were in the form of arterioles and venules with the tendency to decrease. All the 10 experimental animals on the 90th day demonstrated decreased number of fibroblasts as compared to the previous stages (Fig. 1).

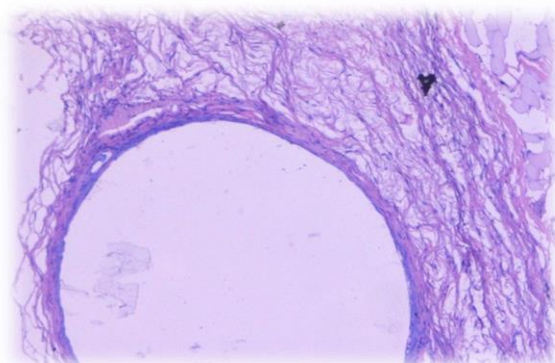


Fig. 1. Morphological picture of the skin tissue sample in the place of implantation of the cosmetic thread Excellence Visage on the 90th day of the experiment. Stained with hematoxylin-eosin. X100

Mallory's histochemical stain showed a low degree of collagen formation in the areas around cosmetic implants; degree of anastomosing also remained rather low; the bundles of collagen fibers remained rather thin and in considerably less as compared to the 30th day (Fig. 2).

The analysis of the results of pathohistological examination determined that intensity of inflammatory infiltration was progressively decreasing in EV implants, and on the 90th day it was not practically determined. However, in case of EV use focal aggregations of eosinophils were present, which can be indicative of a weak allergic reaction in the skin in response to the graft.

The cosmetic suture material Light Lift Thread was used in the second group of the experimental animals. The following morphological manifestation on the skin was found on the 14th day of the experiment after the use of the cosmetic thread Light Lift Thread (they were mostly of the same type in all the ten experimental animals): perifocal peri-implant tissue infiltration mostly by lymphocytes, single plasmocytes, and macrophages. Single aggregations of eosinophils were found.

A "sleeve" is formed around the implantation openings consisting of fibroblasts and fibers of the connective tissue (collagen and elastic); the fibers are mainly directed circularly to the place of opening location; fibroblasts are numerous but located out of order. Aggregations of vessels of a capillary type are present there as well. On the 30th day of the experiment any inflammatory infiltrations were absent. Proliferation of the angiomatous component in the place of alteration was not found. However, it should be noted that bundles of the connective tissue fibers were stratified into more differentiated structures, the number of fibroblasts decreased a little.

90 days later: any inflammatory tissue response in the places of implantation was absent. In certain experimental animals single arteries and small veins with perivascular inconsiderable sclerosis were formed. The number of fibroblasts in comparison with previous stages of the study decreased to the absolute minimum, although it became possible to verify that fibroblasts form bundles. The number of fibroblasts located out of order remains rather low (Fig.3). Mallory's histochemical stain of samples determined a

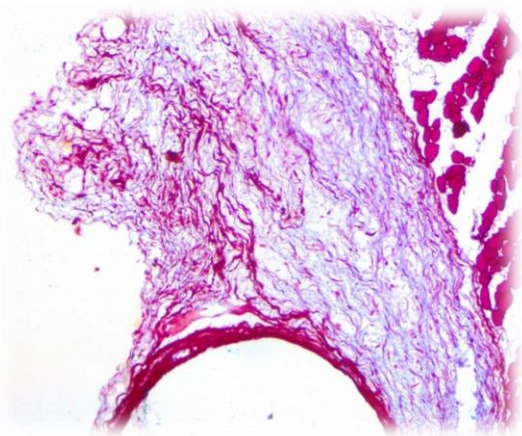


Fig. 2. Collagenogenesis in the peri-implant area (Excellence Visage, 90th day); Mallory's histochemical stain, X100

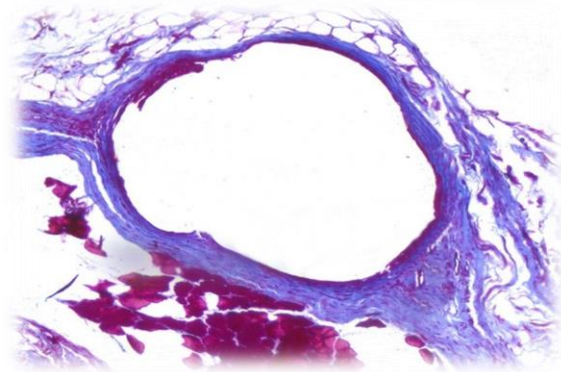


Fig.3. Morphological picture of the necrotic skin tissue sample in the place of implantation of the cosmetic thread Light Lift Thread on the 90th day of the experiment. Stained with hematoxylin-eosin. X100

considerable number of coarse collagen bundles which closely anastomose between themselves, the number of anastomoses is higher in comparison with the previous samples. Direction of collagen fibers in the implantation area was similar. It should be noted that intensity of collagenogenesis remained rather high during all the three stages of the study, and its number did not reduce (Fig.4).

The analysis of the results of pathohistological examination determined, that intensity of inflammatory infiltration in LLT grafts was decreasing progressively, and on the 90th day it was not found practically. However, in case of LLS use focal lymphoplasmocytic infiltrations remained in certain experimental animals on the 90th day since the beginning of the experiment. Degree of angiomatosis was not widely variable and was presented by rather inconsiderable number of small arteries and veins on the 90th day of the experiment. In the group of experimental

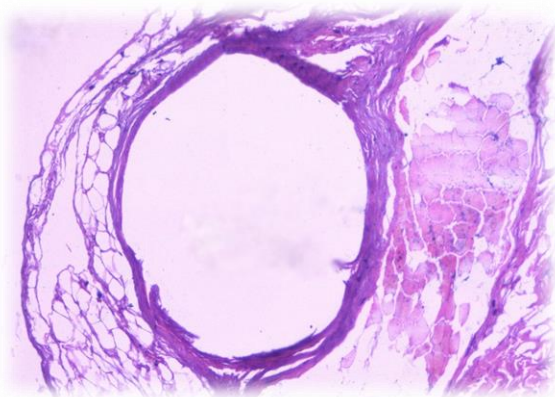


Fig.4. collagenogenesis in the peri-implant area (Light Lift Thread, 90th day); Mallory's histochemical stain, X100

animals where the cosmetic thread Light Lift Thread was used the number of vessels was

considerably less in comparison with another group, although more pronounced collagenogenesis was found.

Examination of resorptive grafts in the skin of the experimental animals determined, that on the 90th day of the experiment none of the threads did not deteriorate completely.

Discussion. On the basis of the above results it was found that collagenogenesis is influenced not only by polylactic acid present in the threads (low collagen formation in EV group; and intensive collagenogenesis with the use of LLT), but mostly by the structure and relief of the cosmetic thread (Table 1). Conducted preliminary statistical estimation of reliability of interrelations between experimental groups is presented in Table 2.

Table 1.

Summary results of morphological changes in the skin of peri-implant areas at different chronological stages

	Infiltration		Infiltrate content		Angiomatosis		Degree of collagenosis	
	LLT	EV	LLT	EV	LLT	EV	LLT	EV
14 th day	F++	D++	L+++ E+ Mp+	L++ E+ Mp++	C+	C+	CCB++ TCB++	CCB+++ TCB+
30 th day	-	F+	-	L++ E+	C+	C+	CCB+ TCB ++	CCB++ TCB+
90 th day	-	-	-	E+	C+	AV+	CCB+ RCB+++	CCB+ TCB+

Notes: D – diffuse; F – focal; L – lymphocytes; E – eosinophils; Mp – macrophages; ISGT – initial signs of the granulation tissue; C – capillaries; AV – arterioles and venules; CCB – cellularity of collagen bundles; TCB – thin collagen bundles; RCB – rough collagen bundles.

Table 2.

Statistically reliable interrelations between the experimental groups

Comparison of differences in groups		Degree of collagen formation	Formed collagen bundles	Degree of anastomosing
LLT	14 day			
LLT	30 day	P _{EV} <0,05;		
LLT	90 day	P _{EV} <0,05;		P _{EV} <0,05;
EV	14 day			
EV	30 day	P _{LLT} <0,05;		
EV	90 day	P _{LLT} <0,05;		

Notes: the table presents only statistically reliable parameters. In all other groups 0,1>p>0,05

Conclusions.

1. The experiment demonstrated that collagen is formed in the peri-implant areas in both groups of experimental animals at different chronological stage with different term of biological degradation

and in different amounts.

2. At the expense of the structure of threads (springs, thread with incisions) and polylactic acid available collagenosis was found to be stimulated considerably.

3. The information provided by the producer concerning the time of disintegration in 365 days correspond to the real facts, since on the 90th day of the experiment resorptive thread was not disintegrated completely; it confirms reasonability of use of these cosmetic threads in esthetic dermatology with the purpose of a long lifting correction of the skin.

4. Polylactic acid available in the content of threads is not an indicator factor of collagenosis efficacy. The structure of the ligature itself must be of greater effect.

Prospects of further scientific studies.

Examination of necrotic skin samples at later chronological stages (180, 365 and 540 days) is planned to be further conducted with additional use of immunohistochemical methods of investigation to determine the type of collagen formed.

The spectrum of examined threads including three more kinds different by their structure and chemical content should be extended.

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RECEPTOR ACTIVITY OF THE EUTOPIC AND ECTOPIC ENDOMETRIUM TO ESTROGEN AND PROGESTERONE MARKERS IN CASE OF ADENOMYOSIS AVAILABLE DURING POSTMENOPAUSE

Abstract. *Objective of the study is to determine ER, PR and AR activity of eutopic and ectopic endometrium in case of adenomyosis available during post-menopause. Materials and methods. 30 cases of adenomyosis have been examined in case of hyperplastic processes in the endometrium and without them in patients aged from 51 to 73 years. Immunohistological examination has been conducted with the purpose to determine the state of receptor apparatus to ER, PR and AR markers. Immunohistological examinations conducted in ectopic foci determined a high mean score of the spread of ER staining of the glandular cells in case of endometrioid adenocarcinoma of the uterus (EACU) and endometrial hyperplasia (EHP) and the stromal cells in the group with EHP. A high mean index of intensity and spread of ER staining of the eutopic endometrium glandular component was found in all the groups. Considerable differences between mean indices of intensity and spread of ER staining of the glandular and stromal cells in adenomyosis foci both between the groups themselves and the examined components are absent ($p>0,05$). An increased level of PR activity was detected respectively to the improvement of differentiation degree of the eutopic endometrium epithelial cells. The comparison of the results of PR activity of the stromal cells and glands of the eutopic and ectopic endometrium did not find any deviations between the examined indices ($p>0,05$). Comparison of the degree of AR expression did not find differences between the groups ($p>0,05$). Conclusions. Adenomyosis during post-menopause period is characterized by prevailed expression level of ER glandular component of the eutopic endometrium over the endometrium in adenomyosis foci in case of EACU and endometrium atrophy available. Increase of ER and PR expression levels occurs respectively to the increase of cellular differentiation degree. Considerable differences by the indices of intensity and staining of PR of the glandular and stromal cells in adenomyosis foci were not found both between the groups themselves and the examined components. Considerable differences by AR expression levels in the eutopic and ectopic endometrium were not found as well. Prospects of further studies: to carry out the analysis of ER, PR and AR expression levels in the ovaries in case adenomyosis available.*

Key words: *adenomyosis; post-menopause; estrogen, progesterone, androgenic receptors.*

Introduction. Endometriosis is benign hormone-dependent inflammatory process associated with structural-functional changes of the female reproductive system, which can be a cause of infertility and disorders in the social, family and economical spheres of life [3, 15]. 176 million of women in the whole world suffer from endometriosis including every 10th one of the reproductive age [15, 16]. In 70-80% of all the

cases endometriosis is manifested by adenomyosis [9, 1, 3]. Women of a reproductive age are mainly affected [15]. At the same time, the disease affects post-menopausal women as well. Occurrence of adenomyosis foci during post-menopausal period is associated with peripheral conversion of androstenediol into estrogen or against the ground of administration of hormonal therapy [15].

Still a considerable progress in investigation of adenomyosis does not enable to formulate completely a clear notion concerning etiopathogenesis of the disease. Metabolic disorders of estrogens are considered to be a leading conception in the development of internal endometriosis [7]. In addition to estrogen and progesterone receptors the variants of their combinations and receptor status of cells are also important [3]. The change of balance of these hormones followed by the development of relative or absolute hyperestrogenemia is the main factor provoking adenomyosis. However, certain scientists state occurrence of endometrioid foci against the ground of hypoestrogenemia and restricted hormonal effect on the heterotopic tissue [6].

16-90% of patients who suffer from internal endometriosis are known to be diagnosed with hyperplastic processes in the endometrium [2]. In recent decades many researchers have indicated the increase of frequency of comorbid (parallel) development of adenomyosis and hyperplastic processes in the endometrium [7, 4].

Investigation of molecular peculiarities of the internal endometriosis foci in combination with other proliferative diseases of the female reproductive system during post-menopausal period is a promising approach, since this group is not completely studied.

Objective: to determine receptor activity of eutopic and ectopic endometrium to the markers of estrogen, progesterone and androgen in case of adenomyosis available during post-menopause.

Materials and methods. The material for the study was surgical material (extracted uteri) from 30 patients (51-73 years of age) with adenomyosis in case of comorbid pathology (endometrioid adenocarcinoma of the uterus (EACU) and endometrial hyperplasia (EHP)) or without it, who were treated in the Center of Restoration and Reconstructive Medicine (University Clinic) of Odessa National Medical University. The selection criteria were the following: amenorrhea over 12 months (post-menopause) and confirmed clinical diagnosis of adenomyosis. The patients were divided into three groups: the first one included 10 women with adenomyosis and simple non-atypical hyperplasia of the endometrium; the second group – 10 women with adenomyosis and

adenocarcinoma of the uterus; the third group (control one) – 10 women with adenomyosis and age changes.

The fragments of the material to be examined were fixed in 10 % neutral formalin with pH 7.0 during 24 hours at the temperature of 37 °C. Then the specimens were processed according to the common unified methods. Immunohistochemical reaction was performed with the use of monoclonal mouse antibodies of estrogen-receptor alpha (ER, clone EP1), progesterone (PR) and androgen antibodies (AR). Expression of estrogen, progesterone and androgen markers was assessed by the numerical score system of the continuous staining method to determine ER- and PR-status (Allred D. C. et al., 1998) (Table 1)[14].

Table 1

Numerical score system to assess continuous staining

Intensity(I)	Spread of staining (Π)
0 = none	(0) – none of stained cells
1 = weak	(1) – stained cells less than 1/100
2 = intermediate	(2) – number of stained cells from 1/100 to 1/10
3 = strong	(3) – number of stained cells from 1/10 to 1/3
	(4) – number of stained cells from 1/3 to 2/3
	(5) – number of stained cells over 2/3

General statistical analysis was made by means of standard methods of mathematical-statistical processing with the use of software MS Office Excel. Means values (M) and error of mean values (m) were estimated. To compare parametric data the method of paired Student criterion was used, probability was determined with p<0,05.

Results. As a result of the immunohistochemical examination conducted on 30 women of a post-menopause period suffering from adenomyosis with and without hyperplastic processes in the endometrium, we have drawn the conclusions concerning the levels of receptor activity of eutopic and ectopic endometrium to the markers of estrogen, progesterone and androgen.

Discussion. Immunohistochemical (IHC) examination determined high mean score of the spread of ER staining of the glandular cells in the

ectopic foci in case of EACU and EHP available, $3,14 \pm 0,6$ and $4,14 \pm 0,5$ respectively (Fig. 1). A similar tendency is characteristic for the stroma of endometrioid heterotropy in the group with EHP. Low indices of ER staining intensity of the stromal component of adenomyosis foci were determined in case of EACU ($0,71 \pm 0,31$) concerning other groups and epithelial cells of ectopic foci in the group itself. A lower level of ER expression of the glandular component of the endometrioid heterotropy was found in case of endometrium atrophy than in case of EHP ($p < 0,05$) (Table 2). Single scientific works are indicative of prevailed receptor activity of the epithelial cells over the stromal ones in adenomyosis foci, where the groups of the study included mainly women of reproductive and pre-menopausal age [11, 13].

Examination of ER activity of the glandular component of the eutopic endometrium presented high mean score of intensity and spread of staining in all 3 groups (Fig. 2) (Table 2). The results of the study of ER expression of the eutopic endometrium are indicative of the absence of considerable differences in the levels of receptor activity depending on comorbid pathology ($p > 0,05$), which agrees partially with conclusions of certain scientists. According to the evidence of certain sources endometrium in case of simple EHP is characterized by a high level of estrogen activity with its gradual decrease in case of combined EHP and EACU [17]. There is an opposite opinion: decreased expression of steroid receptors occurs in the direction of the increased differentiation of cells, the highest indices are found in case of EACU respectively [14, 17]. Assessment of the results of ER activity of the eutopic and ectopic endometrium showed that the 2nd and 3rd groups of women are characterized by higher levels of estrogen expression of the epithelial cells in the eutopic endometrium than in adenomyosis foci (Fig. 3). Such differences are not

peculiar for the stromal component ($p > 0,05$). The data of certain studies indicate that ER activity is lower in endometrioid heterotropy than in eutopic endometrium [9-11]. However, there are opposite opinions concerning prevailing expression of steroid hormones in the foci of internal endometriosis than in eutopic endometrium [11]. Higher estrogen activity of eutopic endometrium concerning ectopic one in the group with atrophy is explained by insufficient amount of ER associated with decrease of their expression in endometrioid heterotropy [9, 17].

Analysis of the activity level of progesterone receptors found a considerable part of stained

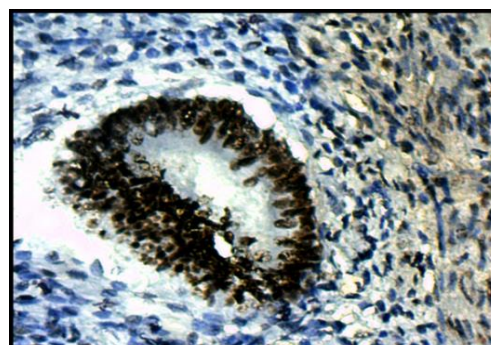


Fig. 1. Adenomyosis focus against the ground of simple non-atypical EHP of a 56-year-old woman. Estimation for glands – 3+++ in 100%; for stroma – 2++ in 60%. IHC reaction with antibodies to estrogen. x400.



Fig. 2. Simple form of endometrial hyperplasia without atypical form in a 73-year-old woman. Estimation for glands – 3+++ y 100%; for stroma – 3+++ y 70%. IHC reaction with antibodies to estrogen. x400.

Table 2

Mean indices of intensity (I) and spread (Π) of staining of ER-receptors in eutopic and ectopic endometrium (M±m, score)

Component	Eutopic endometrium				Ectopic endometrium			
	Glandular		Stromal		Glandular		Stromal	
Index	I	Π	I	Π	I	Π	I	Π
I (n=10)	2,67±0,37	4,5±0,55	2,33±0,37	4,17±0,72	2,14±0,37	4,14±0,5	1,86±0,28	3,57±0,46
II (n=10)	2,56±0,19	4,56±0,26	1,25±0,44	2,63±0,75	1,71±0,31	3,14±0,6	0,71±0,31	2,0±0,85
III (n=10)	2,67±0,41	4,67±0,41	1,67±0,41	3,0±0,71	1,11±0,28	1,78±0,58	1,78±0,24	3,11±0,51

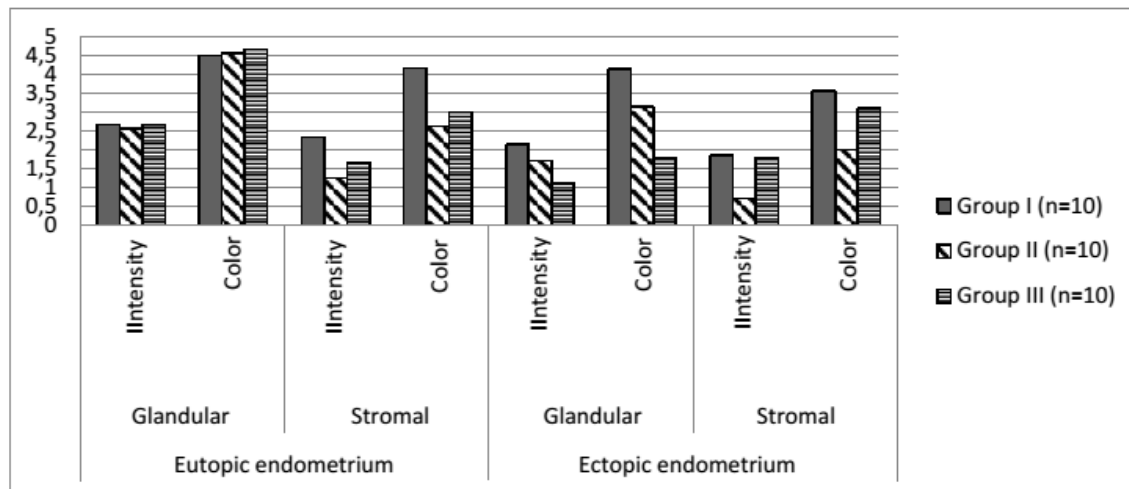


Fig. 3. Mean indices of intensity and staining of ER-receptors in eutopic and ectopic endometrium ($M \pm m$, score).

cells of the glandular and stromal components of eutopic endometrium (Fig. 4). Similar to the cases with ER expression simple non-atypical EHP is characterized by the highest score of intensity—«3» and spread of staining of the epithelial cells - «5» and their decrease in case of complex atypical EHP. The group with EACU is characterized by an increased level of receptor PR activity respectively to the improvement of EACU differentiation degree. The glandular component of low differentiated EACU had minimal score in intensity and spread of staining, stroma according to the given criteria was estimated as “0” in some cases. The mentioned indices in the group were the highest for high-differentiated EACU. The obtained results partially correspond with conclusions of other scientists: the highest degree of PR activity with simple EHP, and the lowest – with complex EHP and EACU [8, 17]. In I and II groups stromal component obtained rather high score by the level of spread of cellular staining – mostly «4» and «5», and as a result mean indices are $4,6 \pm 0,27$ and $4,56 \pm 0,36$ respectively. Analysis of the glandular and stromal components of endometrium by the level of intensity and staining did not find considerable differences ($p > 0,05$) (Table 3), which contradicts the opinion of certain scientists concerning the fact that the number of PR is bigger in the stromal cells irrespective of endometrial state [8]. Considerable difference between mean indices of the degree of intensity and spread of staining of glandular and stromal cells in the internal endometriosis foci (Fig. 5) was not found between the groups themselves and between the examined components ($p > 0,05$) (Table 3).

Results of comparison of PR activity of the stromal cells and glands of eutopic and ectopic endometrium did not find considerable deviations between the examined indices ($p > 0,05$) (Fig. 6), which contradicts statements of other researchers concerning relatively lower level of PR expression in adenomyosis foci than in eutopic endometrium [7, 11]. There is an opposite opinion as well: the components of endometrioid

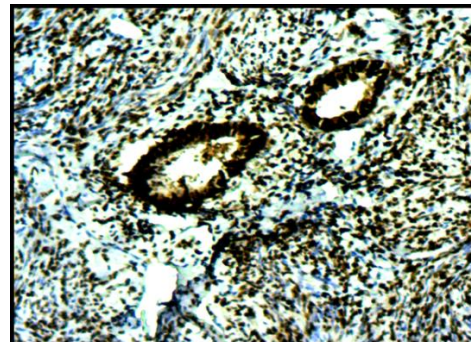


Fig. 4. Endometrioid carcinoma of endometrium G1 in a 70-year-old woman. Estimation for glands – 3+++ y 100%; for stroma – 3+++ in 70%. IHC reaction with antibodies to progesterone. x400.

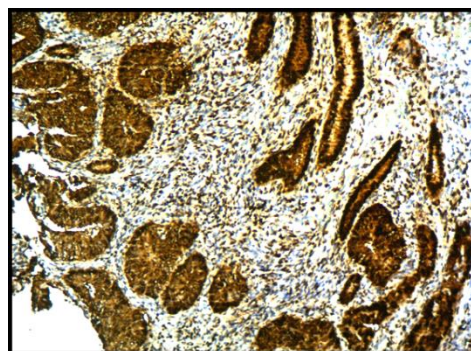


Fig. 5. Adenomyosis focus against the ground of simple non-atypical EHP in a 60-year-old woman. Estimation for glands – 3+++ y 100%; for stroma – 3+++ y 70%. IHC reaction with antibodies to progesterone. x400.

heterotropy have higher PR activity than eutopic endometrium [11].

Analysis of indices of intensity and spread of AR staining showed low score – «0» and «1» mainly (Fig. 7, 8). Comparison of the degree of AR expression did not find considerable differences between the groups of patients examined

($p > 0,05$) (Fig.9) (Table 4), which corresponds to the conclusions of other scientists [7]. However, certain scientists draw attention to the available differences of internal endometriosis foci by the level of receptor expression of steroid hormones, and androgenic in particular, from the surrounding endometrium [5].

Table 3

Mean indices of intensity (I) and spread (Π) of staining of PR-receptors in eutopic and ectopic endometrium (M±m, score)

Component	Eutopic endometrium				Ectopic endometrium			
	Glandular		Stromal		Glandular		Stromal	
Index	I	Π	I	Π	I	Π	I	Π
I (n=10)	2,4±0,27	4,6±0,27	2,8±0,22	4,2±0,42	2,25±0,55	3,75±1,09	2,0±0,47	4,25±0,55
II (n=10)	2,56±0,26	4,56±0,36	2,67±0,35	4,0±0,56	2,2±0,65	3,6±1,1	2,0±0,61	3,4±1,04
III (n=10)	2,67±0,41	4,33±0,82	2,0±0,71	3,33±0,82	2,25±0,33	4,0±0,49	2,63±0,2	4,38±0,2

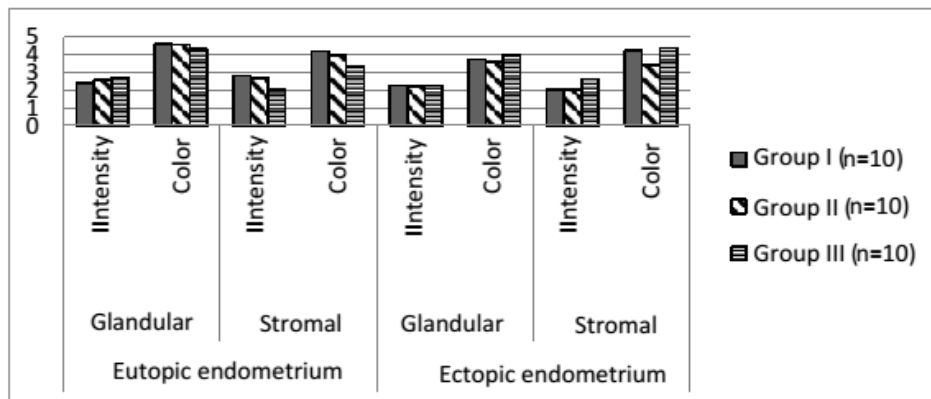


Fig. 6. Mean indices of intensity and staining of PR-receptors in eutopic and ectopic endometrium (M±m, score).

Table 4

Mean indices of intensity (I) and spread (Π) of staining of AR-receptors in eutopic and ectopic endometrium (M±m, score)

Component	Eutopic endometrium				Ectopic endometrium			
	Glandular		Stromal		Glandular		Stromal	
Component	I	Π	I	Π	I	Π	I	Π
I (n=10)	0,29±0,2	0,43±0,32	0,57±0,22	0,71±0,31	0,25±0,17	0,25±0,17	0,85±0,13	0,85±0,13
II (n=10)	1±0	1,33±0,23	1±0	1,17±0,18	0,4±0,27	0,8±0,55	0,8±0,42	0,8±0,42
III (n=10)	0,2±0,22	0,2±0,22	0,4±0,27	0,4±0,27	0,5±0,24	0,83±0,52	0,5±0,24	0,5±0,24



Fig. 7. Age changes (endometrial atrophy) in a 68-year-old woman. Estimation for glands – 1+ in 5%; for stroma – 1+ in 0,5%. IHC reaction with antibodies to androgen. x400.

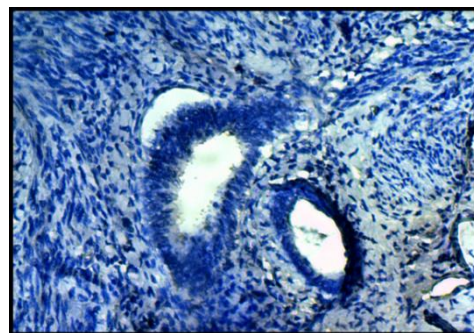


Fig. 8. Adenomyosis focus against the ground of endometrial atrophy in a 68-year-old woman. Estimation for glands – 0 in 100%; for stroma – 0 in 100%. IHC reaction with antibodies to androgen. x400.

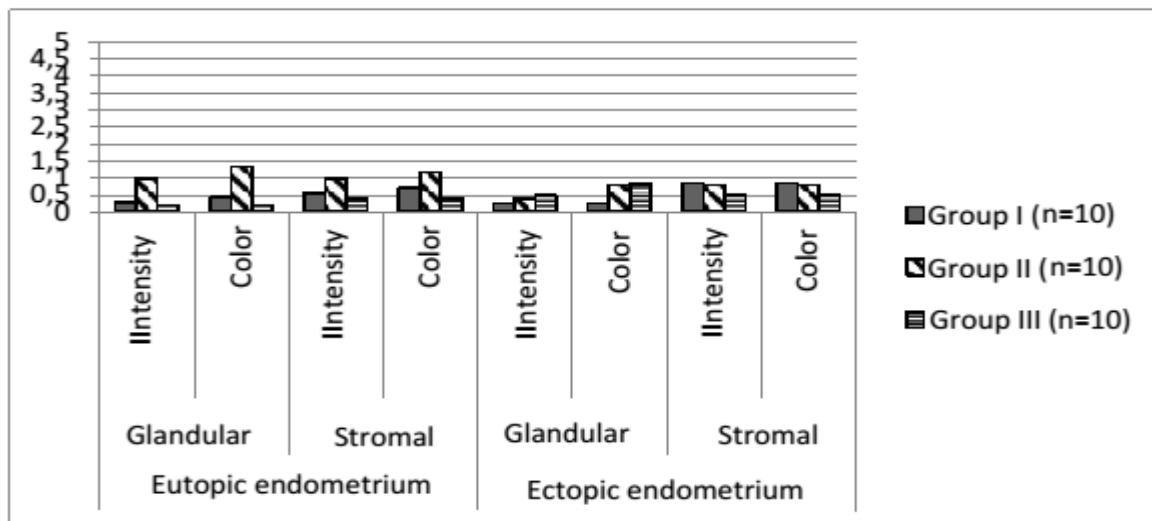


Fig. 9. Mean indices of intensity and staining of AR-receptors in eutopic and ectopic endometrium ($M \pm m$, score).

The difference in opinions concerning immunohistochemical peculiarities of one and the same pathological process can be explained by the presence of comorbid pathology of endometrium (hyperplastic processes of endometrium) and age changes of our patients (post-menopause). Prevalent majority of studies associated with adenomyosis refer to women of a reproductive age and pre-menopausal period without available comorbid pathology of endometrium.

Conclusions. The following immunohistochemical peculiarities were found to be characteristic for adenomyosis during post-menopausal period:

1. Prevalent level of ER expression of the glandular component of eutopic endometrium over the endometrium of adenomyosis foci in case of EACU available and endometrial atrophy. A low level of ER expression in the stromal cells in comparison with the glandular ones both in the foci of endometrioid heterotropy and eutopic endometrium in case of EACU.

2. Increase of levels of ER and PR expression in accordance to the increase of cellular differentiation level.

3. Considerable differences by the indices of intensity and staining of PR of the glandular and stromal cells in adenomyosis foci between the groups and between the examined components are not found.

4. Considerable differences by the levels of AR expression in eutopic and ectopic endometrium are not found.

Prospects of further studies: to carry out the analysis of ER, PR and AR expression levels in the

ovaries in case adenomyosis available.

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PREVENTION OF ATROPHY PROCESS OF THE COLLATERAL CREST BY MEANS OF CONJUNCTIVE APPLICATION OF DISCRETELY STABILIZED MESOSTRUCTURES ON IMPLANTS

Abstract. *The article deals with a topical issue of modern dental implantology: prevention of bone tissue loss after prosthetics using short and angular dental implants. The morphometric analysis in the problem area was carried out: X-ray control - analysis in dynamics during the post-implantation period in the longitudinal aspect. The effectiveness of the prosthetic method on implants, as a means of stabilizing atrophic processes of anatomical structures after teeth loss, was investigated. The influence of external factors on the stabilization process of bone structure remodeling during the post implantation period was studied.*

Key words: *dental implantation, angular implants, periosteum, bone atrophy of implant prosthesis, discreteness, stabilization.*

Introduction. Recently the method of dental implantation has occupied one of the leading places among dental surgery [1, 2]. New implant systems entering the market do not always enable clinicians to achieve a high esthetic result and effective restoration of functions of the human mastication apparatus. The methods and protocols assuming parallel existence of implants between themselves and adjacent teeth did not prevent completely manifestation of dystrophic processes in the periodontal tissues of the adjacent teeth or implants [3]. In Ukraine the offer of implant systems and qualification of dentists-orthopedists concerning implant prosthesis do not always satisfy European requirements [4]. The issues of implant prosthesis require a lot of time from a doctor and a patient. The tendencies of development of medical technologies promote introduction of own clinical methods in Ukraine considering European standards in dentistry [4].

A practical dentist has a task to restore (replace) lost anatomical structures of the collateral process so that complete the result of successful implantation [1-13]. A scientist has a task to find an alternative for classical methods of prosthesis which would prevent development of inflammatory-dystrophic processes in the periodontal tissues of the adjacent teeth or implants [1-3, 5-14].

We consider that one of the ways of their

prevention and preservation of biogenic (osteogenic) potential is introducing modern tendencies of prosthetic methods [1-7].

Numerous studies are indicative of the fact that occlusive load on implants (Fig. 1, 2) is considerably bigger than on natural teeth [5, 6]. And due to the absence of periodontal ligaments round the implant masticating pressure is transmitted directly onto the bone [5]. Implant producers suggest different ways to reduce masticating pressure. For example, IM2-implants have a built-in internal shock absorber (Chapman R, Kirsch A). This design assumes reduced action of only vertical forces transmitting through the implant not changing pathogenic tangential load. Periodical weakening of screw fixation (Fig. 1), abutments and even fractures of screws (Lang M, 2008) are found most often.

In certain cases the liner of prosthetic structures is determined, and the degree of risk increases with the use of angular implants (Zoeller J, 2013; Kasiyanchuk M, 2016). The use of implant supra-structures is a pathogenic factor for the periosteum of the area round the implant [12]. Adjustment of prosthetic structures resting upon numerous implants and abutments in spite of their way of manufacturing – cast or milled by means of CAD / CAM – methods do not pass the test on adjacent precision (White, 1993; Eisenmann 2004; Kasiyanchuk M, 2012, 2016). In

case of dental defects implants as supporting elements, fixators and stabilizers of prosthesis are used as the only measure to satisfy the patient's requirements concerning the quality of prosthesis; and internal bony implants and prosthetic structures with discrete time of fixation on them are preferred. The tendencies of development of medical technologies and scientific research concerning the use of short implants $L < 7\text{mm}$: (Nevins M, 2010; Ewers R, Neugebauer J, 2016) promote introduction of own clinical methods considering European standards in dentistry [1-3, 5-14].

Available methods and materials do not involve possible clinical situations and all the possible factors of pathological effect in the periosteum area. Due to everything mentioned above on the basis of evidential medicine we have analyzed the efficacy of use of additional stabilization (splinting) in case of prosthetics on short



Fig.1. Clinical case: break of mesostructure on the implant.



Fig.2. Clinical case: the use of stabilizing structure of multiple mesostructures on implants (own finding).

multiple implants to perform organ-saving prosthetics when other methods are not guaranteed at the dental clinic (dental implants) and do not assume the possibility of mesostructure service. We have used the methods of prosthetic rehabilitation of a patient based on biophysical regularities considering reparative possibilities of anatomical structures by the principle of discrete fixation and correction of the prosthetic structure [11, 14].

Objective: to determine the efficacy of use of mesostructure stabilization method on multiple implants to perform organ-saving prosthetics, assess correlation dependence of anatomical structures on the external and internal effecting factors.

Methods. The osseous tissue of the collateral process after teeth loss is formed without load occurring during mastication and it is a powerful stimulator of the osseous tissue formation. From the other side, the process of bone remodeling is affected by a number of factors analyzed in our study: age and sex of the patient, characteristics of hormonal state and metabolism, harmful habits, and the quality of food.

Selecting patients for implant prosthetics we have found certain signs (clinical symptoms) indicative of the fact that occlusive load after conjunctive use of implants changes on all the areas of the occlusive surfaces with a certain frequency of occurrence. Odontoglyphic examination in the longitudinal aspect determines the symptoms of hyperfunction of the masticating apparatus. Clinical cases were visualized by means of our own method [11, 15]. Availability of occlusive contacts and occlusive load was determined by Bausch method.

The clinical study included 40 patients after prosthetics on implants examined during 2007-2017 on the base of the private specialized dental clinic in the town of Chernivtsi and Department of Orthopedic Dentistry at the Higher State Educational Establishment of Ukraine "Bukovinian State Medical University". Men-women ratio was 1:2, an average age of patients was $43 \pm 4,7$. Descriptive and analytical design of the study keeping to the requirements of clinical investigations (informed consent) was used. Operative and diagnostic manipulations were performed by one operator under standard

conditions. The stages of laboratory examinations were carried out by one specialist during a short period of time in a specialized dental-technical laboratory. Applying titanium (alloy of the same name) we suppose to reduce the risk to corrosion due to the difference of potentials occurring between the implant and mesostructure (Beliayeva L,1988). In the comparative aspect we have produced a splinting (stabilizing) element (Fig. 3, 4) by means of milling or casting: adjacent precision was compared.

The patients were divided into three groups: the main one (experimental) included patients (10 individuals), the ratio of the supra-structure > infra-structure of the implant, who underwent prosthetics and stabilization of mesostructures according to our own methods, and there is no need to correct orthopedic structure on the implant. For the comparative analysis we have applied a comparable method of prosthetics on implant used in case of narrow location of the adjacent teeth and the implants D 2.8 mm. The mesostructures were lined with polymeric ceramic (composite ceramic, composite).

In this case the covering material performs the role of a shock absorber of masticating pressure.

The patients (10 individuals) from the control group included those who underwent prosthetics according to our own methods: the ratio of supra-structure > infra-structure of the implant who experienced complications (fracture, inadequate wearing of a prosthetic structure), cosmetic defect due to the process of remodeling of anatomical structures of the collateral process in the perigingival area, etc.

The control second group (10 patients) included individuals who underwent prosthetics



Fig.3. Clinical case: odontoglyphic peculiarities during post-implantation period, 8 years (own finding).



Fig. 4. Laboratory stage: adjustment of discrete stabilized prosthetic structure.

according to our own method with the ratio of supra-structure > infra-structure of the implant, although additional stabilization of the orthopedic structure on the implant was not used.

The third control group (10 patients) included those who underwent prosthetics according to the standard methods with the ratio of supra-structure > infra-structure of the implant with complications (fractures, inadequate wearing of the prosthetic structure), cosmetic defect due to the process of remodeling of the anatomical structures of the collateral process in the perigingival area, etc.

In all the cases we have determined the condition of the prosthetic structure (wearing, fracture, fixation). Latent inflammatory process of the soft tissues was determined by means of Shiller-Pisarev test (Pisarev Yu, 1956). To assess the efficacy of the prosthetic method we have examined objective parameters of patients from the main and control groups: the width of the collateral process, degree of vertical resorption of the osseous tissue during 10 years of patients' observation.

According to the data of producers all the covering materials had comparable adhesive properties to titanium frames in case of keeping to technological processes and are intended for covering titanium mesostructures on implants.

To assess the efficacy of the implantation method we have studied objective parameters of the osseous tissue atrophy in the patients of the experimental and control groups: degree of the vertical resorption of the osseous tissue during 12

months of patients' observation. Discrete fixation of the mesostructures enabled us to remove by means of laboratory methods fractures, cosmetic defect due to the process of remodeling of the anatomical structures of the collateral process in the perigingival area, etc. Additional augmentation was performed due to cosmetic causes while assessing vertical resorption of the osseous tissue in 2 patients. Analytical conclusion and statistical analysis were made by the obtained results.

Further observation of patients from the main and control groups during 10 years determined the following complaints: periodical unpleasant sensations in the gums, the need of special hygiene of the inter-implant spaces, etc., the necessity to correct odontoglyphic peculiarities. Clinical cases were visualized by means of our own method [11, 15].

To assess the efficacy of the prosthetics method we have studied objective parameters of the patients from the main and control groups: the width of the collateral process, degree of the vertical resorption of the osseous tissue. The comparative analysis enabled to draw a differential conclusion (stratification of clinical signs), to predict biological regularities of use of discrete orthopedic structures on the angular implants.

Certain parameters of the osseous tissue are always correlatively connected with peculiarities of the life style and harmful habits of patients. And in this aspect it is rather topical. Therefore, analysis of this dependence will enable to predict or avoid possible complications in case of conjunctive use of implant systems. In the dynamics of observations we have followed a number of correlative relations between the parameters of the osseous tissue condition of the collateral process and internal and external factors. We have examined the correlative dependence of the anatomical structures in case of conjunctive use of the implant and mesostructure depending on the sex and age of the patient. These factors included both the factors of a positive effect (intake of calcium containing drugs, sufficient dairy products every day), and the factors of a negative effect (smoking, regular alcohol intake, thyroid diseases and osteoporosis). Both clusters of factors are

connected between themselves by means of a logical link.

Results. Statistical analysis while making examination is the following: in the process of the conjunctive use of implants occlusive load changes on all the links of the occlusive surfaces in 70% of occurrence including those 82% of cases when occlusive load increased (Fig. 2) and requires correction.

In clinical examinations included 40 patients after implant prosthetics: examination of patients from the main group during 3 months did not find complaints. Changes of the gingiva and the problems with further prosthetics were determined with reliably less frequency (Fig. 5). In the cosmetic aspect the patients of the main group also gain benefit from it.

In patients of the main and experimental groups (* - difference probability $p < 0,05$) further examination during 12 months did not find any complaints ($p < 0,05$).

To assess the efficacy of the implant method objective parameters of the osseous tissue atrophy were studied in the patients from the

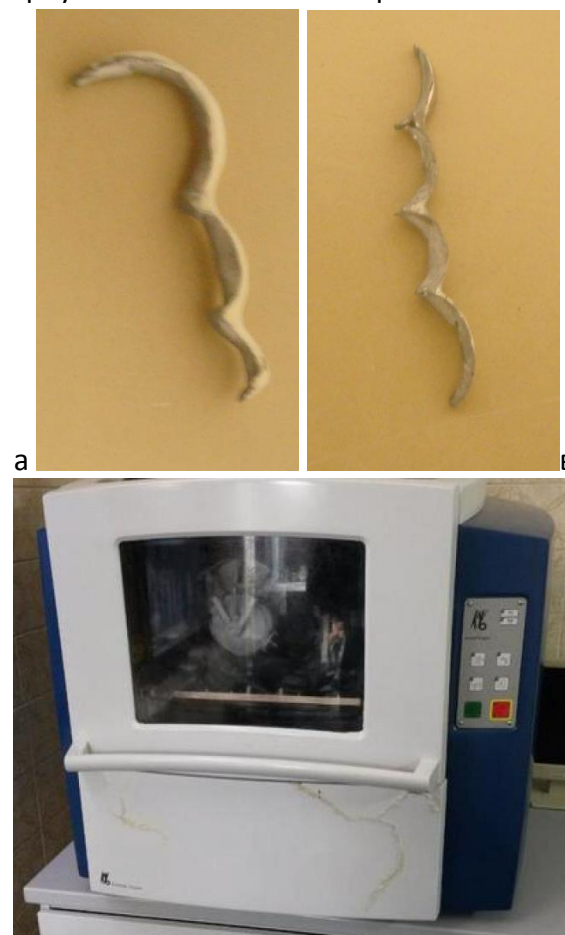


Fig. 5. Stabilizing (splitting) structures of mesostructures on implants produced by means of milling: in CAD / CAM.

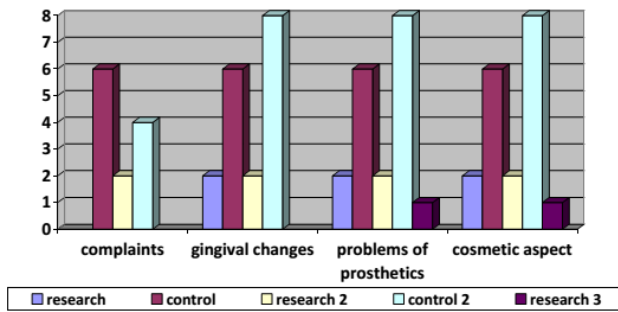


Fig. 6. Frequency of symptoms in the dynamics of post-operative period (3 months) in the patients of the main and experimental groups (difference probability $p < 0,05$).

main and control groups: degree of the vertical resorption of the osseous tissue during 12 months (Fig. 6.) of patients' observation. After assessment of vertical resorption of the osseous tissue, additional augmentation was performed in 2 patients of the main group due to cosmetic causes.

To assess the efficacy of the implant method objective parameters of 10 patients from the following groups were studied: the main one (with splinting) and the control group (without splinting) – the width of the collateral process, degree of the vertical resorption of the osseous tissue during 10 years of patients' observation. In the main and control groups the width of the collateral process after prosthetics differed reliably and was $7,4 \pm 2$ mm for both groups and $4,8 \pm 2$ mm for both groups ($p > 0,05$) now. Similar parameters are found in the assessment of vertical resorption of the osseous tissue.

Latent inflammatory process determined by means of Shiller-Pisarev test was found in 30% of cases in the control group and in 40% of cases of the main (experimental) group. Clinical manifestation of inflammation of the soft tissues was eliminated by the administration of laser therapy by means of the common methods.

We have found fractures of the covering in 20% of cases. Although, fractures of the covering were not diagnosed in case of inconsiderable width of the occlusive surface of the prosthetic structure concerning the diameter of the titanium frame (1/2), or the use of two supporting implants on one prosthetic structure in four patients of the control group (40%). In this group we did not find periodical weakening of the screw fixation of abutments which is the evidence of decreased

tangential dynamic load on the implants and peri-implant structures.

In the dynamics of observations we have followed the following regularities: the parameter of the bone proposition ($r = -0,44$, $p < 0,05$) and plane of the bone cut ($r = -0,39$, $p < 0,05$) had a negative correlation with thyroid diseases. We consider this effect is unlikely to be associated with the thyroid gland itself, but with the parathyroid glands regulating calcium metabolism, and which circulation can suffer due to pathology in this area.

Excessive body weight of the patient or obesity (body mass index (BMI) higher than 25 kg/m^2) had a negative correlation with the vertical size of the cellular bone in the dynamics ($r = -0,43$, $p < 0,05$). It also correlated positively with the bone type – the 3rd and the 4th type of the bone prevailed in those patients ($r = -0,46$, $p < 0,05$). Thus, this type of metabolic disorders correlated with more intensive course of atrophy. Correlative dependence of the collateral process tissue condition and the external and internal effect factors is graphically presented (Fig. 7, 8).

In case of necessity fractures of covering were corrected in laboratories due to discrete fixation (implanting) of the prosthetic structure making

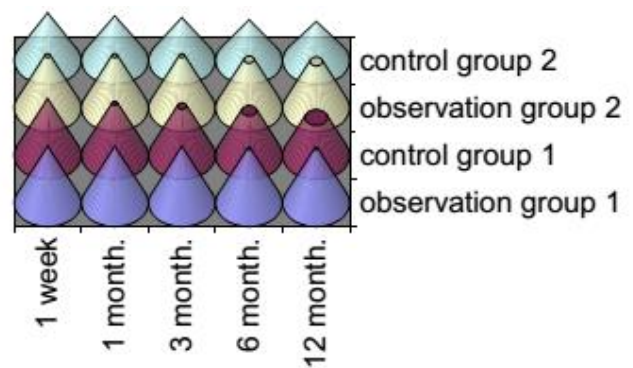


Fig. 7. Degree of vertical resorption (12 months).

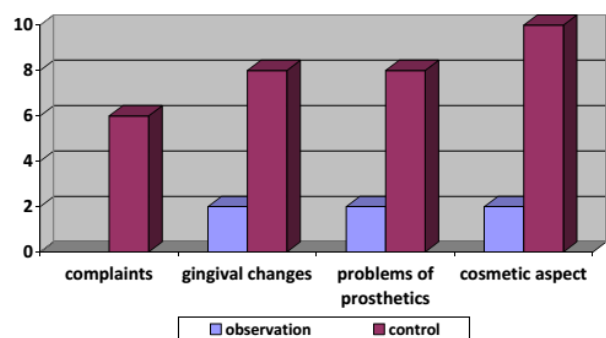


Fig. 8. Frequency of the symptoms in the dynamics in the longitudinal aspect (to 10 years) in patients of the main and experimental groups (difference probability $p < 0,05$).

the term of its service life longer. Unpredictable cementation loss was found in 2 cases of the main group. The problem was eliminated by means of a repeated fixation of the splinting structure.

Conclusion. After prosthetic treatment in the main group probable differences of the collateral process as compared to the control group were determined. After this prosthetic method was used, inconsiderable signs of atrophy of the collateral process in the periosteum area were detected after stabilizing remodeling as compared to the control group.

Conjunctive use of implants with corrected occlusive load of mesostructures can serve as a stabilizer of remodeling the periosteum of the peri-implant zone after teeth loss in the past. The suggested method improves the process of prosthetic rehabilitation, maintenance of favourable conditions of atrophic structures of the collateral process for the realization of the own human osteogenic potential in case of prosthesis of the lost teeth.

Prospects of further studies. Investigation and further development of prosthetic methods based on biophysical regularities considering reparative possibilities of the anatomical structures will enable to create a unique chance to preserve own human tissues, and favourable conditions for the service of dental implants.

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FEATURES OF THE MASTICATORY MUSCLE MICROCIRCULATORY BLOODSTREAM IN THE IN THE EARLY POST-TRAUMATIC PERIOD OF THE EXPERIMENTAL TRAUMA IN THE MAXILLOFACIAL AREA (literature review)

Abstract. *The paper presents the scientific substantiation, development and implementation into clinical practice of the results of experimental studies simulating disorders which tend to occur in the masticatory muscles in the injuries of the maxillofacial area and similar to those in polytrauma. We have studied the peculiarities of hematological changes with the presence of reactive shifts in the microcirculatory bloodstream in the muscles of the jaw apparatus of various functional types under the conditions of posttraumatic hypodynamia, attained through the biodeterioration effect of the experimental modeling. We have obtained new data on the adaptive capabilities of the chewing musculature microcirculatory bloodstream to normal muscular load after posttraumatic changes in the jaw apparatus in a model experiment on laboratory animals.*

Key words: *injuries to maxillofacial area, polytrauma, hematological changes, posttraumatic changes in masticatory muscles.*

Introduction. The problem of the traumatism in the population as a result of road traffic accidents and the presence of polytrauma in the victims are one of the topical areas in interdisciplinary research concerning both medical and social aspects and issues related to road safety [8].

Besides the overall increase in injuries, the frequency and severity of maxillofacial injuries and associated injuries are growing as well. This is evidenced by numerous data of domestic [4, 9] and foreign authors [13]. The proportion of maxillofacial injuries among the total number of bone injuries varies from 3.2 to 3.8% [7, 10]. The number of victims with facial traumas is from 21 to 40% in the structure of inpatients according to the materials of some authors [6]. Many authors note an increase in fractures of the facial bones with injuries to the muscular system by 10-15%, which should be taken into account when organizing specialized inpatient and outpatient care [1, 5, 11].

Methods. Modeling in clinical and experimental surgery has become one of the main methods of scientific research, allowing to accelerate the understanding of the key issues of the pathogenesis of extreme conditions, in particular, traumatogenesis and disorders, including those in the muscular apparatus of the facial skeleton in combined injury of the maxillofacial area, as well as to outline and substantiate the ways of their

prevention and treatment.

To implement the method of experimental modeling, we tested and applied a device for reproducing combined injuries of the face in a series of experiments on laboratory animals.

The proposed experimental model with the reproduction of this trauma in laboratory animals made it possible to trace possible features of hematological changes with the presence of reactive shifts in the system of the microcirculatory bloodstream in the muscles of the maxillary apparatus of various functional types in conditions of posttraumatic hypodynamia during reparative myogenesis similar to those occurring in victims with an identical trauma.

Objective. This paper was aimed at studying the dynamics of the hemogram indices in laboratory animals during reparative myogenesis, which causes a series of hematological changes with the presence of reactive shifts in the blood system, which are an integral part of the body's response to destructive and recovery processes that manifest themselves a great deal and are characterized by their size and duration.

Methods. The study of quantitative parameters of the peripheral blood was carried out on 20 clinically healthy rats-analogs. Before the experiment to study the quantitative changes in peripheral blood cells, 5 laboratory rats had been involved in the study to work out the bench mark (BM). The changes in the period of the reparative

process were studied after causing a complete fracture of the facial skull bones damaging the masticatory muscles of 15 rats. The analysis of the data was carried out with respect to the bench mark characterizing the physiological indices that we obtained in the process of preparing the experiment.

Under experimental conditions, the animals were traumatized to different parts of the facial skull injuring the chewing musculature in a single stage by means of a device for traumatization of laboratory animals (Patent of Ukraine 6548 of May 16, 2005).

By studying the rates of injuries resulting from accidents with maxillofacial trauma in Ukraine and CEE countries, we used the methods of mathematical statistics, econometric ones and methods of time series analysis. For calculations, Statistica and Excel packages were used, as well as the R package, in which the possibilities of using the methods of spatial econometrics and statistics with visual display of calculation results were realized [2, 3, 12].

Results. Analysing the erythroid component of peripheral blood (Table 1) showed that in the injured group, hematological parameters were represented by pronounced dynamics throughout the entire experiment. One day after the beginning of the reparative process of the chewing musculature, the number of red blood cells

decreased by 1.2 times (BM $7.8 \pm 0.2 \times 10^{12}/l$); hemoglobin - by 1,1; hematocrit – by 1.04 times. On the 2nd day of the experiment, we recorded an even greater loss of the red blood component. The quantitative indicator of erythrocytes decreased by 1.73 and amounted to $4.5 \pm 0.16 \times 10^{12} / l$; hemoglobin – by 1,4; The hematocrit decreased by 1.18 times. The color index, which characterizes the ratio of hemoglobin to erythrocyte, increased by 1.12-1.24 times. This process, in our opinion, characterizes the response of the body to blood loss, pain irritation and death of erythrocytes as a result of powerful metabolic stress in the post-traumatic period. Low quantitative values of the erythroid component of the peripheral blood were also determined on the 7-14th day of the experiment.

Erythrocytopenia was associated with the influence of acute-phase proteins and a damage to the erythrocyte membrane. There is a possibility of depositing blood in the central parenchymatous organs and stagnation in the area of trauma. Later on, there was a gradual recovery of red blood values to the level of physiological ones. On the 28th day, the number of erythrocytes increased to $6.0 \pm 0.13 \times 10^{12} / l$, although their level was 1.3 times lower in relation to the bench mark. During this period, the parameters of hemoglobin and hematocrit were restored, and the color index decreased insignificantly.

Table 1

Dynamics of the quantitative indicators of the erythrogram during the period of the rats' reparative myogenesis

Days	Erythrocytes, $x10^{12}/l$		HB, r%		Ht, %		Colour index r%		ESR, mm/h	
	M	m	M	m	M	m	M	m	M	m
BM	7,8	0,20	12,9	0,20	36,9	0,11	0,50	0,05	3,3	0,01
1	6,3	0,13	11,7	0,29	35,2	0,27	0,56	0,06	3,9	0,03
2	4,5	0,16	9,2	0,42	31,4	1,29	0,62	0,07	5,6	0,07
7	4,9	0,18	8,7	0,20	35,4	0,22	0,46	0,07	7,3	1,02
14	5,2	0,11	10,8	0,20	34,3	0,73	0,62	0,12	8,6	1,03
28	6,0	0,13	11,2	0,24	37,7	0,82	0,56	0,09	6,1	1,02
42	7,2	0,33	10,8	0,42	35,8	0,60	0,45	0,06	4,9	0,03
56	7,8	0,18	12,2	0,40	35,6	0,51	0,47	0,08	3,7	0,02

BM – bench mark

The indicators of quantitative changes in the red blood which are presented in Table 1, we regard as a creation of an adaptive buffer in response to a trauma of the maxillofacial area with injuries to the masticatory muscles, as well as a compensation for the acidotic state that occurs after the damage to even a part of the muscle mass. During the final days of the experiment (on the 42-56th days), the index of the erythrocytic link

of the peripheral blood reached the bench mark ($7.8 \pm 0.18 \times 10^{12} / L$). The color index of the peripheral blood on the 42nd and 56th days did not reliably differ from the initial data.

At the same time, the quantitative values characterizing the parameters of hematocrit and hemoglobin reached the physiological values as well. Post-traumatic period, accompanied by the development of the inflammatory reaction, was

recorded by characteristic changes in the rate of erythrocyte sedimentation. On the 14th day after the injury, the erythrocyte sedimentation rate increased by 2.61 times in comparison with the bench mark. Later on, there was a gradual decrease in this index characterizing the weakening of the inflammatory reaction.

Thus, the erythrograms of experimental rats show a direct correlation between the changes caused by a trauma in the maxillofacial region with injuries to the masticatory muscles, which is manifested by loss of erythrocytic mass during 14 days and restoration of the functional activity of the red blood by the 28th day.

In the further stages of the experiment, the leukograms of the peripheral blood (Table 2) revealed a marked leukocyte reaction, which was manifested by an increase in the number of leukocytes on the first day after modeling a trauma in the maxillofacial region by 1.4 times compared to the initial index ($7.2 \pm 0, 24 \times 10^9/l$).

By the second day the number of leukocytes in the blood of rats had decreased, but had significantly exceeded the initial values. A gradual decrease in leukocytes was noted until the 28th day, after which the period of an increase in the quantitative index of leukocytes in the peripheral

blood of experimental animals occurred. The dynamics of stab neutrophils (SNP) in the injured group compared to the bench mark ($6.8 \pm 0.29 \times 10^9 / l$) is as follows: during the first days the increase in this indicator was observed by 2.1 times, and starting with the 7th day, there was a persistent tendency to its reduction. During the final days of the model experiment with the damage to the chewing muscular apparatus of the maxillofacial region, the number of stab neutrophils increased slightly. The content of segmented neutrophils in the injured group in relation to the bench mark ($31.6 \pm 1.6 \%$) was marked by a steady increase through the entire experiment. When studying the dynamics of lymphocytes, we established that on the first and second days their number decreased by 1.3-1.34 times, respectively, relative to the BM ($58.0 \pm 3.11 \%$). The lymphatic cells tended to accumulate starting with the 7th day of the post-traumatic period. A slight decrease on the 42nd day changed into an increase in the percentage of lymphocytes by the 56th day.

The number of eosinophilic granulocytes in the injured group, relative to the bench mark, only increased in the initial post-traumatic period, but later on their dynamics remained rather even. The

Table 2

Dynamics of the leucogram figures in the period of reparative osteogenesis in rats

Days	Leucocytes, $\times 10^9/l$		Lymphocytes, %		Neutrophils, %				Basophils		Eosinophils		Monocytes	
					Stab		Segmented							
	M	m	M	m	M	m	M	m	M	m	M	m	M	m
BM	7,2	0,24	58,0	z,i	6,8	0,29	31,6	1,60	0,6	0,06	2,0	0,06	1,5	0,0
1	10,	0,18	44,0	2,67	15,	0,89	36,0	0,89	-	-•	1,3	0,03	3,3	0,0
2	8,1	0,11	43,1	0,67	14,	0,44	38,3	1,11	0,3	0,03	2,3	0,07	2,0	0,0
7	7,6	0,16	46,3	1,11	12,	0,44	37,7	0,67	0,7	0,03	2,7	0,03	1,2	0,0
14	6,8	0,29	48,3	1,11	9,1	0,44	39,3	1,56	0,7	0,06	1,3	0,04	2,0	0,0
28	6,3	0,38	55,3	1,11	4,2	0,00	38,3	1,56	0,3	0,03	1,5	0,07	0,7	0,0
42	6,8	0,24	49,7	2,89	5,7	0,44	43,3	4,70	-	-	0,9	0,03	0,7	0,0
56	5,6	0,78	58,7	1,56	5,3	0,44	35,3	1,11	-	-	0,3	0,01	1,3	0,0

BM- bench mark

study of the percentage of basophils in the group revealed a decrease in their number on the 2nd day of the experiment, their number increased by the 7th day and the figure remained at this level until the 8th day. In later terms, they were not noticed in the leukogram. The number of monocytes in rats (Table 2) on the 1st day of trauma increased by 2.54 times in comparison with the bench mark ($1.5 \pm 0.04\%$). By the 7th day, the monocyte content decreased and reached the level of the initial data. By the 14th day they increased by 1.54 times in comparison with the bench mark. On the

28th and 42nd days, the level of monocytes started to decrease, and by the 56th day it reached the level of physiological values.

Discussion. The presence of the above changes in the hemogram is regarded as an indicator of an adaptive response of the body, suggesting the development of common processes characterizing the post-traumatic period, which makes it possible to predict and control the course of reparative transformations in the masticatory muscles of laboratory animals.

Conclusions. Thus, in laboratory animals, the

effect of the experimental polytrauma with a damage to the chewing musculature in case of a trauma to the maxillofacial area on the reparative capabilities of muscle tissue in the early post-traumatic period has been studied in detail. When comparing the results obtained in the study, we established the nature of adaptive reaction of the body based on the hematological indices of red and white blood under the influence of the polytrauma.

At the same time, the erythrograms of the experimental rats showed a direct dependence of the changes caused by a trauma to the the maxillofacial area with a damage to the masticatory muscles, manifested by the loss of erythrocyte mass during 14 days and restoration of the functional activity of red blood by the 28th day. Also, a pronounced leukocyte reaction was revealed, which was manifested by an increase in the number of white blood cells on the first day after modeling the trauma of the maxillofacial region, reaching the level of physiological values at the end of the experiment. At the same time, the erythrograms of the experimental rats showed a direct correlation between the changes caused by the trauma of the maxillofacial region and the damage to the masticatory muscles, which is manifested by the loss of erythrocyte mass during 14 days and restoration of the functional activity of red blood by the 28th day. We have also found a pronounced leukocyte reaction, which was manifested by an increase in the number of white blood cells on the first day after modeling the trauma of the maxillofacial region, reaching the level of physiological values at the end of the experiment.

These experimental studies broadened the awareness and confirmed the priority of metabolic disorders in a polytrauma, allowed to provide a prognostic assessment of the further development of the pathological process and to substantiate the tactics of management of such patients in the early period of combined maxillofacial trauma.

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MISCARRIAGE PREVENTION IN MULTIPARA WOMEN IN THE SECOND TRIMESTER OF PREGNANCY

Abstract. *The efficacy of the suggested methods to prevent miscarriage is in reliable and considerable decrease of perinatal complications occurring during pregnancy of multipara women and in particular: gestation anemia (in 2,5 times), threat of preterm delivery (in 4,6 times), placental dysfunction (in 2,6 times), disorders of vaginal microbiocenosis (in 2,2 times), preterm delivery (in 2,8 times), premature rupture of the amniotic sac (in 2,6 times), developmental retardation of the fetus (in 3,2 times), fetal distress (in 3,6 times), and abdominal delivery (in 2,9 times).*

Key words: *miscarriage, multipara women, prevention.*

Introduction. Increase of the number of multipara women is a topical issue for obstetrical and perinatal pathology. It is a well-known fact that women after the third labour constitute a high risk group concerning the development of various complications during pregnancy and labour. Among the main causes of this situation there is a high level of comorbid extra-genital pathology, psycho-emotional tension, and poor economic situation. In spite of numerous publications in modern literature concerning pregnancies and labour in multipara women all the issues cannot be considered solved completely.

One of the most important complications of women from this group is the danger of miscarriage. From one side it is caused by a high risk of preterm labour, and from another side – the absence of an accurate algorithm and specific practical recommendations concerning this issue.

A topical issue to prevent preterm labour in women who had three and more labours in their anamnesis is that it is better to prevent the birth of a preterm child than to fight with its consequences and neonatal complications after delivery. Elaboration of priority effective preventive measures and detection of risk factors promoting the development of preterm labour in women of this group will enable to prevent the birth of deeply preterm children and decrease the number of perinatal complications.

One of the reliable and objective risk factors

promoting development of preterm labour is uterine cervix failure. This risk factor was determined by the data of the retrospective analysis as well as during instrumental methods of examination during pregnancy – vaginal cervicometria. Diagnosed isthmio-cervical failure (ICF) is one of the valuable causes of miscarriage in II and III trimesters of gestation. According to the information suggested by leading specialists, timely diagnostics and treatment of ICF is an important measure to prevent preterm labour.

The necessity to improve the methods of diagnostics and treatment of ICF during pregnancy in multipara women is indicative of the topicality of the investigated scientific issue in solution the problem and decrease of obstetrical and perinatal pathology.

Objective: to reduce the frequency of miscarriage in II and III trimester and perinatal pathology in multipara women on the basis of investigation of anamnesis, echographic, endocrinological, biochemical and organic peculiarities, as well as by means of elaboration and introduction of an advanced algorithm of therapeutic-preventive measures.

Materials and methods. Retrospective analysis and the study were carried out in the dynamics during 2014-2017 on the basis of Maternity Home №2 in Chernivtsi at the Department of Obstetrics, Gynecology and Perinatology. According to the objective set the study was carried out into two stages.

I stage (retrospective study) – 110 women were examined including 60 multipara women with the signs of ICF. This group was divided into 2 subgroups: I subgroup (the main one) – 30 pregnant multipara women with preterm labour in anamnesis, and with an improved algorithm of therapeutic-preventive measures used during pregnancy directed to prevention of miscarriage;

II subgroup (the main one) – 30 multipara women with preterm labour in anamnesis receiving common therapeutic-preventive measures.

The control group included 50 healthy women without preterm labour in anamnesis.

All the pregnant women were comprehensively clinically examined considering their complaints, the data of anamnesis, objective and instrumental methods of examination. Women with signs of inflammatory diseases of the female reproductive organs were not included into the study. The applied methods of examination are safe for the development of pregnancy, mother and fetus, rather informative for objective assessment of the functional state of the uterine-placental-fetal complex. All the women were examined by means of similar methods, and the same devices were used including the influence of a possible error peculiar for any invasive method of examination.

General management of women during pregnancy was carried out according to the recommendations and Orders of the Ministry of Public Health of Ukraine (№417 dated 15.07.2011 «On Organization of Out-Patient Obstetrical-Gynecologic Aid in Ukraine», № 906 dated 27.12.2006 «On Approval of Clinical Protocol in Obstetrical Aid “Perinatal Infection”, and №624 dated 03.11.2008 «On Approval Clinical Protocols in Obstetrics and Gynecology «Preterm Labour»). The women from II subgroup of the main group were treated according to the common therapeutic-preventive measures according to the protocols of the Ministry of Public Health of Ukraine including hormonal correction, vitamins, antioxidants, spasmolytics and anti-aggregants, drugs of tocolytic action, vasoactive drugs and antibacterial drugs according to indications.

We have improved the preventive method, which was the following:

1. The following parameters were used in case of diagnosed ICF by means of trans-vaginal

cervicometria performed at the period of 18-22 weeks of pregnancy: isthmic-cervical coefficient (ICC) and isthmic coefficient (IC). In case of $ICC > 0,22$ and $IC > 1,6$ the diagnosis of ICF was made.

- The obstetrical Arabin cervical pessary is used with the purpose to continue pregnancy. USD (trans-vaginal cervicometria) determined the condition of the uterine cervix and its parameters – the length of the uterine cervix canal, diameter of the internal orifice, the length of isthmus, and on the basis of these parameters ICC and IC were calculated. In case of ICC more than 0,22 and IC more than 1,6 ICF was diagnosed and Arabin cervical pessary was placed. Indications to place Arabin cervical pessary:

- Functional and organic ICF
- Prevention of ICF in pregnant women
- Prevention of suture failure in case of surgical correction of ICF.

2. Administration of a comprehensive maintaining therapy:

- Progesterone (in the dose of 200 mg/day till 37 weeks of gestation)
- A complex medicine: iron hydroxide, polymaltose (100 mg)+ folic acid (0,35 mg) per 1 tablet 2 times a day till hemoglobin level is normal, and then 1 tablet till delivery;
- From the moment of placement of the pessary – vaginal suppositories with chlorhexidine (once a day) and probiotics (vaginal capsules once a day).
- L- arginine (500 mg during 10 days).

The course of the suggested method was 10-14 days in the term of 22-24 weeks and 32-34 weeks of gestation. The terms were selected considering peculiarities of placentogenesis and stages of formation of the fetal-placental complex (FPC) and common critical terms of gestation. The duration and quality of the suggested preventive measures depended on the results of the instrumental methods of examination.

The complex of the examinations performed included statistical, clinical, echographic, cardiotocographic, Doppler, laboratory findings.

Results and discussion. The results of the studies conducted indicate that the main risk factors of development of preterm delivery (in 82,9% in the term to 32 weeks) in multipara women are: infectious diseases of the urinary tract (26,4%), bacterial vaginosis (51,7%),

comorbid extragenital pathology (68,6%), menstrual function disorders (48,9%), early beginning of the sexual life (89,4%), lack of contraceptive methods (94,7%).

It should be noted that pregnancy and labour in multipara women occur with a high frequency of different complications: threat of miscarriage in early terms (61%; $p < 0,05$), threat of preterm delivery (63,4%, $p < 0,05$), bacterial vaginosis (48,6%), gestation anemia (56,7%), ICF (52,3%), placental dysfunction (38,6%), fetal hypoxia (57,6%), developmental retardation of the fetus (31,5%), premature rupture of the amniotic sac (44,3%), preterm delivery (27,8%), causing a high percentage of cesarean sections (27,6%).

Perinatal consequences in multipara women are characterized by rather high percentage of asphyxia of a newborn (31,2%) and posthypoxic encephalopathy (29,8%), causing a high frequency of perinatal loss.

The functional state of the FPC in multipara women is characterized by hormonal disorders: increased cortisone level ($p < 0,01$), decreased progesterone content ($p < 0,05$), placental lactogen ($p < 0,05$) and estriol ($p < 0,01$), increased placental index (PI) in the uterine ($p < 0,05$) and cerebral arteries ($p < 0,05$), reliable increase of circulation flow in the venous duct ($p < 0,05$), and reliable decrease of placental coefficient ($p < 0,05$).

Assessment of microbiological status in multipara women determined dysbiotic changes, especially before delivery in the form of detection of the third and fourth cleanliness of the vagina (26,7%), increase of non-specific vaginitis (21,8%), and bacterial vaginosis (56,8%).

Conclusions. The efficacy of the suggested methods to prevent miscarriage is in reliable and considerable decrease of perinatal complications occurring during pregnancy of multipara women and in particular: gestation anemia (in 2,5 times), threat of preterm delivery (in 4,6 times), placental dysfunction (in 2,6 times), disorders of vaginal microbiocenosis (in 2,2 times), preterm delivery (in 2,8 times), premature rupture of the amniotic sac (in 2,6 times), developmental retardation of the fetus (in 3,2 times), fetal distress (in 3,6 times), and abdominal delivery (in 2,9 times).

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PROGNOSTICATION OF DEVELOPMENT OF PLACENTAL DYSFUNCTION AND GESTATIONAL COMPLICATIONS IN WOMEN WITH VARICOSE VEINS

Abstract. To apply the correlation method of sign with the purpose to prognosticate the development of placental dysfunction and certain complications of pregnancy in women with varicose veins. We have calculated coefficients of sign correlation concerning the possibility to prognosticate the most important parameters of the occurrence of placental dysfunction and certain complications of pregnancy in women of the main group. The correlation method enables to carry out deeper analysis of pathogenic relations existing between different processes during pregnancy in women with varicose veins and obtain new considerable data concerning the efficacy of the applied methods to prevent placental dysfunction.

Key words: placental dysfunction, varicose, veins.

Introduction. To prognosticate placental dysfunction and certain complications of pregnancy in every individual case the correlation method of signs has been applied enabling to assess the degree of relations between certain clinical symptoms (by an appropriate parameter deviated from the norm) when certain pathology occurs. In case of closer correlation it will enable to prognosticate more substantially occurrence of obstetrical pathology in pregnant women with varicose veins. This method assumes calculation of sign correlation coefficient measuring a relative power of relations between two alternative parameters.

Objective: to apply the correlation method of sign with the purpose to prognosticate the development of placental dysfunction and certain complications of pregnancy in women with varicose veins.

Materials and methods. We have calculated coefficients of sign correlation concerning the possibility to prognosticate the most important parameters of the occurrence of placental dysfunction and certain complications of pregnancy in women of the main group.

Availability of correlations was determined with probability level of correlation coefficient $p \leq 0,05$. The form of connection was assessed according to the analysis of the coefficients of the linear and non-linear correlation and its errors. It should be noted that concerning all the examined relations the largest evidence was obtained concerning their linear form, therefore, the value of Pearson linear correlation coefficient was applied as the value of connection strength.

Determined correlation coefficients differed not only in their values but the sign as well, which is marked by means of the symbols «+» (directly proportional relation) and «-» (inversely proportional relation).

Before presenting the results of the study it should be noted that correlation coefficients between the pairs of parameters were determined in every group of the study separately using a certain principle of division into subgroups.

The main group (pregnant women with varicose veins) was divided into two subgroups: I subgroup – pregnant women with varicose veins in compensation stage (n=26), II subgroup – pregnant women with varicose veins in subcompensation stage (n=24).

Results. The data obtained are presented in Table 1. Deviation from the norm of every analyzed parameter enables to prognosticate with high accuracy development of placental dysfunction in pregnant women with varicose veins in the stage of compensation and subcompensation.

The parameters to prognosticate the danger of interruption of pregnancy are presented in Table 2. It should be noted that the danger of interruption of pregnancy is the most prognosticated in case of deviation of systolic-diastolic index in the uterine arteries and decrease of the spiral arteries with complete gestational rebuilding, and both these parameters are directly correlated.

The parameters to prognosticate a partial exfoliation of the chorion and placenta are presented in Table 3.

Table 1

Sign correlation coefficients (SCC) and the value of “positive prognosticated assessment” (PPA) parameter concerning the development of placental dysfunction and the most important parameters of the fetal-placental complex indices in case of varicose veins.

Parameters	Main group (pregnant women with varicose veins in compensation stage) n=26	Main group (pregnant women with varicose veins in subcompensation stage) n=24
Above normal decrease of placental lactogen concentration in the blood serum of a pregnant woman	SCC = +0,751 PPA=0,833	SCC = +0,769 PPA =0,851
Above normal decrease of placental lactogen concentration in the trophoblast of the placental villi	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Above normal decrease of estradiol concentration in the blood serum of a pregnant woman	SCC = +0,724 PPA =0,817	SCC = +0,721 PPA =0,809
Above normal decrease of progesterone concentration in the blood serum of a pregnant woman	SCC = +0,512 PPA =0,642	SCC = +0,509 PPA =0,615
Above normal increase of systolic-diastolic index in the uterine arteries	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Above normal decrease of spiral arteries with complete gestational rebuilding	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Immature chorial tree	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851

Table 2

Sign correlation coefficients (SCC) and the value of “positive prognosticated assessment” (PPA) parameter concerning the danger of interruption of pregnancy and the most important indices of the fetal-placental complex parameters in women with varicose veins

Parameters	Main group (pregnant women with varicose veins in compensation stage) n=26	Main group (pregnant women with varicose veins in subcompensation stage) n=24
Above normal decrease of placental lactogen concentration in the blood serum of a pregnant woman	SCC= +0,512 PPA=0,642	SCC = +0,509 PPA =0,615
Above normal decrease of placental lactogen concentration in the trophoblast of the placental villi	SCC = +0,512 PPA =0,642	SCC = +0,509 PPA =0,615
Above normal decrease of estradiol concentration in the blood serum of a pregnant woman	SCC = +0,512 PPA =0,642	SCC = +0,509 PPA =0,615
Above normal decrease of progesterone concentration in the blood serum of a pregnant woman	SCC = +0,536 PPA =0,671	SCC = +0,539 PPA =0,686
Above normal increase of systolic-diastolic index in the uterine arteries	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Above normal decrease of spiral arteries with complete gestational rebuilding	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Immature chorial tree	SCC = +0,512 PPA =0,642	SCC = +0,509 PPA =0,615

Table 3

Sign correlation coefficients (SCC) and the value of “positive prognosticated assessment” (PPA) parameter concerning a partial exfoliation of the chorion and placenta of the fetal-placental complex condition in women with varicose veins.

Parameters	Main group (pregnant women with varicose veins in compensation stage) n=26	Main group (pregnant women with varicose veins in subcompensation stage) n=24
Above normal decrease of placental lactogen concentration in the blood serum of a pregnant woman	SCC= +0,417 PPA=0,523	SCC = +0,406 PPA =0,509
Above normal decrease of placental lactogen concentration in the trophoblast of the placental villi	SCC = +0,417 PPA =0,523	SCC = +0,406 PPA =0,509
Above normal decrease of estradiol concentration in the blood serum of a pregnant woman	SCC = +0,417 PPA =0,523	SCC = +0,406 PPA =0,509
Above normal decrease of progesterone concentration in the blood serum of a pregnant woman	SCC = +0,417 PPA =0,523	SCC = +0,406 PPA =0,509
Above normal increase of systolic-diastolic index in the uterine arteries	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Above normal decrease of spiral arteries with complete gestational rebuilding	SCC = +0,751 PPA =0,833	SCC = +0,769 PPA =0,851
Immature chorial tree	SCC = +0,417 PPA =0,523	SCC = +0,406 PPA =0,509

According to the data presented in Table 3 a partial exfoliation of the chorion and placenta is most reliably prognosticated in case of disorders of the fetal-placental circulation.

Discussion. The results of the studies presented and the analysis of prognostication of placental dysfunction, danger of interruption of pregnancy enabled to suggest that there are certain interrelations between the investigated processes characterizing different sides of the development of placental dysfunction in the main group of women. With the purpose to analyze possible pathogenic relations the correlation method was applied which enables to determine their availability, form and strength with determined degree of probability.

Conclusion. The correlation method enables to carry out deeper analysis of pathogenic relations existing between different processes during pregnancy in women with varicose veins and obtain new considerable data concerning the efficacy of the applied methods to prevent placental dysfunction.

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HISTOLOGICAL STRUCTURE OF AORTAL WALL IN RATS AND BRANCHES OF ITS BLOOD CIRCULATION AT THE LATE STAGES OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

Abstract. *Diabetes mellitus being considerably spread touches upon the interests of people of different ages and is characterized by early disability and high mortality. Till 2030 the number of patients suffering from diabetes mellitus is expected to reach 552 million (9,9% or 1 diabetic patient per 10 healthy adults), and till 2035 – to 592 million (10,1%). The most common complications of diabetes mellitus are vascular disorders known as “diabetic angiopathy”. This notion includes lesions of small vessels – capillaries, venules and arterioles (diabetic microangiopathy), and large arteries (diabetic macroangiopathy). Investigations of many researchers deal with this pathology, although there is no a universally accepted and generalized view concerning morphogenesis of diabetic angiopathy, pathogenic factors, possibilities of prognostication and early control over the process development and its prevention. Therefore, the **objective** of our study was to perform histological investigation of the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of streptozotocin-induced insulin dependent diabetes mellitus. 26 mature albino male rats of “Wistar” line with the body weight of 100-160 g were used as the material of the study. Experimental diabetes mellitus was simulated by means of a single intraperitoneal injection of streptozotocin produced by “Sigma” in the dose of 7 mg per 100 g of the body weight (prepared on 0,1 mole of citrate buffer, pH = 4,5). The control group included intact albino rats of similar weight, sex and age. The development of diabetes mellitus during 2 weeks was controlled by the increasing glucose level in the blood measured by means of glucose oxidase method. The samples for histological examination were pieces of the ascending portion, arch and descending portion of the aorta. The specimens were stained with azan according to Heidenhain, and the nuclei – with Weigert’s iron hematoxylin. The specimens were examined and photos were made under the microscope МБИ–1 with magnification (ocular 7, objective lens 8), (ocular 10, objective lens 8), (ocular 7, objective lens 20), (ocular 10, objective lens 20). The results of the study were indicative of the fact that in the majority of animals after 6 weeks of streptozotocin-induced diabetes mellitus the aorta preserved its macroscopic structure. Histological examinations of the sections of the aortic wall of rats enables to find the signs of development of diabetic macroangiopathy, that is, atherosclerotic disorders of the endothelial layer of the vascular wall in the form of fatty consolidation (fatty stripes) occupying 30-50% of the internal aortic surface. The end of the sixth week of the experiment was characterized by the signs of arterial hyalinosis and fine inflammatory infiltrations represented by macrophagocytes and lymphocytes. After 8 weeks of streptozotocin-induced diabetes mellitus the internal surface of the aortic wall became dark brown in colour and tuberos, the intima was irregular, whitish in colour, and all its surface containing tubercles and depressions. Elastic fibers of the median membrane are not well-organized, inter-laminar spaces are wide with loss of inter-laminar relations, especially expressed in the external layers of the median membrane. The links of blood circulation are dilated, arterioles with irregular outlines, dilated venules, and swelling round the vessels are found in certain areas.*

Key words: aorta, atherosclerosis, microstructure, blood circulation, streptozotocin, diabetes mellitus, albino rat.

Introduction. Diabetes mellitus (DM) is one of nowadays and it gets priority in the national public the most serious medical-social issues of health systems practically in all the countries of

the world.

In recent years DM has occupied the third position among direct causes of death after cardiovascular and oncological diseases. Social consequences of DM are unfavourable as well, since the control and therapy of the disease require heavy expenses from the health care systems.

According to the data of the World Health Organization (WHO) and International Diabetic Federation (IDF) the number of diabetic patients in the world in 1985 was 30 million among the adult population aged from 20 to 79; in 1995 it was 135 million of the population; in 2000 it numbered 150,9 million (4,6%), in 2003 – 194 million (5,1%), in 2010 – 285 million (6,4%), in 2011– 366 million of the population (8,3%), 2012– 371 million (8,3%), and in 2013 – 382 million (8,3%) of diabetic patients [3, 4, 7, 11, 15, 18]. Till 2030 the number of diabetic patients is suggested to become 552 million (9,9% or 1 diabetic patient per 10 healthy adults), and till 2035 – to 592 million (10,1%) [19].

The most common complications of diabetes mellitus are vascular disorders known as “diabetic angiopathy”. This notion includes lesions of small vessels – capillaries, venules and arterioles (diabetic microangiopathy), and large arteries (diabetic macroangiopathy). Diabetic angiopathy remains the main causes of inability to work of diabetic patients and in the majority of cases it determines the life prognosis for a patient.

Diabetic macroangiopathy are atherosclerotic by its origin.

Damage of the major vessels in diabetic patients occurs in the following forms: a) atherosclerosis – fatty plaques on the intima, b) calcifying Monckeberg’s sclerosis, c) diffuse fibrosis of the intima. These forms of lesions are found in the coronary, cerebral, renal arteries, arteries of the upper and lower limbs. Atherosclerotic lesions of the large and small vessels do not practically differ from atherosclerotic lesions of those individuals who do not suffer from DM [1, 8].

The risk of macroangiopathy is caused by such factors as hypertension, excessive body weight, hyperinsulinemia, smoking, metabolic disorders of cholesterol, lipids and lipoproteids, hemorheological disorders and hyperglycemia [1,

12].

Diabetic microangiopathy is characterized by thickening of the basal membrane, proliferation of the endothelium and accumulation of an excessive amount of PAS-positive substances. Microangiopathy may be of two types: a) hyaline thickening of the arteriole walls, b) dilation of venules and thickening of the capillary walls [9, 10, 20].

Thickening of the basal membrane does not depend on age before the onset of the disease, hyperglycemia degree, residual ability to insulin secretion, frequency of ketoacidosis conditions, hypoglycemia and the type of therapy administered.

Pathogenesis of diabetic microangiopathy is characterized by the following factors: deterioration of the blood circulation resulting in hypoxia and decreased supply of the endothelium; carbohydrate metabolic disorders or a complex of polysaccharides (glycosaminoglycan) in the basal membrane of capillaries and connective tissue; glycosylation of proteins and accumulation of the final glycosylation junctions; decreased ability of the erythrocytes to deformity resulting in increased pressure in the capillaries and thickening of the basal membrane; accumulation of the immune complexes in the basal membrane and extracellular matrix followed by disorders of phagocytic activity of the basal membrane cells and humoral-mediated gene expression of different proteins; increased permeability of the vascular wall for plasma proteins or other macromolecules; microcirculation disorders [16].

In addition, other mechanisms participate in pathogenesis of diabetic microangiopathy: hormonal – elevation and fluctuation of somatogenic hormone, ACTH, cortisol, aldosterone and catecholamine levels in the blood. The role of “local” hormones is not excluded: bradykinin, serotonin and prostaglandins.

Genetic factors play a certain role in the development of diabetic angiopathy. Generalized character of diabetic microangiopathy and its combinations cause different forms of lesions in the clinics.

The problem of macro- and microvascular complications in case of DM is topical and

considerably determines the course and development of the disease. Investigations of many researchers deal with this pathology, although there is no a universally accepted and generalized view concerning morphogenesis of diabetic angiopathy, pathogenic factors, possibilities of prognostication and early control over the process development and its prevention. Therefore, the task of our study was to investigate the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of experimental diabetes mellitus. The article is a fragment of the scientific-research work of Danylo Halytskyi Lviv National Medical University "Morphological peculiarities of the blood circulation in the aortic wall of a rat within the norm and in case of experimental diabetes mellitus".

Objective of the study: to perform histological investigation of the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of streptozotocin-induced insulin dependent diabetes mellitus.

Materials and methods. 26 mature albino male rats of "Wistar" line with the body weight of 100-160 g were used as the material of the study. All the animals were kept in the vivarium, and the study was performed according to the "Regulations of Conducting Studies with the Use of Experimental Animals". Experimental diabetes mellitus was simulated by means of a single intraperitoneal injection of streptozotocin produced by "Sigma" in the dose of 7 mg per 100 g of the body weight (prepared on 0,1 mole of citrate buffer, pH = 4,5). The control group included intact albino rats of similar weight, sex and age. The development of diabetes mellitus during 2 weeks was controlled by the increasing glucose level in the blood measured by means of glucose oxidase method. The study was carried out since the second week of the experiment on animals with glucose level over 13,48 millimole per 1 liter. Three groups of animals were used in the study: 1) 10 intact rats; 2) 8 rats (5+3 control) with DM developed (6 weeks after streptozotocin injection); 3) 8 rats (5+3 control) with DM developed (8 weeks after streptozotocin injection). The material for histological examinations was taken after euthanasia of rats by means of intraperitoneal injection of sodium

thiopental in the dose of 25 mg per 1 kg of the body weight. The samples for histological examination were pieces of the ascending portion, arch and descending portion of the aorta. Before fixation the material was washed in warm physiological solution. The material was fixed in 10% formalin solution during 24 hours prepared directly before its use. After fixation the material was washed under running water.

Numbered and stitched into gauze sacs pieces of the tissue were washed under running water during 24 hours. The material was dehydrated in ethyl alcohol of an increasing concentration during 20 hours: 73 ° ethyl alcohol; 80 ° ethyl alcohol; 86 ° ethyl alcohol; 86 ° ethyl alcohol; 96 ° ethyl alcohol; 96° ethyl alcohol.

Blooming and removing ethyl alcohol was made in organic solutions (xylene or chloroform – 2 portions per 1 hour each).

Paraffin infiltration of the samples was made in 2 dishes-thermostats at the temperature of 56° during 2 hours. Then the material was filled in paraffin blocks. The material filled in blocks was fixed and cut on the sliding microtome – MC–1 5–7 mcm thick. The specimens were stained with azan according to Heidenhain, and the nuclei – with Weigert's iron hematoxylin [9]. Then the stained specimens were placed into Canadian balsam (dissolved in toluene and xylene) and dried in the dry box. The specimens were examined and photos were made under the microscope МБИ–1 with magnification (ocular 7, objective lens 8), (ocular 10, objective lens 8), (ocular 7, objective lens 20), (ocular 10, objective lens 20).

Results and discussion. In the majority of animals after 6 weeks of streptozotocin-induced diabetes mellitus the aorta preserved its macroscopic structure. Histological examinations of the sections of the aortic wall of rats enables to find the signs of development of diabetic macroangiopathy, that is, atherosclerotic disorders of the endothelial layer of the vascular wall in the form of fatty consolidation (fatty stripes) occupying 30-50% of the internal aortic surface. These are massive fatty deposits of pale yellow colour mostly consisting of macrophages containing a number of lipids with a minimal amount of smooth muscular cells (Fig. 1).

According to the data presented in the professional literature [1] such kinds of changes

do not result in vascular occlusion and therefore do not stipulate pronounced clinical manifestation. The end of the sixth week of the experimental DM was characterized by the signs of arteriole hyalinosis and fine inflammatory infiltrations in the form of macrophagocytes and lymphocytes. The walls of the blood circulatory bed are thickened and eosinophilic (Fig. 2).

Microaneurysms of the arterioles are found. The internal membrane of the arterioles is presented by the endothelial cells located on the basal membrane. The loose fibrous connective

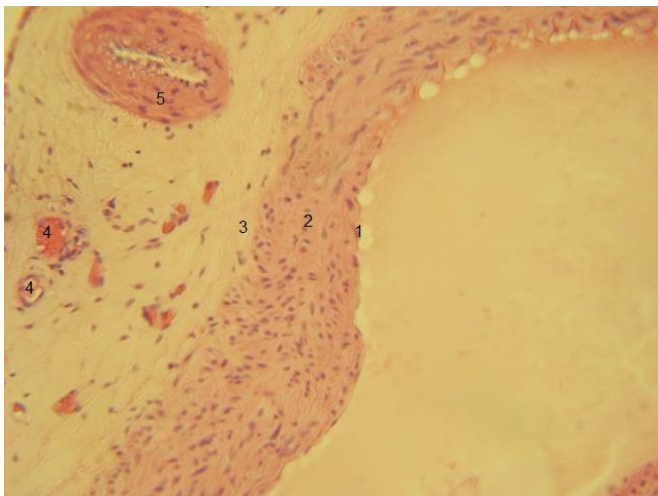


Fig.1. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 200. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – capillary; 5 – arteriole wall.

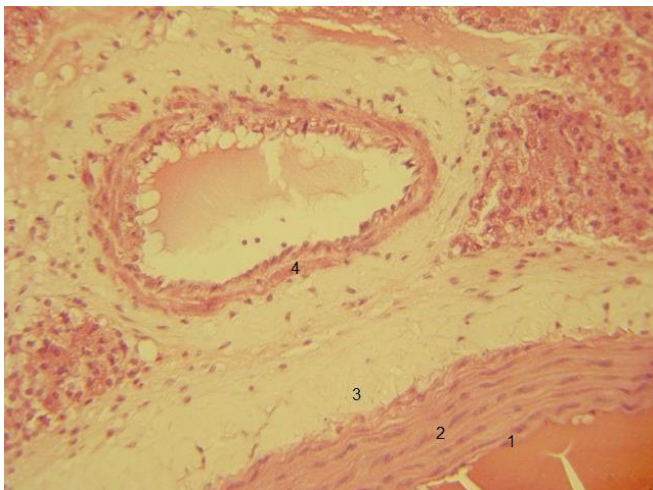


Fig. 2. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – arteriole wall. tissue is located in the subendothelial area with its

penetration into the lumen of vessels. The loose fibrous connective tissue is swollen. Collagen fibers are pale stained with eosin and swollen without clear outline. The venules are full of blood with erythrocytes with their agglutination into fine conglomerates usually located in the center of the lumen (Fig. 3).

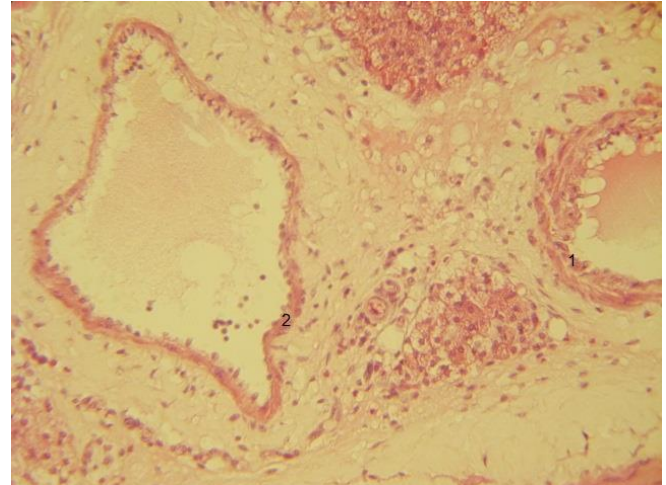


Fig. 3. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – venule wall.

Endothelial cells with flat nuclei form their internal membrane. Endothelial cells are absent on certain intervals where they adhere to the basal membrane. Smooth myocytes form the middle and external membranes, a part of them is located longitudinally, and another part – circumferentially concerning the vascular wall. Loose fibrous connective tissue form adventitial membrane where clear outlines of thin eosinophilic collagen fibers are visualized. The latter penetrate into the interstitial tissue with considerable swelling in the perivascular areas.

After 8 weeks of streptozotocin-induced diabetes mellitus deep destructive aortic changes are found on histological sections. The internal surface of the aortic wall became dark brown in colour and tubercous, the intima was irregular, whitish in colour, and all its surface containing tubercles and depressions (Fig. 4).

The areas of an orange colour are seen on the tubers with white margins, and yellow spots. According to the data of professional literature [5, 6, 8], whitish tubers are fibrous plaques formed due to the growing of the connective tissue into the detritus thickness. Orange spots with white margins are intramural hematomas as the results

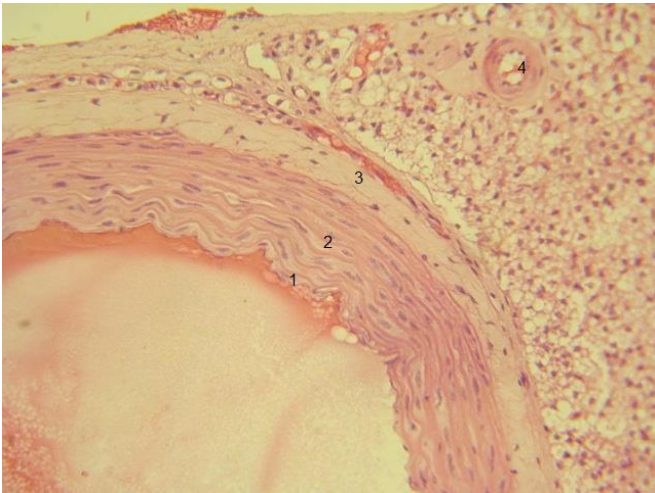


Fig. 4. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 200. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – pre-capillary arteriole.

of plaque destruction or its calcification in case of atheromatosis. The white margin is the area of calcification, and the plaques available are indicative of progressing of atherosclerotic changes and stratification of a new “wave” of lipoidosis on the previous old changes, exfoliation of a part of the endothelial aortic layer is indicative of the formation of exfoliating aneurism [6].

Elastic fibers of the median membrane are not well-organized, inter-laminar spaces are wide with loss of inter-laminar relations, especially expressed in the external layers of the median membrane.

After 8 weeks of streptozotocin-induced diabetes mellitus the links of the blood circulation are dilated, in certain areas arterioles with irregular outlines are found together with dilated venules, swelling is seen round the vessels. The lumen of arterioles is filled with homogenic oxyphilic stained fluid with characteristic signs of hemolysis and absence of cellular elements. Due to plasmatic infiltration of the median and external membranes of the arteriole walls are thickened, they are of homogenous rosy-pink colour with the signs of fibers separation (Fig. 5). One layer of the endothelial cells is visualized in the microstructure of the venules located on the basal membrane. In venules with longitudinal section endothelial cells are located at a distance one from another with acellular areas –

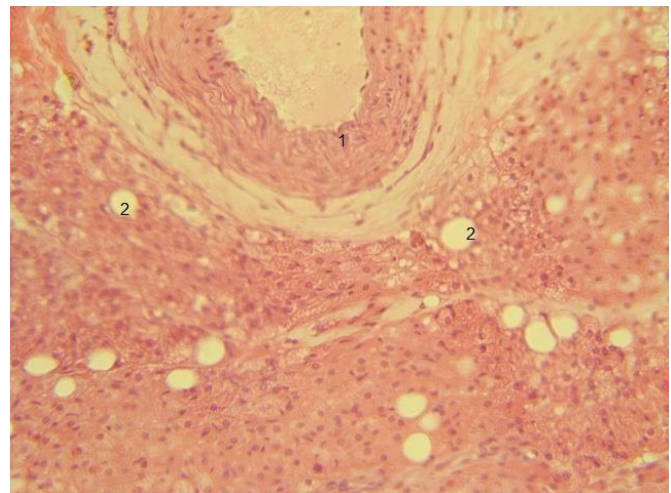


Fig. 5. External aortic wall of an albino rat 8 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – vacuole.

perforations. The shape of endothelial cells is flat and elongated. Erythrocytes and plasmatic fluid are found in the lumen of vessels. The plasmatic fluid has parietal location on a large interval and it penetrates into the perivascular intersticium through the intervals between endothelial cells. In addition, stasis and sludge are found with marginal localization of erythrocytes and formation of agglutination clots, which partially or completely block the lumen of vessels (Fig. 6).

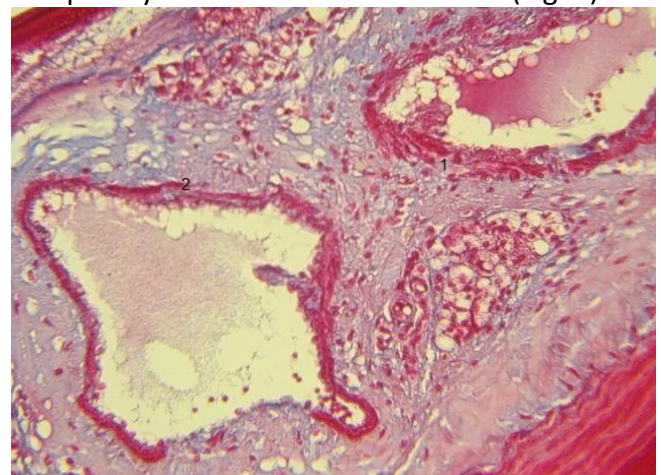


Fig. 6. External aortic membrane of an albino rat 8 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – venule wall.

Conclusions. Our study resulted in determination of morphological peculiarities of the aortic wall on histological level 6 and 8 weeks after streptozotocin-induced diabetes mellitus. After 8 weeks of streptozotocin-induced diabetes mellitus deep changes are found in the aortic wall and vessels of its circulation bed which is

indicative of the development of diabetic arteriosclerosis.

Prospects of further studies. New data obtained concerning microstructural changes of the aortic wall and links of its circulation bed investigated on the experimental DM at the late stages can be of a certain practical value in future investigations, elaboration of new diagnostic and preventive measures concerning this pathology.

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THE SPECIES COMPOSITION AND TROPHIC RELATIONSHIP OF BIRD POPULATIONS COMING TO WINTER IN GYZYLAGAJ BAY AND ADJACENT TERRESTRIAL AREAS, AZERBAIJAN

Abstract. *The species and trophic relations of bird populations coming to winter in Gyzylagaj bay and adjacent lands have been investigated in 2005-2017. It was recorded bird populations coming to winter and belonging to 12 orders, 36 families, 76 genus and 136 species. The diet of 41,2 % of them (56 species) contains animal food, of 51,5% species both animal and floral food (70 species) and of 7.3 % - only vegetal food (10 species). The food ration of 94 bird species contains water invertebrates, of 76 species - terrestrial invertebrates, of 38 species - fishes, of 22 species - amphibians, of 22 species - reptiles, of 20 species - birds, and of 21 species - mammals. The diet of 80 bird species contains seeds of plants, of 56 species vegetative parts of plants, and of 47 species – fruits.*

Key words: *trophic, wintering, sedentary, origin, biodiversity.*

Introduction. It is impossible to characterize the life of animals before knowing the importance of the food resources in their life. Exchange of matters in ecosystems, energy and information flow is only possible in a complex network of interspecific interactions. The strength and quality of interspecific food character provides the necessary level of integrity and completeness of biocenosis.

In 2000 the Strategy and Action Plan for Conservation and Sustainable Use of Biodiversity, in 2010 “Gabala declaration” on Conservation of Biodiversity have been signed in Azerbaijan. The president of Azerbaijan has also signed the order on “Conservation of Biodiversity and Genetic Fond” on 21 December 2002 (1, 2).

The Gyzylagaj bay and adjacent lands are especially important areas for investigation of the species composition, settlement level, interspecific and intraspecific and trophic interactions of wintering bird populations in Azerbaijan. The species composition, settlement level and trophic relations of wintering bird populations are almost not studied yet. However, this area has always been affected by natural (fluctuations of sea level), antropic and antropogenic factors. Trophic relations are important factors in settlements and migration of birds (12, 13). During the wintering period the species composition, settlement level and trophic relations depend on quantity and quality of their

food resources (3). Because food resources can change during the day depending on air temperature. That is why the species composition, settlement level and trophic relations of birds in wintering period have been investigated many years. The settlement of bird populations of the same species in different biotopes and their trophic relationship was considered by us (9).

The main goal is to investigate species composition and trophic relationship by identification of their diet.

Material and methods. The investigations have been carried out in 2005-2017 on transects by using also horses, vehicles, motorized and ordinary boats. The main goal was to study the species composition, settlement level and trophic relations of bird populations coming to winter. Investigations have been carried out every year in December, January and February from 9⁰⁰ to 17⁰⁰, sometimes even in night hours. For observation of birds binoculars and Carl Zeiss telescope were used. In 2005-2010 the professors and students of Vertebrates’ Zoology, High Plants’ Systematics and Biogeography department of Moscow State University have also participated in investigations. The trophic relationship of wintering birds have been studied by analyzing their food remnants, excrements, emesis in their feeding, resting and overnighing grounds by direct visual observations and based on scientific literature. The difference

in importance of these methods for different bird groups, color and smell, more or less deformation of food remnants have been considered. Evaluation of food remnants have been carried out on fodder scale of G.T. Mustafayev and N.A. Sadygova (5, 6). Investigation of food characteristic of owls and seagulls was done on their vommits. Different individuals of the same bird species from populations of sedentary wintering and birds coming to winter can get food from different places, and it is adaptation for increasing of life effect, which has reversible character.

Results and their discussion. The species composition, settlement level, trophic relations of birds coming for wintering in Gyzylagaj bay and surrounding lands are not conditioned by only species diversity, but also with abundance of food (amount of food per area unit), its stability, yearly, daily and biotopically difference, availability. Sometimes, when even the food is abundant, the other negative factors (freezing of water, solid fog, frosts, strong winds, rain etc) change the character of feeding of birds during the day. The abundance of food resources influences not only species composition, settlement and density of bird populations, but also their feeding characteristics. The main food of birds wintering in these areas are animal, vegetable and mixed feeds (Fig. 2).

When comparing the species composition of 136 bird species coming to winter in Gyzylagaj bay and adjacent lands and 367 species of birds recorded in Azerbaijan (4) the number decreases from order to species levels (66.6% - 37.0%) (Fig. 1) (7, 8).

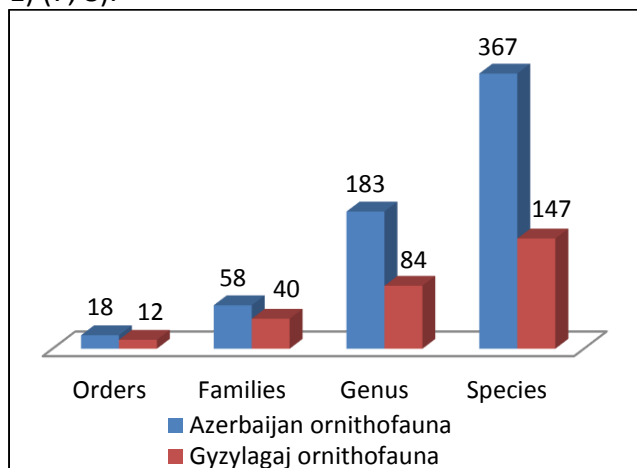


Fig. 1. The taxonomic comparison between Azerbaijan ornithofauna and bird populations coming to winter in Gyzylagaj bay and adjacent terrestrial areas

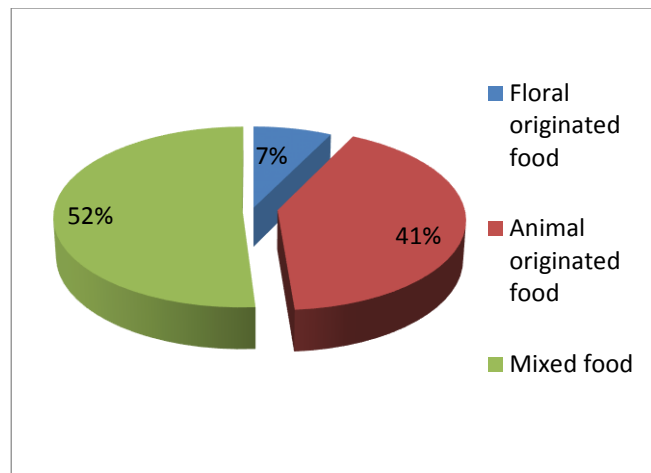


Fig. 2. Food characteristics of bird populations coming to winter in Gyzylagaj bay and surrounding lands

The diet of *Gavia stellata*, *Podiceps ruficollis*, *P.nigricollis*, *P.auritus*, *P.grisegena* coming to winter in Gyzylagaj bay and surrounding lands ~75% contains water plants, and ~25% water invertebrates. It was mainly recorded in Lesser Gyzylagaj bay, in channels connecting Lesser and Greater Gyzylagaj bays (Emergency, Fishpass, Spawning channels). *Pelecanus onocratalus*, *P.crispus*, *Palocrocorax carbo*, *Ph.pygmaeus* (~100%) feed on water invertebrates and fishes (10). *Botarus stellaris*, *Nycticorax nycticorax*, *Egretta alba*, *E. garzetta*, *Ardea cinerea*, *Ciconia ciconia* feed on water and terrestrial invertebrates (~100%). *Phoenicopterus roseus* ~75% feeds on floral, ~25% on animal food; *Rufibrenta ruficollis*, *Anser anser*, *A.albifrons*, *A.erythropus*, *Cygnus olor*, *C.cygnus*, *C.bewickii* feed on floral food (~100%). The diet of *Tadorna ferruginea*, *T.tadorna*, *Anas crecca*, *A.platyrynchos*, *A.strepera*, *A.penolope*, *A.acuta*, *A.querquedula*, *A.clypeta*, *A.anqustirostris*, *Netta rufina*, *Aythya ferina*, *A.nyroca*, *A.fuligula*, *A.marila*, *Busephala clangula* ~75% contains water plants, and ~25% water invertebrates. *Milvus miqrans*, *Melanitta fusca*, *Mergus merganser*, *M. serrator*, *Circus aeruginosus*, *Accipiter nisus*, *A.gentilis*, *Buteo buteo*, *B.laqopus*, *B.rufinus*, *Aquila clanga*, *A.chrysaetos*, *Haliaetus albicilla*, *Falco tinnunculus*, *F.cherrug*, *F.columbarius*, *F.peregrinus*, *Rallus aquaticus* (~100 %) feed on animal originated food. There are 75 % floral and 25 animal food in the diet of *Porzana porzana*, *P.pusilla*, *P.parva*, *Gallnula chloropus*, *Fulica atra*, *Otis tarda*, *Tetrax tetrax*. *Pluvialis apricaria*, *Eudromias morinellus*, *Vanellus vanellus*, *Recurvirosta avocetta*, *Tringa nebularia*, *T.*

ochropus, T. erythropus, Turdus ilicus ~100% feed on animal food. The food ration of *Tringa totanus, T. stangnatilis. Calidris minuta, C. temminski, C. alpina, C.canutus, Calidris alba, Lymnocyptes minimus, Scolopax rusticola, Limosa limosa, Galerida cristata, Calandrella cinerea, C.rufescens, Melanocorypha calandra, M.leucoptera, M. yeltonensis, Alauda arvensis, Anthus pratensis, Sturnus vulgaris, Garrulus glandarius, Corvus frugilegus, Troglodutes troglodutesres, Regulus regulus, Erithacus rubecula, Turdus ruficollis, T. atrogularis, T. pilaris, T. merula, T. vescivorus, Parus major, P. caeruleus, Sitta europea, S.neumayer, Passer hispaniolensis, P.montanus, Fringilla coeleps, F.montifringilla, Serinus pusillus, Chlorius chlorius, Emberiza calandra, E. schoeniculus* contains ~75% vegetal, and ~25% animal food; the diet of *Gallinago gallinago, Numenius arguata, Larus ichthyæetus, L.minitus, L.ridibundus, L.genei, L.argentatus, L.canus, Tuto alba, Asio flammeus, Motasilla flava, M.alba, Lanius exubitor* is ~100% animal food. *Pterocles orientalis, Columba livia* are ~100% vegetarians. The diet of *Pica pica, Corvus cornix* ~ 25% contains plants, and ~75% animals (8, 9).

There are 10 species of birds coming to winter in Gyzylagaj bay and surrounding lands which mainly feed on floral food (~100%) (*Rufibrenta ruficollis, Anser anser, Anser albifrons, Anser erythropus, Cygnus olor, C. cygnus, C. bewickii,*

Pterocles orientalis, Columba livia, Bombycilla garrulus). And 56 species of birds coming to winter, feed on animal food (Fig. 3).

The percentage of floral or animal food in the diet of these bird species coming to winter in Gyzylagaj bay and surrounding lands changes depending of air temperature and availability of food (11). In sunny days with high temperature

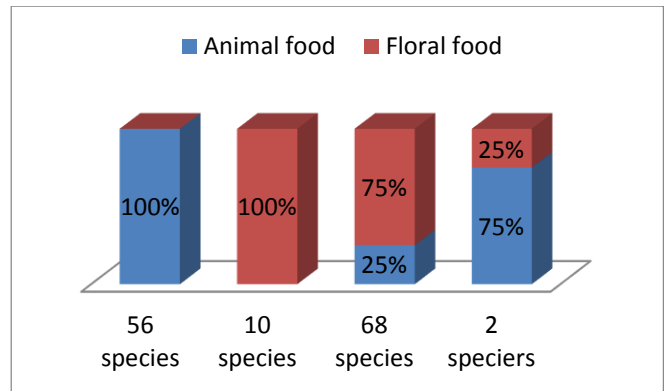


Fig 3. The percentage of floral and animal food in a diet of bird populations coming to winter in Gyzylagaj bay and surrounding terrestrial areas (~%)

birds coming to winter feed mainli 75% on animal food, in cold days floral food dominates (75%). 69 species of birds coming to winter feed on mixed food.

93 species of bird populations coming to winter in Gyzylagaj bay and adjacent lands have in their diet water invertebrates, 77 species – terrestrial invertebrates, 36 species – fishes (Fig. 4).

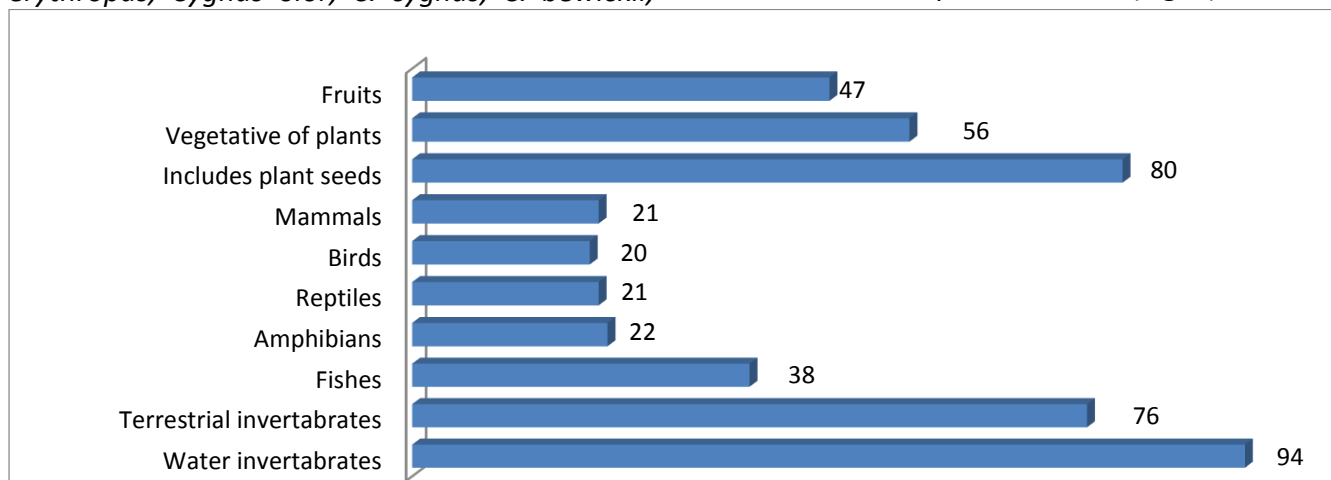


Fig. 4 The diet content of bird populations coming to winter in Gyzylagaj bay and surrounding lands

Predominance of water invertebrates is associated with abundance of these species in biocenosis and being easily available fodder for birds. Plant seeds dominate in the diet of 79 bird species. 55 birds species consume mainly vegetative parts of plants.

Conculision. The species composition and trophic relationship of bird populations coming to winter in Gyzylagaj bay and adjacent terrestrial areas are depending on several factors:

- 1) the intensive poaching in surrounding areas
- 2) grazing by livestock of local population

- 3) amount of annual precipitations
- 4) severe climatic conditions
- 5) changing of fauna and flora species in Greater Gyzylagaj bay after connecting it with Lesser Gyzylagaj bay which has freshwater
- 6) decreasing of plant species Zoosfera minor which is the main food of phytophagous bird species coming to winter

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PROGNOSTICATION OF POLYP LESION STRUCTURE OF THE COLON PORTIONS FOR NEAR-TERM OUTLOOK

Abstract. *to prognosticate the structural parameters of polyp lesions of the colon for 2018 in Lviv region. Retrospective analysis of 51 consulting conclusions concerning the cases of polyp lesion of the colon has been performed. Consulting was conducted at the Department of Pathologic Anatomy and Forensic Medicine, Danylo Halytsky Lviv National Medical University for the period of 2016. With the purpose to prognosticate the structural parameters of polyp lesions of the colon for 2018 we have applied the method of exponential smoothing: Holt two-parametric method. The sense of this method is the following: results of prognostication are constantly adapted to new information obtained, which in its turn increase the accuracy of prognostication reliably. Therefore, the results of prognostication depend mostly on the rates which are most closely to the beginning of a prognosticating period (in our case these are investigations of 2016), and as the parameters are further from the end of the dynamic line they less influence upon the results of prognostication. The study demonstrated a tendency to increase of certain histological kinds of colon polyps and their topographic location. It can be affirmed that for near-term outlook under other unchanged conditions in Lviv region the tendency to increase of five histological kinds will be observed: inflammation polyp, tubular adenoma, papillary adenoma, hyperplastic polyp and juvenile polyposis. As to another nosology for near-term outlook there will be a tendency to their decrease in the structure of polyp lesions of the colon.*

Key words: *structure, polyps, adenomas, colorectal cancer.*

Introduction. the number of patients suffering from colon diseases has been constantly increasing. Every year the WHO estimates over 940 000 new cases of colorectal cancer. Colorectal cancer (CRC) is most often detected on later stages – the frequency of detection of the 3-4th stage is 70% of all the cases found [1]. CRC sickness rate is considerably higher after the age of 50, and its frequency has been increasing in recent twenty years [3]. Survival rate for the last five years has increased from 46 to 62%, although the value of this index is completely determined by the stage of the disease on the moment the diagnosis is made [1, 6]. Considering these data we have to admit that more than in the half of cases the diagnosis is late. At the same time, there are reliable evidences that reduced mortality due to CRC can be achieved by means of detection and treatment of its early forms together with detection and removal of adenomatous polyps [4].

Therefore, close to real cause of polyp frequency can be determined only as the result of

targeted preventive mass examinations of the population or dissection. The majority of cases have the period of latent (asymptomatic) course, followed by the signs of the disease not considered by the patient himself, and most often are characterized as “intestinal discomfort”, while pathomorphologic changes are available in the colon [2, 5, 6]. The commonest lesion of the colon is polyp (colon polyp - CP) including all the processes associated with protrusion of the mucous membrane into the colon lumen [1, 3, 4]. A part of them, adenomas in particular, belong to obligate pre-cancer formations, which in case of untimely diagnostics and inadequate treatment can transform into colorectal cancer [1, 5]. Prognostication of the structural parameters of obligate pre-cancer conditions can help to predict a probable number of cases on colorectal cancer for the nearest future.

Objective: to prognosticate the structural parameters of polyp lesions of the colon for 2018 in Lviv region.

Materials and methods: retrospective analysis of 51 consulting conclusions concerning the cases of polyp lesion of the colon has been performed. Consulting was conducted at the Department of Pathologic Anatomy and Forensic Medicine, Danylo Halytsky Lviv National Medical University for the period of 2016. With the purpose to prognosticate the structural parameters of polyp lesions of the colon for 2018 we have applied the method of exponential smoothing: Holt two-parametric method. The sense of this method is the following: results of prognostication are constantly adapted to new information obtained, which in its turn increase the accuracy of prognostication reliably. Therefore, the results of prognostication depend mostly on the rates which are most closely to the beginning of a prognosticating period (in our case these are investigations of 2016), and as the parameters are further from the end of the dynamic line they less influence upon the results of prognostication.

Results. While making prognostication concerning a part of polyp lesions in the colon (Fig. 1) on the basis of real findings (blue line) we have predicted the indices for 2018 (red line). The presented data demonstrate that the prognosticated indices are mostly effected by real values of the recent years (2014–2016), when the analyzed parameter decreased – even to its complete absence in 2015. Thus, a prognosticated value in 2018 will be 0,24%.

To determine an optimal pattern of prognostication we have determined alpha- (enables to determine the level of rank) and gamma- (enables to determine curve inclination) coefficients for every from the equation. Thus, we have developed an array of equations for every prognosticated parameter (location or histology), and the best ones were determined among them. The criteria of choice were the utmost

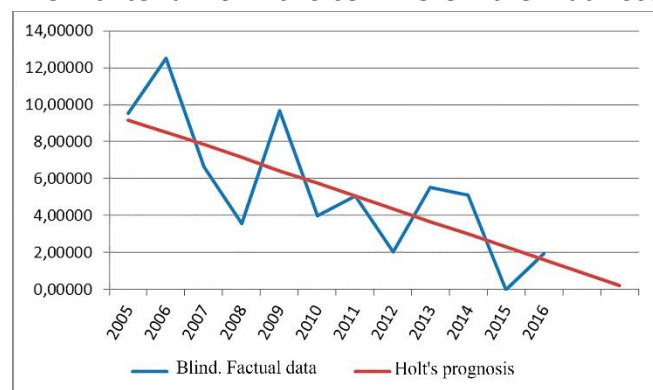


Fig. 1 Real and prognosticated values of a share of polyp lesions in the colon.

coincidence of conditionally prognosticated and real data, and the smallest average values of an absolute residue square. Therefore, in prognostication of a share of polyp lesions in the colon for 2018 an optimal equation has alpha-coefficient 0,01 and gamma-coefficient 0,01.

Discussion. Summarizing the results of work with the array of equations in prognostication of a share of polyp lesion depending on the location in the colon portions, we have designed the Table 1 presenting alpha- and gamma-coefficients to prognosticate a share of every portion for 2018.

Thus, using certain primary real data and obtained alpha- and gamma-coefficients we have prognosticated that in 2018 in Lviv region under other unchanged conditions the structure of polyp lesions of the colon depending on the location of a focus of lesion will be the one presented in Table 2. Therefore, it can be stated that in 2018 under other unchanged conditions in Lviv region a tendency to increase a share of polyp lesion in the transverse-segmented, descending and sigmoid portions of the colon will be found. In other portions there will be a tendency to decrease their share in the structure of polyp lesions in the colon. Similar to the prognostication of a share of polyp lesions depending on their location in the colon we have developed an array of equations to prognosticate the structure of histological types of polyp lesions for the nearest future. The most optimal alpha- and gamma-coefficients for every of such prognosis are presented in Table 3.

Table 1
Alpha- and gamma-coefficients of the obtained equations to prognosticate a share of polyp lesions in different portions of the colon for 2018.

No	Location	Alpha-coefficient	Gamma-coefficient
1	Cecum	0,01	0,01
2	Ileum	0,00	0,00
3	Hepatic flexure	0,00	1,00
4	Transverse-segmented	0,035	1,00
5	Spleen flexure	0,005	1,00
6	Descending portion	0,451	0,00
7	Sigmoid	0,02	1,00
8	Rectal-sigmoid junction	0,01	0,01
9	Rectum	0,034	0,00
10	Anus	1,00	0,00

Table 2

Prognosticated for 2018 structural parameters (%) of polyp pending on their location

No	Location	P±m, %
1	Cecum	0,24±0,05
2	Ileum	4,85±0,68
3	Hepatic flexure	0,02±0,01
4	Transverse-segmented	12,02±1,03
5	Spleen flexure	0,03±0,01
6	Descending	7,47±0,83
7	Sigmoid	38,04±1,54
8	Rectal-sigmoid junction	5,30±0,71
9	Rectum	32,00±1,48
10	Anus	0,03±0,01
	Total	100,00

Table 3

Alpha- and gamma-coefficients of the obtained equations to prognosticate a share of different nosology units of polyp lesions in different portions of the colon for 2018.

No	Nosology unit	Alpha-coefficient	Gamma-coefficient
1	Inflammation polyp	0,05	1,00
2	Fibrous polyp	0,451	0,00
3	Tubular adenoma	0,00	0,00
4	Papillary adenoma	0,012	1,00
5	Tubular-papillary adenoma	0,00	0,00
6	Hyperplastic polyp	0,653	0,00
7	Juvenile polyposis	0,00	0,00
8	Peutz-Jeghers polyposis	1,00	0,00
9	Family polyposis	0,944	0,00
10	Shaped polyp	0,00	0,086

Analogically, using the primary real data and obtained alpha- and gamma-coefficients we have prognosticated that in Lviv region in 2018 under other unchanged conditions the structure of polyp lesions in the colon depending on histological type would have the following presentation as in the Table 4.

Conclusions: therefore, it can be affirmed that for near-term outlook under other unchanged conditions in Lviv region the tendency to increase of five histological kinds will be observed: inflammation polyp, tubular adenoma, papillary adenoma, hyperplastic polyp and juvenile polyposis. As to another nosology for near-term outlook there will be a tendency to their decrease in the structure of polyp lesions of the colon.

Table 4

Prognosticated for 2018 the parameters of structure (%) of polyp lesions in the colon depending on histological type.

No	Histological type	P±m, %
1	Inflammation polyp	16,96±1,19
2	Fibrous polyp	0,18±0,03
3	Tubular adenoma	45,29±1,57
4	Papillary adenoma	20,20±1,27
5	Tubular-papillary adenoma	5,85±0,74
6	Hyperplastic polyp	9,22±0,91
7	Juvenile polyposis	2,25±0,47
8	Peutz-Jeghers polyposis	0,02±0,01
9	Family polyposis	0,00±0,00
10	Shaped polyp	0,03±0,01
	Total	100,00

Prospects of further studies: the results of the study are promising concerning their practical use; they can form a basis for diagnostics and prognostication of polyp lesion occurrence in future and as a result, a part of a probable risk group as to the development of colorectal cancer among the population of Lviv region.

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PECULIARITIES OF PRENATAL DIAGNOSTICS IN THE FIRST TRIMESTER OF GESTATION IN WOMEN WITH COMPLICATED OBSTETRICAL ANAMNESIS

Abstract. *With the purpose to improve prenatal diagnostics in women with habitual miscarriage of pregnancy cytogenetic examination (karyotyping) has been carried out. The investigations conducted and results obtained stipulate the necessity to perform medical-genetic consulting with karyotyping in the 1st trimester of pregnancy enabling to improve early and effective prenatal diagnostics of congenital developmental fetal defects with an accurate prognosis concerning the possibility to prolong pregnancy and manage it.*

Key words: habitual miscarriage of pregnancy, karyotyping.

Introduction. In spite of introduction and fulfillment of state programs in the sphere of public health recent decades in Ukraine are characterized by a considerable decrease of health index among the population resulting in reduction of its number [2, 5, 7]. There are certain reasons to believe that such tendencies are associated with a negative course of genetic processes in the Ukrainian population [1, 3, 6]. Congenital pathology is one of the leading causes of disability and low quality of life of children and adults both in the developed and developing countries of the world [4, 8]. The world medical statistics estimates approximately 5% of newborns diagnosed with congenital pathology [9]. 30-50 infants among those 1000 live births are known to have certain congenital or hereditary diseases. It should be noted that approximately 30% of perinatal and neonatal mortality is caused by the pathology with dominating genetic component.

«Genetization» of medicine resulted in the development of molecular medicine initiating new tendencies in medical science, and one of them is predictive medicine. Contrary to therapeutic and even preventive medicine it should be considered as the first and the earliest stage of an active effect on the human organism with the purpose to correct potentially possible pathology in time [2]. Karyotyping is a cytogenetic method enabling to find deviations in the

structure and number of chromosomes that can be a cause of infertility, congenital disease and birth of a sick child. There are two main especially important types of this examination in medical genetics: examination of chromosomes of patients' blood cells and prenatal karyotyping that is examination of the fetal chromosomes. Karyotype examination is carried out by means of cytogenetic and molecular-cytogenetic methods. The method enables to identify karyotype (peculiarity of structure and the number of chromosomes) by means of karyogram registration. Cytogenetic examination is carried out in a proband, his/her parents, relatives or fetus in case of suspected chromosome syndrome or other chromosome disorder.

Objective. With the purpose to improve prenatal diagnostics in women with complicated obstetrical anamnesis and habitual miscarriage of pregnancy in particular, a comprehensive examination has been conducted.

Materials and methods. At the initial stage of the investigation clinical-statistical analysis of case histories of women with habitual miscarriage of pregnancy was made (n=30, I group), the analysis of medical cards of practically healthy women was made for comparison (n=30, II group). Cytogenetic examination (karyotyping) of both groups was a final stage. Pregnant women of I and II groups differed considerably by age. The majority of women were from 21 to 30 years, although the

age of 33,3% of women with habitual miscarriage of pregnancy was more than 30. Considering the fact that occupation produces a certain effect on miscarriage of pregnancy we have examined peculiarities of working conditions and found that office workers constituted the most numerous group (46,7% and 43,3%), workers of the industrial and agricultural enterprises were on the second position (30,0% and 33,3%), housewives were the rest. Primary disorders of hypothalamic-pituitary regulation have been convincingly proved to play a certain role in the pathogenesis of habitual miscarriage of pregnancy, as a rule manifested by menstrual disorders. To specify the role of this factor in the development of the disease among patients of our region the character of menstrual function since the moment of menarche was studied. The age of menarche was found to range from 10 to 16 in both groups, an average index was $13,5 \pm 1,3$. None of the women was found to have early menarche, although in 13,3% of women from I group sexual maturation delayed and the first menstruation occurred after 15 years of age.

Results. Analysis of the volume of menstrual blood loss found that in 73,3% of patients and 76,7% practically healthy women moderate menstruations occurred that was of no reliable difference. 23,3% women from II group had a tendency to excessive menstruation, while pregnant women with habitual miscarriage presented an opposite situation (26,7% of women indicated hypo- and oligomenorrhea, and irregular menstruations since the moment of menarche). Menstruations were painless in the majority of patients (86,7% and 83,3%, respectively), the rest of them were with painful syndrome, although these data did not differ reliably. Among previously experienced gynecological diseases women with habitual miscarriage of pregnancy suffered from exacerbation of salpingo-oophoritis, cervical erosion, although these findings also did not differ reliably in comparison with the data from II group. It is an interesting fact that polycystic ovary syndrome was found in 23,3% of women from I group without pregnancy, while none of the women from II group suffered from this nosology.

Discussion. Chronic extragenital diseases were found in 66,7% of women with habitual

miscarriage of pregnancy and in 53,3% of pregnant women with uncomplicated anamnesis. It should be noted that the structure of extragenital pathology in both groups was different. Thus, practically healthy women in general suffered from chronic cholecystitis, gastroduodenitis, colitis, pyelonephritis, and pancreatitis. Women with habitual miscarriage of pregnancy since their childhood had been suffering from different degrees of obesity, chronic decompensated tonsillitis, vegeto-vascular disorders of different types (with prevailing hypertensive component), diffuse nontoxic goiter, varicose dilation of veins of the lower limbs.

Karyotyping was conducted for 30 pregnant women with complicated obstetrical anamnesis according to indications from their parents' side. Analysis of karyotyping results determined that all the examined people had a female karyotype – 46, XX. 30,8% of them had deviations in the structure of chromosomes. Changes in the structure of a short arm of the 9th chromosome (9p+) were found in 23,3% of women (46,XX,9qh+; 46,XX,9ph). Polyploid metaphase plates were found with the same frequency which is indicative of mitosis prophase pathology in the form of chromosome conjugation, which initiates multipolar mitosis at the stages of metaphase and anaphase. Polyploid multinuclear cells are formed in the result of pathology of telophase. In single cases the following pathologic karyotypes were found: 46,XX, 1qh; 46,XX, 13stk+s+; 46,XX, homolog heteromorphism; 46,XX, 21pstk, plate with translocation 2:13; 46,XX, 15stk+s+, 22pstk+s+.

Conclusion. Therefore, investigations conducted and results obtained stipulate the necessity to perform medical-genetic consulting with karyotyping in the 1st trimester of pregnancy enabling to improve early and effective prenatal diagnostics of congenital developmental fetal defects with an accurate prognosis concerning the possibility to prolong pregnancy and manage it.

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MORPHOLOGICAL SIGNS OF DESTRUCTION AND REGENERATION OF THE PERIODONTAL TISSUE OF RATS IN EXPERIMENT

Abstract. 80 albino outbred mature male rats were used in the experiment distributed into five groups (16 animals each): two control groups – IC (negative control) and IIC (conditioned positive control) and three experimental ones: IE, IIE, IIIE. The experiment was conducted in two stages. At the 1st stage every day in addition to the main forage rats from the IIC group and experimental groups were introduced per os with 0,04% ammonium chloride solution (400 mg/kg) – acidotic periodontitis, IC group – per os isotonic salt solution (400 mg/kg). At the 2nd stage rats of both control groups were introduced with isotonic salt solution (400 mg/kg), IE group – i/m 5% meldonium dehydrate solution – drug of metabolic action, IIE – per os calcium glycerophosphate – 133 mg/kg; IIIE – i/m 5% meldonium dehydrate solution and calcium glycerophosphate per os simultaneously. After the experiment was completed the blocks of lower jaws with teeth were isolated, the tissues were fixed during 48 hours in 10% neutral formalin solution. Paraffin sections 5-7 microns thick were stained with hematoxylin and eosin according to van Gieson staining method. Purified from paraffin sections after being treated with aniline alcohol were stained with Heidenhain azan stain. Pictures were taken of separate areas of the specimens by means of the digital photo-adaptor under the microscope Leica DFC 420. A comparative histological examination of the mandibular cellular processes fragments stained with hematoxylin and eosin according to Heidenhain azan stain was conducted. Hyperemic, swollen and smoothed dome-shaped interdental papillae were found on the acidotic model of periodontitis (28 days) in rats of IIC group contrary to the animals from IC group. Due to intensified desquamation of cells from the corneal and granular epithelial layers the gingival epithelial layer is partially preserved, erosive areas are covered with granulation tissue. Intercellular spaces of polymorphic cells of the acanthocyte and basal layers are dilated, and on certain areas contacts between cells are absent. Endothelial swelling is found in the blood capillaries of the gums. Resorption of the collagen fibers and osseous tissue of the cellular part in the lower jaw of rats occurred, leukocyte infiltrations were found as the main signs of inflammatory-dystrophic damage. Metabolic correction on the 42nd day of the experiment resulted in an incomplete regeneration of the gingival epithelium and completed reconstruction of the osseous tissue, consolidation of intercellular contacts and basal membrane of the gingival epithelium, appearance of newly formed thin-walled vessels, areas of replacing sclerosis in the sub-epithelial connective tissue, homogeneous structure of the cellular part osseous tissue of the lower jaw in rats.

Key words: periodontal pattern, gingival epithelium, connective tissue, cellular bone.

Introduction. In spite of considerable achievements of modern clinical medicine and newest biotechnological results the problem of restoration and retention of the structural and functional adequacy of the periodontal tissues still remains topical [4, 9, 14]. Thus, according to the data submitted by Komarevtseva I.A. et al (2009) [5] a comprehensive response of the body connective tissue as an integral structural-

functional system performing plastic and trophic functions is an effective natural means to eliminate pathogenic factors and regenerate the damaged tissues [5, 16].

Crucial factors of damage and morphological signs of periodontal restoration are studied at the cellular, sub-cellular and molecular levels [8, 18, 12]. Thus, for example, while simulating periodontitis association of dystrophic and

reparative changes were found in the alternative and proliferative phases of inflammatory process [2, 19, 20]. Generally morphological signs of damage of the mucous and sub-mucous membranes of the gums occur earlier and are more pronounced than those in the osseous tissue of the cellular process [6, 7, 10]. In case of periodontitis collagen protein content decreases in the connective tissue of gums due to prevailing processes of collagen decay over the processes of its synthesis [13, 15], and under conditions of chronic nitrate intoxication in particular [1]. Considering the presented data the condition of the cellular and fibrous elements of the connective tissue attracts special attention while studying the structure of the periodontal tissue in rats with induced periodontitis and after metabolic correction.

Objective: to determine morphological signs of damage and peculiarities of restoration of the mandibular periodontal complex of rats on metabolic pattern of periodontitis.

Materials and methods. The experiment lasted 42 days and was conducted in two stages (the first one – 28 days and the second one – 14 days) on 80 albino outbred male rats aged 2-4 months with the body weight of 170-240 g kept on standard vivarium diet at Danylo Halytskyi Lviv National Medical University. The animals were distributed into five groups (16 animals each): two control groups – IC (negative control) and IIC (conditioned positive control) and three experimental ones: IE, IIE, IIIE. At the 1st stage every day in addition to the main forage rats from the IIC group and experimental groups were introduced per os with 0,04% ammonium chloride solution (400 mg/kg) – acidotic periodontitis, IC group – per os isotonic salt solution (400 mg/kg). At the 2nd stage rats of both control groups were introduced with isotonic salt solution (400 mg/kg), IE group – i/m 5% meldonium dehydrate solution (the drug “Vasonat”, Olainpharm, Latvia) – 0,25 mg/kg; IIE – per os calcium glycerophosphate (PTC “Lugansk Chemical-Pharmaceutical Plant”) – 133 mg/kg; IIIE – i/m 5% meldonium dehydrate solution and calcium glycerophosphate per os simultaneously.

The study was conducted keeping to the requirements of the European Convention of Bioethics (1997), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Studies, general ethical principles of experiments on animals, approved by the First National Congress of Ukraine on Bioethics (2001) [3].

Animals were taken out from the experiment under ether narcosis. The blocks of lower jaws with teeth were isolated, and the tissues were fixed during 48 hours in 10% neutral formalin solution. On completion of fixation with the purpose of decalcification mandibular bones were treated during 4 days in changed solutions of 7% nitric acid, dehydrated in the ascending graded alcohols and filled with paraffin (D.S. Sarkisova, Yu.L. Perova, 1996). Paraffin sections 5-7 microns thick were stained with hematoxylin and eosin according to van Gieson staining method (BlikMedPrep set, RF). Purified from paraffin sections after being treated with aniline alcohol were stained with Heidenhain azan stain (BioVitrum set, RF). Pictures were taken of separate areas of the specimens by means of the digital photo-adaptor under the microscope Leica DFC 420 (Germany) using the eyepiece x 10; 20 and object glass x4; 8.

Results of the study. In rats from IC group a free part of the gums delimited from the neck of the tooth by the gingival slit forms the gingival margin and interdental papillae of an elongated shape (Fig. 1).

The corneal epithelial layer of the gums is formed by nuclear-free flat squamosal cells, the

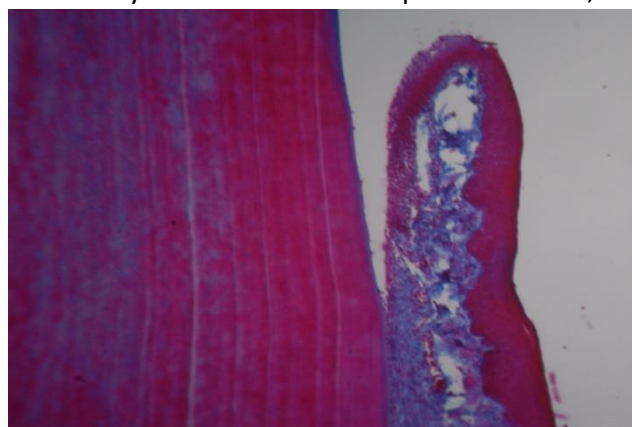


Fig. 1. Mucous membrane of the gums of a rat in the norm (IC). Stained with Heidenhain azan stain.

Magnification: oc. 10, ob. 8.

granular layer – by the layers of flat cells. Acanthocyte layer consists of several (2-3) layers of large cells of a polygonal shape smoothed close to the granular layer, which numerous acanthocyte processes are connected between themselves by means of desmosomes. The basal layer is formed by cubic cells located on the basal membranes. The plate of the gingival mucous membrane proper where single capillaries are located is formed by fibrous connective tissue penetrating in the form of papillae to the epithelial layer (Fig.1).

The cellular process is of a typical plate-like architectonics with homogeneous by the density of location and size beams of the spongy bone. Bundles of the collagen fibers of the periodontal tissue are fixed to the cement of the dental root from one side, and from another – connected with the periosteum (Fig2.).

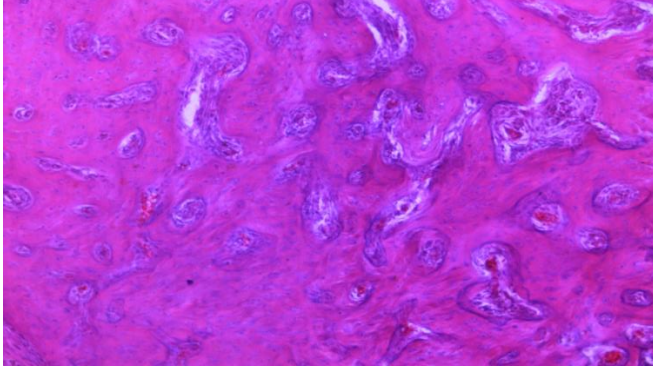


Fig. 2. Osseous tissue of the mandibular cellular part of a rat with osseous trabecula and vascularized cells in the norm. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4.

Hyperemic, swollen and smoothed dome-shaped interdental papillae are determined in rats from IIC group. Intercellular spaces of polymorphic cells of the acanthocyte and basal layers are dilated as well, on certain areas contacts between cells are absent and only desmosomes are found.

In the blood capillaries of gums of a somatic type endothelial swelling is seen, in the pericapillary space and in the gingival connective tissue layer proper – growth of collagen fibers of the connective tissue. In the sub-epithelial connective tissue vessels are sclerosed, surrounded by leukocyte infiltration, microabscesses are found filled with neutrophils, and the areas of the granulation tissue spread deep to the cellular bone. The beams of the spongy bone are different by size, density of location and shape of the inter-beam cells (Fig.3). A free part of the gums in rats from IE group is infiltrated with lymphocytes. Collagen fibrils of the epithelial layer of the gums which are closely connected with the cement of the dental root in the norm and attach gums to the periosteum of the cellular process and form the base of the plate of the sub-mucous layer proper are inhomogeneous by the electron density and diameter, twisted between themselves. Beams of the spongy bone are homogeneous by the shape and size with a regular density of location. Inter-beam spaces contain thin-walled vessels and bone marrow (Fig.4).

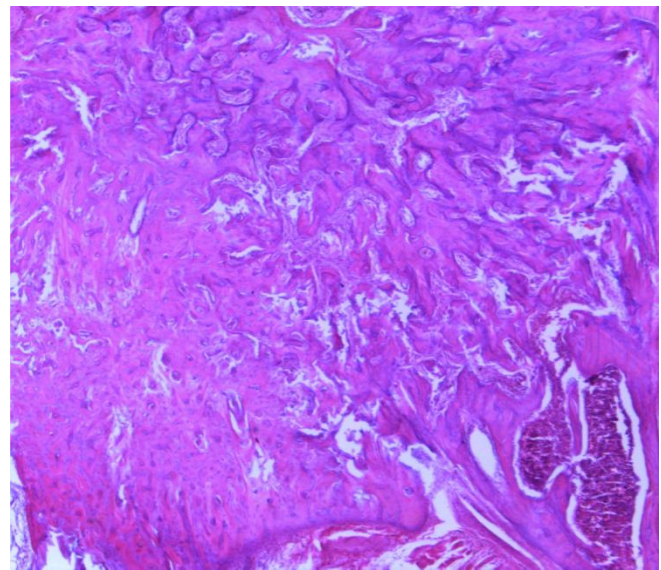


Fig. 3. Microphotograph of a fragment of the mandibular periodontal tissue of a rat from IIC group. фрагменту пародонту нижньої щелепи щура IIC групи. Staining with hematoxylin and eosin. Magnification: oc. 10, ob. 4

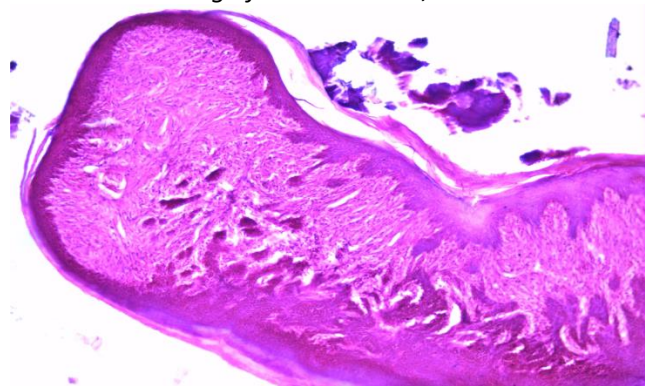


Fig. 4. Mucous membrane of the rat's gums (IE group) with signs of regeneration of the connective tissue and residual signs of inflammatory alteration. Staining with hematoxylin and eosin. Magnification: oc. 10, ob. 4

The connective tissue papillae in animals from IIE group are thickened, deepened into the own plate, acanthosis signs are absent, single intra-epithelial leukocytes are found, basal membrane is preserved. Infiltration and focal sclerosis in the vascular wall are found. Collagen bundles are homogeneous by the shape and size, although they are disorganized in the areas of mixed-cellular infiltration. Beams of the osseous tissue are of a usual structure, similar by the shape and size, although with different degree of density and numerous foci of irregular excessive calcification (Fig. 5).

Epithelial layer in rats from IIIE group is thickened on separate areas, intercellular spaces are narrow, basal membrane is formed by a network of thin reticular fibers. Collagen bundles of the sub-epithelial connective tissue are located in order, without signs of fragmentation, residual

signs of inflammatory process, and single areas of substitutive sclerosis are found. Beams of the spongy bone are similar by the shape and size with regular density of a compact plate. Small inter-beam cells, in addition to vessels, are filled with focally sclerosed connective tissue and bone marrow (Fig. 6).

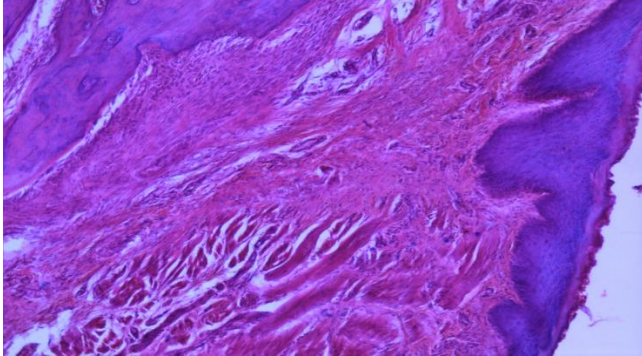


Fig. 5. A fragment of the periodontal tissue of a rat from IIE group. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4

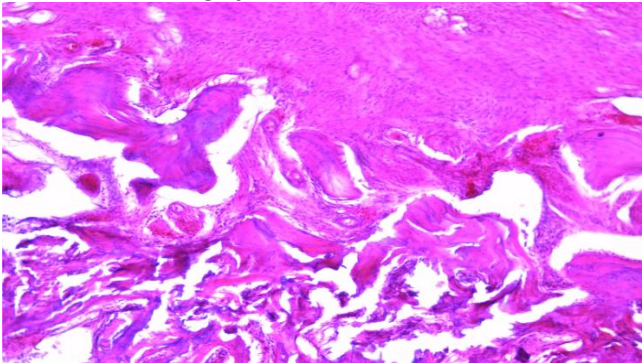


Fig. 6. A fragment of the osseous tissue of the mandibular cellular part of a rat from IIIIE group with regular degree of density. Staining with hematoxylin and eosin. Magnification: oc. 20, ob. 4

Discussion. In rats from the group of a negative control the mucous membrane of gums adhered to the periosteum of the cellular processes is covered with integral laminated epithelium. Numerous papillae of the own plate of the gingival mucous membrane enlarge the square of contact and strengthen connection between the epithelium and adherent connective tissue. Fibrillar proteins are known to be the base of the structure of the basal capillary layer, basal membrane of the epithelium, and the connective tissue of the gingival mucous membrane proper and intercellular substance of the osseous tissue of the cellular process [16].

Integrity of the intercellular connections in rats from the group of a positive control is disturbed due to intensified desquamation of the corneal and granular epithelium. Cornification of the superficial layer of the epithelium is slow, the gingival epithelial layer is partially preserved, and the areas of erosions are covered with the

granulation tissue. Shortened, partially fragmented collagen bundles on separate areas of the pericapillary space as well as in the proper layer of the connective tissue of gums are indicative of an active process of proteolytic resorption. Resorption of collagen fibers according to the data presented by Kolesova N.A., et al (2008) [4] occurs with participation of primary and secondary lysosomes released in case of destruction of leukocytes and fibroblasts. Lacunar resorption of the osseous tissue of the cellular process is associated with formation of periodontal pockets.

In rats of IE group initial signs of perivascular sclerosis of a free part of the gums were seen and erosive defects absent. At the same time, the initial stage of the osseous tissue regeneration was characterized by inter-beam spaces with thin-walled vessels and cells of the bone marrow. Due to hyperplasia of the basal cells and accumulation of keratohyalin in the cells of the granular layer in animals of IIE group the epithelial layer of gums thickened.

In rats of IIIIE group the epithelial layer on certain areas was also thickened, dense intercellular contacts, in formation of which belongs to fibronectin structural protein – a product of fibroblast secretion of the connective tissue [8]. Collagen bundles of the subepithelial connective tissue are located in order, the structure of inter-beam cells is indicative of completion of reconstruction of the mandibular osseous tissue.

Conclusions.

1. Conditions of cells and fibrous structures of the connective tissue of gums and cellular process of the lower jaw of rats in the norm are crucial to assess regenerative ability of the periodontal complex in the experiment.

2. Acidotic pattern of periodontitis (28 days) determined the main signs of inflammatory-dystrophic damages such as erosions of the epithelium, disturbed integrity of the intercellular connections, resorption of collagen fibers and osseous tissue of the cellular part of the lower jaw of a rat, leukocyte infiltrations.

3. Metabolic correction resulted in an incomplete regeneration of the gingival epithelium and completed reconstruction of the osseous tissue, consolidation of intercellular contacts and basal membrane of the gingival epithelium, appearance of newly formed thin-walled vessels, areas of replacing sclerosis in the sub-epithelial connective tissue, homogeneous

structure of the cellular part osseous tissue of the lower jaw in rats.

Prospects of further studies. Presented results of the study are a part of the scientific-research work dealing with investigation of peculiarities of inflammatory diseases of the periodontal tissue against the ground of metabolic disorders. The results obtained in the study can be used to search and analyze effective methods of treatment of periodontitis in patients with metabolic disorders.

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THE EFFECT OF TRACE ELEMENT STATUS OF RATS WITH SIMULATED HYPOTHYROIDISM ON THE STATE OF COGNITIVE FUNCTIONS

Abstract. Hypothyroidism is the second endocrine disease after diabetes mellitus associated with disorders of hormonal homeostasis, imbalance in the content of biological elements, decrease of motor activity, weight gain, dysfunctions of the central nervous system, cognitive functions in particular, and as a result decrease the quality of life. 42 albino male Wistar rats were used in the study. Their body mass (BM), level of thyroid hormones, concentration of copper, zinc and magnesium in the blood serum were evaluated. The state of cognitive functions was determined by means of the tests "Open field", "Social cognition" and detection of "New object". BM of rats with hypothyroidism was found to be more than 25% ($p < 0,05$) as much. T3 and T4 levels in animals with hypothyroidism was found to decrease in 3,9 and 3,3 times ($p < 0,05$), and the content of TSH in the blood serum increased twice as much. The concentration of copper in the blood of animals with hypothyroidism 1,54 times increased ($p < 0,05$), magnesium – 23,36% ($p < 0,05$), and zinc – 6,8% ($p > 0,05$). Under conditions of hypothyroidism development with a longer period of the experiment cognitive-learning functions of animals progressively decreased, as well as memory disorders concerning new and familiar objects or subjects which is indicative of the formation of stable cognitive deficiency.

Key words: hypothyroidism, cognitive functions, trace elements, experiment, rats.

Introduction. Hypothyroidism is one of the most spread endocrine diseases after diabetes mellitus. It is a clinical syndrome associated with continuous decrease of the thyroid hormones action on the target systems. Against the ground of hypothyroidism practically all the organs and systems are afflicted making clinical manifestation rather urgent for the doctor of different specialties. The frequency of primary hypothyroidism in the population is 0,2-2,0%, and subclinical hypothyroidism is diagnosed in 10 - 12% of women and 2-3% of men [6].

Physiological effects of thyroid hormones are rather clearly verified and stipulated not only by their direct effect on the expression of genes controlling synthesis of structural and functional proteins in the cells of different body systems, but indirectly as well – through their interaction with catecholamines providing metabolic processes [1, 10, 13]. The main signs of thyroid hormones include: 1) increase of mitochondrial efficacy and myocardium contraction; 2) providing normal processes of growth, development and differentiation of tissues and organs; 3) increase

of renal blood circulation, glomerular filtration and diuresis; 4) stimulation of heat production and body temperature; 5) maintenance of sexual and reproductive functions; 6) potentiation of sympathetic effects; 7) increase of excitability of the central nervous system and activation of psychic processes [3, 8, 9]. Moreover, thyroid hormones produce a considerable effect on the maturation of specific neuronal populations, therefore their lack during the period of active neurogenesis results in irreversible mental retardation and is associated with multiple morphological changes in the brain [7, 8, 12].

Undoubtedly, changes of the thyroid status and in case of hypothyroidism in particular, produce a direct effect upon the functions of practically all the body systems, and thus the mechanisms of non-specific adaptation can be involved. In its turn, it results in the development of a wide spectrum of symptoms including fatigue, weakness, weight gain and depression, memory deterioration and poor learning progress, progressive decrease of intellectual abilities, psychic disorders, ataxia, muscular weakness and

muscular convulsions, and in severe cases – coma [1, 11].

Modern scientific literature does not present sufficient information concerning the state of cognitive functions and trace elements metabolism in case of hypofunction of the thyroid gland [2, 9]. Thus, investigation of pathogenic aspects and biochemical transformations occurring against the ground of hypothyroidism stipulates a considerable interest to more comprehensive study of the issue.

Objective of the work is to find the effect of changes in trace elements content in rats with simulated hypothyroidism on the state of their cognitive functions.

Materials and methods. 42 albino male Wistar rats were used in the study with their body mass of 300-350 g. The animals were divided into two groups: I – intact; II – with experimental hypothyroidism simulated by means of everyday supplement of Mercazolil (the acting agent Thiamazole) to drinking water of animals (Ltd. Pharmaceutical company “Zdorovya”, Kharkiv, Ukraine) during 30 days.

Efficacy of the suggested model of simulated hypothyroidism was evidenced by the results of examination of the hormonal status. The content of TSH (TSH ELISA, Germany), free triiodothyronine (T3) (T3 EIA KIT, USA), free thyroxin (T4) (T4 EIA KIT, USA) was determined in the blood serum of rats. The body mass of animals was estimated. In addition, reliable changes specific for hypothyroidism were detected by means of morphological examinations of the thyroid gland and published earlier [1].

The whole blood was used as the material for the study. The contents of macronutrient element magnesium (Mg) and trace elements zinc (Zn) and copper (Cu) were determined. The blood was taken for analysis 1 month later since the beginning of the experiment after decapitation performed under ketamine narcosis (40 mg/kg). The obtained 1 ml of blood was placed into the porcelain crucibles and burnt in the muffle furnace at the temperature of 450 °C during 48 hours. Then the ashes received were dissolved in 10% hydrochloric (2 ml) and nitric acids (1 ml), and brought to 10 ml adding bi-distilled water. The solution prepared was analyzed on the atomic-absorptive spectrophotometer C-115ПК

according to the common methods.

To examine and assess cognitive functions the following tests were used: 1) «Open field», enabling to study the animal behavior considering the level of their motor activity (the number of squares in the cage crossed by a rat and rotation movements available), orientation-learning reactions (number of vertical positions and looking into the holes on the floor of the cage), emotional lability (by the number of defecations and urinations), grooming during 2 minutes; 2) test of «Social cognition» during which rats were kept in cages alone for a week, then a juvenile male rat was added, and the time of acquaintance till the moment of losing interest to a young guest was fixed. The latter was taken from the cage for 40 minutes and then returned back; after that the time spent for a repeated acquaintance was fixed again. 3) test to detect «New object» based on the interest of rats to learn new things. The animal was placed in an empty cage containing two similar objects, and the time spent on their learning was fixed. 3 minutes later the objects and the animal were taken away. 40 minutes later the experimental animal was placed back into the cage together with one previously examined object and a new one different by its colour and shape. The time difference in learning new and old objects was fixed.

The data obtained were processed by means of non-parametric Wilcoxon statistical criteria and Sign-test applying the program «Statistica 7» («Statsoft, Inc.» – USA). The differences were considered reliable in case P was 95% and more ($p < 0,05$).

Results. After the first week of the experiment the rats with simulated hypothyroidism developed diminution of physical activity, loss of interest to the environment and satisfactory appetite. 4 weeks later the rats of II group were inert, low-activity, they demonstrate lack of interest to the surrounding factors, the signs of hair loos, in some places hair became yellowish. A part of the animals developed dermatological changes: dry skin with its desquamation, erosions and ulcers.

T3 and T4 levels in the animals from II group with hypothyroidism were found to decrease 3,9 and 3,3 times ($p < 0,05$) respectively as compared to the findings of the intact animals. At the same

time, TSH in the blood serum of this group of animals increased more than twice reliably which may be indicative of the response of the hypothalamic-pituitary system and considerable decrease of thyroid hormones content.

As compared to the control the body mass in II group increased more than on 25% ($p < 0,05$), which is typical for hypothyroidism, while in I group the body mass did not increase reliably.

One of the important and compulsory conditions for normal functioning of the body is stability of the chemical blood content. The blood reflects a current state of element balance in the body. Thus, the concentration of trace elements in the animals with hypothyroidism was the following: Cu increased on 42,93% ($p < 0,05$), Mg – 27,84% ($p < 0,05$), and Zn – remained on the level of that of the control. Similar tendency was found concerning biological elements in the raw substance. In II experimental group Cu concentration increased 1,54 times as much ($p < 0,05$), Mg – 23,36% ($p < 0,05$), and Zn – 6,8% ($p > 0,05$).

Investigation of orientation-learning and cognitive behavior in II group of animals found that the longer the period of the experiment was the more progressive inhibition of cognitive functions was marked. Thus, the number of the examined holes as a signs of hole reflex, which is indicative of the ability of animals to be examined in the open field test, was reliably lower in rats with hypothyroidism. Although the result of the experiment in open field were not only indicative of inhibition of learning functions, but registered less positions on the posterior limbs in the experimental hypothyroidism.

Changes of the character of activity directed to the identification of new subjects were determined, evidenced by the results of the social cognition and new objects tests. They helped to register reduced interest both to unknown rats and unknown objects. The time of identification of old objects was twice as much longer in comparison with that of the control. The changes indicated are the signs of disturbed organization of normal learning behavior and memory, in the situation causing anxiety in particular.

An important part of the experiment was investigation of the emotional status of the animals assessed by the number of defecations

and urinations. Its increase was detected only at the beginning of the experiment which is a sign of anxiety. At the same time, the character of behavior of animals with hypothyroidism at the 3rd week of the experiment is indicative of reduced reactive behavior.

Grooming should be mentioned here as an important characteristics of hygienic behavior of animals. Usually rodents spend a lot of time scratching their hair in comparison with moving in the space which is closely correlated with their motor activity. The experimental animals of II group demonstrated pronounced inhibition of grooming and motor activity while the period of the experiment became longer.

Discussion. Increased concentration of copper and magnesium in case of hypothyroidism attracts certain attention in the analysis of the main blood trace elements. This reaction of biological elements can be explained by compensative-adaptive reactions of the body in response to the development of thyroid dysfunction resulting in the utilization increase of the examined elements in potentiated metabolic processes.

It should be noted that copper is a trace element with antioxidant properties contained in ceruloplasmin, cytochromoxidase, tyrosinase, ascorbinase and other enzymes. Copper participates in hemopoiesis and metabolism of iron, metabolism and oxidation of ascorbic acid, epinephrine, serotonin, regulation of the balance of biogenic amines in the blood, processes of myelinization in the nervous system. Thus, hypothyroidism is characterized by muscle weakness, myalgia, especially in the proximal groups; moreover, typical signs of hypothyroid myopathy – cramps and slow muscular relaxation correlate with dissociation of copper concentration found in the group of experimental animals. This element is important for the maintenance of normal structure of the connective tissue fibers, influences upon carbohydrate metabolism, and tissue respiration. In addition, copper possesses pronounced anti-inflammatory properties, decreases the severity of autoimmune diseases [13].

According to the data suggested by certain authors, the excess of such biologically active elements as Cu and Mg, able to get combined with iodine, can intensify available iodine deficiency

and result in the development of diffuse goiter [2].

Magnesium belongs to the intracellular chemical elements. Its ions participate in carbohydrate and phosphorus metabolism. It is contained in many enzymes: cholinesterase, γ -glutamylcysteamine (control of the first stage of glutathione synthesis), glutamylsynthase (transforms glutamate into glutamine). Mg provides maintenance of energetic (ATP, glucose transport) and plastic (protein synthesis in ribosomes and lipoprotein complexes) processes. It participates in the synthesis of neuromediators: acetylcholine, tyrosine, norepinephrine and neuropeptides in the brain. Its level regulates the balance of lipoproteins of different density and triglycerides [5]. It possesses vasodilatory and antispastic action, stimulates intestinal peristalsis and increases bile secretion. Increased Mg concentrations in the blood can be found in case of kidney diseases with disorders of their excretory function, hypothyroidism, diabetic acidosis and is manifested by sedative effect and respiratory inhibition. Accordingly, increased magnesium content was found in rats of II group with simulated hypothyroidism at the expense of inhibition of metabolic processes which coincides with general notion concerning the content of trace elements in case of thyroid hypofunction [3].

Zinc is known to take the second position after iron concerning its content in the human body and is contained in more than 300 enzymes including alcoholdehydrogenase, DNA- and RNA-polymerase, phosphatase, dehydrogenase, carboxypeptidase, enzymes of tryptophan synthesis, etc. Therefore, its biological role is realized by means of participation of RNA and protein in synthesis, inhibition of free radical oxidation, intensification of the processes of division and differentiation of cells and tissue repair. Moreover, Zn participates in the formation of many links of immune response and performs immune-modulating effect (decreases the intensity of allergic signs). Zinc is contained in insulin, adrenocorticotrophic, somatotrophic and gonadotrophic hormones are zinc-dependent. Zinc participates in the synthesis of thyroid hormones preventing node formation [5, 7].

Since zinc influences upon the growth rate, every next cellular division requires Zn-dependent enzymes of RNA-polymerase, reverse-

transcriptase and thymidine kinase. In spite of the fact that the latter does not contain Zn it is very sensitive to its deficiency as compared to the mentioned above. Thymidine kinase phosphorylates desoxythymidine monophosphate before its involvement in the process of replication to DNA. Moreover, zinc is a component of nuclear receptor T₃ (Zinc fingers), which due to binding with DNA regulates gene expression and synthesis of specific proteins in the cell, with further manifestation of physiological effects of thyroid hormones. On the basis of the mentioned above a decreased Zn concentration in the experimental groups is likely to occur due to its excessive use for reparation requirements of cells. Romaniuk A.M. et al suggested in their research dealing with intake of heavy metals salts that decreased zinc concentration against accumulation of such elements as copper, iron, manganese and others can be explained by their antagonistic interaction [4].

Scientists indicate a negative effect of iodine, iron, manganese, zinc and other trace elements deficiency on the formation and course of the human cognitive functions which is extremely important for children [7, 9, 10]. Moreover, there are certain data that a leading cause of autism can be imbalance between metallothionein and metals [3, 11,12].

A number of clinical studies report about serious cognitive disorders including inability to concentrate, slow thinking, reduced memory concerning the latest events, inability to calculate and understand complicated issues in case of hypothyroidism [8]. Thus, elderly people with hypothyroidism demonstrate worse abilities to learn, visual-spatial skills and attention. In addition, serious deterioration of learning, long-term and short-term memory is found in mature rats after thyroidectomy [3], which correlates with the results of the experiment conducted.

Integrated participation of trace elements in thyroid metabolism should be taken into account. Disorders of any enzymatic link in the process of formation of the thyroid gland hormones can result in changes of chemical content of tissues which in their turn will reflect morphofunctional status of the body on the whole and its cognitive ability in particular.

Conclusions. In case of mercasolil

hypothyroidism threshold and excessive concentrations of such biological elements as copper and magnesium are found in the blood of rats. Zinc content in case of hypothyroidism is found to decrease. By means of the tests "Open field", "Social cognition" and detection of "New object" it was determined that cognitive disorders start to develop in a week after simulation of hypothyroidism in rats. Examination of orientation-learning activity gave the evidence of a negative dynamics concerning cognitive functions and memory inhibition with increased duration of hypothyroidism which is indicative of the formation of stable cognitive deficiency.

Prospects of further studies: to investigate changes of trace elements in the blood serum in order to elaborate pathogenically substantiated schemes of treatment.

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MORPHOLOGICAL PECULIARITIES OF THE GALLBLADDER STRUCTURE AND ITS FUNCTIONAL CHANGES IN HEALTHY INDIVIDUALS OF DIFFERENT SEXES ACCORDING TO THE FINDINGS OF ULTRASOUND EXAMINATION

Abstract. Pathology of the hepatic-duodenal area (HDA) constitutes 25% of all the patients with affliction of the abdominal organs. Making a correct diagnosis in patients with HAD lesions is a complicated issue due to the lack of early pathognomonic symptoms (to the moment jaundice appears), similar clinical manifestation at the stage of jaundice development, absence of specific markers, complicating pre-surgical differential diagnostics of surgical group of diseases. Detection of individual lifetime anatomical structure of a healthy man and finding pathology at early stages of diseases in order to prevent their development have become of a topical value for diagnostic direction of practical medicine. The possibility to apply mini-invasive radiation methods of examination promotes the solution of the task. Objective of our study was to determine the frequency of occurring morphological peculiarities of the gallbladder structure and its functional changes that can be risk factors promoting development of bile expelling structure pathology in healthy adult individuals of different sexes according to the findings of ultrasound examination of the HDA organs. To determine the sickness rate of gallbladder and bile ducts diseases 136 adult people were examined by means of the ultrasound apparatus «Radmir Ultima RU-928» including 60 (44,1%) men and 76 (55,9%) women. The investigation was conducted by means of a convective sensor with the frequency band from 1 to 5 mHz ($\pm 0,5$ mHz) R60 mm. The results of the investigations conducted were indicative of the fact that screening ultrasound diagnostic examinations enable to find both morphological peculiarities of the gallbladder structure and its functional disorders, cholestasis in adult patients without complaints and clinical manifestation of HDA pathology in particular. Among the examined 77 healthy adult individuals including 37 men and 40 women gallbladder flexion was found in 18,2% from the general number of those being examined (21,6% of men and 15% of women). Examination detected asymptomatic cholestasis in 16 people which is 20,8% from general number of those healthy examined ones including 18,9% of men and 22,5% of women. Detection of morphological peculiarities of the gallbladder structure and early diagnostics of cholestasis in individuals without complaints and clinical manifestation of HAD pathology will enable to increase the efficacy of preventive measures directed to maintenance of functional ability of the examined structures.

Key words: gallbladder, morphological peculiarities, functional changes, adult individuals, hepatic-duodenal area.

Introduction. Pathological processes localized in the structures of the hepatic-duodenal area (HDA) are extremely diverse and very often combined. The results of numerous scientific studies indicate that HAD pathology takes the second place by the frequency of lesions after acute appendicitis, or it constitutes one fourth of the general number patients with affliction of the abdominal organs [3, 6].

In spite of rather high technical level of up-to-date diagnostic methods making the correct

diagnosis in patients with HAD pathology remains a complicated issue [4, 8]. The lack of early pathognomonic symptoms (to the moment jaundice appears), similar clinical manifestation at the stage of jaundice development, absence of specific markers, complicating pre-surgical differential diagnostics of surgical group of diseases [5, 7].

In recent years awareness of anatomical peculiarities of a patient depending not only on sex but also age, constitution, individual

anatomical peculiarities of a person, has become of an important value, especially for the diagnostic direction of practical medicine in particular [1, 2].

A wide application of up-to-date mini-invasive diagnostic methods and visualization of organs such as ultrasound diagnostics (USD), computed tomography (CT), and magnetic-resonance imaging (MRI), opens new possibilities for the detection of individual lifetime anatomical norm of the healthy human body and pathology at the early stages of disease with ability to prevent it [9, 10, 11, 12].

Objective of our study was to determine the frequency of occurring morphological peculiarities of the gallbladder structure and its functional changes that can be risk factors promoting development of bile expelling structure pathology in healthy adult individuals of different sexes according to the findings of ultrasound examination of the HDA organs.

Materials and methods. To determine the sickness rate of gallbladder and bile ducts diseases 136 adult people were examined by means of the ultrasound apparatus «Radmir Ultima RU-928» including 60 (44,1%) men and 76 (55,9%) women.

The investigation was conducted by means of a convective sensor with the frequency band from 1 to 5 mHz ($\pm 0,5$ mHz) R60 mm. The study was conducted on the base of USD consulting room at Volyn Regional Infectious Hospital.

The individuals involved in the study were distributed according to their sex and age in the following way:

- 48% of the general number of those examined were men (37 individuals), including 27% of them (10 individuals) at the age of 36 years, and 73% (27 individuals) – older than 36;

- 52% of the general number of those examined were women (40 individuals), including 42,5% of women (17 individuals) aged under 36 years, and 57,5% (23 individuals) – older than 36 (Fig. 1).

The investigations were conducted with the purpose of screening within the frame of planned preventive mass-scale examinations or ultrasound diagnostics of the internal organs according to references not connected with pathology of the hepatic-biliary structures. Anamnesis of the examined patients did not contain any diseases of the liver, gallbladder and bile ducts.

Results. No pathological changes in the

examined structures that could be considered as diseases were found in 77 individuals (56%) (Fig. 2).

Although the examined patients did not complain of disorders from the side of the hepatic-biliary system, certain morphological peculiarities (flexion) and functional disorders (cholestasis) of the examined structures were found (Table 1). The following morphological changes were found among 37 examined men:

- flexion in the neck portion in 3 people (8,1%),
- flexion in the portion of the body – 1 person (2,7%),
- flexion in the portion of the fundus – 2 individuals (5,4%),
- flexion in the portion of the body and neck – 2 individuals (5,4%), (Fig. 3).

Similar changes were detected among the examined 40 women:

- flexion in the neck portion in 4 individuals (10%),(Fig. 4),
- flexion in the portion of the body – 1 person (2,5%),
- flexion in the portion of the fundus – 1 person (2,5%).

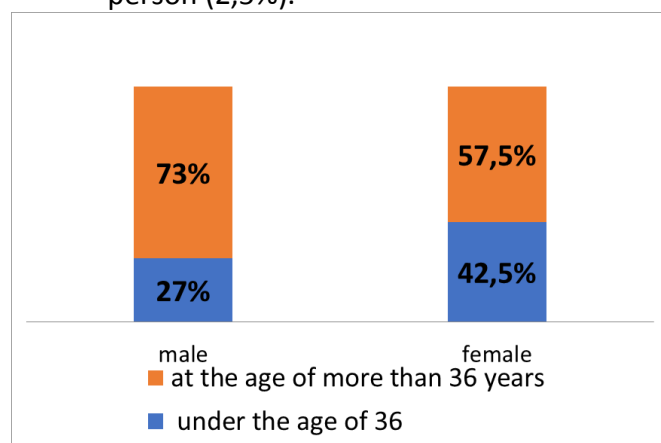


Fig. 1. Distribution of the examined people by sex and age.

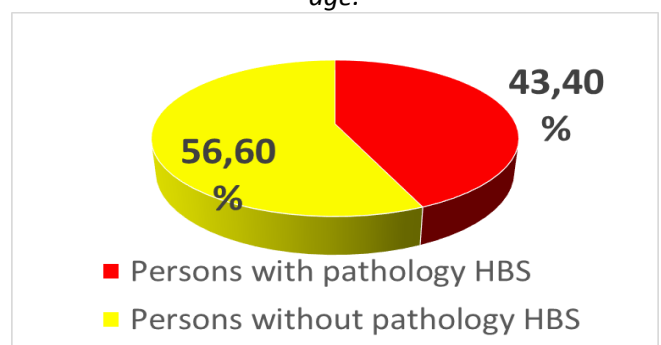


Fig. 2. Distribution of the examined adult individuals according to the frequency of detection of hepatic-biliary system (HBS) pathology.

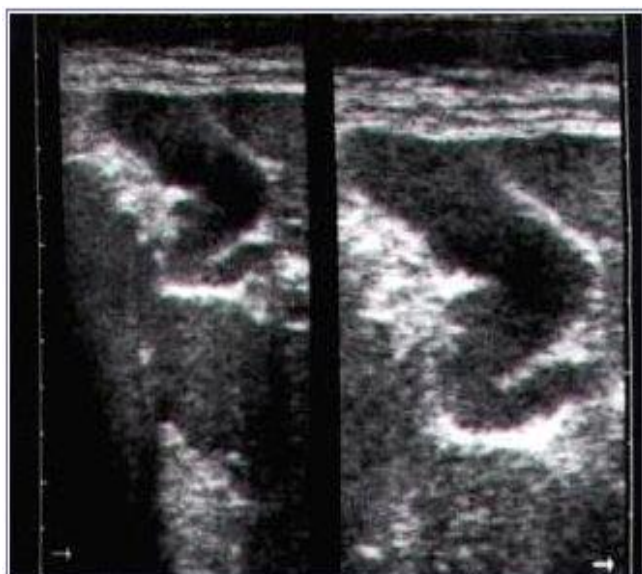


Fig. 3. Ultrasound image of the gallbladder with flexion in the portion of the neck and body.

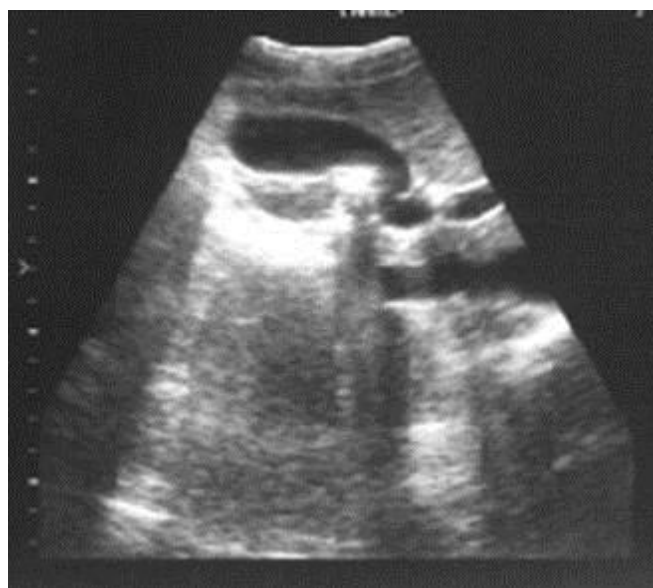


Fig.4 Ultrasound image of the gallbladder with flexion in the portion of the neck.

Therefore, among the group of randomized patients who do not complain of disorders from the side of the liver and bile ducts as well as diseases that could influence on the condition of the hepatic-biliary organs in anamnesis, the frequency of morphological peculiarities in the form of gallbladder flexion constitutes 18,2% from the general amount of the examined people including 21,6% of men and 15% of women among them.

Screening examination of the adult individuals detected functional changes in the examined area – cholestasis in particular, that was not clinically manifested. Asymptomatic cholestasis during examination was found in 16 people which constitutes 20,8% from general number of the

healthy examined people including 7 men (18,9% from all the examined men) and 9 women (22,5% from all the examined women) (Table 2).

Discussion of the results. Numerous sources of scientific literature are indicative of sickness rate increase concerning the organs of the hepatic-biliary system as well as increased possibilities of modern methods of their early diagnostics in individuals without clinical signs of disease [4, 6, 7]. Results of our studies evidenced rather high frequency of changes found in the examined structures that can be considered as diseases in 43,4% randomized adult individuals who underwent screening examinations or ultrasound diagnostics of the internal organs according to

Table 1.

Frequency of occurrence of gallbladder flexion of different localization in adult individuals of different sexes

Localization of flexion	Men				Women			
	Younger than 36		Older than 36		Younger than 36		Older than 36	
	10	100%	27	100%	17	100%	23	100%
Flexion in the neck	1	10	2	7,4	1	5,9	3	13
Flexion in the body			1	3,7			1	4,3
Flexion in the fundus			2	7,4			1	4,3
Flexion in the body and neck			2	7,4				

Table 2.

Frequency of occurrence of asymptomatic cholestasis in adult individuals of different sexes

Sex	Men				Women			
Age	Under 36		Older than 36		Under 36		Older than 36	
Number of examined people	10	100%	27	100%	17	100%	23	100%
Cholestasis	3	30	4	14,8	5	29,4	4	17,4

references not connected with pathology of the structures of the hepatic-biliary system. Ultrasound diagnostics was found to be able to detect morphological peculiarities in the form of flexion localized in different portions of the gallbladder and asymptomatic functional disorders in the form of cholestasis even when patients did not complain of disorders from the side of the liver and bile ducts.

Therefore, the results of the studies conducted enable to draw the following **conclusions**:

1. Screening ultrasound diagnostic examination enable to detect both morphological peculiarities and functional disorders of the gallbladder – cholestasis in particular, in adult patients who do not complain of disorders that were not clinically manifested.

2. Among the examined 77 healthy adult individuals including 37 men and 40 women gallbladder flexion was found in 18,2% from the general number of those being examined (21,6% of men and 15% of women). Examination detected asymptomatic cholestasis in 16 people which is 20,8% from general number of those healthy examined ones including 18,9% of men and 22,5% of women.

3. Detection of morphological peculiarities of the gallbladder structure and early diagnostics of cholestasis in individuals without complaints and clinical manifestation of pathology of HAD organs enable to improve the efficacy of preventive measures directed to maintenance of functional ability of the examined structures.

Prospects of further studies. High sickness rate of the diseases of the gallbladder and bile ducts is a foundation to conduct investigations with the purpose to find morphological peculiarities of the examined structures that can influence on their function. The search for new up-to-date method of early diagnostics of morphological and functional changes with the aim to improve the efficacy of preventive and therapeutic measures becomes especially important.

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CONTENT:

Stefano Martinotti, Elena Toniato, Ganna Maryniuk THE ROLE OF AUTOPHAGY PROCESSES IN MAINTAINING VITAL ACTIVITY OF TUMOUR CELLS UNDER CONDITIONS OF STRESS ON THE PATTERN OF MELANOMA CELL LINE	3
Boiko V.V., Zamiatin P.M., Zamiatin D.P. SURGERY OF CORDIAL DAMAGES	8
Pokotylo V.Y. PECULIARITIES OF MYOCARDIAL ULTRASTRUCTURE OF RATS AT THE LATE TERMS OF OPIOID INTOXICATION	12
Popadiuk O.J., Genyk S.M., Melnyk D.A. WUNDHEILENDE EIGENSCHAFTEN UND STABILITÄT DER NANOHALTIGEN BIODEGRADIERENDEN POLYMERMATERIALIEN IN VERSCHIEDENEN AUFBEWAHRUNGSZEITEN (EXPERIMENTELLE UNTERSUCHUNG)	18
Ganbarov Kh.G., Shafiyeva S.M., Agayeva N.A., Delegan Y.A., Petrikov K.V., Agamaliyev Z.A., Eyvazova G.M. FORMATION OF SILVER NANOPARTICLES USING BIOMASS OF BACTERIUM ACTINOMYCES SPP. NSX-333	23
Turkevych M.O. NON-SURGICAL TACTICS OF LIGATURE CORRECTION OF FACIAL SOFT TISSUE AGE CHANGES AND SUBSTANTIATION OF THEIR USE IN COSMETIC DERMATOLOGY ON THE BASIS OF PATHOMORPHOLOGICAL INVESTIGATIONS CONDUCTED	26
Honcharenko G.Y., Sitnikova V.A., Rosh L.G., RECEPTOR ACTIVITY OF THE EUTOPIC AND ECTOPIC ENDOMETRIUM TO ESTROGEN AND PROGESTERONE MARKERS IN CASE OF ADENOMYOSIS AVAILABLE DURING POSTMENOPAUSE	31
Kasiyanchuk M.V. PREVENTION OF ATROPHY PROCESS OF THE COLLATERAL CREST BY MEANS OF CONJUNCTIVE APPLICATION OF DISCRETELY STABILIZED MESOSTRUCTURES ON IMPLANTS	38
Tovkach Yu.V., Bazik O.N., Andriets V.I. FEATURES OF THE MUSTICATORY MUSCLE MICROCIRCULATORY BLOODSTREAM IN THE IN THE EARLY POST-TRAUMATIC PERIOD OF THE EXPERIMENTAL TRAUMA IN THE MAXILLOFACIAL AREA (literature review)	44
Hoshovska A.V., Hoshovskyi V.M. MISCARRIAGE PREVENTION IN MULTIPARA WOMEN IN THE SECOND TRIMESTER OF PREGNANCY	48
Hoshovska A.V., Hoshovskyi V.M. PROGNOSTICATION OF DEVELOPMENT OF PLACENTAL DYSFUNCTION AND GESTATIONAL COMPLICATIONS IN WOMEN WITH VARICOSE VEINS	51
Tsytoivskyi M.N. HISTOLOGICAL STRUCTURE OF AORTAL WALL IN RATS AND BRANCHES OF ITS BLOOD CIRCULATION AT THE LATE STAGES OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS	54
Tagiyev A.N., Karimova N.A. THE SPECIES COMPOSITION AND TROPHIC RELATIONSHIP OF BIRD POPULATIONS COMING TO WINTER IN GYZYLAGAJ BAY AND ADJACENT TERRESTRIAL AREAS, AZERBAIJAN	60
Varyvoda O., Gutor T. PROGNOSTICATION OF POLYP LESION STRUCTURE OF THE COLON PORTIONS FOR NEAR-TERM OUTLOOK	64
Byrchak I.V., Borsuk O.A. PECULIARITIES OF PRENATAL DIAGNOSTICS IN THE FIRST TRIMESTER OF GESTATION IN WOMEN WITH COMPLICATED OBSTETRICAL ANAMNESIS	67
Kordiyak O.Y., Masna Z.Z., Pupin T.I. MORPHOLOGICAL SIGNS OF DESTRUCTION AND REGENERATION OF THE PERIODONTAL TISSUE OF RATS IN EXPERIMENT	70
Gerasymchuk M.R., Koval T.I. THE EFFECT OF TRACE ELEMENT STATUS OF RATS WITH SIMULATED HYPOTHYROIDISM ON THE STATE OF CONGITIVE FUNCTIONS	75
Zubko L.Yu., Masna Z.Z. MORPHOLOGICAL PECULIARITIES OF THE GALLBLADDER STRUCTURE AND ITS FUNCTIONAL CHANGES IN HEALTHY INDIVIDUALS OF DIFFERENT SEXES ACCORDING TO THE FINDINGS OF ULTRASOUND EXAMINATION	80



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