

**THE LONG-TERM EFFECT OF THE COMPLEX
OF HEAVY METAL SALTS ON THE MORPHOFUNCTIONAL
CHANGES IN THE STRUCTURAL COMPONENTS
OF THE INTERMEDIATE LOBE OF THE MATURE RAT'S
PITUITARY GLAND-THE FEMALE***

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The development of the pathology of individual organs and systems undoubtedly depends on adverse environmental factors. Particular attention of researchers attracts heavy metal salts [1–3]. In some northern regions of Ukraine, an increase in the salts of zinc, chromium, lead, manganese, copper and iron, are found in various combinations depending on the region and have an adverse effect on the health of the population [4, 5]. The endocrine system together with the nervous and immune receives a direct active participation in regulating the homeostasis of the organism [6–8]. It is known from literary sources that the hormones of the intermediate part of the pituitary gland are one of the key places in regulating

and maintaining the basic functions of the organism [7]. Glandular cells of this part of the pituitary gland, in addition to MSG and LTG, may also produce some amount of ACTHs or AKTG-like substances. [6, 9–12]. Information on morphofunctional rearranging the structural components of the intermediate part of the pituitary gland in rats under conditions of prolonged exposure to unfavorable environmental factors is isolated.

Thus, influence of different types of stress on the intermediate lobe of the pituitary gland (nitrates and methylene blue, mobilization, cold, emotional and acoustic) [1, 9, 10, 12–16, 19] has been studied. However, the question of the influence on the intermediate part of the pi-

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pituitary gland of the combination of heavy metal salts, morphological and immune histochemical alterations of the organ's structural components remains relevant, insufficiently studied and requires further research.

MATERIALS AND METHODS

The experiment was conducted on 12 white female rats weighing 250–300 g, 7–8 months, which were divided into 2 groups (control and experimental) according to the estral cycle. Animals of the experimental group used ordinary drinking water, saturated with a combination of salts of heavy metals for 60 days: zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) – 5 mg / l, copper ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) – 1 mg / l, iron (FeSO_4) – 10 mg / l, manganese ($\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$) – 0.1 mg / l, lead ($\text{Pb}(\text{NO}_3)_2$) – 0.1 mg / l and chromium ($\text{K}_2\text{Cr}_2\text{O}_7$) – 0.1 mg / liter. Experimental animals were withdrawn from the experiment after the previous thiopental anesthesia (at a rate of 30–40 mg / 10 g of body weight) on the 60th day of experiment, in accordance with national standards in bioethics. The histological peculiarities of the pituitary gland were studied using serial-stepped sections, stained with hematoxylin-eosin. Functional activity of the structural components of the intermediate lobe of the pituitary gland was assessed according to the following indices: the change

The purpose of the study was to study the morphofunctional rearranging the structural components of the intermediate part of the pituitary gland in mature rats under conditions of long-term influence of heavy metal salts.

in the number of melanotropic and lipotropic cells in preparations, the change in the number of secretory granules in different types of cells, the state of the nuclear apparatus of cells, the state of the vascular bed, immune histochemical and biochemical indicators. Rabbit monoclonal antibodies (clone SP6 for the determination of KI-67, USA) were used for the immune histochemical reaction. The evaluation of KI-67 proliferation marker expression was performed according to [17]. The functional state of the pituitary was evaluated by determining the adrenocorticotrophic hormone ACTH (pg/ml) in the serum of peripheral blood of experimental animals (by the ELISA method). A set of reagents from the Siemens series 252 and 255 was used on the Immulite 1000 Siemens Healthcare Global Immune Chemiluminescent Analyzer. Statistical data processing was carried out in the «Statistic 8.0» program package, using the Student-Fisher test. Significant differences were considered for $p \leq 0.05$.

RESULTS AND THEIR DISCUSSION

The study of the structural organization and histological formation of the intermediate pituitary gland of intact rats showed that the results of their own observations coincide with the data of a number of authors [1, 2, 9, 10]. After 60 days of exposure to the complex of heavy metal salts in the intermediate lobe of the pituitary, dystrophic-destructive rearrangements of the basic structural components of the organ were observed. The hysterectomy of the parenchyma was broken by the particle of the gland, which nevertheless retained the trabecular type of structure. At the same time, there was a pronounced thickening and edema of the stromal component of the gland, presumably due to the growth of perivascular connective tissue in the inter trabecular spaces. (Fig. 1). We found that salts of heavy metals cause noticeable hyper fluidity, hyperemia of

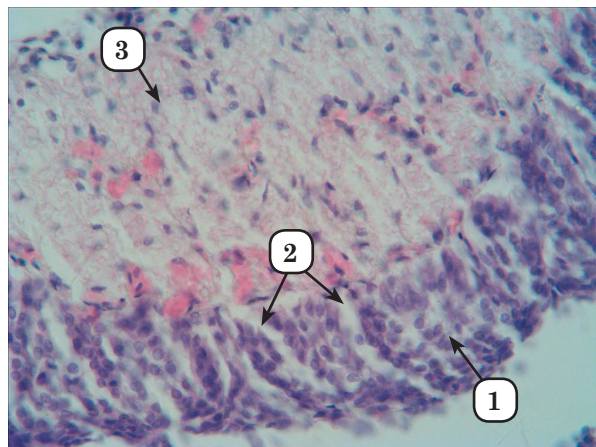


Fig. 1. Intermediate fraction of adenohypophysis and neurohypophysis in rat-females of 60-day trial period.

- 1 — violation of the histo-architecture of cells;
- 2 — thickening and swelling of the stromal component of the gland;
- 3 — neurohypophysis.

Painting: hematoxylin and eosin. $\times 400$.

Results of determining the ACTH hormone in blood serum of experimental and control animals ($M \pm m$), $n = 6$

Indicator	Investigated animal groups	
The content of hormones in serum of blood	Control animals	Experimental animals
ACTH (pg/ml)	$52,6 \pm 0,13$	$281,5 \pm 8,2^{***}$

Note:

the difference between control and experiment $^{***} p \leq 0,001$.

vessels adjacent to the intermediate part from the side of the neuro hypophysis. The vascular wall undergoes noticeable alterations. The vessels were filled with a homogeneous red blood cell mass of bright red color. The boundaries of the blood cells are not clearly contiguous. However, no violations of the rheological properties of blood on these terms of the experiment were observed. Endothelium of capillaries undergoes structural alterations: most of the endothelial cells were swollen; their nuclei were hyper chromic, condensed.

Some of the cores were somewhat reduced in size and appeared in the gaps of the capillaries (Fig. 2). In the parenchyma itself, the intermediate lobe of the pituitary vessel was not visualized. Only in one of the vision fields, fragments of the wall of the hemo capillary was revealed, the lumen of which was empty, which is undoubtedly caused by the features of blood supply to this part of the pituitary gland (Fig. 2). After all, according to literary sources

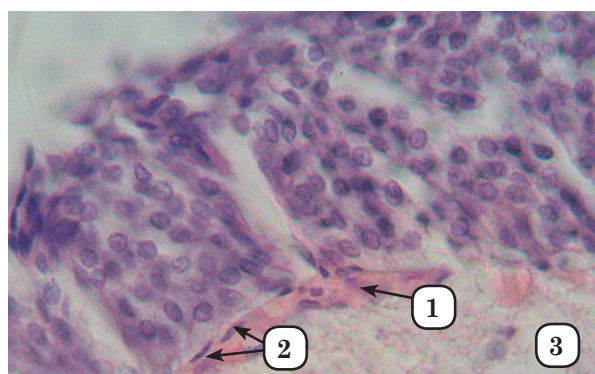


Fig. 2. Intermediate part of the pituitary gland in the rat-female of 60-day trial period:
 1 — full blood pressure, hyperemia of the vessels of the neurohypophysis;
 2 — edema of endothelial cells, hyper chromatosis and nucleus edema;
 3 — neurohypophysis.
 Painting: hematoxylin and eosin. $\times 400$.

[18], the intermediate part of the adeno hypophysis does not have a well-organized blood supply. According to the data there is not a lot of literature [19], the intermediate part of the adeno hypophysis has a vascular system common to the neurohypophysis, as well as a neuro secretory «innervation», since it is infiltrated by the nerve fibers originating from the neurohypophysis and containing the neuro secret. Nerve fibers make synapse contacts with epithelial cells. Cholinergic and amino genic fibers have an inhibitory effect on the secretion of the intermediate part cells of the adeno hypophysis [15]. Investigating the state of the endocrinocytes of the intermediate part of the adeno hypophysis in experimental animals, we found that the cells and their nuclei are oval and relatively small. The nuclei were centrally located and did not have invaginations with nuclear membrane.

The nuclear-cytoplasmic ratio was 1 : 1.6. Chromatin nuclei of the cells was clarified, fine-structure, condensed, and located marginally. A small portion of the nuclei had a well-contoured nucleolus. The other part of the cells nuclei was hyper chromic, with a tendency to pycnosis changes. In different fields of vision, the number of cells with hyper chromic cytoplasm (lipotropic cells) was significantly increased in comparison with the control and the number of light cells (melanotropic). At the same time, the number of melanotropic cells significantly increased in comparison with the control by 8.4% and amounted to 29.5 ± 0.33 ($p < 0.01$, $t = 4.2243282$), whereas the number of lipotropic cells increased in comparison with the control at 10.1% and amounted to 66.17 ± 2.04 ($p < 0.05$, $t = 2.945873$). The level of ACTH, one of the types of melanocortins, in blood serum was increased by 5.4 times ($p < 0.001$, $t = 27.91113$) (Table).

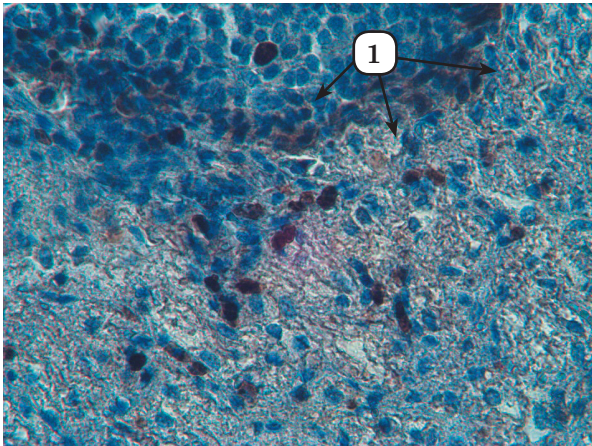


Fig. 3. The peripheral portion of the intermediate lobe of the pituitary gland in a female rat of 60 days of experience.
1 — Expression of Ki-67 (++) from 35 to 40%.
× 400.

According to the results of an immune histochemical study of the tissue of the intermediate pituitary gland marker of cell proliferative activity (Ki-67), we found that on the 60th day of the experiment, the marker KI-67 was localized in single nuclei and basophilic cells with a marked increase in reactivity on the border between the intermediate part of the adenohypophysis and the neurohypophysis. (Fig.3). This indicates the localization of the proliferative processes precisely in the peripheral region of the intermediate particle (35–40%, moderate proliferative activity) in conjunction with the virtually irregularity of the cells in the central part of the parenchyma gland (0–7%, low proliferative activity). The intensity of the coloration of the cells was evaluated as moderate (++), and the cytoplasm is low.

Morph functional alterations in the gland under conditions of influence of salts of heavy metals had a non-specific polymorphic charac-

ter and were characterized by the development in the structural components of the intermediate part of the pituitary gland of the adaptive-compensatory processes with signs of increased functional activity. According to the literature [18, 20], morph functional perturbations are characteristic of increased resistance stage of the general adaptive syndrome.

Conclusions The long-term admission of heavy metal salts to the organism of mature female rats leads to morphological transforming all structural components of the intermediate part of the pituitary gland. Significant thickening and edema of the inter trabecular connective tissue septum, depletion of capillaries and morphological rearranging part of the trophocytes, mainly their nuclear apparatus (condensation of chromatin and its marginal location, initial stages of necrobiotic changes), is manifested. However, a small part of the cell nuclei have well-contoured nucleoli, indicating the activation of protein synthesis in cells. This is firmly confirmed by the results of morphometric and biochemical methods of studying experimental animals, which is manifested in the increasing the number of basophilic cells and the level of ACTH, one of the types of melanocortins. The level of the K67 test is slightly elevated predominantly in the tropic cysts of the peripheral regions of the intermediate portion of the gland and is practically absent in the central regions.

Prospects for further research are based on conducting a wider spectrum of morphological and immune histochemical studies of the intermediate fraction of adenohypophysis in rats under conditions of prolonged exposure to the body of a combination of heavy metal salts.

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ДОВГОТРИВАЛИЙ ВПЛИВ КОМПЛЕКСУ СОЛЕЙ ВАЖКИХ МЕТАЛЛІВ НА МОРФОФУНКЦІОНАЛЬНІ ПЕРЕБУДОВИ СТРУКТУРНИХ КОМПОНЕНТІВ ПРОМІЖНОЇ ЧАСТКИ ГІПОФІЗА СТАТЕВОЗРІЛИХ ЩУРІВ-САМИЦЬ

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Розвиток патології окремих органів та систем безперечно залежить від несприятливих факторів навколишнього середовища. Дисбаланс рівня важких металів негативно впливає на здоров'я населення та може призвести до непередбачуваних наслідків. Гормони проміжної частки гіпофіза займають одне з ключових місць у регуляції та підтриманні основних функцій організму. Питання впливу на проміжну частку гіпофіза комбінації солей важких металів залишається актуальним та недостатньо вивченим. З метою вивчення морфофункціональних перебудов структурних компонентів проміжної частки гіпофіза статевозрілих щурів-самиць був проведений експеримент на 12 тваринах масою тіла 250–300 г, у віці 7–8 місяців. Тварини експериментальної групи протягом 60-ти діб вживали звичайну питну воду, насичену комбінацією солей важких металів. Застосовувалися морфометричні, статистичні, імуногістохімічні, біохімічні та гістологічні методи дослідження. Довготривале надходження до організму статевозрілих щурів-самиць солей важких металів призводить до морфологічних трансформацій усіх структурних компонентів проміжної частки гіпофізу. Виявляється виразне потовщення та набряк міжтрабекулярних сполучнотканинних септ, запустіння капілярів та морфологічні перебудови ядер частини трофоцитів: конденсація хроматину та його маргінальне розташування, початкові етапи некробіотичних змін. Морфофункціональні перебудови у залозі мали неспецифічний поліморфний характер та характеризувалися розвитком пристосувально-компенсаторних процесів з ознаками підвищеної функціональної активності. Наведені перебудови характерні для стадії підвищеної резистентності загального адаптаційного синдрому.

Ключові слова: аденогіпофіз, важкі метали, Ki-67, АКТГ.

ДОЛГОВРЕМЕННОЕ ВЛИЯНИЕ КОМПЛЕКСА СОЛЕЙ ТЯЖЕЛЫХ МЕТАЛЛОВ НА МОРФОФУНКЦИОНАЛЬНЫЕ ПЕРЕСТРОЙКИ СТРУКТУРНЫХ КОМПОНЕНТОВ ПРОМЕЖУТОЧНОЙ ДОЛИ ГИПОФИЗА ПОЛОВОЗРЕЛЫХ КРЫС-САМОК

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Развитие патологии отдельных органов и систем бесспорно зависит от неблагоприятных факторов окружающей среды. Дисбаланс уровня тяжелых металлов негативно влияет на здоровье населения и может привести к непредсказуемым последствиям. Гормоны промежуточной доли гипофиза занимают одно из ключевых мест в регуляции и поддержании основных функций организма. Вопрос влияния на промежуточную долю гипофиза комбинации солей тяжелых металлов остается актуальным и недостаточно изученным. С целью изучения морфофункциональных перестроек структурных компонентов промежуточной доли гипофиза половозрелых крыс-самок был проведен эксперимент на 12 животных массой тела 250–300 г, в возрасте 7–8 месяцев. Животные экспериментальной группы в течение 60-ти суток употребляли обычную питьевую воду, насыщенную комбинацией солей тяжелых металлов. Применялись морфометрические, статистические, иммуногистохимические, биохимические и гистологические методы исследования. Длительное поступление в организм половозрелых крыс-самок солей тяжелых металлов привело к морфологическим трансформациям всех структурных компонентов промежуточной доли гипофиза. Выявлена выразительное утолщение и отек междутрабекулярных

соединительнотканых септ, запустение капилляров и морфологические перестройки ядер части тропоситив: конденсация хроматина и его маргинальное положение, начальные этапы некробиотических изменений. Морфофункциональные перестройки в железе имели неспецифический полиморфный характер и характеризовались развитием приспособительно-компенсаторных процессов с признаками повышенной функциональной активности. Приведенные перестройки характерны для стадии повышенной резистентности общего адаптационного синдрома.

Ключевые слова: аденогипофиз, тяжелые металлы, Ки-67, АКТГ.

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The development of the pathology of individual organs and systems undoubtedly depends on adverse environmental factors. The heavy metal imbalance affects the health of the population and may lead to unpredictable consequences. The hormones of the intermediate part of the pituitary gland are one of the key places in regulating and maintaining the basic functions of the organism. The question of the influence on the intermediate part of the pituitary gland of the combination of heavy metal salts remains relevant and insufficiently studied. In order to study the morph functional rearrangements of the structural components of the intermediate pituitary gland of sexually mature female rats, an experiment was conducted on 12 animals weighing 250–300 g, aged 7–8 months. The animals of the experimental group consumed normal drinking water saturated with a combination of salts of heavy metals for 60 days. Morphometric, statistical, immunohistochemically, biochemical, and histological methods were used. Significant thickening and edema of the inter trabecular connective tissue septum, depletion of capillaries and morphological rearranging part of the troposites, mainly their nuclear apparatus (condensation of chromatin and its marginal location, initial stages of necrobiotic changes), is manifested. Prolonged intake of sexually mature female rats of heavy metal salts leads to morph functional rearrangements of all structural components of the intermediate lobe of the pituitary gland. The above morph functional changes are of a non-specific polymorphic nature and are characteristic of the stage of increased resistance of the general adaptation syndrome.

Key words: adenohypophysis, heavy metals, Ki-67, ACTH.