

лімфаденопатії, гепатоспленомегалії з ураженням шлунково-кишкового тракту. Морфологічні та гістологічні зміни внутрішніх органів у дітей з летальним завершенням захворювання характеризувалися нерівномірним кровонаповненням, набряком і крововиливами в слизову оболонку внутрішніх органів, дистрофічними змінами паренхіматозних органів, утворенням конгломератів лімфатичних вузлів з ділянками некрозу. Отримані дані свідчать, що летальні випадки кишкового ерсиніозу зустрічалися у дітей раннього віку на тлі обтяженого преморбідного фону і супутньої патології.

**Ключові слова:** кишковий ерсиніоз, патоморфологічні зміни, гістологічні дослідження, діти.  
Стаття надійшла 12.12.18 р.

лімфаденопатии, гепатоспленомегалии с поражением ЖКТ. Морфологические и гистологические изменения внутренних органов у детей с летальным завершением заболевания характеризовались неравномерным кровенаполнением, отеком и кровоизлияниями в слизистую оболочку внутренних органов, дистрофическими изменениями паренхиматозных органов, образованием конгломератов лимфатических узлов с участками некроза. Полученные данные свидетельствуют, что летальные случаи кишечного ерсиниоза встречались у детей раннего возраста на фоне отягощенного преморбидного фона и сопутствующей патологии.

**Ключевые слова:** кишечный ерсиниоз, патоморфологические изменения, гистологические исследования, дети.

Рецензент Старченко І.І.

DOI 10.26724/2079-8334-2019-3-69-209-213

UDC 611.12-076:611.013:616-092.9:669.018.674

O.O. Nefodova, V.F. Shatorna, O.I. Halperin, O.O. Nefodov,  
G.A. Yeroshenko<sup>1</sup>, I.V. Tverdokhib, V.I. Harets  
SI "Dnipropetrovsk Medical Academy of Ministry of Health of Ukraine", Dnipro  
<sup>1</sup> Ukrainian Medical Stomatological Academy, Poltava

## CARDIOGENESIS CHANGES UNDER THE IMPACT OF CADMIUM CHLORIDE IN RAT EMBRYOGENESIS

e-mail: elenanefedova1803@gmail.com

The purpose of the experimental study was to determine the impact of cadmium chloride on the overall course of cardiogenesis with intragastric administration throughout the entire pregnancy period in rats. Experimental studies were carried out on female Wistar rats. To simulate the effect of cadmium chloride throughout the pregnancy, Wistar female rats were daily administered the aqueous solution of cadmium chloride *per os* via a probe at the dose of 1.0 mg/kg. The cardiofetal index growing in the group of exposure to cadmium chloride indicates an increase in the cardiac weight in the group when the embryo body weight is reduced due to intoxication. Microscopically, in 12.3% of rats the atrium endocardium hyperplasia was detected in the group exposed to cadmium chloride at the indicated dose. In 26-29% of rats cadmium chloride also led to changes in the formation of the embryo heart ventricles wall: thickening of both the compact and trabecular myocardium layers and the formation of abnormal forms of trabeculas.

**Key words:** cardiogenesis, myocardium, heart ventricle, atrium, cadmium.

*The study is a fragment of the research project "Morphofunctional state of organs and tissues of experimental animals and humans at ontogenesis in the normal and under the influence of external and internal factors", state registration No. 0117U003181.*

The urbanization growth inevitably leads to the environmental conditions complication in the areas occupied by industrial enterprises, transport highways, as well as in the adjacent territories. The environmental changes in industrialized countries necessitate intensive studies of the environmental factors impact on biological objects. In most industrialized countries, the priority eco-toxicants are heavy metals and, in particular, cadmium compounds, which are easily digested by plants in sufficiently large amounts, enter the bio-systems and have the property to accumulate in the body [1, 2]. Despite significant advances in the diagnosis and treatment of many cardiovascular system (CVS) diseases, in the whole world, including our country, their incidence continues to grow in adults and children [3, 10]. Over the past 25 years, the prevalence of cardiovascular pathology among the population of Ukraine has grown by 3 times, and its mortality rate has grown by 45% [7]. At the same time, according to the World Heart League, Ukraine is among the first in the European countries in terms of mortality from blood circulation and stroke diseases.

In Ukraine, since the mid-1970s and until 2016 inclusive, over half of deaths are due to blood circulatory system diseases. In 2016, mortality from the causes of this class reached 920.3 per 100,000 of population [7]. On average, today up to 73.3% of all fatal cases in Ukraine fall under three main causes of death: circulatory system diseases, external causes of death and neoplasm [7].

The results of numerous studies confirm that one of the etiopathogenetic causes is the impact of environmental factors: emissions from industrial enterprises and vehicles, radiation pollution, agricultural chemistry, use of dyes, preservation agents and other chemical additives in food production [6, 9].

However, the influence of cadmium compounds on the development of cardiovascular systems is an underinvestigated field, both in experimental morphology and in medicine. In a number of studies by

M.P. Chekunov et al. [5] it was found that with a prolonged presence of the increased concentrations of the potentially toxic stibium element in the body, there occurs a change in the QRS complex, increased voltage of P and T waves, and shift of the ST interval above the isoline. The authors distinguish the studied metals by the mechanism of pathogenetic action on the myocardium: 1) those who do not possess selective cardiotoxicity and are characterized mainly by thiol mechanism of action (lead and copper); and 2) those with the pronounced cardiotoxic action through the catecholamine mechanism (cadmium and cobalt).

In sub-acute and chronic experiments on the cadmium salts impact in animals, disorders of the cardiovascular system were observed as well as the fact that the observed functional disorders are based on biochemical changes, in particular, reduction of the glycogen level, enhancing of glycolysis processes, growth of the pyruvic acid concentration in the myocardium, decrease of the lactate dehydrogenase activity, growth in the activity of lysosomal hydrolases, phosphorylase, acid phosphatase, disorders of the nor adrenaline and adrenaline metabolism, significant increase in the activity of monoamine oxidase, acidic ribonuclease and phosphatase, phosphorylase and cathepsins [8, 4].

Thus, the topical field of morphological experimental studies is the detection of a range of disorders in the embryo cardiac morphogenesis under the impact of cadmium chloride on the pregnant female.

**The purpose** of the study was to experimentally determine the cadmium chloride impact on the overall course of cardiogenesis with intragastric administration throughout the entire period of pregnancy in rats.

**Materials and methods.** Experimental studies were performed on female Wistar rats ("Dali" vivarium, Kyiv). The choice of these laboratory animals as a study subject is due to the low level of spontaneous developmental defects (0.02-0.85%) compared to mice (0.04-15.7%) and rabbits (0.74-4.2 %) [1].

To simulate the effect and the toxic impact of the cadmium chloride exposure throughout the pregnancy period, the female Wistar rats were administered daily with a cadmium chloride solution (1.0 mg / kg) *per os* via the probe. We have selected a dose approaching the one that can enter the body from the environment when it is polluted with cadmium.

In compliance with the conditions and requirements of embryonic experiments, we have provided a complete diet, drinking water and due care for the females; the of metal solutions administering (via the probe) was carried out from the first day of pregnancy daily, at the same time of day (from 10 to 12 hours). For embryonic study, females with a dated pregnancy term were obtained using the vaginal smear method, which permitted to determine the oestrus cycle stages.

At the stage of proestrus and oestrus, males were planted in cages with females in the amount of 1: 3. The first day of pregnancy was established based on the detection of sperm in a vaginal smear. On the 13th and the 20th day of pregnancy, an operative slaughter was performed. The embryo rats were taken from the uterus, checked for the "live-dead" test, weighed, photographed and fixed by the 10% neutral formalin solution (fig. 1).

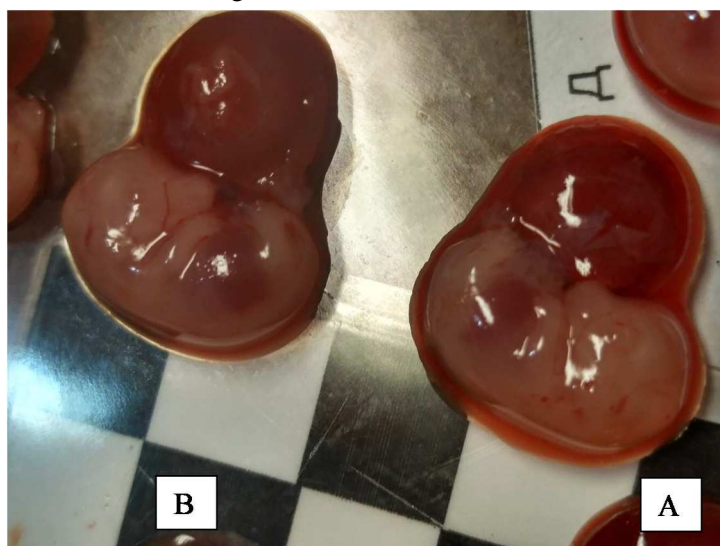


Fig. 1 Rat embryos with placenta on the 13th day of development, removed from the uterus: A - control group; B - group of exposure to cadmium chloride. The photo is made on a gauging surface to compare the size: the length of the square wall is 10mm.

To determine the impact of cadmium chloride on the course of cardiogenesis, the removal of the heart was performed in fixed embryos of rats of the 20th day in the control and experimental groups. The heart was examined from the outside for compliance with the stage of development: the atrial auricula formation, the heart sulcuses and its shape and location in the thoracic cavity were determined. Then the determination of the heart weight and its filling into paraplasm were performed according to the above factors on the course of cardiogenesis on the 20th day of cardiogenesis, we calculated the cardiofetal index: the ratio of the heart weight to the fetus weight, expressed as a percentage.

The obtained results were processed by the variation statistics method. The reliability of statistical studies was assessed using the Student's t-criterion.

Animal studies were carried out in accordance with the “General Ethical Principles of Animal Experiments” (Kyiv, 2001), which comply with the European Convention for the Protection of Experimental Animals (Strasbourg, 1985).

**Results of the study and their discussion.** Formation of the ventricle walls and the heart shape-forming processes in rats on the 13th day of embryogenesis is due to the ventricular wall delamination. The process of heart septation begins by stratification (delamination) of the ventricular myocardium’s upper part, and in the cusps formation, a significant role is played by endocardial pads: provisional organs for future atrial-ventricular valves. Simultaneously with the formation of the atrial-ventricular lumen valves formation of the heart papillary-trabecular apparatus occurs. The myocardium, as a result of delamination is divided into 2 layers: the internal one, which, together with the endocardial pads remnants, gives rise to the valve cusp, forms the primary chordae tendinae, and the primary papilliform muscles; and the myocardium external layer, which becomes a substrate forming the compact myocardium and heart trabeculae.

Thus, the formation of chordae tendinae, trabecular myocardium, and the ventricular papilliform muscles is the unitary process that occurs with the separation of the valve cusps in the process of delamination. It is during this period that cardiogenesis is actively undergoing rebuilding of the embryonic heart’s primary myocardial ventricular layer and formation of the compact and the trabecular layers of the heart wall. Under the cadmium chloride impact, on the 13th day of embryogenesis, the thickness of the ventricle wall was 26-29% compared to that in the control group (fig. 2). Such significant thickening occurred both in the compact and in the trabecular layers of the myocardium.

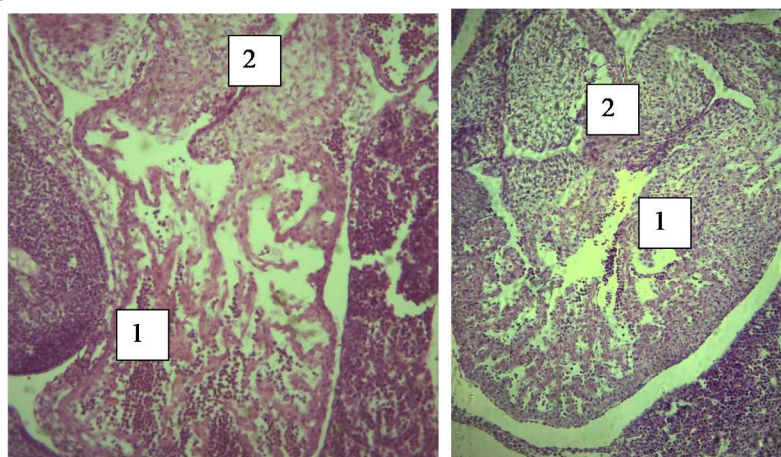


Fig. 2. Microphotography of the histological section of the embryo rat’s heart of the 13th day of embryogenesis. A - the heart of the control group; B - the heart of the embryo group exposed to cadmium chloride. Magn. x 400. Staining by hematoxylin according to Heidenhain. Labelling: 1 - heart ventricle, 2 - endocardial pads.

With an external examination of the removed embryonic hearts on the 20th day of embryogenesis, we have not detected external developmental defects of the heart, though the study of the cadmium chloride effect on the heart development revealed certain deviations in cardiogenesis compared to the control group. Disorder of the cardiogenesis course has already been detected at the level of determining the rat embryos’ heart weight, i.e. in the cadmium intoxication group, not only the embryos’

body weight reduction itself, but also a reliable decrease in the heart weight was observed. In order to exclude errors concerning the effect of embryos’ weight and their hearts weight reduction on the cardiotoxicity indices, we calculated the cardiofetal index on the 20th day of embryogenesis, i.e., at the end of the rat prenatal development (fig. 2).

Table 1

**Values of the cardiofetal index and the number and weight of rat embryos on the 20th day of development in the experiment**

Index	Control	Group exposed to the cadmium chloride impact
Number of live fetuses per 1 female	9.50±0.13	7.88±0.40*
Body weight of 1 fetus, g	2.76±0.07	2.72±0.08
Embryo heart weight, mg	34.08±0.53	34.80±0.58
Cardiofetal index	1.23±0.02	1.33±0.04

Notes: \* -  $p < 0.05$ ; compared to the control group.

As it is shown by the analysis of the results obtained with the cadmium chloride impact on pregnant females, there are changes not only in the number of live embryos in the litter, but also changes in the massometric indices of the embryos themselves. Thus, compared to the control group, a reliable reduction in the number of live embryos was determined in the group exposed to cadmium chloride, though the mean indices of fetus weight and the heart weight had no reliable difference (table 1). An increase in the

cardiofetal index in the group exposed to cadmium chloride indicates an increase in the cardiac weights in the group when the body mass of the embryo is reduced due to the intoxication.

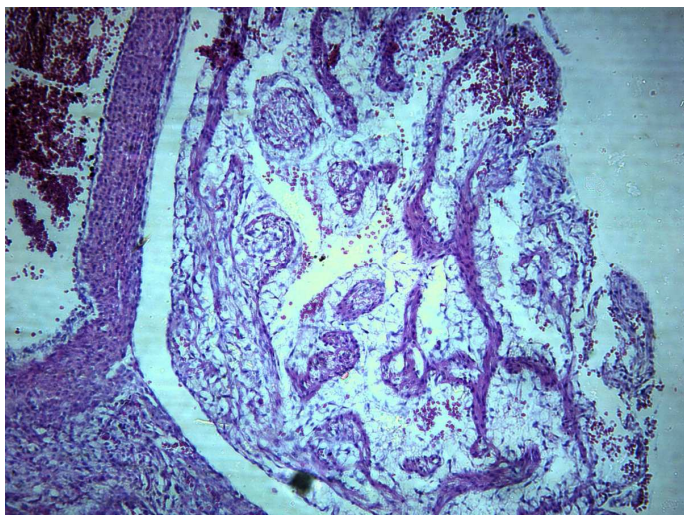


Fig. 3. Microphotography of the rat embryo heart atrium on the 20th day of embryogenesis under the exposure to cadmium chloride. Hyperplasia of the atrial endocardium. Magn. x 400. Staining by hematoxylin according to Heidenhain.

myocardium in 26-29% of the experimental group. In addition to the thickening, the formation of stratifications in the trabecular myocardium and the formation of abnormal forms of “claviform” trabeculae were also determined (fig. 4).

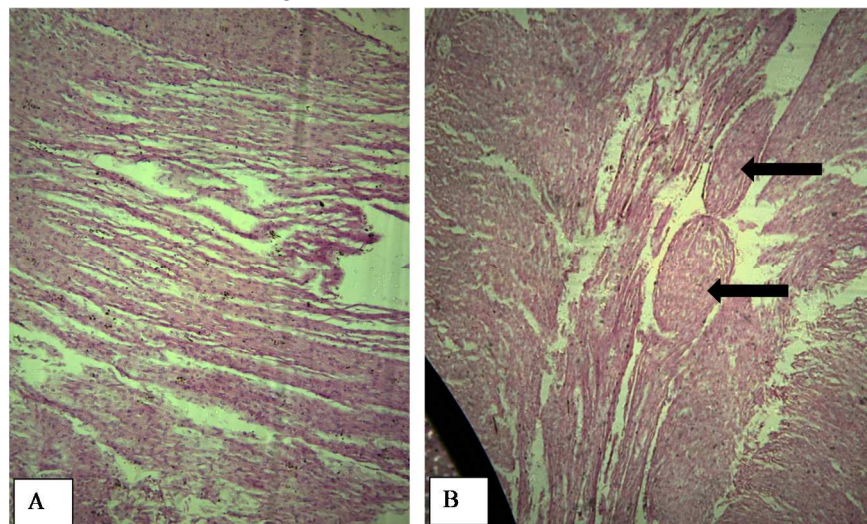


Fig. 4. Microphotography of the rat embryo heart ventricle on the 20th day under the cadmium chloride impact. A. Stratification of trabeculae; B. Formation of “claviform” thickenings in the ventricle trabeculae (indicated by arrows). Magn. x 400. Staining by hematoxylin according to Heidenhain.

Bioavailability and toxicity of metals depend not only on their overall concentrations, but also on the form in which they exist. Therefore, a complete understanding of the behavior of metals and the prediction of their potential risk in biological systems and organisms is possible only when all geochemical forms in which they exist in the environment and in different doses and methods of administration are identified. The influence on the physiological processes of a living organism of various salts of cadmium has not only a dose-dependent nature, but also differently affects embryogenesis and development of the heart. Increasing importance in the development of heart diseases in children and adolescents is given to the influence of negative environmental factors [8], which is confirmed by the results of our experiment. Comparison of the results obtained in the experiment with the data of experimental studies of other authors not only complemented the information on the negative effects of cadmium compounds on the state of peripheral blood in the formation of adaptive-protective reactions from the embryo itself in chronic effects of cadmium [2,5], but also determined the degree of damage to the heart of the embryo on macro- and microlevels at the effect of these doses of the test compound.

Histological studies have shown that changes in the atria and ventricles structure have been detected in the cadmium chloride impact group. In 12.3% of the examined sections, hyperplasia of the atrial endocardium was determined (fig. 3), with the ventricular endocardium remaining normal. The hyperplasia processes may indicate the formation of compensatory mechanisms by the endothelium in response to the toxic effects of cadmium chloride during embryogenesis.

Cadmium chloride also caused changes in the development of the ventricles, namely, we observed a thickening of both the compact and trabecular layers of the both ventricles

Such disorders in the formation of the compact and trabecular myocardium indicated the negative effects of the substance under study at the early stages of cardiogenesis, in particular on the processes of myocardial delamination, resulting in the formation of not only trabeculae, but also the papilliform muscles of atrial-ventricular valves.

Thus, the study of the cadmium chloride impact on the overall course of

cardiogenesis with intragastric administration during the entire period of pregnancy in rats has resulted in

changes of the embryonic heart structure at micro- and macro-levels.

**Conclusion**

The cardiofetal index growth in the group exposed to cadmium chloride indicates an increase in the cardiac weights in the group when the body mass of the embryo was reduced due to the intoxication.

Microscopy has revealed the atrial endocardium hyperplasia in 12.3% of the embryos in the group exposed to cadmium chloride in the indicated dose. Cadmium chloride also caused changes in 26-29% expressed in the embryos' heart ventricles wall formation: thickening of both the compact and trabecular layers of the myocardium and the formation of abnormal forms of trabeculae.

*Prospects for further research, in our opinion, include the immunohistochemical study of embryos and their organs after exposure to cadmium chloride is promising in order to predict possible changes in the basic histogenetic processes with cadmium intoxication of pregnant females.*

**References**

1. Skalnyy AV, Zaytseva IP, Tinkov AA. Mikroelementy i sport. Personalizirovannaya korrektsiya elementnogo statusa sportsmenov. M.: Sport; 2018. 288 s. [in Russian]
2. Slyuzova OV, Stepanova YEV, Bucharskaya AB. Razvitiye adaptatsionnykh mekhanizmov u samok belakh kryis v otvet na vozdeystviye ionov kadmiya. Toksikologicheskiy vestnik. 2008; 3: 23–27. [in Russian]
3. Oberlis D, Skalniy AV, Skalnaya MG. Patofiziologiya mikroelementozov. Patogenez. 2016; 14(2): 20-27. [in Russian]
4. Fomenko OZ, Shaulskaia OE, Kot YG, Ushakova GA, Shevtsova AI. Vliyaniye raznykh doz kadmiya na aktivnost matriksnykh metalloproteinaz v serdtse, mozge i syvorotke krovi kryis. Zhurnal Grodnenskogo gosudarstvennogo meditsinskogo universiteta. 2016; 3: 103-7. [in Russian]
5. Frolova NA, Mirzoyev EB., Kobyalko VO., Gubina OA. Otvetnaya reaktsiya organizma kryis pri khronicheskom vozdeystvii kadmiya v antenatal'nyy i molochnyy period vskarmlivaniya. Aktualnyye problemy ekologii i prirodopol'zovaniya. Sbornik nauchnykh trudov. - M.: IPTS "Luch". 2010; 12: 226-230. [in Russian]
6. Shatorna VF, Harets' VI, Kononova II. Porivnyannya effektiv vplyvu soley svyntsyu ta kadmiyu na embriohenez u shchuriv. Ukrayinskyi zhurnal medytsyny, biolohiyi ta sportu. 2018; T. 3, 6(15): S.310-314. [in Ukrainian]
7. Shchorichna dopovid pro stan zdorovya naselennya, sanitarno-epidemichnu sytuatsiyu ta rezultaty diyalnosti systemy okhorony zdorovya Ukrayiny. 2016 rik. MOZ Ukrayiny, DU «UISD MOZ Ukrayiny». Kyiv; 2017. 516 s. [in Ukrainian]
8. Gidding SS. Cardiovascular risk factors in adolescents. Curr. Treat. Options Cardiovasc. Med. 2006; 8 (4): 269275.
8. Dourson M, Becker RA., Haber LT, Pottenger L H., Bredfeldt T, Fenner-Crisp PA. Advancing human health risk assessment: Integrating recent advisory committee recommendations // Critical Reviews in Toxicology. 2013 Jul; 43(6): 467–492.
9. Järup L, Åkesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol [Internet]. 2009 [cited 2018 July 17];238(3):201-8.

**Реферати**

**ЗМІНИ КАРДІОГЕНЕЗУ  
ПІД ВПЛИВОМ ХЛОРИДУ КАДМІЮ  
В ЕМБРІОГЕНЕЗІ ЩУРА**

**Нефьодова О.О., Шаторна В.Ф., Гальперін О.І.,  
Нефьодов О.О., Єрошенко Г.А., Твердохліб І.В.,  
Гарець В.І.**

Мета експериментального дослідження: визначити вплив на загальний хід кардіогенезу хлориду кадмію при внутрішньошлунковому введенні впродовж всього періоду вагітності у щурів. Експериментальні дослідження були проведені на самицях щурів лінії Wistar. Для моделювання впливу хлоридом кадмію протягом всієї вагітності самицям щурів лінії Wistar щодня per os через зонд вводили водний розчин хлориду кадмію в дозі - 1,0 мг/кг. Збільшення показника кардіофетального індексу в групі впливу хлоридом кадмію вказує на збільшення вагових показників серця в групі при зниженні маси тіла ембріона внаслідок інтоксикації. Мікроскопічно виявлено у 12,3% гіперплазія ендокарду передсердь в групі впливу хлоридом кадмію в зазначеній дозі. Хлорид кадмію також у 26-29% призводив до змін в формуванні стінки шлуночків серця ембріонів: потовщення як компактного так і трабекулярного шарів міокарду та формування аномальних за формою трабекул.

**Ключові слова:** кардіогенез, міокард, шлуночок серця, передсердя, кадмій.

Стаття надійшла 15.10.18 р.

**ИЗМЕНЕНИЯ КАРДИОГЕНЕЗА  
ПОД ВОЗДЕЙСТВИЕМ ХЛОРИДА КАДМИЯ  
В ЭМБРИОГЕНЕЗЕ КРЫСЫ**

**Нефедова Е.А., Шаторная В.Ф., Гальперин А.И., Нефедов  
А.А., Ерошенко Г.А., Твердохлеб И.В.,  
Гарец В.И.**

Цель экспериментального исследования: определить влияние на общий ход кардиогенеза хлорида кадмия при внутрижелудочном введении в течение всего периода беременности у крыс. Экспериментальные исследования были проведены на самках крыс линии Wistar. Для моделирования влияния хлоридом кадмия в течение всей беременности самкам крыс линии Wistar ежедневно per os через зонд вводили раствор хлорида кадмия (в дозе - 1,0 мг / кг). Исследование влияния на общий ход кардиогенеза хлорида кадмия показало изменения в строении эмбрионального сердца на макро- и макроуровне. Увеличение показателя кардиофетального индекса в группе влияния хлоридом кадмия указывает на увеличение весовых показателей сердца в группе при снижении массы тела эмбриона вследствие интоксикации. Микроскопически обнаружено в 12,3% гиперплазия эндокарда предсердий в группе влияния хлоридом кадмия в указанной дозе. Хлорид кадмия также в 26-29% приводил к изменениям в формировании стенки желудочков сердца эмбрионов: утолщение как компактного так и трабекулярного слоев миокарда и формирования аномальных по форме трабекул.

**Ключевые слова:** кардиогенез, миокард, желудочек сердца, предсердия, кадмий.

Рецензент Старченко І.І.