

**DYNAMICS OF CADMIUM ACCUMULATION IN THE BLOOD AND SMALL INTESTINE
IN A CHRONIC EXPERIMENT ON RATS**

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The purpose of the experiment was to determine the degree of accumulation of cadmium and copper in the small intestine and blood of adult females and in rat embryos with isolated intragastric administration of cadmium chloride and with combined administration with copper succinate in a chronic experiment. Female rats were exposed to the studied factors daily during pregnancy by intragastric injection of the solution. The level of accumulation of cadmium and copper was determined by the method of polyelement analysis by atomic emission with electric arc atomization, carried out at the State Enterprise "Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine" (Odesa).

Isolated chronic administration of cadmium chloride increases the level of cadmium accumulation in the studied biological samples (blood, small intestine of females, embryos), and combined administration of copper succinate with cadmium chloride reduces the level of cadmium accumulation compared to the group of isolated cadmium administration in a chronic experiment on rats.

The combined administration of copper succinate with cadmium chloride has a modifying effect on the accumulation of cadmium chloride both in the blood and intestine of female rats, and in embryos, and copper succinate itself can be considered as a possible bioantagonist of cadmium.

Key words: rats, accumulation, cadmium, copper, small intestine, embryo.

Connection of the publication with planned research works.

The experimental study was carried out as part of the research work of the Department of Medical Biology, Pharmacognosy, Botany and Histology of the Dnipro State Medical University "Biological bases of morphogenesis of organs and animals under the influence of microelements and ultramicroelements in the experiment" (state registration number 0118U006635).

Introduction.

Cadmium (Cd) is a heavy toxic metal that is common in the environment of industrial regions, capable of accumulation and poses a threat to human and animal health. Excessive intake of heavy metal salts can cause severe damage to the small intestine, which is the main site of cadmium absorption and the main target organ after oral administration. Cadmium disrupts protein metabolism, limits iron assimilation, increases the removal of calcium, zinc and copper from compounds with metallothionein – a protein containing 30% cysteine, with the -SH groups of which cadmium forms very stable compounds [1, 2, 3]. In fact, one of the toxic actions of cadmium in the body is the provocation of trace element imbalance.

Modern scientific and experimental studies on the search for possible new bioantagonists have shown that zinc chelate of the hydroxy analogue of methionine (Zn-HMTB) has the ability to reduce the accumulation of Cd in the liver and kidneys of experimental animals in a chronic experiment. Studies were conducted on piglets to investigate the effects of Zn-HMTB on Cd absorption and Cd-induced toxicity in the small intestine when receiving cadmium at a daily dose of 30 mg/kg. Gastrointestinal tract tissues were collected, also samples of liver, kidney, spleen, heart, lung and long muscle and feces. The concentrations of Cd and metal trace elements in the gastrointestinal tract and organs were analyzed, the relative expression of matrix RNA (mRNA), inflammatory cytokines and transporters of metal ele-

ments in the small intestine, epithelial apoptosis in the small intestine. The obtained results suggest that Zn-HMTB may be useful in reducing the accumulation of Cd in the gastrointestinal tract and organs of piglets and reducing the toxicity caused by Cd in small animals [3]. Modern experimental searches for new potential means to reduce or prevent trace element imbalance are held back by the lack of knowledge about the feature of the exchange of trace elements in the body of healthy people and the norm in conditions of increased man-made load, also by the lack of experimental data on the balance, forms and types of interaction of trace elements and ultra-trace elements when they are simultaneously received [4, 5, 6, 7]. Thus, the search for new forms of trace elements that may have potential bioantagonistic properties in relation to the toxicity of cadmium compounds is relevant today. Promising are biotechnological preparations, which include trace elements in the form of succinates, which have recently been increasingly used in medical and biological experimental work and pharmacy [8, 9].

The aim of the study.

To determine the level of cadmium and copper accumulation in the small intestine and blood of adult females and in rat embryos with isolated intragastric administration of cadmium chloride at a dose of 2.0 mg/kg and with combined administration with copper succinate in a chronic experiment on pregnant female rats.

Object and research methods.

The study was conducted on 48 young female rats of the Wistar line ("Dali 2000" kennel, Kyiv), weighing 180-300 g. To perform the tasks, females with a dated gestation period were obtained, which allowed to obtain embryos with a certain date of development. In the experiment, all pregnant females were divided into the following groups: the first group – control; the second group – isolated injection of cadmium chloride solution at a dose of 2.0 mg/kg; the third group – combined injection of cadmium chloride solution at a dose of 2.0

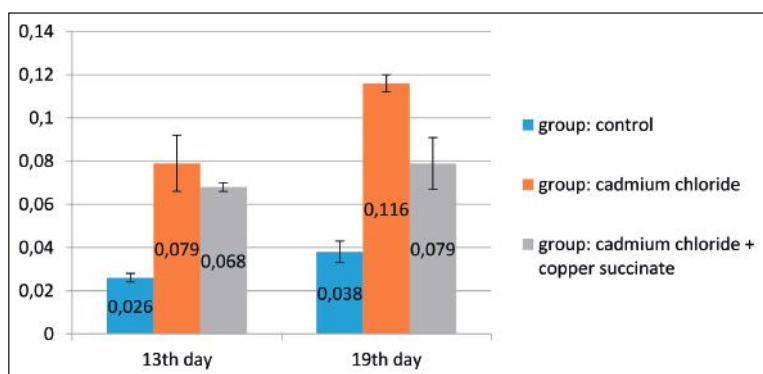


Figure 1 – Dynamics of indicators of the level of cadmium (µg/g) in the blood of pregnant females on the 13th and 19th days of the experiment.

mg/kg + copper succinate 0.1 mg/kg. The number of experimental animals was 16 females in each group, which were further divided into 2 subgroups by the time of withdrawal from the experiment. The experiment was conducted in the vivarium of the Dnipro State Medical University with observance of all necessary conditions for keeping animals.

The influencing factors were cadmium chloride (ionic solution) and copper succinate. Copper succinate aquachelate was nanosized, that is, we used copper succinate nanoaquachelate, which was obtained under the agreement on scientific and technical cooperation at the Scientific Research Institute of Nanobiotechnologies and Resource Conservation of Ukraine (Kyiv). The influence of the studied factors on female rats was carried out daily from the 1st to the 19th day of pregnancy by injecting the solution intragastrically, through a probe once a day, on the 13th and 19th days of pregnancy, the females were surgically slaughtered. To fulfill the purpose of the research, during the experiment blood was taken, the small intestine of pregnant females and embryos were removed, which were to be frozen without fixation to determine the degree of cadmium accumulation.

Determination of the level of cadmium accumulation was carried out using polyelement analysis. Sample measurements were carried out at the State Enterprise “Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine” (Odesa) in accordance with the agreement on scientific and technical cooperation. Sample preparation and measurement of metal content was carried out in accordance with GOST 30823-2002. Quantitative measurement of the metal content in the samples was carried out on the Emas-200 CCD atomic emission spectrometer, which is a modern analytical device, controlled by a computer and performs all the necessary calculations independently with minimal operator involvement. A standard spectral buffering mixture according to GOST 30823-2002 was used as a solvent. Quantitative determination of cadmium in the analyzed objects was carried out at a wavelength of 228.802 nm, zinc – 213.856 nm, copper – 324.754 nm. The current strength in the arc in all cases was 15 A, the size of the optical gap of the device when measuring cadmium and zinc was equal to 0.4, copper – 0.1. Atomic emission analysis with arc atomization allows qualitative and quantitative elemental analysis of samples of almost any nature.

Research was carried out in accordance with the principles of the Declaration of Helsinki, adopted by the

General Assembly of the World Medical Association (2000), the Convention of the Council of Europe on Human Rights and Biomedicine (1997), the relevant provisions of the WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics (1983), “General ethical principles of experiments on animals” approved by the First National Congress on Bioethics (Kyiv, 2001) in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Educational Purposes” (Strasbourg, March 18, 1986).

Statistical processing of the obtained data was performed using the standard Microsoft Excel program. Univariate regression analysis with a 95% confidence interval was used to identify possible associations between the analyzed factors. Differences at $p < 0.05$ (5% significance level) were considered statistically significant.

Research results and their discussion.

To solve the tasks we determined the level of accumulation of cadmium and copper in two directions. The first direction is the determination of the accumulation of these trace elements in the small intestine and in the blood of females. The second direction is determining the accumulation of cadmium and copper in embryos. The obtained data made it possible to determine the dynamics of microelement composition changes during the female’s pregnancy and embryogenesis, because the material was collected twice: on the 13th day and at the end of embryogenesis (the 19th day).

The analysis and comparison of the obtained results showed the following. Even in the control group during embryogenesis the level of cadmium in the blood of females increases unreliably. On the 13th day of embryogenesis, with the isolated introduction of cadmium chloride, the content of this trace element in the blood increases 3 times compared to the control, which is logical. In the group of combined administration of cadmium with copper succinate, despite the identical dose of cadmium administration, the level of cadmium in the blood is only 2.6 times higher than in the control. At the end of embryogenesis the level of cadmium in the blood of females of the isolated administration group exceeded the control by 3.1 times, and in the combined administration group it was only 2.1 times (fig. 1).

That is, the combined administration of cadmium with zinc succinate reduces the level of cadmium in the blood at both time points, which may indicate the possible bioantagonistic character of copper succinate relative to cadmium chloride.

We also investigated and analyzed shifts in the trace element balance due to copper accumulation. On the 13th day of embryogenesis, with the isolated introduction of cadmium, the copper content in the blood increased by 1.4 times compared to the control group. In the group of combined administration of cadmium with copper succinate, the content of this metal in the blood increased by 1.7 times, as expected, according to the control, and only by 1.2 times compared to the group of isolated administration of cadmium chloride. On the 19th day of embryonic development, the level of copper

in the blood of the females of the group of isolated administration was 1.1 times higher than that of the control group, and in the group of combined administration of cadmium chloride with copper succinate, the level of the heavy metal increased by 3.3 times compared to the control indicators and by 2.9 times compared to the group of isolated introduction of cadmium, this was predicted.

Analysis of indicators of cadmium accumulation in the small intestine of pregnant rats showed the following. As in the previous blood test, it was determined that the level of cadmium in the structures of the small intestine increases in the control group at the end of embryogenesis. On the 13th day in the group of isolated cadmium chloride administration, the level of cadmium accumulation increases by 33.7 times and on the 19th day by 68.9 times. The obtained results are logical, because the introduction of cadmium chloride took place intragastrically by probing, and the wall of the stomach and small intestine was the first barrier to the penetration of cadmium into the body. However, the combined administration of the same dose of cadmium with copper succinate demonstrated a decrease in the level of cadmium in the wall of the small intestine compared to the group of isolated administration, both on the 13th and on the 19th day of the chronic experiment (fig. 2).

Thus, the combined administration of copper succinate with cadmium chloride reduces the level of cadmium accumulation in the wall of the small intestine, compared to the group of isolated cadmium administration at both time points.

Analysis of the dynamics of copper accumulation in the small intestine of females of the experimental groups demonstrated the following. On the 13th day of the experiment, there was a 2.1-fold decrease in the copper level in the cadmium chloride group compared to the control, and a 1.4-fold increase in the isolated cadmium chloride with copper succinate group, relative to the group of isolated introduction, the indicator increased by 2.9 times, respectively. On day 19, copper levels also decreased 1.2-fold to control, but increased 1.8-fold in the combined administration group, this was expected. Thus, the isolated introduction of cadmium chloride reliably reduces the level of accumulation of copper in the structures of the small intestine compared to the control group, and the combined introduction of cadmium chloride with copper succinate increases the level of accumulation of copper in the wall of the small intestine, despite the fact that the amount of cadmium received by females remains the same as the group of isolated introduction of cadmium chloride.

The second direction of research was the determination and comparison of the level of accumulation of cadmium in embryos at the investigated terms of the experiment. The obtained results in the control group showed an unreliable increase in cadmium levels in the embryos. On the 13th day of embryonic development in the

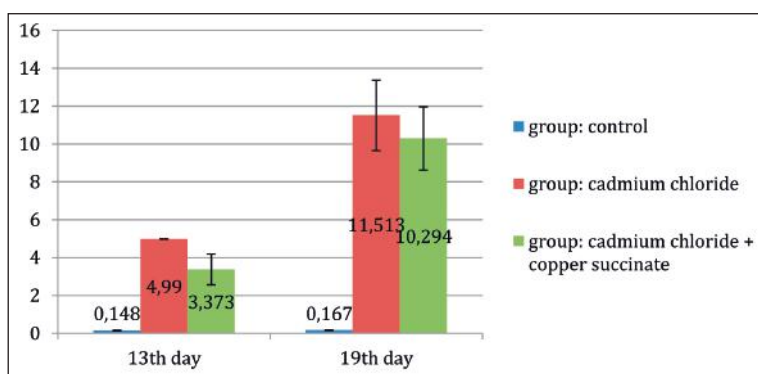


Figure 2 – Dynamics of cadmium level indicators (µg/g) in the small intestine of pregnant females on the 13th and 19th days of the experiment.

group of isolated introduction of cadmium chloride, an increase in indicators was recorded by 8.5 times, and on the 19th day of embryogenesis by 9.3 times in accordance with the control, which was expected. In the group of combined administration of cadmium chloride with copper succinate, a decrease in indicators of accumulation of heavy metals was ascertained, compared to the group of isolated administration, both on the 13th and on the 19th day. On the 13th day, the increase of the experimental data in comparison with the reference indicators of accumulation exceeded 6.3 times, and on the 19th day it exceeded the experimental data by 6.8 times (figure 3).

The obtained results of the level of copper accumulation in embryos in the control group demonstrated an increase in copper indicators at the end of embryogenesis, which is logical, because embryos are actively growing, their bone system is being formed. In the group of isolated introductions of cadmium chloride on the 13th day of the experiment, a decrease of these indicators by 1.4 times was ascertained, and on the 19th day of embryogenesis by 1.2 times, according to the control.

Thus, the analysis of the obtained results of cadmium accumulation in all studied biological samples (blood, small intestine of female rats, embryos) demonstrated that in the groups of combined administration of cadmium chloride with copper succinate, a decrease in the level of cadmium accumulation was determined in comparison to the group of isolated administration of cadmium, despite the identity of the dose by cadmium. The analysis of the obtained results of copper accumulation in all studied biological samples (blood, small intestine of females, embryos) demonstrated that

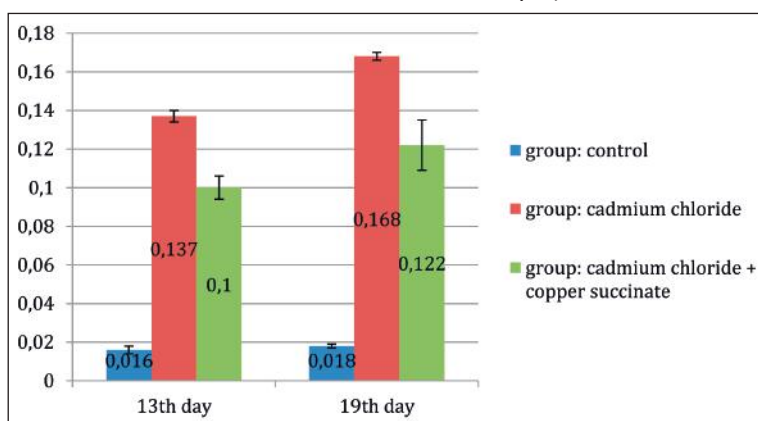


Figure 3 – Dynamics of indicators of the level of cadmium (µg/g) in embryos at both studied developmental periods.

in the groups of combined administration of cadmium chloride with copper succinate, an increase in the level of copper accumulation was determined, compared to both the group of isolated administration of cadmium and to the control.

Conclusions.

1. Isolated chronic intragastric administration of cadmium chloride at a dose of 2.0 mg/kg increases the level of cadmium accumulation in the studied biological samples (blood, small intestine of female rats, embryos), and the combined administration of copper succinate with cadmium chloride reduces the level of cadmium accumulation in comparison with the group isolated introduction of cadmium at both studied periods (on the

13th and 19th day of embryogenesis) in a chronic experiment on rats.

2. The combined administration of copper succinate with cadmium chloride has a modifying effect on the accumulation of cadmium chloride both in the blood and intestine of female rats and in embryos, and copper succinate itself can be considered as a possible bioantagonist of cadmium.

Prospects for further research.

A promising direction of further research is the detection of morphological changes in the structure of the small intestine of female rats and embryos after chronic intragastric administration of the studied substances.

References

1. Shatorna VF, Krasnov OO. Effect of cadmium chloride on kidney morphology in rats in isolated introduction and in combination with copper and zinc succinates. *Vistnyk problem biolohiyi i medytsyny*. 2022;1(163):97-102.
2. Shafran LM, Pykhteyeva YeG, Bol'shoy DV. *Metallotioneiny*. Odessa: Izdatel'stvo "Chornomor'ya". 2011. 428 s.
3. Ni HJ, Liu FF, Liang X, Yin YL, Liu G. The role of zinc chelate of hydroxy analogue of methionine in cadmium toxicity: effects on cadmium absorption on intestinal health in piglets. *Animal (The Animal Consortium)*. 2020;1:1-10.
4. Shatorna V, Kononova I, Rudenko K. Investigation of the effect of cadmium and kuprum on the digestive system of living organisms (literature review) *Modern Science – Moderní věda*. 2020;3:142-149.
5. Khopta NS, Yerstenyuk AM. *Metabolíchní zmíni v kístkoví tkaniní tvarin dlya umov yeksperimental'nogo kadmiozu*. *Science Rise. Biologicheskaya nauka*. 2018;5:31-5. [in Ukrainian].
6. Arustamyan OM, Tkachishin VS, Aleksiyshuk OYu. *Vpliv spoluk kadmíyu na organizm lyudini*. *Medytsina neotlozhnykh sostoyaniy*. 2016;7:109-14. [in Ukrainian].
7. Shamelashvili K, Ostrovska S, Shatorna V. The toxic effect of cadmium on a living organism and its detoxification by zinc ions. *Modern Science – Moderní věda*. 2020;3:150-157. DOI: [10.29254/2077-4214-2020-3-157-58-61](https://doi.org/10.29254/2077-4214-2020-3-157-58-61).
8. Orlov YUP, Govorova NV. *Rol' suksinatov pri kriticheskikh sostoyaniyakh*. *Obshchaya reanimatologiya*. 2014;10(6):65-78.
9. Yevglevskiy AA, Ryzhkova GF, Yevglevskaya YEP, Vanina NV, Mikhaylova II, Denisova AV, et al. *Biologicheskaya rol' i metabolicheskaya aktivnost' yantaroy kisloty*. *Vestnik Kurskoy gosudarstvennoy sel'skokhozyaystvennoy akademii*. 2013;9:65-69.

ДИНАМІКА НАКОПИЧЕННЯ КАДМІЮ В КРОВІ ТА ТОНКІЙ КИШЦІ В ХРОНІЧНОМУ ЕКСПЕРИМЕНТІ НА ЩУРАХ

Шаторна В. Ф., Тимчук К. М.

Резюме. Актуальним на сьогодні є пошук нових форм мікроелементів, які можуть мати потенційні біоантогоністичні властивості щодо токсичності та накопичення сполук кадмію. Перспективними є біотехнологічні препарати, до складу яких включають мікроелементи у формі сукцинатів, які останнім часом все більше використовуються в медико-біологічних експериментальних роботах.

Метою експерименту було визначення ступеню накопичення кадмію та міді в тонкій кишці і крові дорослих самиць та в ембріонах щурів при ізольованому внутрішньошлунковому введенні хлориду кадмію та при комбінованому введенні з сукцинатом міді в хронічному експерименті. Вплив досліджуваних чинників самицями щурів проводили щоденно з 1-го по 19-й день вагітності введенням розчину внутрішньошлунково, на 13-й та 19-й день вагітності самиць проводили оперативний забій. Для виконання поставленої мети дослідження під час експерименту проводили забір крові, вилучали тонку кишку вагітних самиць та ембріонів, які підлягали заморожуванню без фіксації для визначення ступеню накопичення кадмію. Проводили визначення рівню накопичення кадмію та міді в двох спрямуваннях: перший напрямок – накопичення зазначених мікроелементів в тонкій кишці та в крові самиць. Другий напрямок визначення накопичення кадмію та купруму в ембріонах. Для визначення зсувів мікроелементного балансу використовували метод поліелементного аналізу біологічних матеріалів шляхом атомної емісії з електродуговою атомізацією.

Ізольоване хронічне введення хлориду кадмію збільшує рівень накопичення кадмію в досліджуваних біологічних зразках (кров, тонка кишка самиць, ембріони), а комбіноване введення сукцинату міді з хлоридом кадмію знижує рівень накопичення кадмію в порівнянні до групи ізольованого введення кадмію в хронічному експерименті на щурах.

Комбіноване введення сукцинату міді з хлоридом кадмію має модифікуючий вплив на накопичення хлориду кадмію як в крові та кишці самиці щура, так і в ембріонах, а сам сукцинат міді може розглядатися як можливий біоантогоніст кадмію.

Ключові слова: щури, накопичення, кадмій, мідь, тонка кишка, ембріон.

DYNAMICS OF CADMIUM ACCUMULATION IN THE BLOOD AND SMALL INTESTINE IN A CHRONIC EXPERIMENT ON RATS

Shatorna V. F., Tymchuk K. M.

Abstract. The search for new forms of microelements, which may have potential bioantagonistic properties regarding the toxicity and accumulation of cadmium compounds, is relevant today. Promising are biotechnological preparations, which include trace elements in the form of succinates, which have recently been increasingly used in medical and biological experimental work.

The purpose of the experiment was to determine the degree of accumulation of cadmium and copper in the small intestine and blood of adult females and in rat embryos with isolated intragastric administration of cadmium chloride and with combined administration with copper succinate in a chronic experiment. Female rats were exposed to the studied factors daily from the 1st to the 19th day of pregnancy by intragastric injection of the solution; on the 13th and 19th days of pregnancy, the females were surgically slaughtered. To fulfill the purpose of the research, during the experiment blood was taken, the small intestine of pregnant females and embryos were removed, which were to be frozen without fixation to determine the degree of cadmium accumulation. The level of accumulation of cadmium and copper was determined in two directions: the first direction is the accumulation of these microelements in the small intestine and in the blood of female rats; the second direction is determining the accumulation of cadmium and copper in embryos. The method of polyelement analysis of biological materials by atomic emission with electric arc atomization was used to determine shifts in the trace element balance.

Isolated chronic administration of cadmium chloride increases the level of cadmium accumulation in the studied biological samples (blood, small intestine of female rats, embryos), and combined administration of copper succinate with cadmium chloride reduces the level of cadmium accumulation compared to the group of isolated cadmium administration in a chronic experiment.

The combined administration of copper succinate with cadmium chloride has a modifying effect on the accumulation of cadmium chloride both in the blood and intestine of female rats, and in embryos, and copper succinate itself can be considered as a possible bioantagonist of cadmium.

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Conflict of interest:

The authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

Received 17.08.2022

Accepted 28.01.2023