In previous studies it has been established that chronic ethanol intoxication affects the resistive link of the hemomicrocirculatory bed of the rat's submandibular gland lobules, which is determined by vascular changes in saliva whose functions are diverse. First of all, it is established that quantitative and qualitative changes in saliva protein and mucosal secretion, but is also associated with osmotic transmural transfer of large fluid volumes ± 0.41 µm at the early stages of observation. Reduction in the external diameter and the lumen diameter with the reduced epithelial cells height growth up to 15.44 µm at the late stages of the experiment, and is confirmed by reduction in their size, the number of secretory granules and the increased optical density of cells.

Key words: chronic intoxication with ethanol, rats, submandibular salivary glands.

The study is a fragment of the research project “Experimental morphological study of cryopreserved placenta transplants action and other exogenous factors on the morphofunctional status in a number of internal organs”, state registration No. 0113U006185.

The alcohol consumption situation in Ukraine is currently quite threatening. Today, the level of alcohol consumption in Ukraine is one of the highest in the world and is about 12-13 liters of absolute alcohol per capita in a year (unofficial statistics reports 20 l). In Ukraine, over 40 thousand people die yearly due to alcohol [9, 6]. Chronic ethanol intoxication is manifested by a wide range of various negative factors effects on the body [3]. Specific receptors sensitive to ethanol do not exist. However, it interacts with many cell components, including extracellular and intracellular receptors located in the membranes of many organs, with secondary mediators of receptors and enzymatic cell systems, which are reflected in the clinical picture of intoxication [1].

All salivary glands of both humans and rats, according to the literature, are based on a single principle and are complex branched, alveolar-tubular glands consisting of end or secretory lobules, and of the outflow ducts system [14]. The function of salivary glands acini lies not only in the production of protein and mucosal secretion, but is also associated with osmotic transmural transfer of large fluid volumes into the end pieces lumens from the surrounding interstitium [5]. The secret of the salivary glands is saliva whose functions are diverse. First of all, it is established that quantitative and qualitative changes in saliva largely determine the teeth resistance to caries [2, 7].

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spasm at the early stages of the observation, with an increase in the vascular wall’s thickness [6]. The capacitive link of the submandibular salivary gland lobules at the early stages of observation responds by the venules expansion and is confirmed by a significant increase in the external diameter and the lumen diameter with a reduction of the vascular wall thickness [16].

Consequently, studying the patterns of the salivary glands reaction to various stimuli is of great importance, due to the diagnostic value of saliva as a highly informative object for the clinical status assessment of the whole body.

Application of the morphometric method permits an objective assessment of changes in the structural elements of organs after the action of various endogenous and exogenous factors [15].

**The purpose** of the present study was to establish structural changes of the submandibular glands’ end pieces of rats in normal and in chronic ethanol intoxication.

**Materials and methods.** The work was performed on 45 white outbred rats. The total of 5 animals constituted the control group, being administered isotonic sodium chloride solution 4 times a day, and 40 experimental ones, which were injected intragastrically 4 times a day with 12 mg/kg of 40 ABV ethanol per day (in terms of pure alcohol) [11].

Animals were sacrificed on the 5th, 9th, 12th and 30th days by the thiopental anesthesia overdose (25 mg / kg). Fragments of the submandibular glands were embedded in epon-812 according to the generally accepted procedure [4]. The semi-thin sections were stained with methylene blue [12].

The mean values of the external diameter, lumen and height of the epithelial cells were determined using the Biorex 3 BM-500T microscope with a digital DCM-900 photomicrographic attachment with the research program adapted for the present study. Statistical processing of morphometric data was performed using the Exel software [13].

Animal management and experiments were carried out in compliance with the “General Ethical Rules for Animal Experiments” adopted by the 1st National Congress on Bioethics and with the requirements of the International Principles of the European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes [10].

**Results of the study and their discussion.** It was found by the morphometric study, that in the control group rats, the mean value of the external diameter in the submandibular glands end pieces was $36.86 \pm 1.11 \mu m$, the lumen diameter was $9.17 \pm 0.33 \mu m$, and the height of the epithelial cells was $14.74 \pm 0.65$ microns (tab.).

<table>
<thead>
<tr>
<th>Table</th>
<th>Morphometric indices of the submandibular glands end pieces (μm)</th>
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<tbody>
<tr>
<td></td>
<td>End pieces</td>
</tr>
<tr>
<td>Control group (n=5)</td>
<td>36.86±1.11</td>
</tr>
<tr>
<td>The 5th day (n=5)</td>
<td>37.00±1.06</td>
</tr>
<tr>
<td>The 9th day (n=5)</td>
<td>38.45±1.23</td>
</tr>
<tr>
<td>The 12th day (n=5)</td>
<td>34.07±2.23</td>
</tr>
<tr>
<td>The 30th day (n=5)</td>
<td>31.15±1.19 <em>,</em>*</td>
</tr>
</tbody>
</table>

Note. * - P <0.05 in compared to the control group; ** - P <0.05 compared to the previous observation period.

In the histological study the end parts of the submandibular gland in the control group rats have a tubular shape and are separated by thin layers of interstitial connective tissue. In the end pieces, two types of cells are defined: seromucous and myoepithelial (fig. 1), it should be noted that the structural feature of these glands is the absence of clearly expressed mucous and serous secretory cells in the end pieces, as all epitheliocytes synthesize mainly a mixed secret.

On the fifth day of ethanol intoxication, the mean external diameter of the end pieces of the submandibular gland does not significantly change and amounts to $37.00 \pm 1.06 \mu m$. The lumen diameter is significantly reduced by 13.63% (p <0.05), which makes 7.92 ± 0.42 μm. The height of the epithelial cells does not change, its mean values are 14.38 ± 0.56 μm (table) and do not differ from the values in the control group.

On the ninth day of the experiment, the values of the end pieces external diameter tend to increase up to 38.45 ± 1.23 μm. The lumen diameter on the ninth day is 7.86 ± 0.02 μm, which is 14.29% lower than that in the control group rats (Fig. 2). The height of the epithelial cells compared to that in the control group remains unchanged, and increases by 7.37% (p <0.05) compared to the previous period of the experiment, its mean values making 15.44 ± 0.41 μm (table).
On the twelfth day of study, the external diameter mean values of the submandibular salivary glands end pieces are 34.07 ± 2.23 μm, which is by 11.39%, reliably less than the value of the previous experiment and by 7.57% less than that in the control group rats. The lumen diameter is 7.83 ± 0.12 μm, which is by 14.61% reliably less than that in the control group and does not differ from the results of the previous day of the study. The epitheliocytes height amounts to 13.84 ± 0.81 μm and is by 10.36% reliably less than on the ninth day of the experiment (p <0.05), and its results are less by 6.11% than the values in the control group of animals (table). Seromucous cells are densely located; clearly visible light and dark areas are observed in the cytoplasm; the acini lumen is very small or almost undetectable; increased are interstitial layers of connective tissue in which the mastocytes degranulation and the organelles induration in the cytoplasmic matrix are observed (fig. 3).

On the thirtieth day of ethanol intoxication there occurs a significant reduction in the external diameter of the submandibular salivary glands end pieces, their lumen diameter and the decrease in the epithelial cells height. The external diameter is 31.15 ± 1.19 μm, which is by 22.07% less than that on the 12th day of the experiment, being by 15.49% less than the similar value in the control group (p <0.05). The lumen diameter is reduced by 0.52% compared to the twelfth day of the experiment and amounts to 7.79 ± 0.22 μm, which is also by 15.05% less than in the control group. The epithelial cells height on the thirtieth day is 11.58 ± 0.38 μm, being by 31.24% reliably lower than the similar value of the previous study period, and by 21.44% lower than that in the control group rats (p <0.05) (table). The serocytes are smaller in size, on the basal parts of the cells there are interspaces with clefts, strongly developed interstitial connective tissue and well-visible parts of the acini, which have undergone complete sliming, got flattened and adjacent to the basement membrane by nuclei, turbid and foamy cytoplasm. Between them and the normal serocytes, a group of cells is noted with a dark cytoplasm, almost invisible dark nuclei and a fuzzy boundary between the cells, which are clearly a transitional stage to complete sliming (fig. 4).
**Conclusion**

Chronic ethanol intoxication causes structural changes in the parenchyma of the submandibular salivary gland, which are manifested at the early stages of observation by the increased secretory activity of the end pieces cells. Inhibition of secretion is determined by the twelfth day of the experiment, which is morphometrically confirmed by the reliable decrease of the epithelial cells height by 6.11%, as compared to the control group. On the thirtieth day of observation, dystrophic changes in glandular cells and rearrangement of the secretory apparatus for the carbohydrates synthesis were established.

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