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ACTIVITY OF THE CITRIC ACID CYCLE DEHYDROGENASES UNDER THE CONDITIONS OF SEPARATE AND COMBINED ACTION OF CADMIUM CHLORIDE AND SODIUM NITRITE.

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АКТИВНОСТЬ ДЕГИДРОГЕНАЗ ЦИКЛА ТРИКАРБОНОВЫХ КИСЛОТ В УСЛОВИЯХ РАЗДЕЛЬНОГО И СОЧЕТАННОГО ДЕЙСТВИЯ ХЛОРИДА КАДМИЯ И НИТРИТА НАТРИЯ.

Summary. Due to the regulatory reactions of high-energy processes in the cell, in particular the citric acid cycle, different types and structures of xenobiotics act. To date, the mechanisms of separate and combined effects of cadmium and nitrites on energy metabolism remain insufficy understood. In this regard, studies of Krebs cycle enzymes under the conditions of cadmium, nitrite and cadmium-nitrite intoxication in the brain, tissues of the myocardium and liver of experimental animals (rats) are relevant.

The intoxication was modeled as follows: cadmium chloride was administered intramuscularly, sodium nitrite was administered with drinking water at a dose of 1/10 LD50 once daily for 10 days. Sampling was performed after decapitation under thiopental anesthesia at 1-, 14-, 28-days after the completion of the toxicant administration.

The results obtained indicate that the activity of succinate dehydrogenase increased throughout the study period in all organs of experimental animals studied by us under the conditions of separate and combined action of cadmium chloride and sodium nitrite. In the brain and liver of all our studies, a decrease in isocitrate dehydrogenase activity was observed throughout the observation period.

Аннотация. Через регуляторные реакции высокоэнергетических процессов в клетке, в частности цикла трикарбоновых кислот, действуют разного рода и структуры ксенобиотики. На сегодняшний день недостаточно изученными остаются механизмы раздельного и сочетанного воздействия кадмия и нитритов на энергетический обмен. В связи с этим, актуальными являются исследования энзимов цикла Кребса в условиях кадмиевой, нитритной и кадмиево-нитритной интоксикаций в главном мозге, тканях миокарда и печени экспериментальных животных (крыс).

Интоксикацию моделировали следующим образом: кадмий хлорид – внутримышечно, натрий нитрит - с питьевой водой в дозе 1/10 LD_{50} один раз в день в течение 10-ти суток. Забор материала проводили после декапитации под тиопенталовым наркозом на 1, 14-, 28 день после завершения ввода токсикантов.

Полученные результаты свидетельствуют о росте активности сукцинатдегидрогеназы в течение всего периода исследования во всех исследуемых нами органах экспериментальных животных в условиях раздельной и сочетанного действия хлорида кадмия и нитрита натрия. В главном мозге и печени в условиях всех исследуемых нами интоксикаций отмечено снижение активности изоцитратдегидрогеназы на протяжении всего периода наблюдения.

Keywords: energy metabolism, cadmium chloride, sodium nitrite, alpha-ketoglutarate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase.

Ключевые слова: энергетический обмен, кадмий хлорид, натрия нитрит, альфа-кетоглутаратдегидрогеназа, изоцитратдегидрогеназа, сукцинатдегидрогеназа, малатдегидрогеназа.

INTRODUCTION. One of the defining processes that takes place in a cell and provides its energy is the tricarboxylic acid cycle. The regulatory reactions of this process are those that are accompanied by the formation of energy [14, 23]. Xenobiotics, different in chemical structure, can influence the activity of the regulatory enzymes of the citric acid cycle and thus the energy supply of cells. It is known from the scientific literature [3] that Cadmium ions are able to form complexes with high molecular weight compounds (proteins, nucleic acids) as a result of substitution of sulfhydryl groups, which in turn leads to disruption of redox reactions, energy supply of cells [16, 19, 21], the permeability of cell membranes and the processes of transport of metals in the body [3, 5], cell division, etc. Nitrites cause tissue hypoxia [1, 20, 9], which causes respiratory chain inhibition and the separation of oxidation and phosphorylation processes. The xenobiotics studied by us [16, 20, 21] cause hypoxia due to the formation of a large amount of methemoglobin. However, the mechanisms of separate and combined effects of cadmium and nitrite on energy metabolism remain insufficiently studied to date. In this regard, studies of tricarboxylic acid cycle enzymes under the conditions of cadmium, nitrite and cadmium-nitrite intoxication are relevant. In view of the above, the purpose of this study was to determine the activity of Krebs cycle enzymes in the brain, tissues of the myocardium and liver of experimental animals (rats) under the conditions of separate and combined action of Cadmium chloride and sodium nitrite.

MATERIALS AND METHODS. The studies were performed on white outbred laboratory rats-males weighing 180-220 g, which were kept on a standard diet of vivarium. Toxic damage was caused by Cadmium chloride ($CdCl_2$) and Sodium nitrite ($NaNO_2$). The intoxication was modeled as follows: Cadmium chloride was administered intramuscularly at a dose of 1.2 mg / kg body weight of the animal (1/10 LD_{50}), and Sodium nitrite was administered with drinking water at a rate of 2.1 mg / kg body weight of the animal at a dose of 1 / 10 LD_{50} [3] once a day for 10 days. Intact animals were simultaneously administered an appropriate amount of 0.9% sodium chloride solution. The animals studied were divided into 4 groups: group I – intact animals; group II – animals intoxicated with Cadmium chloride; group III – animals intoxicated with Sodium Nitrite; group IV – animals intoxicated with cadmium chloride and sodium nitrite. Brain, heart, and liver homogenates were used for the study. Material was collected according to the rules of the European Convention on the Humane Treatment of Laboratory Animals (Strasbourg, 1986), after decapitation under thiopental anesthesia for 1-, 14-, 28 days after the completion of toxicants. The activity of alpha-ketoglutarate dehydrogenase [17, 23], isocitrate dehydrogenase [13, 23], malate dehydrogenase [15, 23], succinate dehydrogenase [22, 23] was determined spectrophotometrically. The results obtained were subjected to statistical analysis by conventional methods [2] using the Student's t-test (Statstica 8).

RESULTS and DISCUSSION. Cadmium is one of the most toxic metals. When it comes to food and drinking water, it gradually accumulates in the organs and tissues of living organisms. In humans, Cadmium ions combine with carboxyl, amine, and sulfhydryl groups, which are contained in protein-enzyme molecules [3, 23], thereby disrupting metabolic processes, including energy metabolism.

Comparative analysis of enzyme activity in the test organs and blood plasma was performed on relatively intact animals. Brain tissue is highly dependent on oxygen supply. It is known from the literature that Cadmium has a high ability to cross the blood-brain barrier, causing severe neurological damage [21]. Under the conditions of cadmium intoxication, isocitrate dehydrogenase activity decreased 1.5-2 folds during the whole study period (Fig. 1). At the same time, we observed an increase of succinate dehydrogenase activity 33 fold at 1 and 14 days and 17 fold at 28 days after the end of toxicant administration. The increase in the activity of malate dehydrogenase was established 3.8 fold in the late period of the study (Fig. 1).

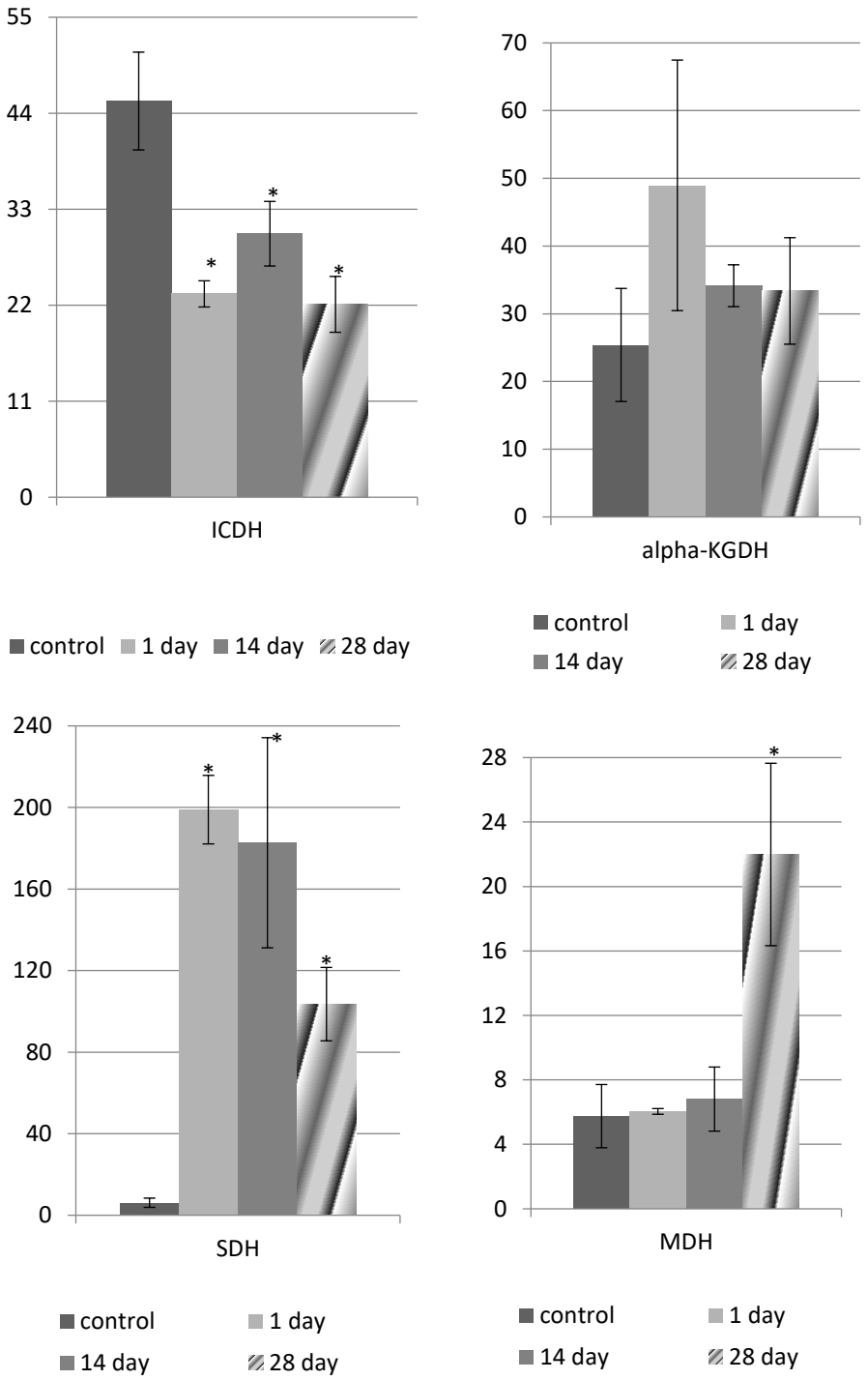

Figure 1. Activity of citric acid cycle enzymes in the brain of experimental animals under the conditions of cadmium intoxication. Note: here and in the following figures: α-ketoglutarate dehydrogenase activity (alpha-KGDH) (μmol NADH / min • mg protein), isocitrate dehydrogenase (ICDH) (μmol NADH / min • mg protein), succinate dehydrogenase (SDH) (NADH / min • mg protein)) and malate dehydrogenase (MDH) (μmol NADH / min mg protein).
** - different from control with p≤0,001 by Student's test.*

In myocardial tissue, tricarboxylic acid cycle enzymes play a decisive role in the energy supply to the heart muscle [5, 16]; under conditions of cadmium intoxication, there was a slight decrease in the activity of isocitrate dehydrogenase; growth of α-ketoglutarate dehydrogenase – on the 14th day by 1.7 fold (Fig. 2); a significant increase of succinate dehydrogenase activity in the early and late periods of the study by 26 and 30 fold, respectively, and a decrease in the activity of malate dehydrogenase by 3.5 fold on the 1st and 14th days and 2 fold on the 28th day of the study (Fig. 2).

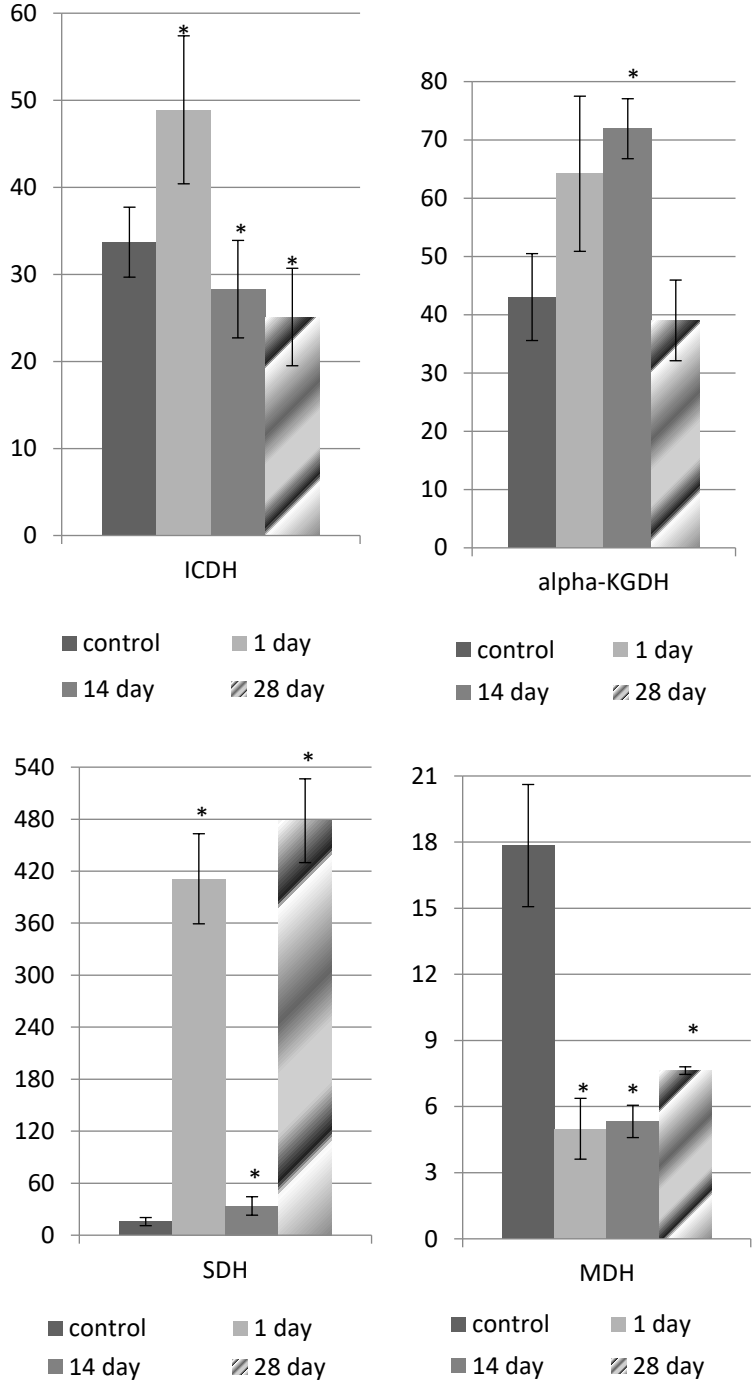

Figure 2. Activity of citric acid cycle enzymes in the myocardium of experimental animals under the conditions of cadmium intoxication.

It is known that 80% of cadmium in the liver is bound by endogenous metallothionein, but no excess accumulation in this organ occurs [6, 19]. A study of the activity of the citric acid cycle enzymes in the liver homogenate showed a maximum increase in the early period of α-ketoglutarate dehydrogenase – 35 fold and succinate dehydrogenase 21 fold (Fig. 3). Isocitrate dehydrogenase activity decreased throughout the study period: 2 fold in the early period and 8 fold in other periods of the study (Fig. 3).

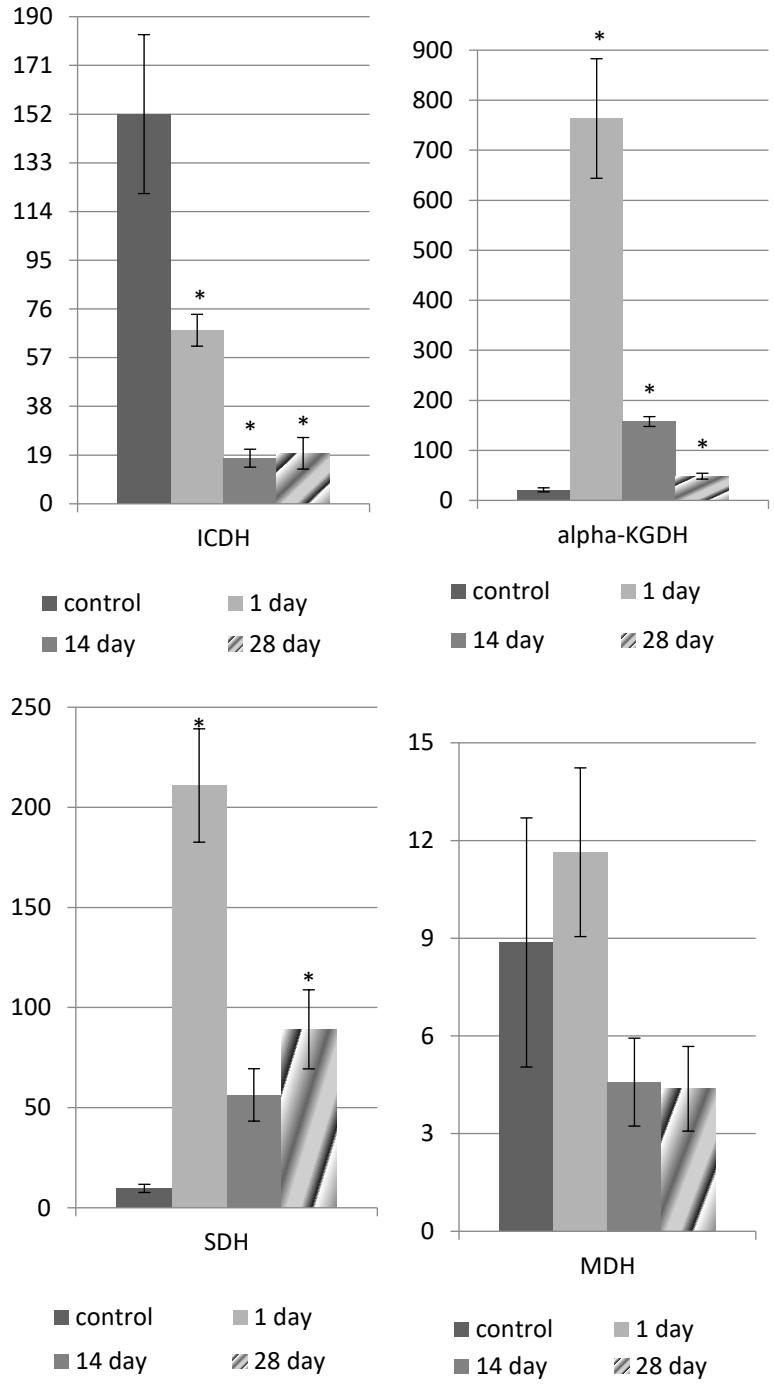

Figure 3. Activity of citric acid cycle enzymes in the liver of experimental animals under the conditions of cadmium intoxication.

Cadmium binds to blood proteins and is transmitted to organs [16, 19]. The study of the activity of the citric acid cycle enzymes in blood plasma showed: in the early period of decrease in the activity of all investigated NAD-dependent dehydrogenases: α-ketoglutarate dehydrogenase – 3 fold; isocitrate dehydrogenase – 7 fold: malate dehydrogenase – 14 fold (Fig. 4). Only the activity of isocitrate dehydrogenase until the late period of the study increased slightly relative to the indicators of the control group of animals. Regarding FAD-dependent dehydrogenase (succinate dehydrogenase), it should be noted that growth during the entire study period with maximum values on the 28th day is 13 fold higher than intact animals (Fig. 4).

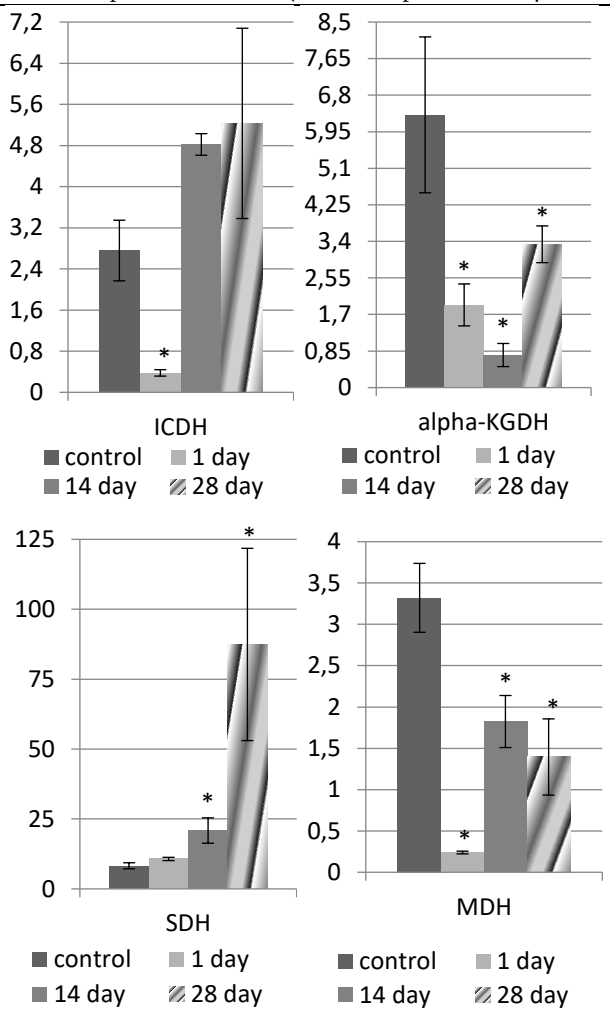

Figure 4. Activity of citric acid cycle enzymes in the blood of experimental animals under the conditions of cadmium intoxication.

From the scientific literature it is known [1, 10, 20, 4, 9} that nitrites adversely affect the nervous and cardiovascular systems, causing the development of methemoglobinemia, resulting in impaired oxygen supply to the tissues [11, 12]. The development of hypoxia leads to impaired functioning of the respiratory chain of mitochondria and Krebs cycle enzymes, which give protons and electrons to the respiratory chain [8, 14].

A study of the activity of such enzymes in brain homogenate under conditions of nitrite intoxication showed an increase in the activity of α-ketoglutarate dehydrogenase on the 14th day of the study by 3 fold and succinate dehydrogenase during the entire observation period by 4-16 folds (Fig. 5). The activity of NAD-dependent isocitrate dehydrogenase decreased 1.6-3.7 folds during the whole study period (Fig. 5).

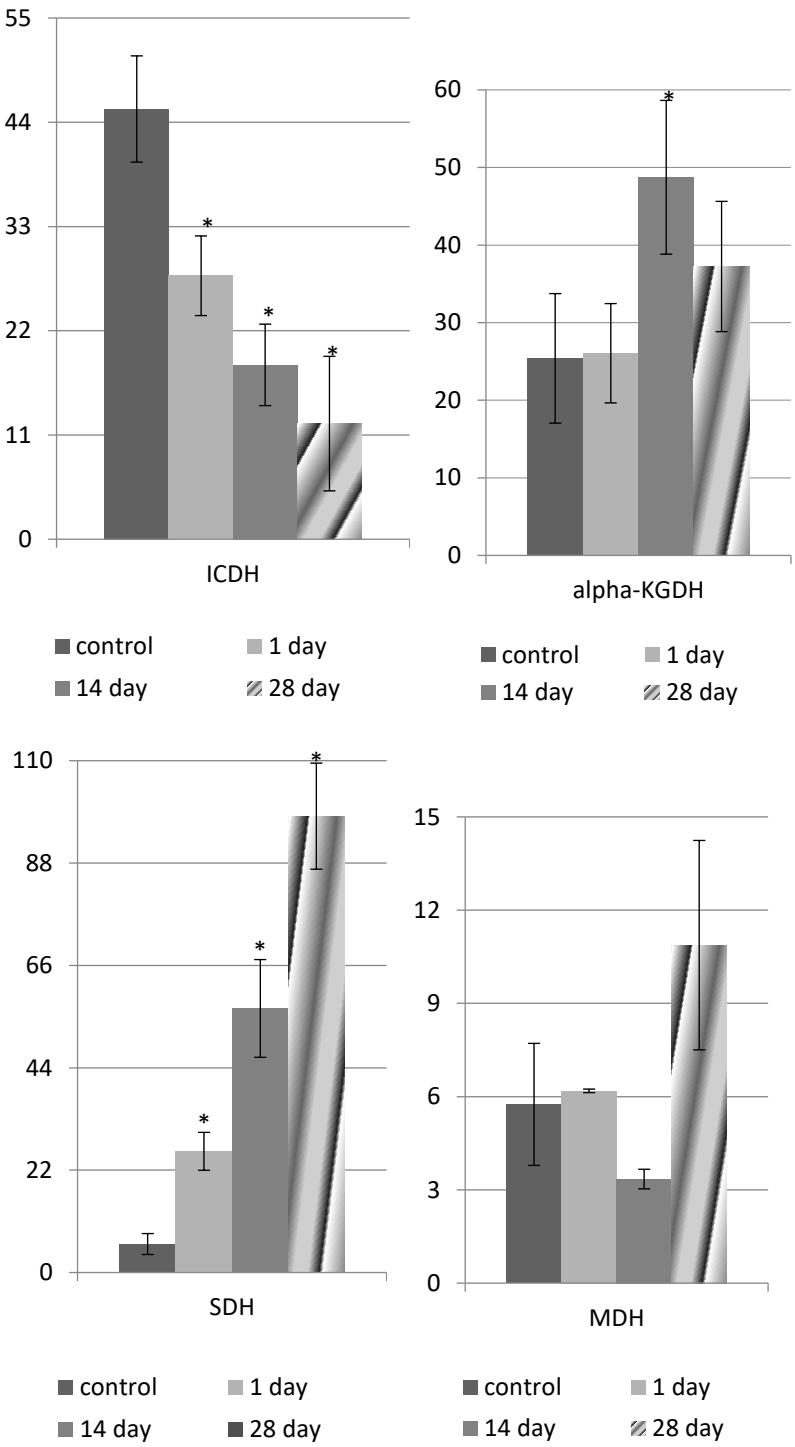

Figure 5. Activity of citric acid cycle enzymes in the brain of experimental animals under the conditions of nitrite intoxication.

In the heart tissue under the conditions of nitrite intoxication the activity of the investigated NAD-dependent dehydrogenases decreased specifically: isocitrate dehydrogenase-2 fold on the 14th day and 1.5 fold on the 28th day (Fig. 6); α-ketoglutarate dehydrogenase in the late period – 3.5 fold (Fig. 6); malate dehydrogenase – 2-5.5 folds throughout the study. (Fig. 6). Only succinate dehydrogenase activity increased throughout the study period and was maximal in the early period – 35 fold higher (Fig. 6) than in intact animals.

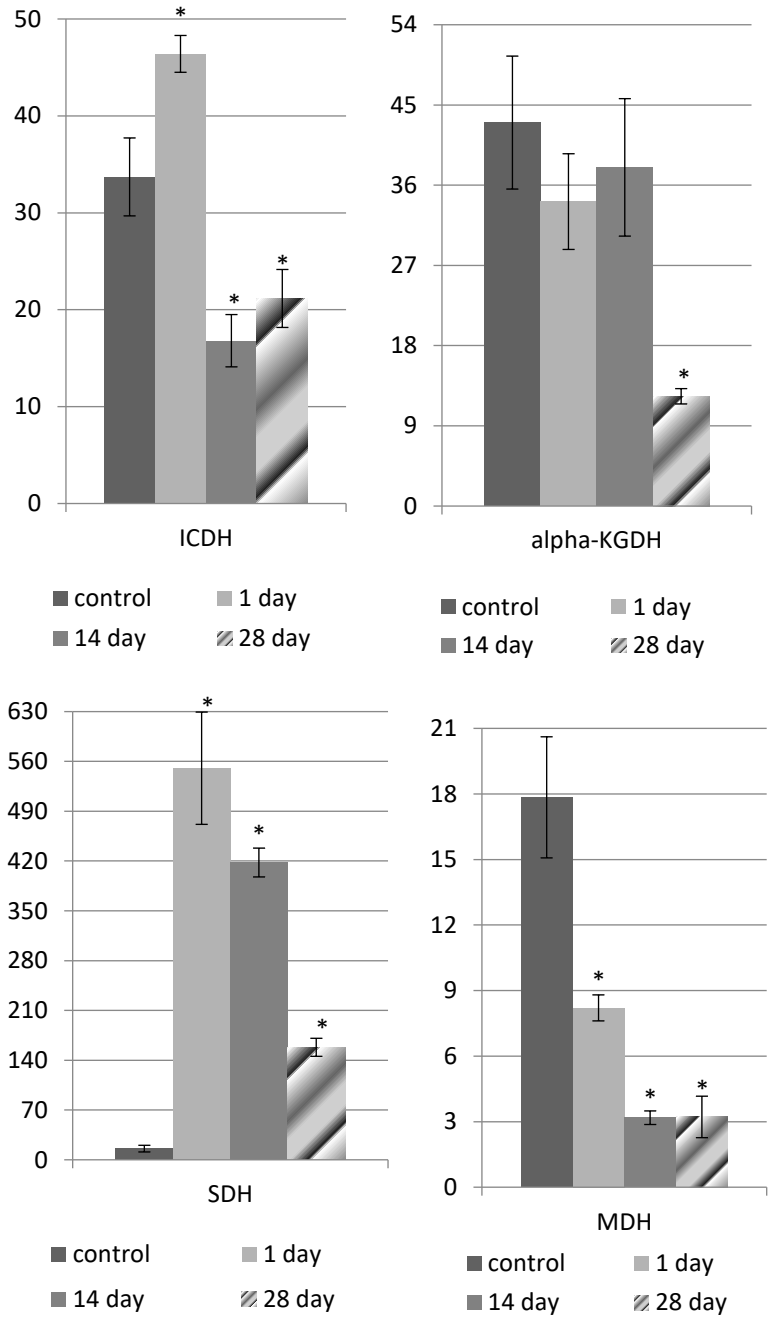

Figure 6. Activity of citric acid cycle enzymes in the myocardium of experimental animals under conditions of nitrite intoxication.

In the liver, as well as in the brain under conditions of nitrite intoxication, similar results were obtained. Activity isocitrate dehydrogenase decreased by 3.5-7 folds throughout the study period and α-ketoglutarate dehydrogenase activity increased by 15 fold on the 14th day (Fig. 7).

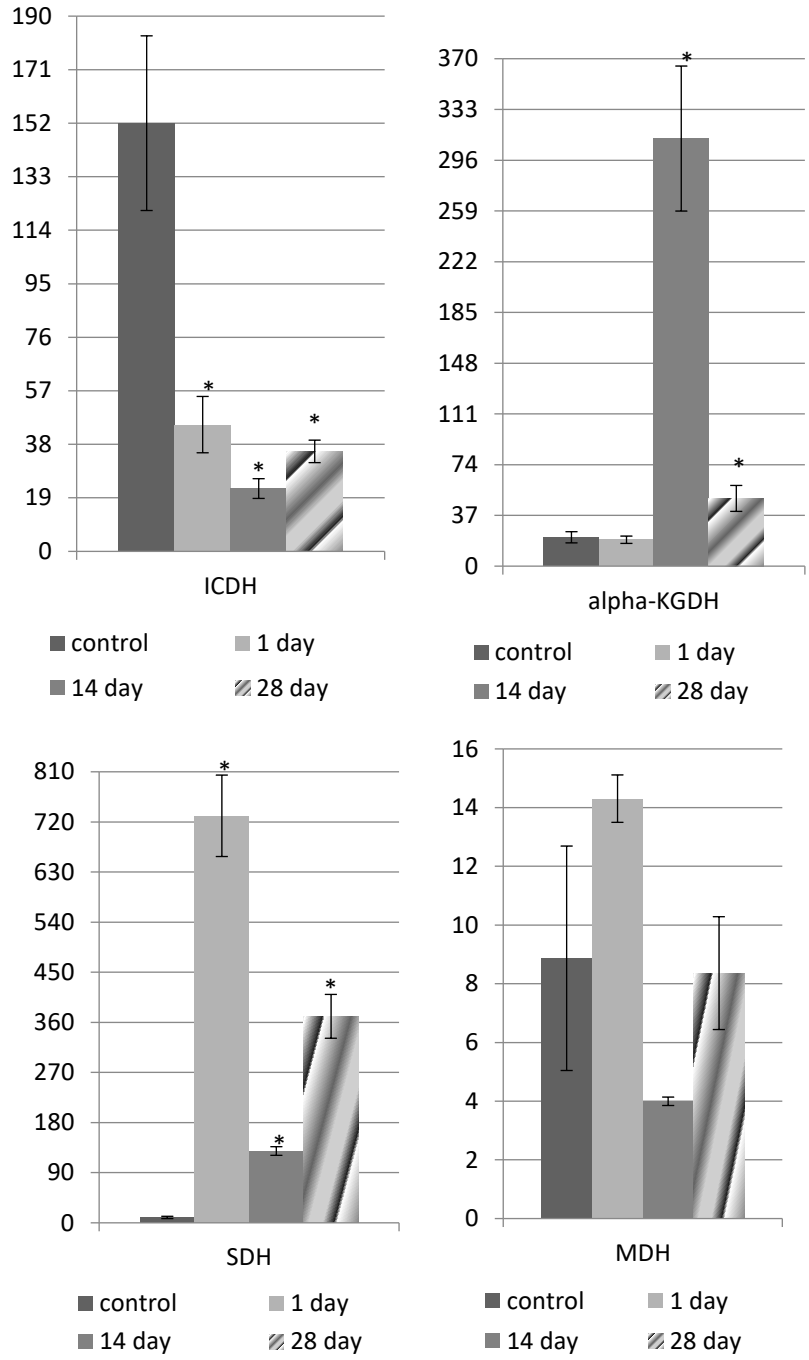

Figure 7. Activity of citric acid cycle enzymes in the liver of experimental animals under the conditions of nitrite intoxication.

In the blood plasma, nitrite intoxication in the early period of the study decreased the activity of all four investigated dehydrogenases. In other periods of the experiment, the activity of α-ketoglutarate dehydrogenase and malate dehydrogenase increased slightly relative to day 1, but did not reach the values of the control group of animals (Fig. 8). At the same time, it should be noted that the activities of succinate dehydrogenase and isocitrate dehydrogenase in other periods of the study significantly exceed the values of the intact group of animals – 2-3 fold (Fig. 8).

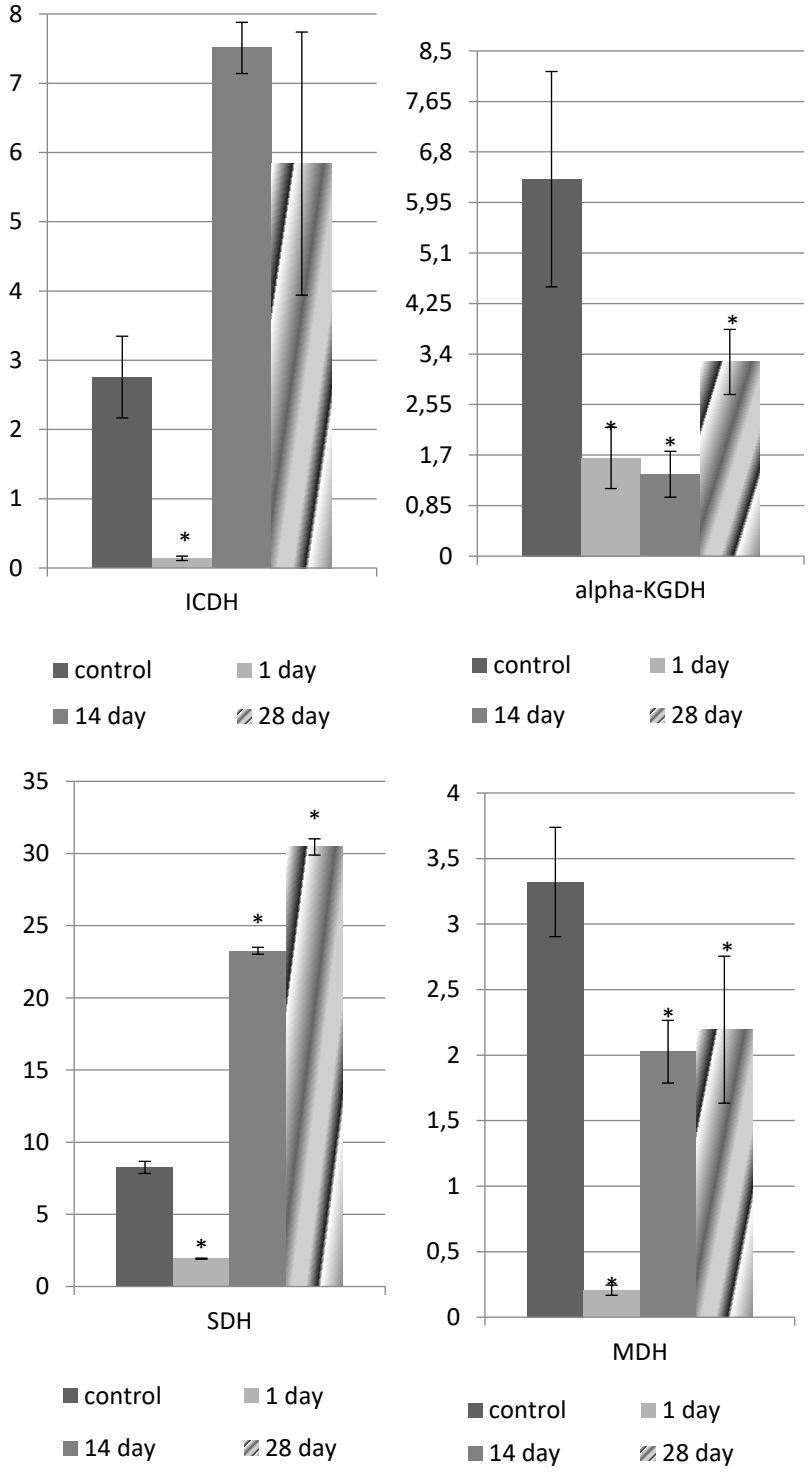

Figure 8. Activity of citric acid cycle enzymes in the blood of experimental animals under conditions of nitrite intoxication.

Living organisms are constantly affected by several factors at the same time. The combined effect of cadmium and nitrite [7, 18] has been poorly described in the scientific literature, so it was important to find out the features of energy metabolism when these xenobiotics are combined.

In the brain under the conditions of cadmium-nitrite intoxication we observed: a decrease in the activity of isocitrate dehydrogenase 2 times during the whole period of the study and an increase in the activity of α-ketoglutarate dehydrogenase 1.6-2 folds (Fig. 9). The activity of succinate dehydrogenase maximized in the early period of the study - 18 fold (Fig. 9).

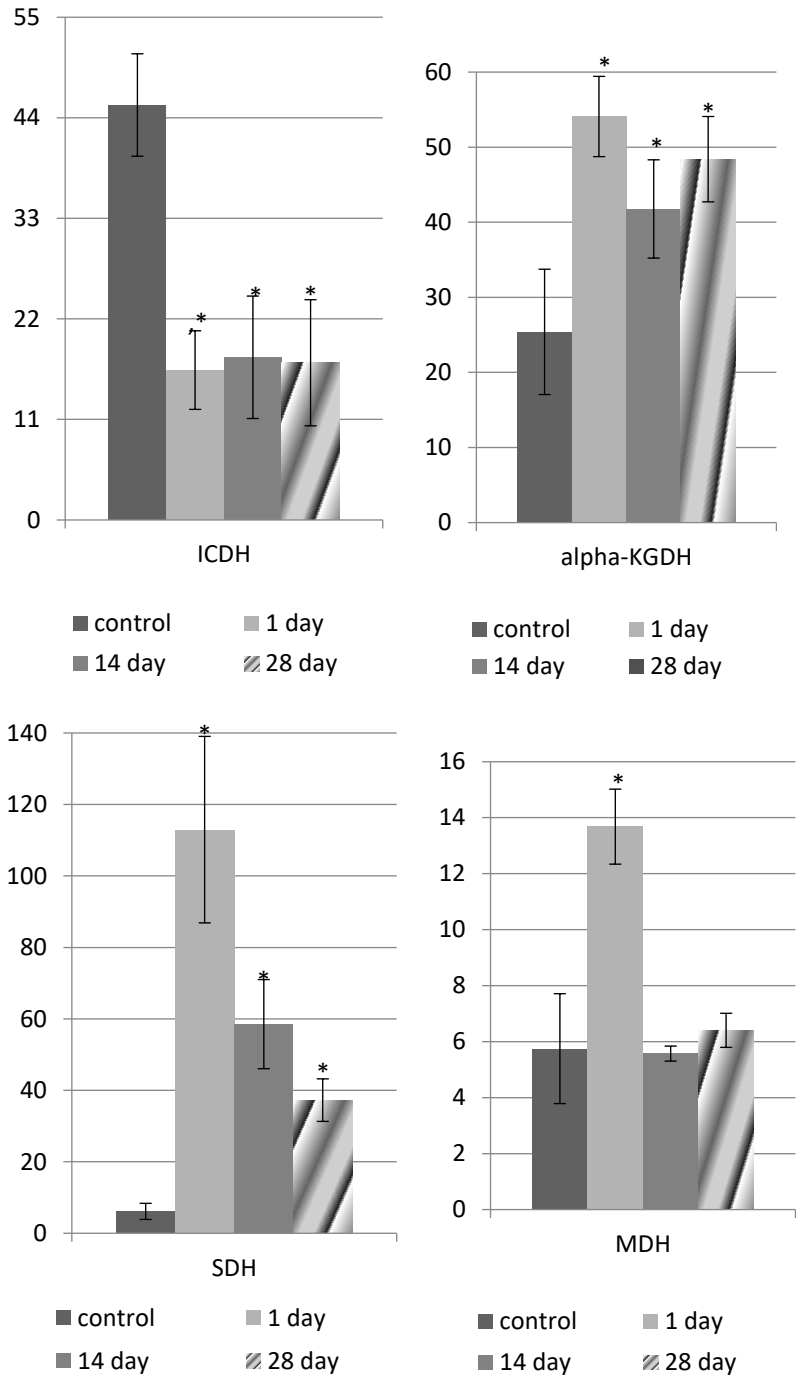

Figure 9. Activity of citric acid cycle enzymes in the brain of experimental animals under the conditions of cadmium-nitrite intoxication.

In the myocardium under conditions of cadmium-nitrite intoxication the activity of isocitrate dehydrogenase and malate dehydrogenase decreased in the late period 2 fold (Fig. 10). While in the same period, the maximal activity of α-ketoglutarate dehydrogenase increased twice and succinate dehydrogenase increased 21 fold (Fig. 10).

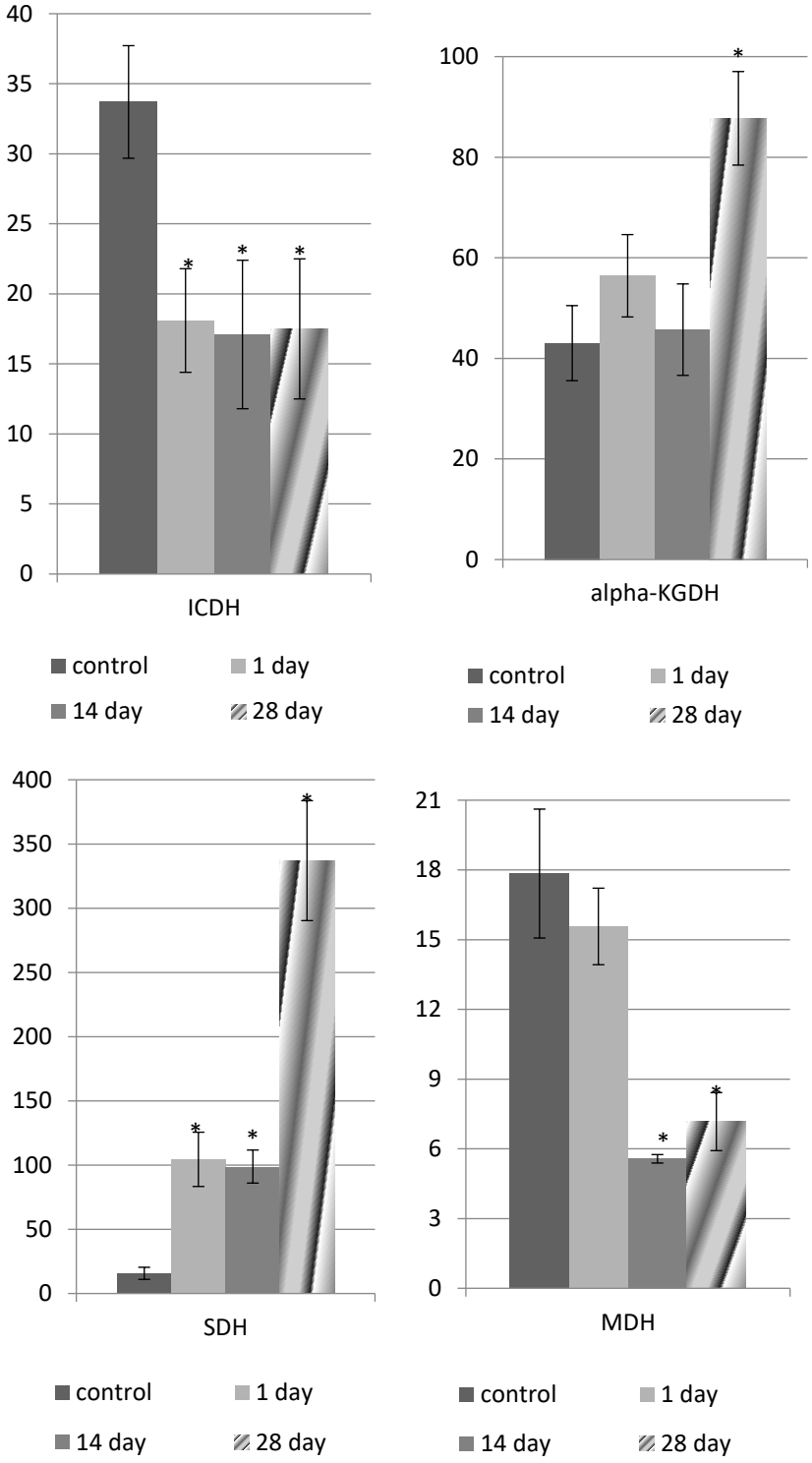

Figure 10. Activity of citric acid cycle enzymes in the myocardium of experimental animals under the conditions of cadmium-nitrite intoxication.

In the liver under the conditions of cadmium-nitrite intoxication, the activity of isocitrate dehydrogenase decreased by 4.5-9 folds throughout the study period (Fig. 11). The activities of α-ketoglutarate dehydrogenase and succinate dehydrogenase increased throughout the study period (Fig. 11).

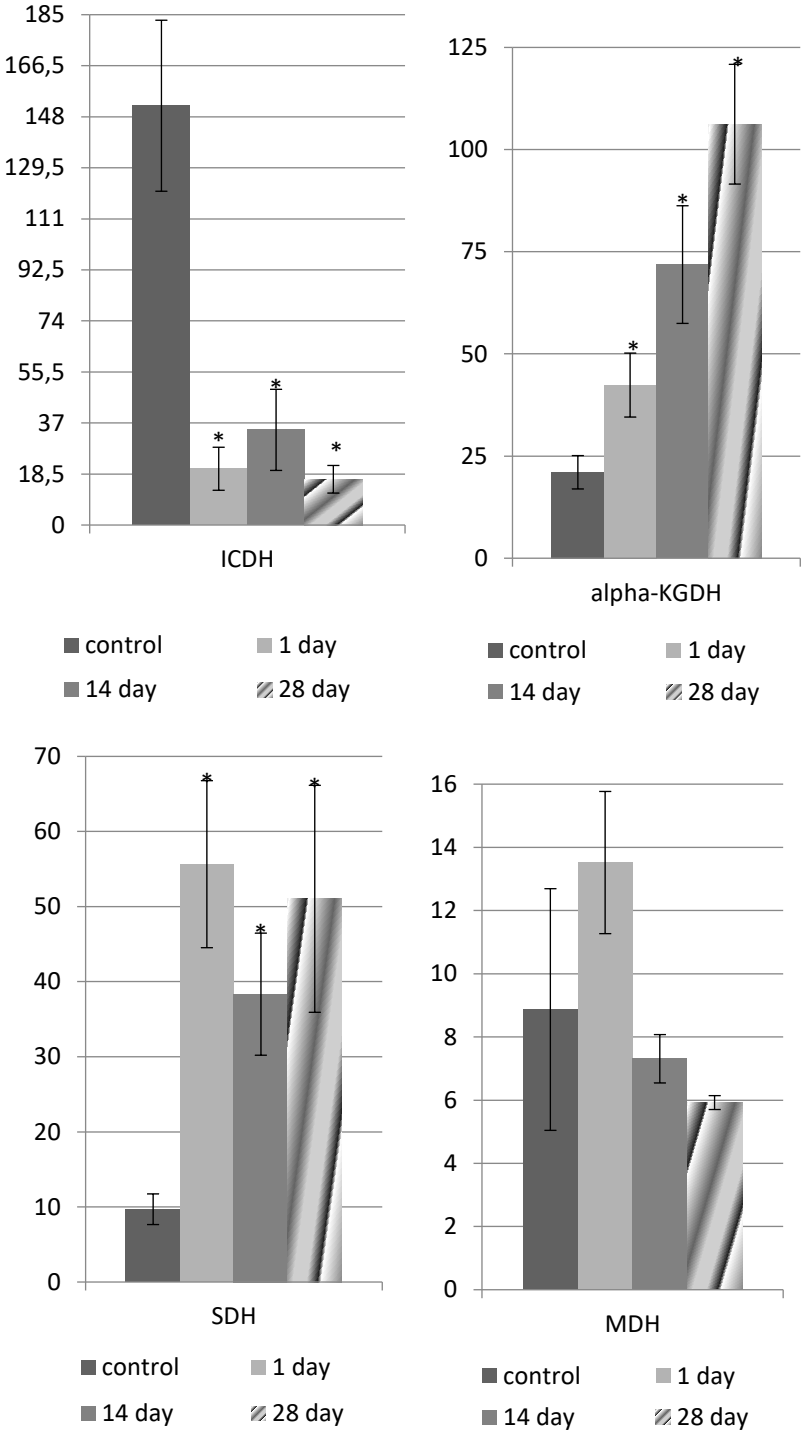

Figure 11. Activity enzymes of cycle citric acid in the liver of experimental animals under the conditions of cadmium-nitrite intoxication.

In the blood plasma of this group of experimental animals, we found a decrease in the activity of all the enzymes studied in the early period: 2 fold isocitrate dehydrogenase and succinate dehydrogenase, 6 fold α-ketoglutarate dehydrogenase and 10 folds malate dehydrogenase (Fig. 12). The late period of combined exposure of Cadmium chloride and Sodium nitrite was characterized by an increase in the activity of the following enzymes: α-ketoglutarate dehydrogenase – 3 fold, isocitrate dehydrogenase – 3.7 fold and succinate dehydrogenase – 7 fold (Fig. 12).

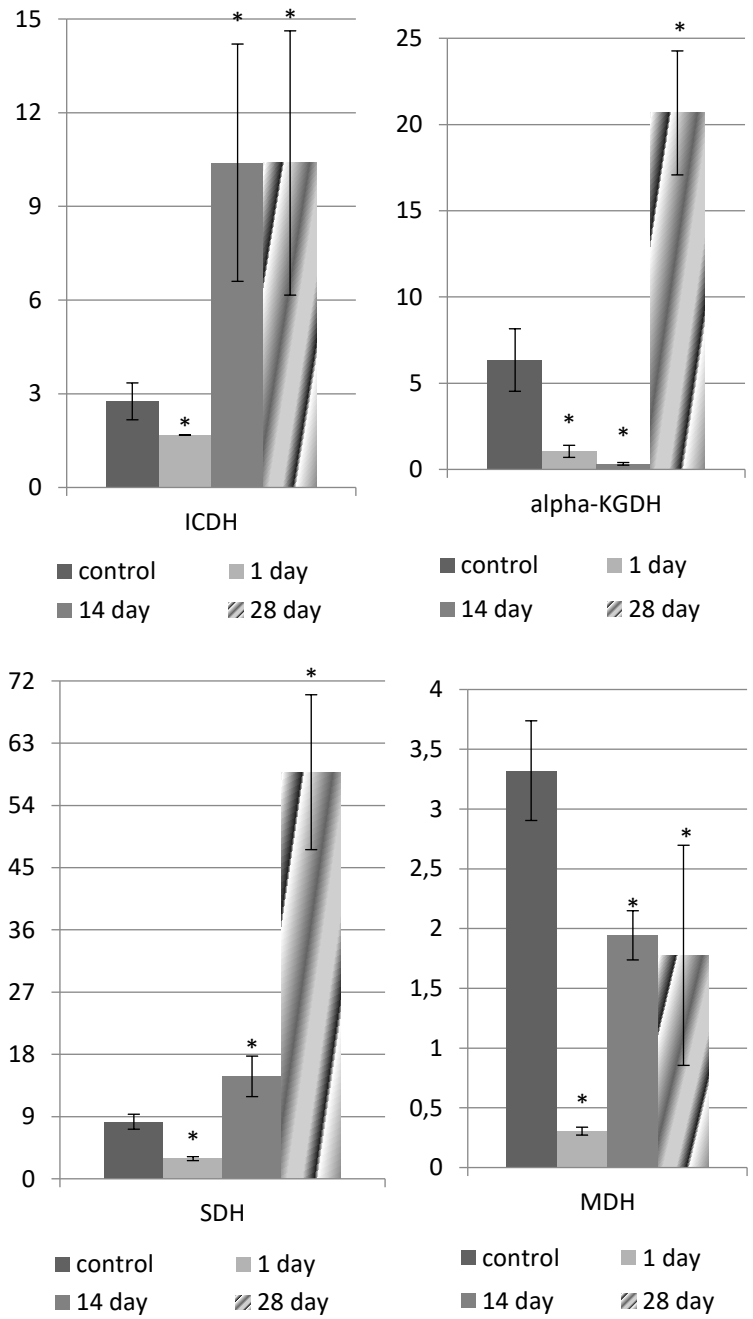

Figure 12. Activity of citric acid cycle enzymes in the blood of experimental animals under conditions of cadmium-nitrite intoxication.

Conclusions.

1. Our studies have to establish the multidirectional nature of changes in the citric acid cycle dehydrogenases in the organs and tissues of experimental animals under the conditions of separate and combined intoxication with Cadmium chloride and Sodium nitrite.

2. The activity of FAD-dependent dehydrogenase (succinate dehydrogenase) increased in the liver, brain, and blood of experimental animals throughout the study period.

3. In the blood of experimental animals in the early period of the study there was a decrease in the activity of all the enzymes we studied under the conditions of cadmium, nitrite and cadmium-nitrite intoxication.

4. The activity of isocitrate dehydrogenase decreased during the whole period of the study in the brain and liver, in the myocardium, we found an increase in this indicator in the early period of cadmium and nitrite intoxication, and under conditions of combined action of xenobiotics, the activity of the study enzyme was reduced.

5. The results obtained can serve as a basis for understanding the peculiarities of energy metabolism in organs and tissues under the influence of Cadmium chloride and sodium nitrite and approaches to the metabolic correction of such conditions.

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