## Findings

Thus, taking into account the organospecific features of the brain (soft, jelly-like consistency, the watery character) and the complexity of visualization of the liquor system associated with them, we propose some methodological approaches to a study of the brain of fetuses and newborns, which includes special methods of fixation and a complex evaluation of pathomorphologic changes at all levels of the structural organization. An evaluation of the rates of gyrification and their correspondence to the gestational age of a fetus or a newborn, a perfusion of the cerebral vessels with the aid of Karavanov's fixator are original, informative, and may be used successfully by morphologists in their research work.

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# Morphofunctional changes in the structure of rats' adrenal glands cortex zones by alcohol intoxication and the effect of antioxidant


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Studies have shown that prolonged involuntary alcohol intoxication causes significant structural and functional reorganization of the cortex of the adrenal glands. Natural antioxidant α-tocopherol has a modifying influence on changes in the cells of the adrenal glands cortex in case of alcoholization, with the intensity and direction of this effect depends on whether the use of alcohol is being continued.



**Key words:** the cortex of adrenal glands of rats, alcohol, antioxidant.


### Морфофункциональные изменения в структуре зон коры надпочечников крыс под влиянием алкогольной интоксикации и антиоксиданта


Исследования показали, что длительная непроизвольная алкогольная интоксикация приводит к значительной структурной и функциональной реорганизации коры надпочечников. Природный антиоксидант α-токоферол имеет модифицирующее влияние на изменения в клетках коры надпочечников железы в случае алкоголизации, интенсивность и направленность этого влияния зависит от того, продолжается ли употребление алкоголя.



**Ключевые слова:** кора надпочечников крыс, алкоголь, антиоксидант.

## Introduction

Exogenous influences cause changes in basic physiological systems of the body in much greater extent than the endogenous biological irritants. Therefore, studying the influence of these factors on various tissues and organs is becoming more and more important.

One of the most important glands that regulates the activity of the body and response to the biological effects of exogenous irritants is the adrenal gland, in particular the adrenal cortex. Unfortunately, the literature has almost no data obtained from the use of a systematic approach to characterize the features of the structural organization of the morpho-functional system "the adrenal cortex", performing the function of the effector component of adaptation. Research of the past decades, devoted to the study of the adrenal glands, showed that their morphological state could assess the adequacy of adaptive response of the organism to various stressors. The effect of weak stressors causes a reaction in the adrenal cortex glomerular zone at first. The action of long-term stressors leads to the highest reaction manifestations of the beam part and reticular zones. The action of strong stressors leads to an almost simultaneous, but different in severity, involvement of all the structural and functional zones of the adrenal glands in the implementation of the adaptive response of the organism.

The aim of the study is to evaluate the influence of alcohol intoxication of rats due to morphometric parameters of the cortex of adrenal glands in the treatment by antioxidants. Experimentation was preceded by a selection of animals with different tolerances to alcohol. Ethyl alcohol, because of its physicochemical properties and characteristics of biological and toxic effects is sharply distinguished from a wide variety of psychoactive compounds with the potential to cause the development of dependency. Systematic use of the ethyl alcohol, unlike most other psychoactive substances, with high probability leads to the development of pathological processes in different organs and tissues of the body. Information accumulated to date on the mechanisms of toxic action of ethanol can clearly mark out its direct and indirect toxic effects.

## Material and method

Experimental studies were conducted at the Department of the normal anatomy of the Voronezh N.N. Burdenko State Medical Academy, and the Department of Human and Animal Physiology, Voronezh State University in the autumn-winter period of 2007-2010. Selection of animals with different tolerances to alcohol preceded the pilot studies. At the initial stage, 510 male outbreed albino rats weighing 200-250 g were provided free access to food, water, and a 15% ethanol solution for 3 weeks. Daily measurements were related to each animal's consumption of water and alcohol solution.

At the end of the observation period, according to the amount of consumption of ethanol and water, three major groups of 72 rats were formed that consumed only water and no more than 10% ethanol: Group 1 – 76 rats – alcohol in a total volume of fluid drunk per day; Group 2 – preferred only solution with a share of alcohol consumption 90-97% of the total fluid intake; Group 3 – 362 rats who consumed throughout the day, both liquid (10% to 85% ethanol solution and, correspondingly, from 90% to 15% water). Thus, in the studied population of outbreed male albino rats, the proportion of animals not consuming alcohol amounted to 14.1 ± 1.5%, and the animals that had switched to the constant consumption of alcohol – 14.9 ± 1.6%. For the next major experimental research, animals were taken from the 1st and 2nd groups.

Then, after a week "recovery" period, during which the animals selected for the experiment were kept on a standard diet of water and no alcohol, all rats were transferred to the previous contents of the food consumption that included a 15% ethanol solution with a total exclusion of water for 30 days.

The choice of a 15% ethanol solution was determined by the instructions as to the optimization of such a concentration for forming the changes corresponding to chronic alcohol intoxication among experimental animals during a relatively short period.

Thus, one part of the rats subjected to the forced alcoholization, and the other part – to alcohol intoxication, which can be roughly defined as "voluntary". Half of the animals in both groups, during the 10 days of alcohol abuse, were injected intraperitoneally with α-tocopherol in an amount of 0.1 mg per 100 g of body weight (20% oil solution).

Therefore, according to the scheme of the main experiment, the material was investigated out of 188 animals, which were formed into 6 groups: Group 1 (vivarium control) consisted of 20 intact rats kept on a general diet, the rats in group 2 (α-tocopherol control) were injected by α-tocopherol in the amount similar to the previous ones (20 rats) for 10 days before the end of the experiment, group 3 – animals after forced alcoholization (36 rats), Group 4 – animals after the "voluntary" alcoholization (38 rats), Group 5 – rats after forced alcoholization and

the introduction of α-tocopherol (36 rats), Group 6 - rats after a "voluntary" alcohol abuse and the introduction of α-tocopherol (38 rats).

For morphological studies of the rats' adrenal glands cortex they were subjected to decapitation, abdominal dissection and removing the adrenal glands under ether anesthesia at the end of the period of alcoholization.

The animals were carried out under ether anesthesia at the same time. The brain, pituitary, and adrenal glands were removed after decapitation and weighed. The mass of the organs was assessed in absolute and relative terms and then calculated: the relative weight of the adrenal gland weight – in milligrams per 100 g body weight.

Excised tissue blocks of adrenal glands were fixed for 24 hours in Bouin's mixture, and then, after dehydration in ascending alcohol concentrations and posting I-II dioxane were poured into the filling mixture "Gistomiks." Morphological studies of zones of the cortex of adrenal glands were performed on serial 6 microns thick paraffin sections. For the analysis of the histological and morphological changes, they were stained with hematoxylin and eosin. After staining, the sections were dehydrated by alcohols of increasing concentration, enlightened by meta-xylene and embedded into balsam. The state of the cortex was assessed by visual microscopy and morphometry, in which the width of the cortical areas and the volumes of the nuclei were measured. Also, a histochemical study of the functional state of the adrenal cortex was performed.

In determining the amount of cores, given their elliptical shape, the diameter was measured twice and then converted into mkm$^3$ ellipsoid formula $V = lb^2\pi/6$, where $l$ – a larger diameter, $b$ – a smaller diameter. Statistical processing of the results was performed using the package Statistica 6.0 (Stat Soft). The results are presented as $M \pm \sigma^2$ (M – arithmetic mean; σ-standard deviation). Evaluation of reliability indices of mean values of the comparison groups was performed using Student's t-test and, if abnormal distributions of nonparametric Mann-Whitney test (significance level $p < 0.05$).

## Results

During the experiment, the mass of the rats of the control group increased by 45.5%, the weight of animals subjected to forced alcoholization – by 15.3%, the relative increase in weight after the "voluntary" alcohol abuse was 25.6%. However, if the dynamics of mass in the control group was linear (fig. 1), the same dynamics of the animals of the 2nd and especially 1st group deviated considerably from linearity.

For the animals of the control group, the mass of the right adrenal gland was 22.3 ± 1.4 mg, left – 22.6 ± 1.1 mg; the rats treated for 80 days with 15% ethanol were expressed with the asymmetry: the right adrenal gland weighed 26.75 ± 2.1 mg, and the left – 30.0 ± 2.4 mg, which was significantly ($p < 0.05$) more than in the group. The right adrenal gland of animals of group 2 weighed 24.0 ± 2.3 mg, and the left – 26.0 ± 2.6 mg, in the third group, the right adrenal gland weighed 26.0 ± 2.8 mg, and the left – 26.0 ± 2.6 mg.

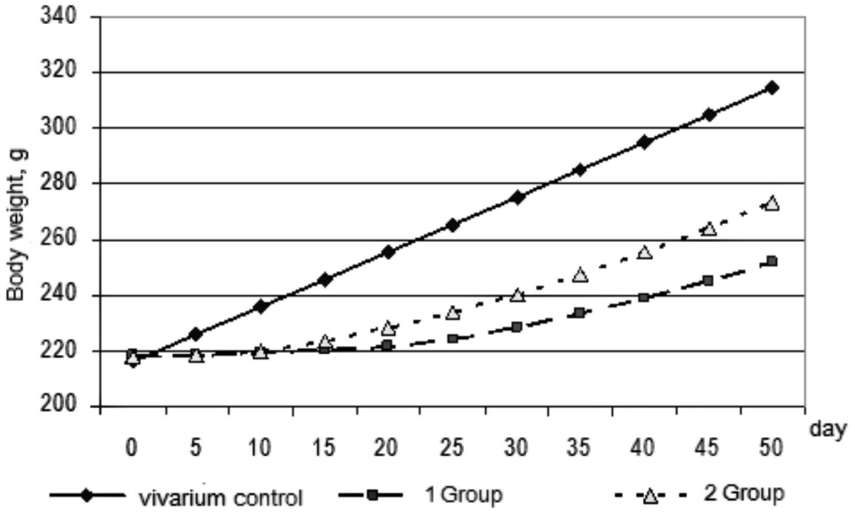


**Fig. 1. Dynamics of changes in body weight of alcoholized and the control group animals.**

One of the indicators of the functional activity of the cells is the size of their nuclei. Studies have shown that in the control group the volume of the nuclei of the glomerular zone was 89.6 ± 2.3 mkm$^3$, the beam and the reticular zones 132.6 ± 2.4 mkm$^3$ and 79.0 ± 2.7 mkm$^3$ respectively. After the forced alcohol intoxication during the 80 days, the amount of cell nuclei in the glomerular area was significantly increased ($p < 0.05$) and amounted to 94.7 ± 2.5 mkm$^3$ in the beam area – 138.8 ± 2.4 mkm$^3$ and the netted area – 83.0 ± 2.2 mkm$^3$.

Introduction of an antioxidant during 20 days in the background of ongoing alcohol abuse led to an even greater increase in the volume of the nuclei. In the glomerular zone, the volume of nuclei was 98.6 ± 2.5 mkm$^3$ in the beam area – 152.7 ± 3.2 mkm$^3$, and in the netted area – 81.3 ± 2.8 mkm$^3$.

In the animal group, which began to be given water and administered antioxidant after 60 days of alcohol abuse, karyometric indicators of the glomerular cells and the reticular zones (91.5 ± 2.3 mkm3 and 79.6 ± 2.2 mkm3, respectively) did not differ significantly from the respective figures of the control, and the increase of cell nuclei of the beam area was less significant (139.6 ± 2.4 mkm$^3$).

Histology of adrenal glands after forced alcohol intoxication during the 60 days was as follows: connective tissue capsule thinned with signs of dissociation and edema. The endocrine cells of the glomerular zone had sharply vacuolated cytoplasm; some cells were close to a vacuolar degeneration. The sudan negative zone practically has not been determined. The beam zone was represented by strands of hypertrophied endocrine cells; some cells underwent the vacuolar degeneration. This picture reflects the state of the body in the phase of maladjustment.

The histological picture of the adrenal glands of rats that had used alcohol during the 70 and 80 days showed thinning of the connective tissue capsule. The endocrine glomerular zone had vacuolated cytoplasm. Sudan zone has not been determined. In some places, the lumen of vessels had a "gape." These indicators are the morphological equivalent of a deep depletion of adaptation.

The histological picture of the adrenal glands of rats of groups 4 and 5 reflected the structural restoration of the organs. The structure was determined by the preservation of the adrenal glands and clear architectonic of all zones. The connective tissue capsule was thinned, but there were no signs of edema. Signs of the glomerular cell hypertrophy and the beam areas were preserved. The sudan negative area was determined by individual sites. In a number of visual fields, the pyronin positive cytoplasm and nucleoli of cells in glomerular and beam zones were marked. There is a tendency towards a normalization of the structure and ultrastructure of all three zones of the cortex. However, on the border of the cortex and medulla in the individual sections there is micro cystic cavity, apparently caused on the site of cell death after stress. Structural and functional assessment of the width (thickness) of the cortical areas in the study of histological sections showed significant changes, as their absolute values and the ratio of the width of each zone to the total width of the cortex. The results showed that the forced alcoholization leads to an overall increase in the width of the cortex, particularly pronounced in animals treated with α-tocopherol. This increase was primarily the result of the expansion of beam zone, while the relative width of the glomerular was significantly reduced.

One of the indicators of the functional activity of cells is the size of their nuclei. Studies have shown that in the control group the volume of the glomerular nuclei, the beam, and the reticular zones corresponded normally. After the forced alcohol intoxication during the 80 days, the amount of cell nuclei in glomerular area was significantly increased, the same pattern was observed in the beam, and in the reticular zones.

Introduction within 20 days of an antioxidant in the background of ongoing alcohol abuse resulted in even more significant increase in the volume of the nuclei.

In the group of animals, which after 60 days of alcohol abuse began to be given water and administered antioxidant karyometric indicators of glomerular cells and reticular zones did not differ significantly from those of the control parameters, and the increase of cell nuclei of the beam area was less significant.

## Derivation

The research results reveal the possibility of developing methods for the directed correction of changes occurring in the cortex of the adrenal glands in the compulsory and voluntary intoxication of the stress through antioxidant effects of α-tocopherol. But, at the same time, at this stage of the research, we can conclude a need for careful monitoring of corrective action of antioxidants in alcohol intoxication, amplifying the destructive effects in organs exposed to the toxic stress.

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# Acțiunea câmpului electromagnetic pulsat de frecvență joasă asupra proliferării şi morfologiei celulelor stem mezenchimale

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## Action of low frequency pulsed electromagnetic fields on proliferation and morphology of the mesenchymal stem cells

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Stimulation of cell divisionis the source of physiological recovery that provides the most reliable perspective in tissue engineering. A non-invasive and accessible method of amplifying the process of cell division is using electromagnetic fields. Our purpose was to analyze the oscillating electromagnetic fields capacity to influence the cellular proliferation *in vitro*. For this purpose, were used cell cultures of mesenchymal stem cells, derived from 14 days aviary embryos. Cells were subjected toaquasi-rectangular pulse del ectromagnetic field with duration of 300μs, a frequency of 7.5Hz, 2hours each day for 7days. The results indicate arise with 25% of the number of cells subjected to the magnetic field, and this report was not influenced by the cell density. The cell morphology showed no difference between groups. These results suggest the possibility of using low frequency pulsed electromagnetic fields in tissue engineering with the purposes to accelerate mesenchymal stem cell division, which can be applied in bone regeneration therapy.

**Key words:** Stem cell, pulsed electromagnetic field, cell culture, tissue engineering, bone regeneration.

## Действие низкого частотного импульсного электромагнитного поля на пролиферацию и морфологию мезенхимальных стволовых клеток

Стимуляция физиологическго деления клеток является источником восстановления клеток, который обеспечивает наиболее надежные перспективы в тканевой инженерии. Использование электромагнитных полей является неинвазивным и доступным методом усиления процесса деления клеток. Целью этой работы был анализ способности пульсных электромагнитных полей влиять на клеточную пролиферацию *in vitro*. Для этой цели использовали культуру мезенхимальных стволовых клеток, полученных из птичьих эмбрионов. Клетки подвергались квази-прямоугольных пульсных электромагнитных полей с длительностью 300 μs, частота 7,5 Гц, 2 часа каждый день в течение 7 дней. Полученные результаты указывают на 25% рост количества клеток в группе подвергнутом магнитному полю чем в контрольной, и результат не зависит от плотности клеток. Морфология клеток не выявило различий между группами. Эти результаты свидетельствуют о возможности использования низкочастотных пульсных электромагнитных полей в тканевой инженерии с целью ускорить деление мезенхимальных стволовых клеток.

**Ключевые слова:** стволовые клетки, импульсные электромагнитные поля, культура клеток, тканевой инженерии, стволовые клетки.

Câmpurile electromagnetice pulsate (CEMP) sunt aplicate clinic pentru a stimula regenerarea osoasă [15]. Inițial, eficiența CEMP era asociată cu accelerarea formării matricei osoase din cauza apariției locale a unui curent electric slab, indus de câmpul magnetic [4,11].

Studiile ulterioare au indicat că câmpurile electromagnetice pot vindeca fracturile osoase şi încetinesc pierderea de matrice osoasă la animale [3,9,13,19], aceasta a orientat aplicarea clinică în domeniul regenerării osoase. În pofida aplicării cu succes a câmpurilor electromagnetice au fost raportate şi studii cu privire la efectele negative asupra proliferării şi a diferențierii osteoblastelor [20, 21]. Sub influența câmpurilor magnetice specifice a fost determinat că activitatea fosfatazei alcaline creşte, însă proliferarea osteoblastică este limitată [19]. Studiile actuale au demonstrat că aprovizionarea tisulară fiziologică cu celule funcționale mature este asigurată de proliferarea şi diferențierea celulelor stem tisulare [16].