DOI 10.29254/2077-4214-2023-4-171-337-341
UDC 616.24-091.8:616-001.17-085.23]-092.9

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MORPHOMETRIC ANALYSIS OF THE LUNGS PARAMETERS OF WHITE RATS WITH EXPERIMENTAL THERMAL INJURY AND UNDER THE CONDITIONS OF SURFACTANT USE

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Thermal injury is one of the most urgent medical and social problems, and it is advisable to find pulmonary protectors that increase the resistance of the lungs to the influence of pathological factors. Compensation of the endogenous surfactant deficiency, promotes recovery of damaged lung tissue in thermal injuries, in this connection, special attention is paid to various types of surfactants. The study was conducted on 70 adult male rats, which were divided into a group of animals with thermal injury (40), animals with intratracheal administration of an exogenous surfactant (30). Preparation of material for microscopic and morphometric study of lung structure was carried out according to generally accepted methods. In the late stages of the study (14th, 21st days), a gradual decrease in the average values of the relative proportion of vessels, bronchi, and lymphoid tissue was determined morphometrically, compared with similar indicators in the group of animals without correction of thermal injury with surfactant. Morphometrically, this was confirmed by the decrease on the 14th day of the average values of the portions of dys- and atelectasis and emphysematously changed lung tissue to (15.22±0.46) %, (15.17±0.52) % and (24.58±0.96)).) % (in all cases p<0.001) in accordance with the average values in the group of animals without correction of thermal injury with the surfactant. The positive effect of the use of the surfactant in the early period after a thermal injury on the structural components of the lungs was moderate. However, already on the 7th day of the experiment, the organization of the respiratory portion improved, the areas of dys- and atelectasis, emphysema zones decreased, and the area of unchanged lung tissue increased. In the late period after burn injury under the conditions of administration of the surfactant, there was a lower degree of destruction of blood vessels, bronchi and components of the respiratory portion.

Key words: *lungs, thermal injury, surfactant, morphometry.*

Connection of the publication with planned research works.

The work is a fragment of the research work «Remodeling of blood vessels of internal organs and tissues in various pathological conditions in the experiment», state registration number 0111U008026.

Introduction.

Thermal damage is one of the most urgent medical and social problems of modern medicine in the world and in Ukraine, in particular from the point of view of man-made disasters and military conflicts. Thermal injuries are one of the most severe types of military injuries. In local wars and military conflicts, thermal burns occur in 1.5-3.6% of all casualties [1-4].

Stable and fairly high mortality in burn shock and especially in the period of acute burn toxemia forces us to admit that the problem of thermal burns remains relevant even in the present [5]. A burn injury is specific, as a rule, it is not sufficiently familiar even to surgeons, traumatologists or intensive care doctors and has significant differences from all other types of injuries – the prevalence of tissue damage, severity, duration of shock and intoxication, frequency of development of generalized infection and other complications of burn disease [6, 7]. Burn disease is the body's response to a severe burn injury, as a result of which numerous skin receptors are irritated, damaged and destroyed, with subsequent changes in the vital activity of all internal organs and systems and disruption of all types of metabolism. Burn disease has a complex, multi-component pathogenesis, the individual links of which become more important at different time intervals after receiving a burn: hypovolemia and impaired blood circulation, especially microcirculation, in the first day after receiving a burn; acute intoxication – in the first 1–2 weeks; infection – in the following weeks [8, 9].

Burn injury, among other etiological factors, causes the development of ARDS – acute respiratory distress syndrome. It is advisable to search for pulmonary protectors that increase the resistance of the lungs to the influence of pathological factors. Compensation of the endogenous surfactant deficiency contributes to the recovery of damaged lung tissue in case of thermal injuries; in this connection, special attention is paid to various types of surfactants. Therefore, it is important to study the morphometric analysis of the structural components of the lungs in the dynamics after thermal injury and under the conditions of use of the surfactant.

The aim of the study.

To establish changes in the morphometric parameters of the lungs of white rats during experimental thermal injury and under the conditions of use of the surfactant.

Object and research methods.

Experimental studies were performed on 70 adult white male rats with a body weight of (190±20) g. The animal care and all manipulations were carried out in accordance with the recommendations of the «European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes» (Strasbourg, 1986), as well as in accordance with the provisions of the «General Ethical Principles of Animal Experiments» adopted by the First National Congress on Bioethics (Kyiv, 2013). Burns were simulated under sodium thiopental anesthesia with copper plates heated in boiled water to a temperature of 97-100 °C

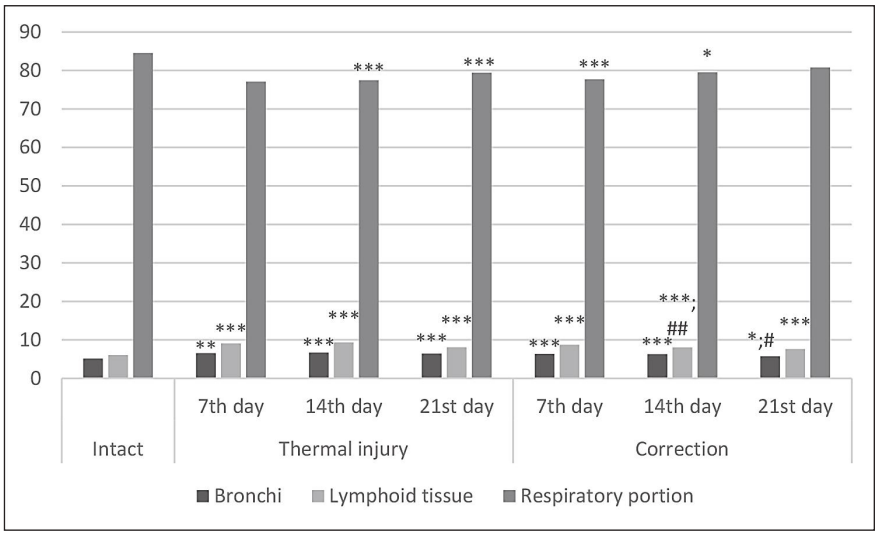

Figure 1 – The ratio of the structural components of the lungs of animals at different stages after thermal injury and under the conditions of use of the surfactant drug.

Notes: * – values that are statistically significantly different from the indicators of animals of the intact group (* – p<0.05; ** – p<0.01; *** – p<0.001); # – values that are statistically significantly different from the indicators of the control group of animals (# – p<0.05; ### – p<0.001).

on the epilated surface of the skin of the back and side surfaces of the animal's body for 15 seconds. The size of the affected area was 18-20% of the body surface. Animals of the second experimental group, 1 day after the thermal injury under general anesthesia, were given a single intratracheal injection of the exogenous surfactant «Kurosurf»® (registration certificate in Ukraine No. UA/10170/01/01) at a dose of 300 mg/kg of animal body weight.

Lungs were then collected for morphological examination. After fixing pieces of tissue in a 10% neutral formalin solution, dehydration in alcohols of increasing concentration and embedding in paraffin, histological sections with a thickness of 5-6 µm were made, which were stained with hematoxylin and eosin [10]. Morphometric studies were performed using a system of visual analysis of histological slides. Images from histological preparations were displayed on the computer monitor from the microscope "MICROmed SEO SCAN" and with the help of the video camera "Vision CCD Camera" and the program SEO ImageLAB Bio, and Microsoft Excel, "STATISTICA" 12.0 on a personal computer.

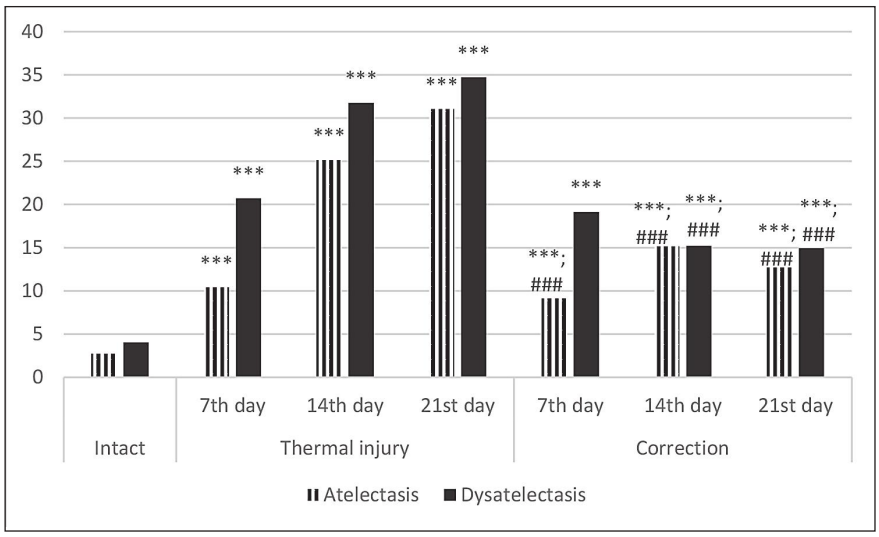

Figure 2 – Dynamics of changes in the structural components of the respiratory portion of the lungs at different stages after experimental thermal injury and under conditions of correction.

Notes: * – values that are statistically significantly different from the indicators of animals of the intact group (* – p<0.05; ** – p<0.01; *** – p<0.001); # – values that are statistically significantly different from the indicators of the control group of animals (# – p<0.05; ### – p<0.001).

Research results and their discussion.

Microscopic examinations of the lungs on the 7th day after the burn injury in the conditions of administration of the surfactant revealed hemodynamic disorders, changes in the structural organization of the bronchi and components of the respiratory portion, but these changes were not as significant as in the group of animals without correction of thermal injury. Morphometrically, in this term of the experiment, under the conditions of surfactant use, there was a decrease in the average values of the relative areas of blood vessels, bronchi, and lymphoid tissue by (7.25±0.31)%; (6.34±0.25) % and (8.71±0.34) % compared to the average values in the group of animals without correction of thermal injury, but at this time they were not yet reliable **(fig. 1)**. Therefore, the average value of the relative share of the respiratory portion increased and it was (77.70±1.98)%, which indicated the initial restoration of the structural organization of their components. during the morphometric examination, the average values of the parts of dys- and atelectasis, emphysematous dilated alveoli decreased by (9.13±0.34)% (p<0.01); (19.13±0.60)% and (33.12±1.30)% (p<0.01), and the average value of the area of lung tissue with unchanged histoarchitectonics significantly increased by 1.37 times compared to the average value of the group animals without correction and it was (42.62±1.62)% (p<0.001) **(fig. 2).**

In the late stages of the experiment, a gradual decrease in the average values of the relative share of blood vessels, bronchi, and lymphoid tissue was established morphometrically compared to similar indicators in the group of animals without correction of thermal injury with the surfactant use. They were equal, respectively, on the 14th day: (6.19±0.24)%, (6.27±0.29)% and (8.04±0.31)% (p<0.01) and on the 21st day: (5.91±0.23)%, (5.72±0.23)% (p<0.01) and (7.59±0.31)%. Due to this, there was an increase in the average value of the relative area of the respiratory portion in relation to the indicators of the group of animals to which the corrective drug was not applied on the

14th day – (79.50±2.01)% and on the 21st day – (80.78±2.05)%. Morphometrically, this was confirmed by the decrease on the 14th day of the average values of the portions of dys- and atelectasis and emphysematously changed lung tissue to (15.22±0.46)%, (15.17±0.52)% and (24.58±0.96)% (in all cases p<0.001) compared to the average values in the group of animals without correction of thermal injury with surfactant. On the 21st day, their average values significantly decreased to 2.39; 2.44 and 1.63 times relative to similar indicators of a group of animals without the use of a corrective drug.

Table – Morphometric parameters of the medium-sized arteries of the lungs and the Vogenvoort index at different times after thermal injury under the conditions of the use of the surfactant drug (M±m)

Term of experiment		Parameters, μm			
		Outer diameter of vessels	Inner diameter of blood vessels	The thickness of the vessel wall	Vogenvoort index
Intact		107,01±2,12	65,40±1,37	20,81±0,63	167,8±3,7
Burn	7th day	117,14±3,08**	52,14±1,27***	32,50±0,90***	404,7±9,6***
	14th day	118,07±2,5**	53,47±1,34***	32,30±1,17***	387,6±8,9***
	21st day	115,61±2,50**	54,08±1,51***	30,77±0,95***	357,0±9,1***
Burn + correction	7th day	115,2±2,31**	55,81±1,68***	29,70±0,80***,#	326,1±9,5***,###
	14th day	113,7±2,92	56,30±1,94***	28,69±0,77***,##	307,9±8,6***,###
	21st day	111,6±3,01	60,71±2,05##	25,45±0,73***,###	237,9±7,4***,###

Notes: * – values that are statistically significantly different from the indicators of the intact group of animals (* – p<0.05, ** – p<0.01, *** – p<0.001); # – values that are statistically significantly different from the indicators of the group of untreated animals (# – p<0.05, ## – p<0.01, ### – p<0.001).

According to morphometric studies of medium-sized arteries already in the stage of toxemia under the conditions of administration of the surfactant, the average values of their outer diameter and wall thickness decreased to (115.2±2.31) μm and (29.70±0.80) μm (p<0.05), and the average value of their outer diameter increased and it was (55.81±1.68) μm, so the value of the Wogenvoort index decreased by 24.1% and was equal to 326.1±9.5 (p<0.001).

A similar tendency to a decrease in morphometric parameters was also characteristic of the small-sized arteries of the lungs of animals under the conditions of correction of a burn injury with surfactant. Thus, there was a decrease in the average value of their wall thickness to (16.61±0.50) microns due to a decrease in the average value of the outer diameter to (62.13±2.36) microns and an increase in the inner diameter – (28.91±0.98) μm relative to the indicators in the group of animals without the use of a corrective drug. The average value of the Wogenvoort index of such vessels was 361.9±8.3 (p<0.001), which was 13.4% less than in the group of animals without correction **(table).** Thus, in medium-size arteries, the average values of their outer diameter and wall thickness on the 14th day were reduced to (113.7±2.92) μm and (28.69±0.77) μm (p<0.01); on 21st day – (111.6±3.01) μm and (25.45±0.73) μm (p<0.001), respectively, compared to similar values in the group of animals without correction.

The average value of the internal diameter of such arteries on the 14th day increased by 5.3% relative to the average value of the group of animals without the use of the corrective drug and it was (56.30±1.94) μm. And on the 21st day, the average value of their internal diameter was equal to (60.71±2.05) μm (p<0.01), which was 12.3% less compared to the similar indicator of the group of animals without correction. Therefore, the Wogenvoort index of such arteries decreased on the 14th day and it was 307.9±8.6, and on the 21st – 237.9±7.4 (in both cases p<0.001), which was reliably 1.26 and 1.5 times less similar values in the group without thermal injury correction.

When conducting morphometric studies of small-sized arteries, it was also established that the average values of the outer diameter and wall thickness on the 14th day decreased to (61.80±2.22) μm and (16.09±0.47) μm (p<0.01), and at 21 – (59.45±2.30) μm and (13.64±0.38) μm (p<0.01) relative to similar values in the group of animals without correction of thermal injury. The average value of the internal diameter increased significantly by 8.4% on the 14th day, and by 10% on the 21st day compared to the indicators of the group of animals without the use of the corrective drug. Therefore, the Wogenvoort index of such arteries decreased by 1.34 times on the 14th day, and by 1.38 times on the 21st, respectively.

Conclusions.

The positive effect of using the surfactant in the early stage after a thermal injury on the structural components of the lungs was moderate. However, already on the 7th day of the experiment, the organization of the respiratory portion improved, the areas of dys- and atelectasis, emphysema zones decreased, and the area of unchanged lung tissue increased.

On the 14th and 21st days after applying the burn under the conditions of administration of the surfactant drug, there was a lower degree of destruction of blood vessels, bronchi and components of the respiratory portion. The average values of the areas of the unchanged lung tissue of the respiratory portion of the lungs increased significantly compared to the indicators of the group of burned animals, respectively by 4.48 times on the 14th day and by 7.99 times on the 21st day (p<0.001). The average values of the areas of atelectasis, acute emphysematous expansion decreased by 2.44 and 1.63 times (p<0.001) on 21st day compared to the indicators of the group of animals with burns without correction.

Prospects for further research.

These studies provide a scientific basis for establishing correlations between other types of corrective factors of thermal skin injury.

References

1. Yakupu A, Zhang J, Dong W, Song F, Dong J, Lu S. The epidemiological characteristic and trends of burns globally. BMC Public Health. 2022;22(1):1596. DOI: 10.1186/s12889-022-13887-2.
2. Yeroshenko GA, Donets IM, Shevchenko KV, Grigorenko AS, Ryabushko OB, Klepets OV. Structural features of rat lungs and their remoding after the action of various exogenous factors. Bulletin of problems biology and medicine. 2021;2(160):26-29. DOI: 10.29254/2077-4214-2021-2-160-26-29.

3. Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S. Burn injury. Nat Rev Dis Primers. 2020;6(1):11. DOI: 10.1038/s41572-020-0145-5.
4. Cheng YJ, Chan KC, Chien CT, Sun WZ, Lin CJ. Oxidative stress during 1-lung ventilation. The Journal of Thoracic and Cardiovascular Surgery. 2006;132(3):513-518. DOI: 10.1016/j.jtcvs.2006.03.060.
5. Popko SS. Morphological features of the respiratory part in guinea pigs lung in dynamics of experimental allergic inflammatory process. Reports of Morphology. 2022;28(1):54-58. DOI: 10.31393/morphology-journal-2022-28(1)-08.
6. Davenport ML, Sherrill TP, Blackwell TS, Edmonds MD. Perfusion and Inflation of the Mouse Lung for Tumor Histology. J Vis Exp. 2020;(162):10.3791/60605. DOI: 10.3791/60605.
7. Lykhatskyi PH, Ohinska NV, Getmanyuk IB, Nebesna ZM, Trach Rosolovska SV. Morphometric analysis of lungs parameters under conditions of simulated burn injury. Reports of Morphology. 2023;29(1):35-39. DOI: 10.31393/morphology-journal-2023-29(1)-05.
8. Weibel ER. Lung morphometry: the link between structure and function. Cell Tissue Res. 2017;367(3):413-426. DOI: 10.1007/s00441-016-2541-4.
9. Usenko OY, Sydiuk AV, Sydiuk OY, Klimas AS, Savenko GY, Teslia OT. Morphometric indicators for selection of dual endobronchial tube in thoracic anesthesiology. Reports of Morphology. 2022;28(1):64-68. DOI: 10.31393/morphology-journal-2022-28(1)-10.
10. Goralskiy LP, Homich VT, Kononskiy OI. Fundamentals of histological technique and morphofunctional methods of research in norm and in pathology. Zhitomir: Polissya; 2011. 288 p. [in Ukrainian].

МОРФОМЕТРИЧНИЙ АНАЛІЗ ПАРАМЕТРІВ ЛЕГЕНЬ БІЛИХ ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ТЕРМІЧНІЙ ТРАВМІ ТА ЗА УМОВ ЗАСТОСУВАННЯ ПРЕПАРАТУ СУРФАКТАНТУ

Небесна З. М., Клімик В. Б., Огінська Н. В.

Резюме. *Вступ.* Термічні ураження – одна з найактуальніщих медико-соціальних проблем і доцільним є пошук пульмопротекторів, які підвищують стійкість легенів до впливу патологічних факторів. Компенсація дефіциту ендогенного сурфактанту, сприяє відновленню ушкодженої тканини легень при термічних травмах у зв'язку з цим надається особлива увага різним видам сурфактантів.

Об'єкт і методи. Дослідження проведено на 70 статевозрілих щурах самцях, які поділено на групу тварин з термічною травмою (40), тварини з інтратрахеальним введення екзогенного препарату сурфактанту (30). Підготовку матеріалу для мікроскопічного та морфометричного досліджень структур легень проводили за загальноприйнятною методикою.

Результати і обговорення. У пізні терміни досліду (14, 21 доба) морфометрично встановлено поступове зниження середніх значень відносної частки судин, бронхів, лімфоїдної тканини порівняно із аналогічними показниками у групі тварин без корекції термічної травми препаратом сурфактанту. Морфометрично це підтверджується зменшенням на 14 добу середніх значень часток дис- та ателектазів і емфізематозно зміненої легеневої тканини до (15,22±0,46)%, (15,17±0,52)% і (24,58±0,96)% (у всіх випадках $p<0,001$) порівняно із середніми значеннями у групі тварин без корекції термічної травми препаратом сурфактанту. На 21 добу їх середні значення достовірно знижуються у 2,39; 2,44 та 1,63 раза відносно аналогічних показників групи тварин без застосування коригуючого препарату.

Висновки. Позитивний вплив застосування препарату сурфактанту в ранній термін після термічного опіку на структурні компоненти легень помірний. Проте вже на 7 добу експерименту покращується організація респіраторного відділу, зменшуються ділянки дис- та ателектазів, зони емфіземи, збільшується площа незміненої легеневої тканини. У пізні терміни після нанесення опіку в умовах введення препарату сурфактанту наявний нижчий ступінь деструкції судин, бронхів та компонентів респіраторного відділу.

Ключові слова: легені, термічна травма, сурфактант, морфометрія.

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Abstract. *Introduction.* Thermal trauma is one of the most urgent medical and social problems, and it is advisable to search for pulmonary protectors that increase the resistance of the lungs to the influence of pathological factors. Compensation of the endogenous surfactant deficiency contributes to the recovery of damaged lung tissue in case of thermal injuries; in this connection, special attention is paid to various types of surfactants.

Object and methods. The study was conducted on 70 adult male rats, which were divided into a group of animals with thermal injury (40), animals with intratracheal administration of an exogenous surfactant (30). Preparation of material for microscopic and morphometric studies of lung structures was carried out according to generally accepted methods.

Results and discussion. In the late stages of the experiment (14th, 21st days), a gradual decrease in the average values of the relative proportion of blood vessels, bronchi, and lymphoid tissue was determined morphometrically compared to similar indicators in the group of animals without correction of thermal injury with the surfactant use. Morphometrically, this was confirmed by the decrease on the 14th day of the average values of the portions of dys- and atelectasis and emphysematously changed lung tissue to (15.22±0.46)%, (15.17±0.52)% and (24.58±0.96)% (in all cases $p<0.001$) compared to the average values in the group of animals without correction of thermal injury with surfactant. On the 21st day, their average values significantly decreased to 2.39; 2.44 and 1.63 times relative to similar indicators of a group of animals without the use of a corrective drug.

Conclusions. The positive effect of using the surfactant in the early stages after a thermal burn on the structural components of the lungs was moderate. However, already on the 7th day of the experiment, the organization of the respiratory department improved, the areas of dys- and atelectasis, emphysema zones decreased, and the area of unchanged lung tissue increased. In the late stages after the burn under the conditions of administration of the surfactant, there was a lower degree of destruction of blood vessels, bronchi and components of the respiratory portion.

Key words: lungs, thermal injury, surfactant, morphometry.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received 01.06.2023
Accepted 10.11.2023

DOI 10.29254/2077-4214-2023-4-171-341-350
UDC 615.099:[546.48:546.81]:616.36-092.9-07-085.243.3

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EXPERIMENTAL STUDY OF CADMIUM ACCUMULATION IN THE LOWER JAW OF RATS UNDER CONDITIONS OF CORRECTION WITH ZINC AND IRON SUCCINATE

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One of the most dangerous, long-lasting and persistent pollutants is heavy metals, the most typical anthropogenic associations of which are lead Pb, cadmium Cd, nickel Ni, as well as tin Sn, cobalt Co, mercury Hg, antimony Sb and bismuth Bi. Currently, the most common environmental toxicants in the group of heavy metals are mercury, cadmium and lead salts, which, when ingested by mammals, stimulate oxidative stress and compete with biogenic metals (zinc, manganese, copper, iron, calcium, etc.) for binding to the active site of many proteins and enzymes, causing their dysfunction. Accumulating in the body, cadmium has both direct and indirect adverse effects, mainly on the reproductive, endocrine, immune systems, blood, kidneys and bone tissue, causing significant changes in them and inducing degeneration and transmutation of the cellular apparatus. The peculiarities of cadmium accumulation in rat bones under isolated administration and under correction conditions with zinc and ferrous succinates were determined using polyelement analysis of biological materials and objects by atomic emission with electric arc atomisation.

Chronic intragastric administration of cadmium chloride at a dose of 2.0 mg/kg leads to a decrease in the zinc level in the mandible's bone tissue compared to the control values both on the 20th and 30th day of the study. Zinc succinate reduces the cadmium level in the mandible's bone tissue with combined intragastric administration in a chronic experiment on rats. During the 30 days of the experimental study, no significant difference in the effect of the studied factors on the calcium level of the mandible was determined.

Key words: *cadmium intoxication, ferrous succinate, zinc succinate, mandible, atomic emission.*

Connection of the publication with planned research works.

The work was performed within the framework of the research work of the Department of Human Anatomy, Clinical Anatomy and Operative Surgery of Dnipro State Medical University "Morphological and functional state of organs and tissues under the influence of external and internal factors" (state registration number 0120U105219).

Introduction.

The fundamental task of the state environmental policy is to protect the environment in the interests of public health, particularly to reduce the risk of adverse effects of the negative impact of pollution of various natural objects on humans [1]. This activity is becoming increasingly relevant and is gaining growing recognition as environmental and health issues take an increasingly important place in politics both globally and in the European context.

One of the most dangerous, long-lasting and persistent pollutants is heavy metals, the most typical anthropogenic associations of which are lead Pb, cadmium Cd, nickel Ni, as well as tin Sn, cobalt Co, mercury Hg, antimony Sb and bismuth Bi [2, 3]. Currently, the most common environmental toxicants in the group of heavy metals are mercury, cadmium and lead salts, which, when ingested by mammals, stimulate oxidative stress and compete with biogenic metals (zinc, manganese,