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# MORPHOLOGY AND FUNCTIONAL STATE OF THE RAT PINEAL GLAND IN CHRONIC ETHANOL INTOXICATION

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The purpose of the study was to establish the features of the morpho-functional state of the rat pineal gland in conditions of chronic ethanol intoxication. The results of the study showed that after 30 days of exposure to ethanol, the total number of pineal cells decreases significantly. An increase in the volume of the cytoplasm is determined due to vacuolation processes of varying degrees. Along with the decrease in the number of pinealocytes, the content of glial cells increases, which indicates a decrease in the functional activity of the pineal gland under experimental conditions. The structural organization of dark pinealocytes is also changing. Dark cell nuclei were found to lose a high degree of basophilia due to heterochromatin decondensation. Such morphological changes may indicate the transformation of dark cells in the light.

**Key words:** ethanol, pineal cells, gliocytes, vacuolar dystrophy, apoptosis, rats.

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## МОРФО-ФУНКЦІОНАЛЬНИЙ СТАН ЕПІФІЗУ ЩУРІВ ЗА УМОВ ХРОНІЧНОЇ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

Метою дослідження було встановлення особливостей морфо- функціонального стану епіфізу щурів в умовах хронічної інтоксикації етанолом. Результати дослідження показали, що після 30-добового впливу етанолу суттєво зменшується загальна кількість пінеальних клітин. Визначається збільшення об'єму цитоплазми, за рахунок процесів вакуолізації різного ступеню. Поряд зі зменшенням кількості пінеалоцитів збільшується вміст гліальних клітин, що свідчить про зниження функціональної активності епіфізу в умовах експерименту. Структурна організація темних пінеалоцитів також зазнає змін. Виявлено, що ядра темних клітин втрачають високий ступінь базофілії внаслідок деконденсації гетерохроматину. Подібні морфологічні зміни, можливо, свідчать про трансформацію темних клітин на світлі.

**Ключові слова:** етанол, пінеалоцити, етанол, вакуольна дистрофія, апоптоз, щури.

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Modern society is characterized by a significant increase in the impact of stressors due to socio-economic and psychological tensions, especially in quarantine and quarantine restrictions associated with the COVID-19 pandemic, which has led to a sharp increase in alcohol consumption and alcoholism. The WHO considers alcohol abuse as a global medical and social problem of the XXI century [12].

It is known that ethanol has chronobiological effects that are associated with inhibition of melatonin synthesis and secretion, with the development of stress reactions, including oxidative stress, which occurs due to increased production of reactive oxygen species and leads to the accumulation of excessive free radicals, causing in its turn tissue injury and exacerbates inflammation [6, 7, 9, 13]. The effect of ethanol and its metabolites on the body is accompanied by a complex of morpho-functional changes in almost all organs and systems, which leads to homeostasis impairment. Our analytical review of the literature shows that the effect of ethanol on various visceral organs, especially the liver and kidneys, has been studied in detail and described by many researchers [4, 15].

However, the effect of ethanol on the morphological and functional state of various parts of the brain has not been studied enough. Existing publications are more devoted to the effect of ethanol on the structure of the cerebellum [5, 10]. At the same time, changes in the morphology of the pineal gland, which is one of the main organs that adapts to the action of stress factors through the rapidly emerging nonspecific hormonal response and finding ways to correct this pathological condition, are insufficiently studied [11]. In this regard, a promising field is the study of morphology and functional activity of the pineal gland under the influence of ethanol.

**The purpose** of the study was to establish the features of the structural organization and functional state of the rat pineal gland in the physiological norm and in chronic ethanol intoxication.

**Materials and methods.** The experimental studies involved 20 sexually matured male Wistar rats weighing 180–220 g. The animals were kept in standard vivarium conditions. Experimental animals were divided into 2 groups: the control and the experimental ones. The control group included 6 individuals, the experimental group – 14. The first group consisted of intact rats. The second group is rats, which were simulated alcohol intoxication by administering 40 % ethanol solution at a rate of 12 mg/kg body weight intragastrically 4 times a day [14, 15]. The control group was under normal conditions without the influence

of additional factors. On day 30 after the start of the experiment, rats were euthanized with thiopentane anaesthesia at a rate of 25 mg/kg body weight. After completion of the decapitation procedure, the pineal gland was isolated together with the adjacent blood vessels [2] and fixed in a 10 % solution of neutral formalin. In order to the timely and complete penetration of the fixing solution into the pineal gland, the soft membrane of the animal brain, in the places closest to the area of the pineal gland, was previously dissected. Without deviating from standard methods, the material was embedded in paraffin blocks, of which sections with the thickness of 4–5 μm were made and stained with hematoxylin and eosin.

The number of pinealocytes in the field of view of the microscope was counted on the obtained histology specimens of the pineal gland. In our studies, the field of view of the microscope at magnification × 20 and binocular × 10 was taken as a conventional unit of area in which the number of pineal cells was counted. Cell counting was performed by analogy with the counting chamber and following Egorov's rule, (pinealocytes were counted in the field of view and on the border of the upper and right sectors, and cells located on the lower and left border of the microscope field of view were not counted). To obtain statistically significant data, the number of cells in 10 fields of view of the microscope was determined. 10 histological preparations of the studied organ of each experimental group were analyzed. Histology specimens of the control and experimental groups were studied in parallel.

All stages of the experiment, manipulations and euthanasia of experimental rats were carried out in accordance with the general principles of work with experimental animals in accordance with the following standards: Convention on Bioethics of the Council of Europe (1997); European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001); Law of Ukraine "On Protection of Animals from Cruelty" (2006) and other international agreements and current national legislation in the field of medical and biological research.

**Results of the study and their discussion.** Optical examination of histology specimens of the pineal gland of male rats under the conditions of 30-day exposure to ethanol revealed that the pineal gland retained its inherent morphological features, including oval shape, close connection with the vascular plexus of the brain's third ventricle, capsule integrity and corpuscle integrity (fig. 1). The only thing should be noted that the pineal gland looked swollen, and its size was increased compared to the intact group of experimental animals.

It is established that the capsule of the pineal gland is not thickened. From the capsule into the organ a large number of septa branches off, which divide the organ's parenchyma into a large number of corpuscles of different sizes. It was noted that the interparticle membranes looked thickened and swollen. In the course of further microscopic examination of the pineal gland's specimens of intact and experimental rats with a small overall magnification of the microscope, its differentiation into stroma and parenchyma was clearly visualized. The pineal gland's parenchyma of both control and experimental groups was represented by two populations of specialized cells: light and dark pinealocytes (fig. 2).

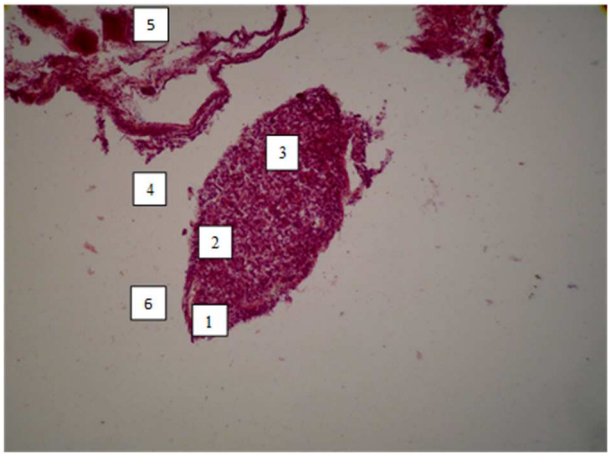


Fig. 1. Photoreconstruction of the pineal gland's shape (section through the centre of the organ). Magn. × 100. Staining hematoxylin and eosin.

Symbols: 1 – the upper part of the pineal gland; 2 – middle part (body of the organ); 3 – the base of the pineal gland; 4 – subarachnoid space; 5 – blood vessels; 6 – lamination of the capsule of the pineal gland.

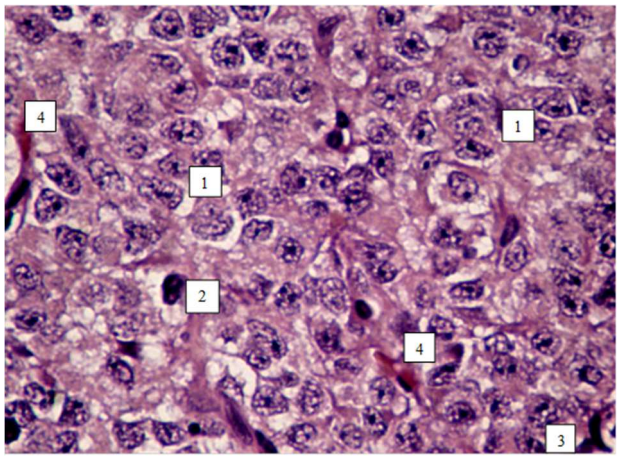


Figure 2. Fragment of the pineal gland's parenchyma in the control group rat. Staining with hematoxylin and eosin. Magn. × 400.

Symbols: 1 – light pinealocytes; 2 – dark pinealocyte; 3 – glial cell; 4 – blood vessels.

In the study of histology specimens of a group of animals that were under chronic exposure to ethanol, it was found that light cells with clarified, vacuolated cytoplasm dominated.

A comparative analysis of histological specimens of two experimental animals' groups found that rats exposed to 30 days of ethanol significantly reduced the total number of pineal cells in each of the studied fields of view of the microscope. Thus, if in the control group of animals the number of cells in one field of view of the microscope at a total magnification of×200 was 198.4±12.0, in the group of experimental animals under chronic ethanol intoxication the number of pinealocytes in one field of view of the microscope decreased to the level of 119.8±10.6. This rarefaction of the pineal gland's parenchyma due to the decrease in the density of pinealocytes can be explained by the fact that almost all pineal cells (both peripheral and central zone of the organ's parenchyma) were increased in volume compared to control cells. Because of cell hypertrophy, fewer cells were placed in the field of view of the microscope. Another reason for the sparse location of pineal cells was the presence of a large number of vacuoles, which also increased the distance between neighbouring pinealocytes, and their number in the field of view decreased accordingly (fig. 3).

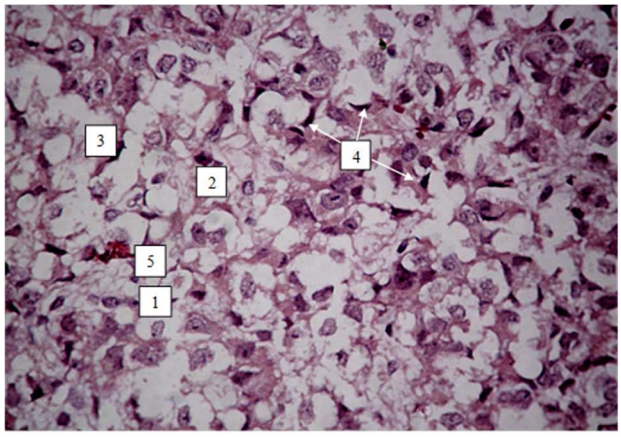


Fig. 3. Fragment of the parenchyma of the rat pineal gland under conditions of chronic ethanol intoxication. Staining with hematoxylin and eosin. Magn.:×200.

Symbols: 1 – light pinealocytes in the state of vacuolation; 2 – dark pinealocyte; 3 – vacuoles filled with cytoplasmic fluid; 4 – glial cells; 5 – blood vessel.

It was found that the cytoplasm gained a special increase due to the processes of vacuolation of varying severity. It was found that some pineal cells had a moderate degree of vacuolation, other cells were characterized by a significantly increased volume of cytoplasm, and in the place of other pinealocytes in general vacuoles of different calibers filled with cytoplasmic fluid could be seen. It is noted that vacuolation of cellular elements was accompanied by a significant increase in the volume of the cytoplasm and, in connection with it, stretching of the cytolemma. It was found that in some cases, vacuolation of the cytoplasm caused a rupture of the cytolemma, resulting in cytoplasmic fluid spilled into the corpuscles of the organ. On some histological specimens, we found accumulations of cytoplasmic fluid, even under the capsule of the pineal gland, which led to its exfoliation in these areas.

It was found that with standard methods of staining histological specimens, the cytoplasm of light pinealocytes in rats exposed to ethanol was optically transparent. Only occasionally, a basophilic substance could be seen in the cytoplasm of light cells, which was randomly arranged around the nucleus.

The presence of a large number of vacuoles indicates that the cells functioned with increased load and as a result underwent lysis and apoptosis, which indicates a loss of cellular composition in the pineal parenchyma and because of reduced synthetic processes. It should be noted that along with the decrease in the number of pinealocytes the content of glial cells increased. Taking into account the support-mechanical properties of gliocytes, the increase in their number is evidence of a decrease in the functional activity of the pineal gland under experimental conditions.

In a further study of histology specimens of a group of rats that were in conditions of chronic ethanol intoxication, it was found that the nuclei of light pinealocytes had a predominantly oval shape. Occasionally there were round shape nuclei. It should be noted that the nuclei were visually larger than the nuclei of light cells of animals in the control group. On the studied specimens, we found cells with signs of karyolysis and karyorrhexis, which were manifested by gradual discolouration of nuclear substance and nucleoli, their disintegration into fragments and the formation of "shadows" of nuclei with their subsequent dissolution.

It was found that in pinealocytes, in which the volume of the cytoplasm was significantly increased, the nucleus was almost completely discoloured and turned into a homogeneous mass. Due to the bright basophilia of the nuclear substance, nucleoli of various sizes and round shapes were well identified in the nucleus. It was found that the nucleoli occupied both central positions in the nucleus and could be shifted to its periphery.

It should be noted in that the histology specimens of this group of experimental animals, the vast majority of pineal cells were in the above condition. Intact pinealocytes were either not detected or were detected as single cells only in certain fields of view of the microscope. A very small number of pineal cells had nuclei with signs of karyopyknosis. It should be noted that in vacuolated pinealocytes, the nuclei, or their "shadows" occupied both central positions and could be located eccentrically, i.e. pushed to the periphery of the pineal cell.

It was found that in contrast to light cells, the structural organization of dark pineal cells remained virtually unchanged. It was noted that the nuclei retained the intensity of the basophilia degree. The nuclei basophilia was due to the high content and condensation of the functionally inactive type of chromatin

(heterochromatin), which was chaotically scattered throughout the karyoplasm, masking the presence of nucleoli. The cytoplasm of dark cells was a narrow, often light, unstructured space around the nucleus. Only on some histology specimens we found dark pinealocytes, the nuclei of which lost a high degree of basophilia due to decondensation of heterochromatin. Such morphological changes may indicate the transformation of dark cells in the light. It was found that the cytoplasm of dark pinealocytes was not subjected to vacuolation processes and therefore these cells were located at a short distance from other cells or structures. At the same time, it was found that the location of dark pineal cells did not have a certain structural order. They were found both in the peripheral regions and in the center of the pineal gland's parenchyma. It was noted that dark cells were located both in singles between glial cells and light pinealocytes, and in the form of small clusters consisting of 2–3 cells.

We also found changes in the proportion between dark and light pineal cells. It was found that in the animals of the control group the percentage of light cells from the total number of pinealocytes was 82.35±0.68 %, and the percentage of dark cells was 17.65±0.73 %. It was determined that in rats exposed to the chronic effect of ethanol, the number of light pineal cells increased compared to the control group, and dark, on the contrary, decreased. It was found that the percentage of light cells in the experimental group was 89.08±0.91 %, and the percentage of dark cells decreased to 10.92±1.21. The revealed changes in the ratio of the two types of pinealocytes indicate the possible transformation of dark pinealocytes into light cells, which occurs due to the loss of overloaded cells.

Information on the influence of ethanol on the morphology and functional state of the pineal gland in our domestic and foreign literature sources are limited [12]. However, the data obtained on the morpho-functional state of the pineal gland are consistent with the data of researchers who studied changes in morphology and functional state of the pineal gland in response to other pathological factors of endogenous and exogenous origin [1, 3, 8]. Thus, the identified morphological disorders, according to literature sources, can be characterized as manifestations of parenchymal vacuolar dystrophy, which indicates apoptosis of pinealocytes, as evidenced by the sparse location of cells in the parenchyma of the pineal gland and a decrease in the number of pinealocytes by 39.62 % compared to the control group of rats. At the same time, in addition to destructive processes in the parenchyma of the organ, we revealed adaptive-compensatory changes in response to the toxic effects of ethanol, manifested by the inclusion of pineal reserve in synthetic processes and hypertrophy of pinealocytes to compensate for their progressive loss.

Morphologically, this fact is confirmed by changes in the ratio of the two types of pinealocytes and by the fact that all pineal cells were in a state of pronounced vacuolation of the cytoplasm. According to the literature, this state of the structural components of the parenchyma indicates the active synthesis and secretion of indolamines in response to damaging factors to maintain the adaptive capacity of the body and maintain its homeostasis [8].

## Conclusion

Because of histological examination, it was found that chronic ethanol intoxication is accompanied by pronounced destructive changes in the parenchyma of the pineal gland. It was noted a decrease in the total number of pinealocytes by 39.62 % and their sparse placement in the parenchyma, indicating apoptosis of pinealocytes. It was found that pinealocytes undergo vacuolation processes with the subsequent development of lysis of intracellular structures, which indicates the development of vacuolar dystrophy.

*Prospects of further research. In the future, it is planned to study the morphometric changes of rat pineal cells in conditions of chronic ethanol intoxication.*

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# STUDY OF STRENGTH OF FUMERAL BONE OF RATS AFTER FILLING BONE TISSUE DEFECTS WITH BONE CEMENT BASED ON TRICALCIUM PHOSPHATE

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There are bone defects that do not heal on their own, despite the ability of bone tissue to fully regenerate. These defects occur due to degenerative-dystrophic diseases, injuries, resections of tumors and tumor-like formations, metastatic lesions, as a result of the development of aseptic instability after primary arthroplasty. Autogenous material for transplantation is the most acceptable, but its use depends on the quality of the bone. Allografts have compensated for the shortcomings of autografts, but the risk of infection and ethical issues limit the use of this material. The group II cement that we offer retains the properties of tricalcium phosphate-based materials: high biocompatibility, osteoconductive and osteointegrative qualities, and, due to strengthening by HA whiskers, it has higher strength and slower biodegradation time.

**Key words:** bioceramics, α-tricalcium phosphate, calcium phosphate cement, orthopedic surgery, bone regeneration.

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# ДОСЛІДЖЕННЯ МІЦНОСТІ СТЕГНОВИХ КІСТОК ЩУРІВ ПІСЛЯ ЗАПОВНЕННЯ ДЕФЕКТІВ КІСТКОВОЇ ТКАНИНИ КІСТКОВИМ ЦЕМЕНТОМ НА ОСНОВІ ТРИКАЛЬЦІЙФОСФАТУ

Існують дефекти кістки, які не загоюються самостійно, незважаючи на здатність кісткової тканини до повної регенерації. Ці дефекти виникли внаслідок дегенеративно-дистрофічних захворювань, травм, резекцій пухлин і пухлиноподібних утворень, метастатичних уражень, у результаті розвитку асептичної нестабільності після первинного ендопротезування. Аутогенний матеріал для трансплантації є найбільш прийнятним, але його використання залежить від якості кістки. Аллотрансплантати скомпенсували недоліки аутотрансплантатів, але ризик інфекційного ураження й етичні питання обмежують застосування цього матеріалу. Цемент II групи, який пропонуємо ми, лишив у собі характеристики, що притаманні матеріалам на основі трикальційфосфату: високі біосумісні, остеокондуктивні й остеоінтегративні якості, та, за рахунок зміцнення гілками гідроксіапатиту отримав вищу міцність, і більш повільний час біодеградації. Ці характеристики як час перебудови імплантованого матеріалу та його витривалість при навантаженні, напряму мають вплив на можливість здійснення навантаження в більш ранній післяопераційний період.

**Ключові слова:** біокераміки, α-трикальційфосфат, кальцій-фосфатний цемент, ортопедичні операції, регенерація кістки.

*The study is an initiative.*

The relevance of the study of osteoplastic materials is due to the occurrence of such bone defects that do not heal on their own, despite the ability of bone tissue to fully regenerate. These include defects caused by degenerative-dystrophic diseases, injuries, resections of tumors and tumor-like formations, metastatic lesions, as well as in the case of aseptic instability after primary arthroplasty [1–3]. Autogenous material for transplantation is the most suitable, mainly because it meets certain requirements: it contains