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Morphofunctional Changes of the Adrenals at Chronic Exposure to Magnesium Chlorate

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⁵ Assistant of the Department of Obstetrics and Gynecology №1, Samarkand State Medical University, 0007.hp@mail.ru **Abstract:** With chronic daily administration of threshold doses of magnesium chlorate (1/100 LD50) to rats in the early stages (3-7 days) of the study, an increase in the functional activity of the rat adrenal cortex zones is noted. At a later date (14-90 days), the functional activity of the glomerular and reticular zones is reduced, and the hyperfunction of the fascicular zone is preserved.

An increase in the number of noradrenaline-containing cells was noted from days 3 to 14 of observation, on day 30 the number of adrenaline-containing cells prevailed, and on days 60 and 90 the number of norepinephrinecontaining cells again increased. A change in the ratio of epinephrine- and noradrenaline-containing cells may be the result of both an increased release of adrenaline and a slowdown in its synthesis. The increase in structural changes in the parenchyma and stroma of the gland, as the duration of exposure to magnesium chlorate increased, occurred in parallel with the increase in hemodynamic disorders. Along with impaired vascular permeability, hemo- and lymphostasis is also noted.

Key words: cronic poisoning, magnesium chlorate, morphology, adrenal glands.

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Introduction. The widespread use of pesticides in agriculture contributes to environmental pollution, the ingress of toxic substances and their metabolites into the air, soil, water bodies, and through them into the human and animal body. Chemicalization of agriculture creates new environmental factors that must be taken into account.

In recent years, the adverse effect of many pesticides on the body has been proven, which leads to the development of pathological conditions in it. The relevance of studying the state of the organs of the digestive, endocrine systems, immune and reproductive systems under the influence of various exogenous factors is substantiated [1; 11].

The adrenal glands are involved in many metabolic processes in the body, provide regulatory connections, and are actively involved in the reactions that occur when the body's homeostasis is disturbed. Their role in the development of various diseases and pathologies has been proven [9].

In stressful situations (adaptation to new environmental conditions, irradiation, physical inactivity, hypovitaminosis, infectious diseases), a reaction of the adrenal cortex zones is observed, which manifests itself in an unequal relationship between glucocorticoid, mineralocorticoid and androgenic functions, and the medulla [19].

At the same time, magnesium chlorate is still used in agriculture as a defoliant and desiccant. The conducted studies indicate that when mammals are exposed to magnesium chlorate, inflammatory, degenerative changes in internal organs, a decrease in efficiency, excitability of nerve receptors, immunity, and a slowdown in bone repair are noted. There is not enough literature data on the state of adaptive reactions in the adrenal glands after exposure to pesticides, including magnesium chlorate [3]. That contributes to a detailed study of morphofunctional changes in the adrenal glands with repeated exposure to the body of various doses of magnesium chlorate, and is in demand in its value.

The purpose of the study was to identify morphological changes in the adrenal glands with repeated exposure to magnesium chlorate.

Material and research methods. The study was carried out on 78 adult outbred male rats weighing 150-200 g. Magnesium chlorate at a dose of 41 mg/kg of animal body weight was administered intragastrically, in the form of a 4.1% solution, on an empty stomach. The pesticide was dissolved in distilled water. The indicated dose corresponds to 1/100 LD50. A conventional syringe with a metal probe was placed deep into the oral cavity of rats and the drug was slowly injected. The oral route of administration of magnesium chlorate was chosen taking into account the fact that, according to a number of authors, in 85-90% of cases, pesticides enter the body with food and water.

Multiple injections were carried out within 3 months, which corresponds to 1/10 of the animal's lifespan. For repeated administration, a dose of 1/100 LD50 of magnesium chlorate was used.

Animals of the first series (6 rats) were intact. Animals of the second series (36 rats) served as controls. Under similar conditions to rats exposed to magnesium chlorate, they received distilled water. The state of the adrenal glands was studied in 36 rats that were orally administered distilled water for 3, 7, 14, 30, 60 and 90 days. In the third series (36 rats), magnesium chlorate was administered daily, once at a dose of 1/100 LD50 for 3 months. To study the morphofunctional state of the adrenal glands, rats were killed 3 hours after the administration of the drug, on days 3, 7, 14, 30, 60, and 90 of the experiment.

All animals (intact, control and experimental) were kept in the same vivarium conditions. No animal mortality was observed. Prior to the start of the experiment (within one month) and throughout the entire period of drug administration, the rats were constantly monitored: the general condition was noted, weight, stool, and animal mobility were monitored. Animals were weighed before and after the

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experiment. Rats treated with magnesium chlorate, as well as control and intact rats, were sacrificed simultaneously by instantaneous decapitation using a specially constructed guillotine. The adrenal glands were removed immediately after the slaughter of the animal and weighed on a torsion balance.

To assess possible reactive and compensatory changes in the state of the adrenal glands after the administration of magnesium chlorate, we used histological and histochemical research methods. After weighing along the conditional transverse axis of the organ, the adrenal glands were divided in half so that the state of the cortical and medulla could be assessed.

For fixation of the adrenal glands, 12% neutral formalin solution, Carnoy's liquid, Becker's liquid, 10% silver nitrate solution and Sevka's liquid were used. After appropriate processing of the material according to the selected methods, it was passed through alcohols of increasing concentration and embedded in paraffin. Sections 5-7 microns thick were prepared from paraffin blocks. The sections were stained with hematoxylin-eosin, picrofuchsin according to Van Gieson, impregnation according to the Foot method. The PAS reaction was also carried out to detect glycogen with amylase control, the Brachet reaction for the detection of RNA with ribonuclease control, Sudan black staining to determine lipids, the detection of ascorbic acid by the Giroud-Leblon method, and the Sevka reaction (a type of chromaffin reaction).

The selected methods make it possible to assess the state of the zones of the cortical substance and the medulla of the adrenal glands, which make up their cellular and fibrous structures, the localization and spread of the pathological process in the adrenal glands. In addition, histochemical methods make it possible to present the morphofunctional characteristics of adrenocorticocytes and chromaffin cells of the organ.

The histochemical methods used by us make it possible to judge not only the functional state of the adrenal gland, but also to determine the violation of the stages of the secretory cycle in its glandular cells. Thus, the RNA content is an indicator of the intensity of the synthesis of enzymes of steroidogenesis. Glycogen is necessary to initiate the processes of synthesis of hormones of the adrenal cortex. Lipids are the material for hormone formation in the cells of the cortex of the gland. Ascorbic acid most of all reflects the stage of hormone release from cortical cells.

Statistical processing of the obtained digital material was carried out with the calculation of average errors for arithmetic means (M \pm m). The degree of significance of the difference was calculated based on the definition of t-Student's test. Then, according to the Student's criterion table, the probability (P) of a possible error was determined. Such differences between control and experiment were considered significant when $0.001 \le P \le 0.05$.

Research results and discussion. Repeated administration of magnesium chlorate to rats at a dose of 1/100 LD50 leads to changes in the adrenal glands, which are determined over the course of 90 days of the study. Already in the early periods of observation (3-7 days) there is a plethora of blood vessels and the presence of hemorrhages. Dystrophic changes occur in adrenocorticocytes. Along with this, the adaptive reaction of the adrenal glands in experimental animals is sharply expressed, since the weight of the adrenal glands and the width of the cortex are significantly higher than in the control ones. Among the zones of the cortex, the fascicular zone is subject to pronounced hypertrophy, the width of which is significantly higher than the control level in all periods of the study. The glomerular and reticular zones are also expanded, however, significant differences are observed only on days 3-14. In the adrenocorticocytes of the fascicular zone, hypertrophy of the nuclei is observed. In the glomerular zone, the nuclei of glandular cells were also hypertrophied at all periods of the study, except for day 60. The reticular zone is characterized by an increase in the volume of nuclei only in the early stages (3.7 days), later the nuclei of this zone are significantly smaller than in the control. Apparently, the fascicular zone has the greatest adaptive capabilities, compared with the glomerular

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and reticular zones. Expansion of the fascicular zone of the adrenal cortex is also described in the dynamics of long-term arterial hypertension (Alyabyeva S.Yu. et al., 2015) [2].

It is known that sub capsular cells and the sudanophobic layer play an important role in the regeneration of the adrenal cortex in various injuries (Memetova E.Ya., 2014) [6]. Under the influence of magnesium chlorate, changes in the cells of both the sub capsular and sudanophobic layers are observed in the adrenal glands of rats. They are expressed in the expansion of these layers, in the disordered arrangement of cells, their hyperplasia, the presence of karyopyknosis in some of them, high pyroninophilia, and violation of the argyrophilic stroma. The data found seem to indicate the processes of rearrangement occurring in the subcapsular and sudanophobic layers and their possible participation in the adaptive responses of the cortex to the impact of the pesticide. It was found that partial dedifferentiation of subcapsular cells precedes their proliferation and occurs 3 days after thermal burn (Dzevulsky I.V. et al., 2014) [5]. As is known, these areas of the cortical substance of the organ provide regeneration processes after various injuries (Nikki M., 2015) [12]. In these areas of the cortical substance, some researchers observe more significant structural changes. So, already 30 minutes after acute heat stress, pycnotic nuclei appear in the sudanophobic layer, and 45 minutes later, islands of small immature cells near the capsule appear in the glomerular zone (Koko V. et al., 2004) [10]. Violation of the mechanisms of lipid release from adrenocorticocytes in the adrenal cortex was also observed in suicide victims (Willenberg H.S., 2009) [17].

Histochemical parameters in the adrenal cortex of rats subjected to prolonged exposure to magnesium chlorate at a dose of 1/100 LD50 also changed in comparison with the control. We found that in experimental rats in adrenocorticocytes there is a violation of both synthetic processes and the release of a secretory product from them. The content of RNA and glycogen, which are indicators of synthesis in the glandular cells of the cortex, in the glomerular zone during all periods of the study is less than in the control group. In the beam zone, in most periods of the study, these substances are also less than in the control, only on days 3 and 90 the RNA content is significantly higher. However, the content of glycogen in all periods of the study is small, including on the 3rd and 90th days. In the reticular zone, a higher content of RNA than in the control is observed on days 7, 14, and 90, at other times it is less. The content of glycogen in all terms is reduced. The detected changes indicate that synthetic processes are disturbed in all zones.

Analysis of histochemical parameters characterizing the phase of excretion in the secretory cycle of adrenocorticocytes (lipids and ascorbic acid) showed the following. In the glomerular zone, there is a reduced content of lipids and vitamin C, compared with the control, from days 3 to 7 of the study. In the future (14-90 days), the amount of lipids is also reduced, and ascorbic acid in the adrenocorticocytes of this zone becomes much larger. In the beam zone, the content of lipids and ascorbic acid is below the control level in all periods of the study. In the reticular zone, as well as in the glomerular zone, lipids and vitamin C are less from 3 to 7 days than in the control. On the 14-90th day, the amount of lipids is reduced, and on the 60th day it increases. Ascorbic acid on the 14-90th day in the glandular cells of the reticular zone contains more than in the control.

Comparing the data obtained with the help of morphometry and histo chemistry, we can conclude on the functional activity of the zones of the adrenal cortex in rats subjected to repeated exposure to magnesium chlorate at a dose of 1/100 LD50. On the 3rd-7th day of observation, there is an increase in the functional activity of all zones of the cortical substance of the organ, on the 14-90th day, high functional activity of only the fascicular zone remains. In the glomerular zone during these periods there is an inhibition of functional activity, accompanied by the disappearance of compensatory hypertrophy. In addition, on the 60th day there is a decrease in the volume of nuclei of adrenocorticocytes in this zone. A decrease in functional activity on the 14-90th day of exposure also occurs in the reticular zone, it is accompanied by a decrease in the volume of the nuclei of

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adrenocorticocytes. However, some increase in synthetic processes by the end of the observation apparently leads to the maintenance of hypertrophy in this zone. It should be noted that a multidirectional reaction of the zones of the adrenal cortex was found when exposed to other stimuli (Odo R.I. et al., 2019) [13]. Thus, an increase in the size of the fascicular zone and a decrease in the reticular zone in the adrenal glands of rats are observed when modeling acute postnatal hypoxia in them (Gubina-Vakulik G.I., et al.) [4].

Studies of the medulla of the adrenal glands of rats exposed to magnesium chlorate at a dose of 1/100 LD50 showed an increase in the number of norepinephrine-containing cells from 3 to 14 days of observation, on the 30th day the number of adrenaline-containing cells predominates, and on the 60th and 90th days the number of norepinephrine-containing cells again increases. A change in the ratio of epinephrine- and noradrenaline-containing cells may be the result of both an increased release of adrenaline and a slowdown in its synthesis. According to a number of authors, hormones of the adrenal cortex have a regulatory effect on the last phase of adrenaline biosynthesis, especially when exposed to noise (Gannouni N. et al., 2014) [8]. The entry of cortical hormones into the medulla is possible due to the presence of a common blood supply system between them. Under the influence of corticosteroids, norepinephrine is methylated and converted into adrenaline.

In our studies, it was found that when exposed to magnesium chlorate at a dose of 1/100 LD50, hyperfunction of the beam zone is observed throughout all periods of observation. In this regard, it can be assumed that a decrease in the number of adrenaline-containing cells is associated with an increase in the release of adrenaline from them. Only on the 30th day, the release of adrenaline was slowed down compared to other periods of the study and control. In most chromaffin cells, there is an increase in functional activity, which reaches a maximum on the 90th day of seeding, this is evidenced by an increase in the volume of cell nuclei, the presence of small-drop vacuolization of chromaffin cells, which gradually increases both in number and size. Violation of the functional state of the adrenal medulla was also noted under the action of acute heat stress (Petrovic-Kosanovic D., 2012) [14]. In response to any stress impact, an increase in the functional activity of the medulla is further supported by the cortical part of the adrenal gland, which contributes to its continuation and preservation (Utiger, Robert D., 2021) [16].

An increase in structural changes in the parenchyma and stroma of the gland with an increase in the duration of exposure to magnesium chlorate occurred in parallel with an increase in hemodynamic disorders. Along with impaired vascular permeability, hemo- and lymphostasis is also noted. Violation of hemodynamics and the development of destructive processes in the adrenal glands is observed with prolonged exposure to exogenous factors (Stelnikova I.G., 2007) [7], especially when they are chronically stressed (Ulrich-Lai Yv.M. et al., 2006) [15]. The pathological effect of magnesium chlorate on the body is associated precisely with a violation of the microcirculatory bed. In particular, in the occurrence of necrosis in the cortex of the gland, paramount importance is given to an increase in intravascular coagulation and an increase in lipid peroxidation (Zaki S.M. et al., 2018) [20]. Microcirculatory disorders are a key factor in the emerging dystrophic processes in adrenocorticocytes, ending in gland necrosis, in endotoxin shock (William F., 2006) [18]. Apparently, the violation of the state of the intraorgan vascular bed of the adrenal glands is one of the important reasons in the pathogenesis of destructive processes in the adrenal cortex, leading to focal sclerosis in it.

Our results indicate that with repeated administration of magnesium chlorate at a dose of 1/100 LD50 for a long time, synthetic processes are reduced in most adrenocorticocytes. However, adaptive reactions lead to an increase in the functional activity of all zones of the adrenal cortex on the 3rd-7th day of pesticide administration. In the future (14-90 days), hyper function of only the beam zone remains. In the glomerular and reticular zones during these periods, there is a decrease in the

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functional activity of adrenocorticocytes. A decrease in the functional activity of the cortical substance occurs both with age and under the action of exogenous agents (Yiallouris A. et al., 2019) [19]. It is possible that under the action of other pesticides, the histological structures responsible for the reserve capacity of the gland also turn out to be untenable. Chromaffin cells are activated in the medulla with increased release of adrenaline in most periods of the study. Although pathomorphological changes in the medulla without adrenal enlargement have been noted with exposure to septic shock (Jung B. et al., 2011) [9].

According to the results of our studies, suppression of the secretory activity of only the glomerular and reticular zones is observed during this period. The methods we have chosen make it possible to detect damage to each of the zones of the adrenal cortex. In addition, our data allow us to determine the pathogenesis of inhibition of the functional state of the zones.

Conclusions. Thus, the combination of these changes indicates that with daily administration of threshold doses of magnesium chlorate (1/100 LD50) to rats in the early stages (3-7 days) of the study, there is an increase in the functional activity of the zones of the adrenal cortex of rats. At a later date (14-90 days), the functional activity of the glomerular and reticular zones is reduced, and the hyperfunction of the fascicular zone is preserved.

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