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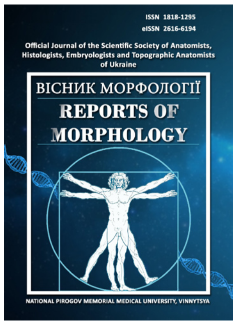

Dynamics of changes in the morphometric parameters of the ductal system of the submandibular salivary glands in rats under the influence of a food additive complex

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The ductal system of the salivary glands not only ensures saliva transport but also participates in its formation and modification; therefore, alterations in its structural parameters may impair salivary secretion. The aim of the study was to determine the dynamics of morphometric parameters of the intercalated, striated, granular, and intralobular collecting ducts of the submandibular salivary glands in rats under normal conditions and following the combined action of food additives. The study was performed on 84 sexually mature male rats. Animals of the control group received drinking water and physiological saline, whereas rats of the experimental group were administered 0.6 mg/kg sodium nitrite, 20 mg/kg monosodium glutamate, and 5 mg/kg Ponceau 4R in 0.5 mL of distilled water orally once daily. The animals were removed from the experiment after 1, 4, 8, 12, and 16 weeks. Fragments of the glands were fixed in 10 % neutral formalin and glutaraldehyde and embedded in paraffin and Epon-812. Microphotography and morphometric analysis were performed using a Levenhuk D740T digital microscope, and statistical processing was carried out in Excel. In the intercalated ducts, a significant decrease in the outer diameter, lumen diameter, and epithelial cell height was observed as early as week 1, and by week 16 these parameters remained lower than the control values. At the initial stages of the experiment, the striated ducts responded with an increase in outer diameter, lumen diameter, and epithelial cell height; however, subsequent narrowing of the lumen and a decrease in some parameters were observed. In the granular ducts, an increase in the outer diameter and epithelial cell height predominated up to week 12, whereas by week 16 the epithelial cell height decreased below the control level. The intralobular collecting ducts were characterized by an early increase in morphometric parameters followed by a decrease in lumen diameter and epithelial cell height at later stages. The obtained data indicate wave-like remodeling of the ductal system, in which early compensatory-adaptive responses were replaced by signs of dystrophic alterations. The identified morphological changes indicate impairment of the functional state of the ductal system and disturbances in the processes of saliva formation, transport, and modification.

Keywords: *salivary glands, excretory ducts, saliva, rats, food additives, histological study, morphometric method, monosodium glutamate, sodium nitrite, Ponceau 4R.*

Introduction

The oral cavity is moistened by saliva, which is a highly complex biological fluid produced by the salivary glands [23, 24, 27, 30]. The mechanism of salivary gland function can be readily understood by envisioning a system of tubules originating from the secretory end pieces that convey primary saliva formed as a result of blood filtration from capillaries into the interstitial tissue. This interstitial fluid subsequently penetrates the basement membrane, either transcellularly or juxtacellularly, into the lumina, where, while passing through the ductal system, it undergoes substantial modification [6, 9, 18, 21].

The ductal system of the submandibular salivary glands is represented by a network of tubules with a tendency toward increasing diameter and consists of intercalated, striated, and intralobular collecting ducts; in rats, it also includes granular ducts whose cytoplasm contains a large number of basophilic granules. Thus, the excretory duct system not only transports saliva but also actively participates in its formation and modification of its properties [3, 17]. With the development of modern manufacturing technologies, the use of food additives has become an important component of technological processes. These substances play a significant role not only in enhancing the flavor properties of food products but also in preservation processes and in imparting an attractive color. This issue has generated considerable controversy both within the scientific community and among the general public due to the lack of comprehensive studies regarding their effects on the human body, particularly under conditions of combined exposure.

Previous studies concerning the potential health hazards of monosodium glutamate have been conducted by researchers who associated its consumption with hepatotoxicity, neurotoxicity, low-grade inflammation, and metabolic disorders [4]. In addition to its well-known effect on the palatability of food, monosodium glutamate enhances salivary secretion and interferes with carbohydrate metabolism, whereas its effects on satiety and postprandial hunger recovery varied depending on the composition of the diet. At present, a review of the available literature has demonstrated that many reports describing adverse health effects of monosodium glutamate are insufficiently informative, as they are based on excessive dosages that do not correspond to the levels typically present in food products [25, 29].

Experimental data regarding the effects of sodium nitrite in rats [12] demonstrated the development of membrane-destructive processes within the organism and, consequently, suppression of tissue respiration processes, particularly the activity of mitochondrial enzymes (succinate dehydrogenase and cytochrome oxidase), ultimately leading to the development of hypoxia [16].

Numerous allergic reactions associated with the use of food colorants have been described in relation to food additives [13, 14, 19, 28]. Therefore, evaluation of the effects of azo dyes remains necessary due to complications such as genotoxicity, which may ultimately lead to carcinogenicity in the long term [7, 10, 20].

Thus, the studies conducted to date do not provide a definitive explanation for the varying degrees of human susceptibility to the effects manifested under exposure to food additives, while data regarding their combined effects remain unavailable [15].

The aim of the study was to determine the dynamics of changes in the morphometric parameters of the intercalated, striated, granular, and intralobular collecting ducts of rat submandibular salivary glands under normal conditions and under the combined action of food additives (monosodium glutamate, sodium nitrite, and Ponceau 4R).

Materials and methods

The study involved 84 sexually mature male rats. Animals of the control group consumed drinking water and received physiological saline orally. Rats of the experimental group, under conditions of free access to water, received sodium nitrite at a dose of 0.6 mg/kg, monosodium glutamate at a dose of 20 mg/kg, and Ponceau 4R at a dose of 5 mg/kg dissolved in 0.5 ml of distilled water once daily by oral administration. Notably, the administered doses of the food additives were twofold lower than the maximum permissible levels. Adaptive behavior in rats was assessed using the open-field test. The animals were sacrificed after 1, 4, 8, 12, and 16 weeks by overdose of thiopental anesthesia. Following euthanasia, fragments of the submandibular salivary glands were fixed in a 10 % formalin solution. Subsequently, tissue samples of the submandibular salivary glands were embedded in paraffin and Epon-812 according to standard protocols [1]. Sections were obtained using an ARM 3600 microtome and a UNTP-7 ultramicrotome. After staining with hematoxylin and eosin, methylene blue, and polychrome dye, the sections were mounted in polystyrene resin and examined under a light microscope. Photomicrography and morphometric analysis were performed using a Levenhuk D740T digital microscope equipped with software adapted for the present study. Statistical processing of the morphometric data was carried out using Excel software with the integrated VBA Analysis ToolPak, specifically the "Descriptive Statistics" tool. The Shapiro-Wilk test was applied to assess normality of variance distribution, and when normal distribution was confirmed, comparisons were performed using Student's t-test for independent samples. Differences were considered statistically significant at $p<0.05$ [11, 26].

All animal experiments were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), in accordance with the rules for the maintenance of laboratory animals established by Directive 2010/63/EU of the European Parliament and of the Council, as well as Order No. 249 of the Ministry of Education and Science, Youth and Sports of Ukraine dated March 1, 2012, "On Approval of the Procedure for Conducting Experiments and Scientific Studies on Animals by Scientific Institutions". The study also adhered to the recommendations of the First National Congress on Bioethics (2001) and was approved by the Biomedical Ethics Commission of Poltava State Medical University (Protocol No. 220 dated October 25, 2023).

Results

During the morphometric analysis of the intralobular ducts of the rat submandibular salivary glands, it was established that the external diameter of the intercalated ducts was 17.32±0.22 μm, the lumen diameter was 5.023±0.152 μm, and the epithelial cell height was 6.352±0.112 μm (Table 1).

The administration of a complex of food additives after 1 week of the experiment led to a statistically significant

Table 1. Morphometric parameters of the intercalated ducts of rat submandibular salivary glands.

Groups	External diameter	Lumen diameter	Epithelial cell height
Control group	17.32±0.22	5.023±0.152	6.352±0.112
1st week	15.11±0.16*	3.742±0.101*	5.851±0.082*
4th week	16.22±0.16*.**	4.201±0.104*.**	6.011±0.082*
8th week	13.49±0.17*.**	3.491±0.083*.**	4.743±0.072*.**
12th week	12.84±0.17*.**	3.332±0.071*.**	4.692±0.061*
16th week	12.80±0.10*	3.011±0,042*.**	4.673±0.081*

Notes:* – p<0.05 compared with the control group; ** – p<0.05 compared with the previous observation period.

decrease in the mean external diameter of the intercalated ducts by 12.76 %, reaching 15.11±0.16 μm. The lumen diameter also decreased by 25.20 %, amounting to 3.742±0.101 μm. In addition, epithelial cell height decreased by 7.87 %, with mean values of 5.851±0.082 μm (p<0.05).

At the 4th week of the experiment, the external diameter of the intercalated ducts showed a statistically significant increase of 7.35 % compared with the previous observation period, reaching 16.22±0.16 μm; however, this value remained significantly lower than in the control group by 6.35 % (p<0.05). The internal diameter (lumen diameter) also demonstrated a significant increase of 12.30 % compared with the 1st week results, although it remained 16.33 % lower than the control values (p<0.05), with mean lumen diameter of 4.201±0.104 μm. The mean epithelial cell height increased to 6.011±0.082 μm; however, this change was not statistically significant compared with the previous experimental period and remained 5.35 % lower than the control group values (p<0.05).

The combined exposure to monosodium glutamate, sodium nitrite, and Ponceau 4R at the 8th week resulted in a decrease in the mean external diameter of the intercalated ducts by 16.83 % compared with the results obtained at the 4th week, which was also significantly lower than the control values by 22.11 % (p<0.05). The external diameter of the intercalated ducts of the submandibular salivary gland lobules at the 8th week was 13.49±0.17 μm. Morphometric parameters of the lumen diameter of the intercalated ducts also significantly decreased both compared with the previous observation period by 16.90 % and in comparison with the control group by 30.48 %, amounting to 3.491±0.083 μm (p<0.05). Epithelial cell height at the 8th week showed a statistically significant decrease to 4.743±0.072 μm. These values were 21.13 % lower than those of the previous experimental period and 25.35 % lower than the control group values (p<0.05).

As a result of food additive exposure at the 12th week, the morphometric parameters of the external diameter of the intercalated ducts were 12.84±0.17 μm, which was 4.82 % significantly lower than the values of the previous experimental period and 25.87 % lower than the control group (p<0.05). The mean lumen diameter of the intercalated ducts significantly decreased to 3.33±0.07 μm. These values were 4.58 % lower than those recorded at the 8th week and significantly lower than the control group by 33.67 % (p<0.05). The mean epithelial cell height decreased to 4.692±0.061 μm, which did not differ significantly from the previous observation period but was 26.14 % lower than the control group values (p<0.05).

Exposure to the food additive complex at the 16th week resulted in a decrease in the external diameter of the intercalated ducts, which at this time point was 12.80±0.10 μm. This value did not differ significantly from the previous observation period; however, it was significantly lower than that of the control group by 26.10 % (p<0.05). The mean lumen diameter significantly decreased compared with the previous experimental period by 9.61 % and remained significantly lower than the control values by 5.44 % (p<0.05), reaching 3.011±0.042 μm. A persistent decrease in epithelial cell height was also observed, with mean values of 4.673±0.081 μm. This parameter did not differ significantly from the 12th week values but was significantly lower than in the control group by 26.46 % (p<0.05).

The intercalated ducts of the rat submandibular salivary glands represent a direct continuation of the secretory end pieces. In the control group, the duct wall was formed by epithelial cells predominantly of cuboidal shape, with weakly basophilic cytoplasm and centrally located nuclei. The duct lumen was clearly visualized (Fig. 1). In the experimental group, the intercalated duct epithelial cells acquired a flattened shape, and the cytoplasm showed low optical density with areas of rarefaction. A marked reduction in the duct lumen was observed, which evidently indicates active involvement of these structures in the initial stages of saliva secretion, ultimately leading to depletion of the secretory epithelium. The narrowing of the lumen also confirms impaired outflow of primary saliva from the secretory end pieces into the ductal system of the rat submandibular salivary glands (Fig. 2).

During the morphometric study of the striated ducts of the lobules of rat submandibular salivary glands in the control group, it was established that the external diameter was 33.57±0.46 μm, while the lumen diameter was 9.401±0.192 μm. The epithelial cell height was 12.38±0.14 μm (Table 2).

Table 2. Morphometric parameters of the striated ducts of rat submandibular salivary glands.

Groups	External diameter	Lumen diameter	Epithelial cell height
Control group	33.57±0.46	9.401±0.192	12.38±0.14
1st week	47.32±0.34*	17.16±0.41*	15.00±0.13*
4th week	37.88±0.58*.**	12.60±0.14*.**	12.75±0.19*.**
8th week	40.26±0.47*.**	4.051±0.062*.**	17.65±0.08*.**
12th week	32.71±0.26*.**	4.401±0.132*.**	14.35±0.16*.**
16th week	30.10±0.25*.**	5.491±0.132*.**	12.06±0.15*.**

Notes:* – p<0.05 compared with the control group; ** – p<0.05 compared with the previous observation period.

At the 1st week of the experiment, a statistically significant increase in the external diameter of the striated ducts by

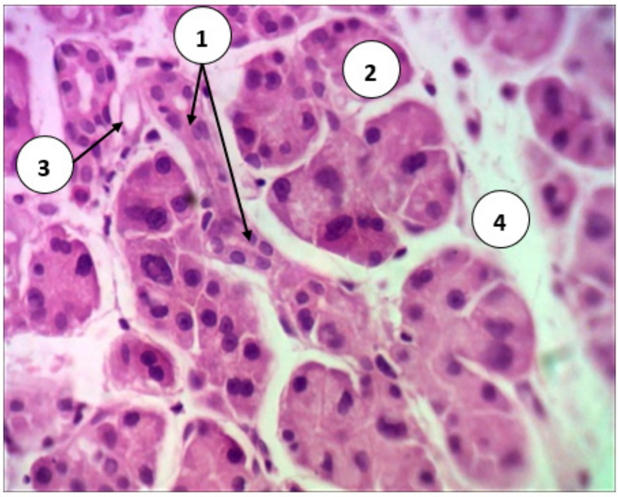

Fig. 1. Intercalated duct of the rat submandibular salivary glands in the control group. 1 – intercalated duct; 2 – secretory end piece; 3 – capillary; 4 – interstitial tissue. Hematoxylin and eosin stain. ×400.

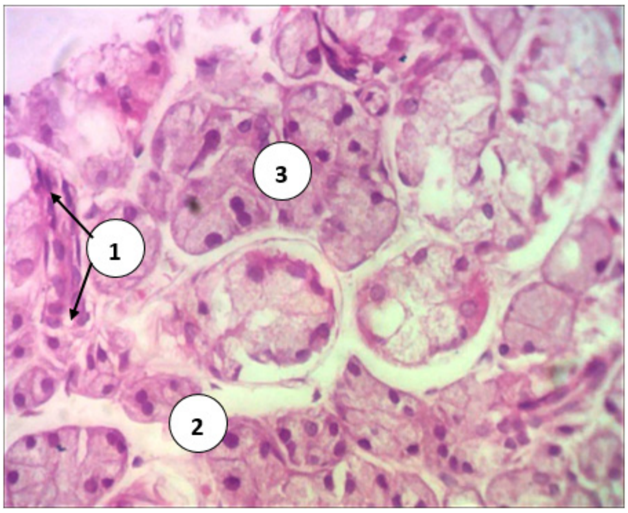

Fig. 2. Intercalated duct after 16 weeks of exposure to a complex of food additives. 1 – intercalated duct; 2 – interstitial tissue; 3 – secretory end piece. Hematoxylin and eosin stain. ×400.

40.96 % was observed, reaching 47.32±0.34 μm ($p<0.05$). The mean lumen diameter was 17.16±0.41 μm, which was significantly higher than in the control group by 88.55 % ($p<0.05$). Epithelial cell height also increased significantly by 21.16 % compared with the control group and amounted to 15.00±0.13 μm ($p<0.05$).

Under the influence of the complex of food additives, at the 4th week of the experiment, the external diameter of the striated ducts of the lobules of rat submandibular salivary glands was 37.88±0.58 μm, which was significantly lower than the previous observation period by 19.95 % but remained 12.84 % higher than the control group values ($p<0.05$). The lumen diameter significantly decreased by 26.57 % compared with the previous period, with mean values of 12.60±0.14 μm, which was still 34.04 % higher than in the control group ($p<0.05$). Epithelial cell height was 12.75±0.19 μm, which was 15.00 % lower than at the 1st week of the experiment but 2.99 % higher than in the control group ($p<0.05$).

At the 8th week, the mean external diameter of the striated ducts was 40.26±0.47 μm, which was significantly higher than the 4th-week values by 6.28 % and also significantly higher than the control group by 19.93 % ($p<0.05$). The lumen diameter of the striated ducts decreased significantly compared with the previous results by 67.86 %, reaching 4.051±0.062 μm, which was also significantly lower than the control group values by 56.91 % ($p<0.05$). The epithelial cell height of the striated ducts of the submandibular glands was 17.65±0.08 μm, which was 38.43 % lower than the previous observation period but 42.57 % higher than in the control group ($p<0.05$).

At the 12th week of exposure to the complex of food additives, a significant decrease in the mean external diameter of the striated ducts was observed, with values of 32.71±0.26 μm. This parameter was 18.75 % lower than at the 8th week of the experiment and 2.56 % lower than in the control group ($p<0.05$). The lumen diameter of the striated ducts showed an increase, with mean values of 4.401±0.132 μm, which was 8.64 % higher than the previous experimental period; however, it remained significantly lower than in the control group by 53.19 % ($p<0.05$). The epithelial cell height at the 12th week was 14.35±0.16 μm, which was significantly lower than the previous experimental period by 17.29 % but remained 15.91 % higher than the control group values ($p<0.05$).

At the 16th week of exposure to the complex of monosodium glutamate, sodium nitrite, and Ponceau 4R, a decrease in the mean external diameter of the striated ducts of the rat submandibular salivary glands was observed. The parameter measured 30.10±0.25 μm, which was significantly lower than both the previous experimental period by 7.98 % and the control group values by 10.34 % ($p<0.05$). The lumen diameter of the striated ducts increased compared with the 12th week by 24.77 %, but remained significantly lower than in the control group by 41.60 % ($p<0.05$). The mean lumen diameter at the 16th week was 5.491±0.132 μm. Epithelial cell height was 12.06±0.15 μm, which was significantly lower than at the 12th week of the experiment by 15.96 % and also significantly lower than in the control group by 2.58 % ($p<0.05$).

Identification of striated ducts in histological preparations of the control group rats presents no difficulty due to their low optical density. The ductal epithelial cells were of a prismatic (columnar) shape with clearly visible basal striations formed by broad infoldings of the basal plasmalemma, which, by local narrowing, formed vesicle-like structures extending almost to the apical surface of the cells (Fig. 3). At the 4th week of the experiment, signs of increased secretory activity were observed as a compensatory and adaptive response, manifested by the positioning of optically dense nuclei in the apical part of the cells. The striations were formed by narrow infoldings, accompanied by increased cytoplasmic basophilia, which was observed not only in the epithelial cells of the striated ducts but also in the seromucous cells of the secretory end pieces. The basal contour of the striated ducts appeared smooth and regular. Adjacent to the striated ducts, microcirculatory vessels, predominantly capillaries, were identified and showed signs of congestion, with erythrocytes present in their lumina. The duct lumina were dilated and contained secretion of low electron density (Fig. 4).

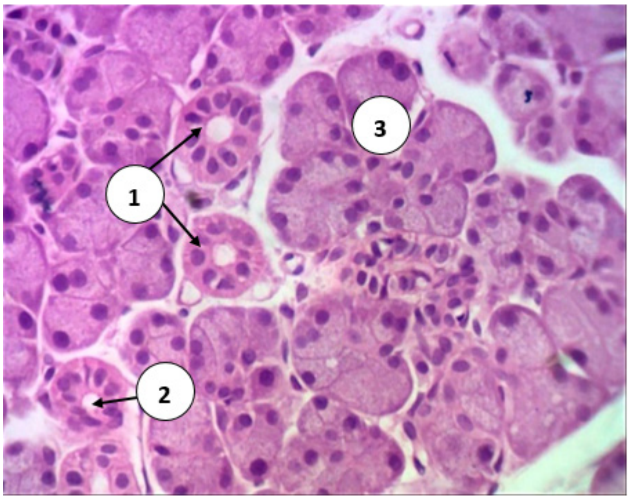

Fig. 3. Striated ducts of the lobules of rat submandibular salivary glands in the control group. 1 – striated duct; 2 – lumen of the striated duct; 3 – secretory end piece. Hematoxylin and eosin stain. ×400.

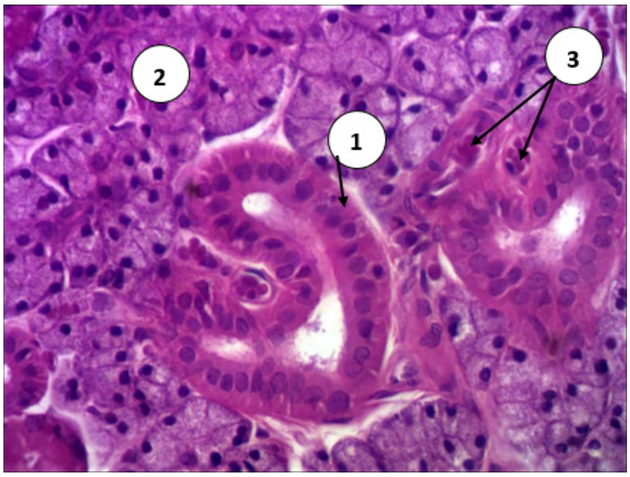

Fig. 4. Increased secretory activity of the striated ducts after 4 weeks of exposure to a complex of food additives. 1 – increased oxyphilia of epithelial cells of the striated ducts; 2 – secretory end piece; 3 – vascular congestion of the microcirculatory bed. Hematoxylin and eosin stain. ×400.

During the morphometric study of the rat submandibular salivary gland, it was established that the external diameter of the granular ducts in the control group was 35.55±0.20 μm, while the internal (lumen) diameter was 9.212±0.131 μm. The epithelial cell height was 13.22±0.12 μm (Table 3).

Table 3. Morphometric parameters of the granular ducts of rat submandibular salivary glands.

Groups	External diameter	Lumen diameter	Epithelial cell height
Control group	35.55±0.20	9.212±0.131	13.22±0.12
1st week	39.75±0.54*	8.881±0.162*	15.28±0.14*
4th week	40.18±0.49*	9.292±0.161 **	15.55±0.13*
8th week	41.38±0.49*.**	9.491±0.163	15.95±0.13*.**
12th week	41.81±0.45*	9.883±0.141*.**	16.48±0.14*.**
16th week	38.82±0.31*.**	9.524±0.152*.**	11.52±0.13*.**

Notes:* – p<0.05 compared with the control group; ** – p<0.05 compared with the previous observation period.

At the 1st week after exposure to the complex of food additives, a statistically significant increase in the external diameter of the granular ducts was observed by 11.81 %, with mean values of 39.75±0.54 μm (p<0.05). The lumen diameter significantly decreased (8.881±0.162 μm) compared with the corresponding control group value (p<0.05). The mean epithelial cell height was 15.28±0.14 μm, which was 15.58 % lower than in the control group (p<0.05).

At the 4th week of the experiment, the mean external diameter of the granular ducts was 40.18±0.49 μm, which did not differ significantly from the 1st week values and remained significantly higher than in the control group by 13.02 % (p<0.05). The lumen diameter increased significantly by 4.62 % compared with the previous experimental period and reached 9.292±0.161 μm, showing no significant difference from the control parameters (p<0.05). The mean epithelial cell height of the granular ducts of rat submandibular salivary gland lobules did not differ significantly from the previous experimental period; however, it was significantly higher than in the control group by 17.62 %, reaching 15.55±0.13 μm (p<0.05).

At the 8th week of exposure to the complex of food additives, the mean external diameter of the granular ducts was 41.38±0.49 μm, which was significantly higher than at the 4th week (by 2.99 %) and also significantly higher than in the control group (by 16.40 %) (p<0.05). The lumen diameter did not differ significantly from the previous experimental period and was 9.491±0.163 μm, also showing no significant difference from the control group mean values. The epithelial cell height of the granular ducts of the submandibular salivary glands was 15.95±0.13 μm, which was 2.57 % higher than in the previous period and significantly higher than in the control group by 20.65 % (p<0.05).

At the 12th week of exposure to the complex of food additives, the mean external diameter of the granular ducts was 41.81±0.45 μm, which did not differ significantly from the values obtained at the 8th week of the experiment and was significantly higher than in the control group by 17.61 % (p<0.05). The lumen diameter of the granular ducts increased, reaching mean values of 9.883±0.141 μm, which was 4.11 % higher than in the previous experimental period and significantly higher than the corresponding control values by 7.27 % (p<0.05). The epithelial cell height at the 12th week was 16.48±0.14 μm, which was significantly higher than in the previous experimental period by 3.22 % and also significantly higher than in the control group by 24.66 % (p<0.05).

At the 16th week of exposure to the complex of monosodium glutamate, sodium nitrite, and Ponceau 4R, a decrease in the mean external diameter of the granular ducts of the rat submandibular salivary glands was observed. The parameter was 38.82±0.31 μm, which was significantly lower than the values of the previous experimental period by 7.15 %, but remained significantly higher than in the control group by 9.20 % (p<0.05). The lumen diameter of the granular ducts significantly decreased compared with the 12th week results by 3.64 %, but, in comparison with the control group values, remained significantly higher by 3.37 % (p<0.05). The mean lumen diameter of the granular ducts at the 16th week was 9.524±0.152 μm. The epithelial cell height was 11.52±0.13 μm, which was significantly lower than the parameters obtained at the 12th week of the experiment by 30.10 % and also significantly lower than the mean epithelial cell height in the control group by 12.86 % (p<0.05).

The granular ducts of the control group rats were lined by a single layer of high columnar epithelial cells, whose cytoplasm was filled with numerous basophilic granules of variable size and differing optical density. These granules were predominantly located in the apical portions of the ductal epithelial cells and contained mainly kallikrein, contributing to local regulatory mechanisms of blood flow within the microcirculatory bed of the lobules of the rat submandibular salivary glands (Fig. 5). At the 8th week of the experiment, cells within the ductal epithelium that lacked granules were identified; their cytoplasm contained optically clear, predominantly round areas. Within the epithelium of the granular ducts, cells with optically pale nuclei were observed, positioned near the basal surface of the cells. The surrounding interstitium showed signs of hyperhydration. These histological features of remodeling of the granular ducts represent morphological evidence of increased functional activity of ductal epithelial cells in response to vascular spasm at early stages of the experiment, which undoubtedly reduced the amount of primary saliva under the influence of the complex of food additives (Fig. 6).

During the morphometric study of the intralobular collecting ducts of rat submandibular salivary glands, it was established that the external diameter was 36.10±0.46 μm, the lumen diameter was 3.672±0.161 μm, and the epithelial cell height was 18.96±0.13 μm (Table 4).

Table 4. Morphometric parameters of the intralobular collecting ducts of rat submandibular salivary glands.

Groups	External diameter	Lumen diameter	Epithelial cell height
Control group	36.10±0.46	3.672±0.161	18.96±0.13
1st week	47.14±0.25*	6.681±0.162*	21.74±0.14*
4th week	43.78±0.52*,**	5.281±0.152*,**	19.88±0.19*,**
8th week	49.38±0.49*,**	2.572±0.151*,**	23.95±0.13*,**
12th week	40.71±0.26*,**	2.453±0.112*	19.35±0.16*,**
16th week	37.80±0.25*,**	2.991±0.132*,**	17.06±0.15*,**

Notes:* – p<0.05 compared with the control group; ** – p<0.05 compared with the previous observation period.

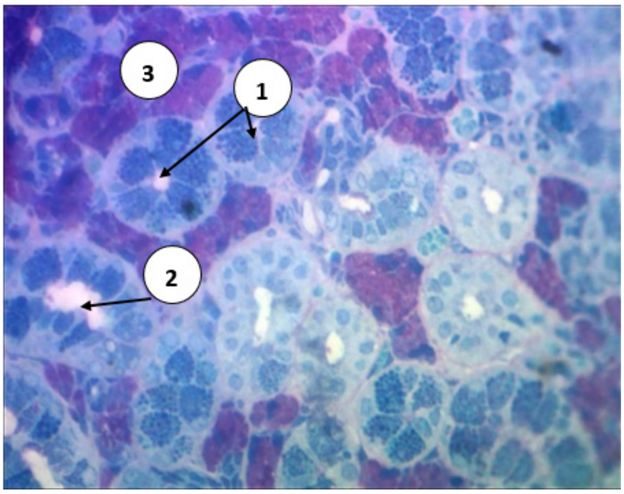

Fig. 5. Granular ducts of the lobules of rat submandibular salivary glands in the control group. 1 – granular duct; 2 – lumen of the granular duct; 3 – secretory end piece. Polychrome stain. ×400.

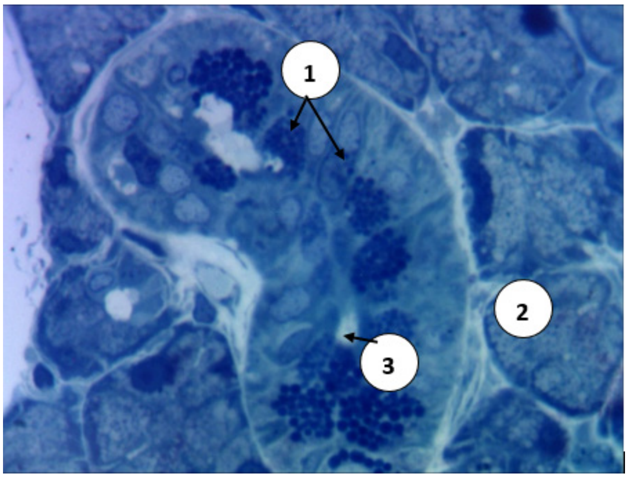

Fig. 6. Degranulation of granular ducts after 8 weeks of exposure to a complex of food additives. 1 – signs of degranulation; 2 – secretory end piece; 3 – lumen of the granular duct. Methylene blue stain. ×800.

Exposure to the complex of food additives after 1 week of the experiment resulted in a statistically significant increase in the mean external diameter of the intralobular collecting ducts by 30.58 %, reaching 47.14±0.25 μm. The lumen diameter increased by 82.02 %, amounting to 6.681±0.162 μm. In addition, epithelial cell height increased by 14.66 %, with mean values of 21.74±0.14 μm (p<0.05).

At the 4th week of the experiment, the external diameter of the intralobular collecting ducts showed a statistically significant decrease by 7.13 % compared with the previous observation period, reaching 43.78±0.52 μm. However, this value remained significantly higher than in the control group by 21.27 % (p<0.05). The internal (lumen) diameter also showed a significant decrease by 20.96 % compared with the values obtained at the 1st week of the experiment; nevertheless, it remained significantly higher than in the control group by 43.87 % (p<0.05). The mean lumen diameter of the intralobular collecting ducts of rat submandibular salivary glands at the 4th week was 5.281±0.152 μm. The mean epithelial cell height decreased to 19.88±0.19 μm, which was significantly lower than the values of the previous experimental period by 8.56 %. However, it remained 4.85 % higher than in the control group ($p<0.05$).

At the 8th week, the combined exposure to monosodium glutamate, sodium nitrite, and Ponceau 4R resulted in an increase in the mean external diameter of the intralobular collecting ducts by 12.79 % compared with the values obtained at the 4th week, which was also significantly higher than in the control group by 36.79 % ($p<0.05$). The mean external diameter of the intralobular collecting ducts of the lobules of rat submandibular salivary glands at the 8th week was 49.38±0.49 μm. Morphometric parameters of the lumen diameter of the intralobular collecting ducts significantly decreased both compared with the previous observation period by 51.33 % and in comparison with the control group by 29.97 %, amounting to 2.572±0.151 μm ($p<0.05$). Epithelial cell height at the 8th week showed a statistically significant increase to 23.95±0.13 μm. These values were 23.58 % higher than those of the previous experimental period and 26.32 % higher than in the control group ($p<0.05$).

At the 12th week of exposure to food additives, the morphometric parameters of the external diameter of the intralobular collecting ducts were 40.71±0.26 μm, which was significantly lower by 17.56 % compared with the previous experimental period but remained significantly higher than in the control group by 12.77 % ($p<0.05$). The mean lumen diameter of the intralobular collecting ducts decreased to 2.453±0.112 μm, which did not differ significantly from the values recorded at the 8th week of the experiment but was significantly lower than in the control group by 33.24 % ($p<0.05$). The epithelial cell height decreased to 19.35±0.16 μm, which was significantly lower by 19.21 % compared with the previous observation period, but remained 2.06 % higher than in the control group ($p<0.05$).

At the 16th week of exposure to the complex of food additives, a decrease in the external diameter of the intralobular collecting ducts was observed, reaching 37.80±0.25 μm. This parameter was significantly lower than the values recorded at the 12th week of the experiment by 7.15 %, while its mean values remained significantly higher than those of the control group by 4.71 % ($p<0.05$). The mean lumen diameter significantly increased compared with the previous experimental period by 22.04 %, yet remained significantly lower than in the control group by 18.53 % ($p<0.05$). The lumen diameter of the intralobular collecting ducts was 2.991±0.132 μm. The mean epithelial cell height decreased to 17.06±0.15 μm, which was 11.83 % lower than the values obtained at the 12th week of the experiment and also 10.02 % lower than in the control group ($p<0.05$).

Discussion

Thus, the ductal system of the rat submandibular salivary glands comprises a network of intercalated, striated, granular, and intralobular collecting ducts, all of which play an important role in glandular function. These structures are not only involved in the transport of secretory products but also directly participate in saliva modification and its fluid enrichment. The secretion produced by seromucous cells of the secretory end pieces is hypertonic in terms of salts and isotonic in terms of protein composition. The application of morphometric methods enables an objective assessment of structural changes in organ components following exposure to various endogenous and exogenous factors.

Analysis of the morphometric findings indicates a structural remodeling of the ductal system of the rat submandibular salivary glands under the influence of prolonged intake of a complex of food additives. The obtained data demonstrate a distinct trend of wave-like changes, characterized by an initial acute response at the 1st week, most likely triggered by the primary synergistic endogenous action of the components of the food additive complex, followed by the development of periductal interstitial edema in response to hypoxia and mast cell activation [1]. This is consistent with previously obtained data on the effects of various exogenous factors on the structural components of rat submandibular salivary glands [5, 8], reflecting an attempt at transient adaptation at the 4th week and subsequent progressive suppression of morphometric parameters starting from the 8th week of the experiment. The detected changes are dynamic in nature and manifest as differential responses of individual duct types at different experimental time points. This indicates complex adaptive and compensatory processes occurring in response to the action of the studied substances, followed by exhaustion of the secretory apparatus of ductal epithelial cells.

The most vulnerable component was the intercalated ducts, where a significant decrease in the external diameter, lumen diameter, and epithelial cell height was observed at the 1st week of the experiment. At the 4th week, partial recovery of the parameters was noted; however, they remained lower than the control values. Starting from the 8th week, a gradual and persistent decrease in all morphometric parameters was observed, persisting until the 16th week. This suggests a reduced influx of primary saliva into the ductal system, as intercalated ducts are not only involved in the regeneration of cells of intralobular ducts and their terminal segments but also regulate saliva transport within the ductal system. Such changes may indicate the development of dystrophic processes in the epithelial cells of the intercalated ducts and a decrease in their functional activity. Considering that intercalated ducts participate in the initial stages of secretion transport from the secretory end pieces, their morphological alterations adversely affect subsequent processes of saliva formation and modification.

The study of the striated ducts revealed a different pattern of response to the complex of food additives. At the initial stage of the experiment, a marked increase in the external diameter, lumen diameter, and epithelial cell height was observed. These changes reflect a compensatory and adaptive tissue response to the action of exogenous factors.

Striated ducts play an important role in active ion transport and modification of the primary secretion; therefore, an increase in epithelial cell height may indicate enhanced functional activity of the cells in response to reduced inflow of primary saliva, resulting from the dynamic narrowing of the intercalated duct lumen. However, at later stages of the experiment, starting from the mid-term observations, a gradual decrease in most morphometric parameters was noted. These changes may be attributed to the exhaustion of cellular adaptive mechanisms and the development of structural alterations within the ductal tissue.

The response of the granular ducts was characterized by a predominant increase in the external diameter and epithelial cell height throughout the first twelve weeks of the experiment. This may indicate the development of compensatory cellular hypertrophy in response to exposure to food additives. It is well established that the granular ducts of rat submandibular salivary glands play an important role in the synthesis and secretion of biologically active substances; therefore, the increase in their morphometric parameters may reflect an activation of secretory activity in response to the development of hypoxia [2]. The diameter of vessels in the resistive and exchange segments decreases, as confirmed by previously published data [22], which leads to an increased blood inflow to the microcirculatory bed and compensatory restoration of tissue homeostasis in the salivary gland. At the 16th week of the experiment, a significant decrease in epithelial cell height and a reduction in other morphometric parameters were observed, indicating the development of dystrophic changes in the cells and impairment of their functional state.

Analysis of the morphometric parameters of the intralobular collecting ducts also demonstrated a phase-dependent pattern of changes. At the initial stage of the experiment, a marked increase in the external diameter, lumen diameter, and epithelial cell height was observed, likely reflecting an adaptive response of the ductal system to the action of the investigated substances. However, a gradual decrease in these parameters was subsequently noted. A particularly pronounced narrowing of the ductal lumen was observed in the mid-term stages of the experiment, which may indicate impaired secretion transport processes and altered functional status of the epithelium. These findings suggest the development of compensatory vascular and tissue reactions or the emergence of sclerotic changes in the periductal connective tissue, leading to thickening of the overall duct profile alongside degradation of its functional epithelial layer. By the end of the experiment, most parameters, although showing a tendency toward stabilization, still remained different from the control values. Overall, the dynamics of morphometric parameters confirm that the synergistic action of monosodium glutamate, sodium nitrite, and Ponceau 4R exerts a pronounced toxic effect, leading to disorganization of the ductal system and depletion of its adaptive reserves by the 16th week of exposure. The detected morphological changes indicate impairment of saliva formation, transport, and modification processes in the rat submandibular salivary glands under conditions of long-term combined exposure to food additives.

The obtained results justify further investigation of the effects of food additives on the oral cavity organs and the salivary secretion system. Future studies should focus on the mechanisms of prevention and correction of structural and functional disorders of the salivary gland ductal system that arise under conditions of prolonged intake of flavor enhancers, preservatives, and synthetic food colorants.

Conclusions

1. Prolonged administration of a complex of food additives containing monosodium glutamate, sodium nitrite, and Ponceau 4R leads to a gradual structural remodeling of the ductal system of the rat submandibular salivary glands.

2. At the early stages of the experiment, the observed changes were predominantly compensatory-adaptive in nature. They were manifested by an increase in selected morphometric parameters of the striated, granular, and intralobular collecting ducts, which may indicate an increased functional load on the ductal epithelium.

3. With prolongation of the experimental period, a trend toward exhaustion of the adaptive capacity of the ductal system was identified, characterized by a reduction in duct diameter, narrowing of the lumen, and a decrease in epithelial cell height.

4. The most pronounced signs of dystrophic remodeling were observed in the intercalated ducts, where a persistent decrease in the main morphometric parameters was recorded throughout the experiment. This may indicate impairment of the initial stages of secretion transport from the secretory end pieces to the ductal system.

5. The striated, granular, and intralobular collecting ducts demonstrated a wave-like response to the complex of food additives: an initial increase in selected parameters was followed by their subsequent decline or instability. This dynamic pattern confirms a progressive deterioration of the structural and functional state of the ductal apparatus.

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ДИНАМІКА ЗМІН МОРФОМЕТРИЧНИХ ПАРАМЕТРІВ ПРОТОКОВОЇ СИСТЕМИ ПІДНИЖНЬОЩЕЛЕПНИХ СЛИННИХ ЗАЛОЗ ЩУРІВ ПІД ВПЛИВОМ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

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Система вивідних проток слинних залоз не лише забезпечує транспорт слини, а й бере участь у її формуванні та модифікації, тому зміна їх структурних параметрів може порушувати слиновиділення. Метою роботи було встановити динаміку морфометричних показників вставних, посмугованих, гранулярних та внутрішньочасточкових колекторних проток піднижньощелепних слинних залоз щурів у нормі та за умов комплексної дії харчових добавок. Дослідження проведено на 84 статевозрілих щурах-самцях. Тварини контрольної групи отримували питну воду та фізіологічний розчин, експериментальної групи – 0,6 мг/кг нітриту натрію, 20 мг/кг глутамату натрію та 5 мг/кг Понсо 4R у 0,5 мл дистильованої води перорально один раз на добу. Тварин виводили з експерименту через 1, 4, 8, 12 та 16 тижнів. Фрагменти залоз фіксували у 10 % нейтральному формаліні та глютаровому альдегіді, ущільнювали у парафін та Епон-812. Мікрофотографування і морфометричне дослідження проводили за допомогою цифрового мікроскопа Levenhuk D740T, статистичну обробку виконували в Excel. У вставних протоках уже з 1-го тижня визначалося достовірне зменшення зовнішнього діаметра, просвіту та висоти епітеліоцитів, а до 16-го тижня ці показники залишалися нижчими за контрольні значення. Посмуговані протоки на початку експерименту реагували збільшенням зовнішнього діаметра, просвіту та висоти епітеліоцитів, однак надалі спостерігалося звуження просвіту і зниження окремих показників. У гранулярних протоках до 12-го тижня переважало збільшення зовнішнього діаметра та висоти епітеліоцитів, але на 16-й тиждень висота клітин зменшилася нижче контрольного рівня. Внутрішньочасточкові колекторні протоки характеризувалися раннім збільшенням морфометричних параметрів з подальшим зменшенням просвіту та висоти епітеліоцитів у пізні терміни. Отримані дані свідчать про хвилеподібну перебудову протокової системи: ранні компенсаторно-адаптаційні реакції змінювалися ознаками дистрофічних порушень. Виявлені морфологічні зміни вказують на порушення функціонального стану протокової системи та процесів формування, транспорту і модифікації слини.

Ключові слова: *слинні залози, вивідні протоки, слина, щури, харчові добавки, гістологічне дослідження, морфометричний метод, глутамат натрію, нітрит натрію, Понсо 4R.*

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