форми свинцю $Pb(NO_3)_2$. Токсичний ефект наночастинок свинцю спостерігався в основному в кардіоміоцитах міокарда передсердь, в той час як стінка аорти залишалася майже інтактною. Найбільш виражені структурні зміни спостерігалися в легенях та бронхах, що може бути обумовлено способом введення токсикантів.

Ключові слова: свинець, наночастинки, інтратрахеальна інтоксикація, морфологічні зміни, міокард, аорта, легені.

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ионной формы свинца $Pb(NO_3)_2$. Токсический эффект наночастиц свинца наблюдался в основном в кардиомиоцитах миокарда предсердий, в то время как стенка аорты оставалась почти интактной. Наиболее выраженные структурные изменения наблюдались в легких и бронхах, что может быть обусловлено способом введения токсикантов.

Ключевые слова: свинец, наночастицы, интратрахеальная интоксикация, морфологические изменения, миокард, аорта, легкие.

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ULTRASTRUCTURAL REMODELING OF RAT SUBMANDIBULAR GLANDS IN CHRONIC ETHANOL INTOXICATION

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The study presents a detailed analysis of the ultramicroscopic structure of the rat submandibular glands in chronic ethanol intoxication at the later stages of the experiment. It has been established that prolonged exposure to ethanol at the later stages of the experiment was characterized by the appearance of signs of adaptive-compensatory responses of the parenchymal elements □nd v□ss□ls □f th□ bl□□d m□r□v□s□ul□r s□st□m w□th th□ □m□rg□n□□ □f d□str□h□□ □h□ng□s □nd □□□t□s□s □f du□t□l □□th□l□um; however, no complete regeneration of the structure was detected.

Keywords: ethanol, salivary glands, acini, ducts, rats.

The work is a fragment of the research project "Experimental morphological study of the effect of cryopreserved cord blood products and embriofetoplacental complex (EFPC), diphereline, ethanol and 1 % methacrylate on the morphofunctional condition of several internal organs", state registration No. 0119U102925.

Chronic ethanol intoxication causes multiple alterations in the structure and functions of the oral cavity organs; however, the study was focused on the prevalence and intensity of diseases of the oral mucosa and periodontal diseases. The findings have shown that alcohol consumption is accompanied by salivary dysfunction, as well as the destructive changes in the salivary glands; however, these data are often fragmentary and sometimes ambiguous [6]. Ethyl alcohol, due to its physicochemical properties, is able to easily penetrate through the cell membranes and have both direct and indirect toxic effects, which leads to the matrix and transport dysfunction of membranes and the formation of adaptive changes in long-term effect of ethanol, manifested by elevated cholesterol, thickening of the phospholipid layer and higher membrane density, which in turn is accompanied by alterations in the mode of functioning of enzyme, receptor and immune complexes [2].

Electron microscopic method, used to investigate ultrastructural changes in tissues, has established that at the early stages of the experiment, chronic ethanol intoxication causes significant changes in both parenchymal elements and vessels of the blood microvascular system, which is expressed by intensification of secretion in the acini and increased functional activity of the ductal system; therefore, the study on the effect of ethanol at the late stages enables disclosure of a full picture of restructuring of the submandibular glands during the experiment.

The purpose of the work was to study structural changes in the elements of the rat submandibular glands' lobules in chronic ethanol intoxication at late stages of the experiment.

Materials and methods. 20 outbred albino rats were involved into electron microscopic study. The rodents were administered with 12 mg/kg 40° ethanol 4 times a day directly into the stomach [4]. The animals were killed under 25 mg/kg thiopental anesthesia overdose on day 12 and 30 of the experiment. The fragments of submandibular glands were embedded into epon-812 according to standard procedure [1].

Ultrathin sections were made on the LKB-3 (Sweden) ultramicrotome. Contrast staining of the sections were performed first with 1% uranyl acetate solution in methanol, and then with lead citrate according to Reynolds [1].

The sections were studied in the PEM-125K electron microscope (serial number 38-76, TU 25-07-871-70) at accelerating voltage (50 - 75) kW.

Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics and the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [7].

Results of the study and their discussion. On day 12 of the experimental model of chronic ethanol intoxication in the cells of the acini an eccentric position and compaction of nuclei of irregular shape was noted and increase in the amount of heterochromatin was observed. The number of secretory granules in the cytoplasm decreased. They were of different diameters, different densities of contents, oblong and conjoined in places. In some epitheliocytes of the acini there were electron transparent vacuoles, which were located near the nucleus. In the intercellular spaces, cytoplasmic evaginations on the lateral surfaces and extended fissures near the basal surface were detected (fig. 1a).

Venules with signs of edema of the vascular wall were found in the periductal interstitium. Endotheliocytes had elongated shape. The lumen of the vessels was completely filled with blood corpuscles, as evidenced by previously obtained data from the morphometric study, where on day 12 the thickness of the vascular wall significantly increased by 20.1% compared with day 9 of the experiment and was by 6.3% greater compared to the value in controls ($p < 0.05$) [3]. Interstitial tissue was represented by amorphous substance and collagen fibers (fig. 1b).

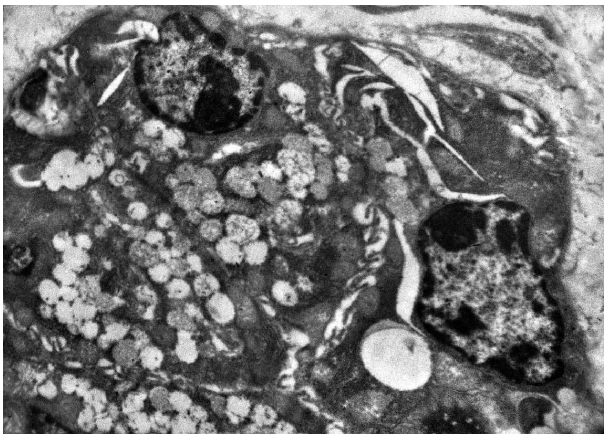

Fig. 1a. Extension of fissures in the seromucous cells of the acini of rat submandibular glands on day 12 of the experiment. Electronogram. 6000×magnification.

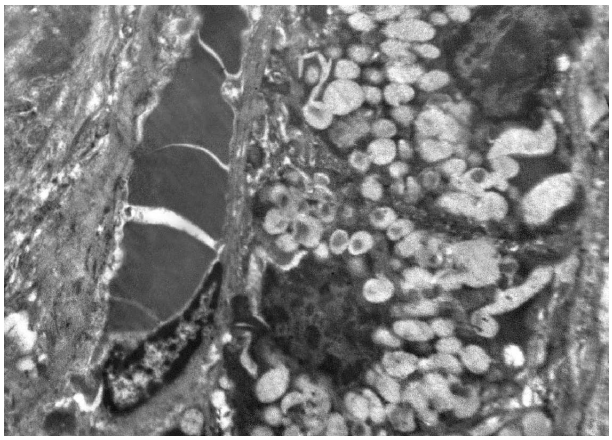

Fig . 1b. Venule in the interstitial tissue on day 12 of the experimental model of chronic ethanol intoxication. Electronogram. 7000×magnification.

The cells of the striated ducts had a prismatic shape. The nuclei were enlarged, oblong with a predominance of euchromatin. A decrease in the width of the folds of the basal striation, a decrease in the number of mitochondria was noted. The basement membrane was smooth. In the periductal interstitium, amorphous substance predominated over the collagen fibers, and capillaries with endothelial cells of elongated irregular shape were found. The cytoplasm of endothelial cells was filled with different electron density contents and transport vesicles. In the lumen of the capillaries erythrocytes were oblong (fig. 2a).

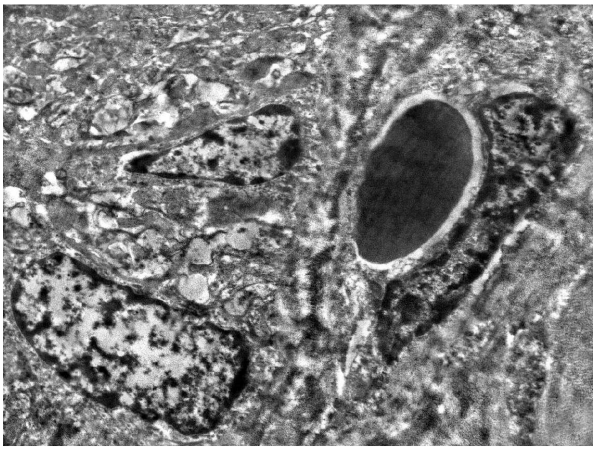

Fig. 2a. Capillary in the periductal interstitium of submandibular glands in rats of the experimental group on day 12 of the experiment. Electronogram. 8000×magnification.

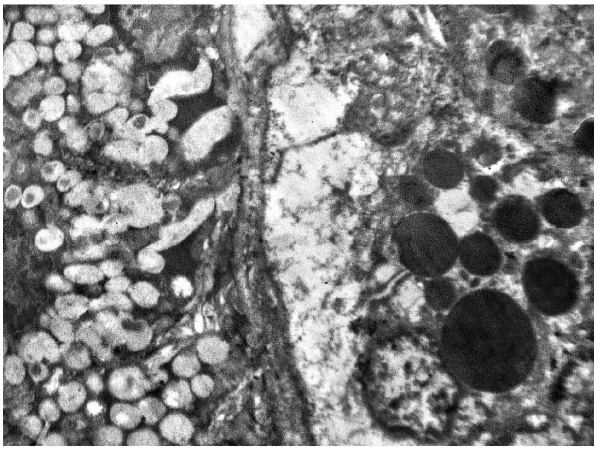

Fig. 2b. Detachment of plasmalemma of the granular ducts of rat submandibular glands on day 30 of the experiment. Electronogram. 7000×magnification.

On day 30 of the experiment in the cells of the acini, the nucleus moved to the center of the cells and numerous electron-light granules of various shapes were detected. Dark electron-dense gaps of the cytoplasm were found on the basal surfaces. Granular ducts in rats are responsible for maintaining and providing a system of local mechanisms for regulating vascular blood supply. Their epitheliocytes had basally located nuclei with a pronounced polymorphism of the granules, which were different in size and mostly electron-dense. Large fissures of various shapes were detected between the plasma membrane and the basement membrane (fig. 2b). In some cells of the ductal epithelium, heterogeneity of the cytoplasm with electron-light vacuole-like structures was noted; the nuclei were of various shapes, hyperchromic, and cells with crescent-shaped nuclei were detected, the central part of which was filled with transparent content (fig. 3a). Elongated fissures were noted in the cells of the striated ducts at the border with the basement membrane. Fibroblasts and adjacent bundles of collagen fibers were detected among the amorphous substance in the surrounding periacinar interstitium. Small lymphocytes and macrophages were noted (fig. 3b).

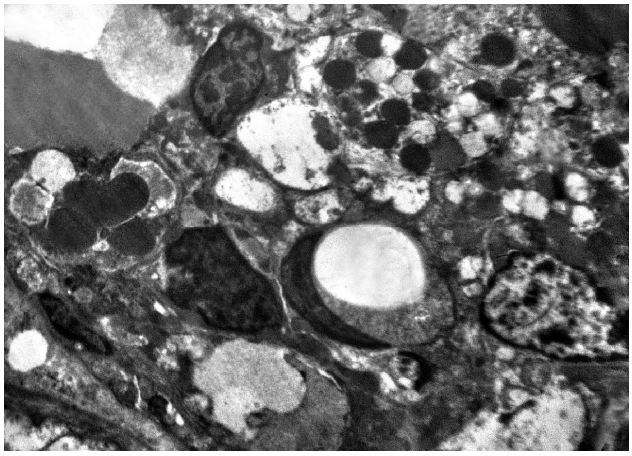

Fig. 3a. The apoptosis phenomena in granular duct cells of rat submandibular glands on day 30 of the experiment. Electronogram. 7000×magnification.

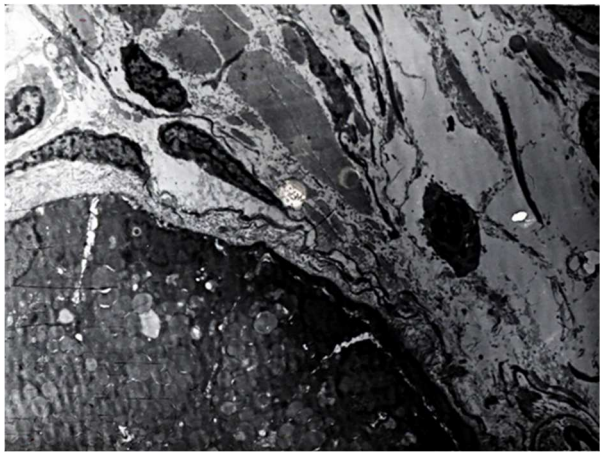

Fig. 3b. Striated duct and interstitial periductal tissue on day 30 of the experiment. Electronogram. 6000×magnification.

Thus, on day 12 of ethanol effect, the signs of decreased secretion by seromucocytes of the acini was detected, which was confirmed on the electronograms by compaction of nuclei, a decrease in the number of secretory granules and the appearance of cytoplasmic ptojections on the intercellular surfaces. The cells of the striated ducts took on a typical prismatic shape, a decrease in the number of mitochondria and folds of the basal labyrinth indicated a decrease in the saliva modification processes by epitheliocytes. Due to the decrease in hydration of the interstitium, contributed by hypersalivation, at the early stages of the experiment, the vessels of the blood microvascular system of the interstitial connective tissue increased in volume and blood corpuscles were visualized.

On day 30 of the experimental model of chronic ethanol intoxication the formation of adaptive-compensatory changes of parenchymal elements of the submandibular glands in response to the long-term effect of ethanol. In the seromucous cells of the acini, compaction of the basal parts of the cytoplasm was detected, expressed by dark areas on the electrogram. The nuclei moved to the center and were compacted. The granules were polymophic, sometimes rod-shaped, electron-light, and conjoined in some areas. The above changes in the acini indicated a compensatory adjustment after hypersalivation at the early stages of the experiment and restructuring of the secretory apparatus mainly in the direction of carbohydrate synthesis. The epitheliocytes of the striated ducts had numerous folds of basal striation oriented perpendicular to the basal surface of the cells, were narrow and high with numerous vacuole-like structures in the cytoplasm. Clear catenate fissures were visible in the intercellular spaces. The presence of large electron-dense granules and the presence of optically bright areas in the basal parts starting from the basement membrane to almost a third of the cells were noted in the granular ducts. Heterogeneity of the cytoplasm and the emergence of the nuclei of the irregular shape indicate the occurrence of apoptosis. The established changes in the ductal epithelium indicate partial depletion of cells with the appearance of signs of dystrophy, and the full use of their adaptation to support salivation processes during prolonged exposure to ethanol. The increased number of macrophages and lymphocytes in the periductal connective tissue was detected, indicated about their active role in providing a local protective barrier.

In the previous experiments on the rat submandibular glands exposed to the effect of other stimuli, namely 1% methacrylate, parenchymal components showed signs of inflammation and

decompensation of the microcirculation, which led to significant vasodilation. Enlargement of the diameters of the duct lumen was observed at the final stages of the experiment. The number of plasma cells in the periacinar and periductal epithelium increased significantly. There was no tendency to recover [5], which obviously proves a more toxic effect on the parenchymal elements of the stimulus, to which the salivary glands respond by inflammation with dysfunction, while the effect of ethanol, as a metabolite, causes less reactive outcomes with the emergence of dystrophic changes and apoptotic phenomena without inflammatory process. The observations indicate that the reaction of the structural components of the submandibular glands depends on both the modality of the active stimulus and its chemical affiliation.

Conclusion

Chronic ethanol intoxication at the later stages of the experiment is characterized by the appearance of signs of adaptive-compensatory reactions of parenchymal elements and vessels of the blood microvascular system with the emergence of dystrophic changes and apoptosis of ductal epithelium caused by prolonged effect of ethanol and its products. However, no complete regeneration of the structure was detected.

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Реферати

УЛЬТРАСТРУКТУРНЕ РЕМОДЕЛЮВАННЯ ПІДНИЖНЬОЩЕЛЕПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПРИ ХРОНІЧНІЙ ІНТОКСИКАЦІЇ ЕТАНОЛОМ

Єрошенко Г.А., Шевченко К.В., Лисаченко О.Д., Вільхова О.В., Якушко О.С., Скотаренко Т.А., Білаш В.П.

В даному дослідженні було проведено детальне вивчення ультрамікроскопічної будови піднижньощелепних слинних залоз щурів за хронічної інтоксикації етанолом на пізніх термінах експерименту та було встановлено, що тривала дія етанолу на пізніх стадіях експерименту характеризується появою ознак адаптивно-компенсаторних реакцій паренхіматозних елементів та судин гемомікроциркуляторного русла з виникненням дистрофічних змін та явищами апоптозу протокового епітелію, однак повного відновлення структури не відбувалось.

Ключові слова: етанол, слинні залози, кінцеві відділи, протоки, щури.

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УЛЬТРАСТРУКТУРНОЕ РЕМОДЕЛИРОВАНИЕ ПОДНИЖНЕЧЕЛЮСТНЫХ СЛЮННЫХ ЖЕЛЕЗ КРЫС ПРИ ХРОНИЧЕСКОЙ ИНТОКСИКАЦИИ ЭТАНОЛОМ

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В данном исследовании было проведено детальное изучение ультрамикроскопического строения поднижнечелюстных слюнных желез крыс при хронической интоксикации этанолом на поздних терминах эксперимента. Было установлено, что длительное воздействие этанола на поздних стадиях эксперимента характеризуется появлением признаков адаптивно-компенсаторных реакций паренхиматозных компонентов и сосудов гемомикроциркуляторного русла с возникновением дистрофических изменений и явлениями апоптоза протокового эпителия, однако полного восстановления структуры не происходило.

Ключевые слова: этанол, слюнные железы, концевые отделы, протоки, крысы.

Рецензент Старченко І.І.