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Morphological Changes in the Upper Part of Digestive Tract in a Case of Experimental Duodenogastric Reflux

Ye.G. Romanenko[1,a], M.P. Komskyi[2,b]

[1]Dnepropetrovsk Medical Academy of the Ministry of Public Health of Ukraine, Dental Faculty, Dniper, Ukraine

[2]Dniprovskyi Medical Institute of Traditional and Non-Traditional Medicine, Dental Faculty, Dniper, Ukraine

[a]helenromanenko2017@gmail.com, [b]rolex-50@yandex.ru

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Abstract. Experimental reproduction of duodeno-gastric reflux was performed among Wistar line 28 rats, which were injected with 50 % solution of medical bile by intragastric way. Morphological study had been shown significant structural changes in the gingival tissues, mucous membrane of an esophagus, stomach and duodenum. In the epithelial layer of gingiva and esophagus was observed numerous infringements in a process of differentiation cells, in the stroma was shown phenomena of fibrosis in a papillary layer, microcirculation disorders. In the gastric mucosa had been found out multiple erosions, the glandular cells were increased. It was demonstrated vacuolization of the basal and thorn layers of the stomach epithelium. In the stroma defined phenomena of fibrosis and a dense lymphocyte infiltration with eosinophils. In the duodenum was determined desquamation of a glandular epithelium, superficial erosions of the villi, lympho–histio–plasmocytosis infiltration in a stroma, microvascular disorders. The given experiment demonstrates a role of functional disorders in the pathogenesis of combined pathology in the upper gastrointestinal tract.

Introduction

Bile reflux is a symptom of several diseases that affect the upper digestive tract, such as chronic gastritis, gastroesophageal reflux disease, peptic ulcer of a stomach and duodenal ulcers [1–3]. The bile or alkaline reflux is a well–known retrograde spillage of bile from duodenum into the anatomically highly situated organs – stomach, esophagus and oral cavity. If reflux of the gastric contents into esophagus is considered being normal phenomena – the so–called physiological reflux, a bile reflux is associated with diseases [4–7]. However, some of authors consider duodeno-gastric reflux (DGR) to be a compensatory adaptive mechanism in a case of high pH in the stomach [8–10]. Medical bile injected intragastric way, caused gastritis and duodenitis at the animals, which closely connected with humans by a pathomorphogenesis [11–12]. Pepsin and combination of pepsin with the conjugated bile acids in an acidic environment are the basic aggressive factors, which destroy a protective barrier of mucous membrane in the stomach and duodenum. However, a role of bile at the appearance of esophagus and oral cavity lesions in a case of DGR is uncertain [13–16].

Purpose of Research – to identify morphological changes in the upper digestive tract tissues in a case of experimental model of duodeno–gastric reflux.

Material and Methods

The model of DGR was reproduced with 28 immature rats of both sexes of Wistar's line. Their weight was 70–90 g. The control group consisted of 12 intact animals. Laboratory rats in the experimental group were administered for 30 days with 50 % solution of medical bile by intragastric way in the amount of 1 ml per 100 g of animal weight once a day. Rats were on the fully balanced diet. Their access to water was not limited. This period provided the preliminary

adaptation to the next stage of the model. Second stage of the experiment lasted 10 days. During the second stage rats' diet was restricted by 1/3 and they were injected with 50% solution of medical bile. Feeding the animals was performed twice daily. The animal had been given 1/3 of the cut down feed in the morning, 6–7 hours before the injection of bile and water was given without any restrictions. Water dishes had been taken before 1 hour till the bile injection. The last part of a diet was given 1 hour after the bile injection and then the rats were given water without any restrictions. Animals were sacrificed under thiopentone anesthesia. It was conducted in two stages: 30 days after experiment (12 animals: 6–from control group, 6–from experimental group) and to the next day after finishing a model (28 animals: 6–from control group, 22–from experimental group). Morphological condition of the gums, mucous membrane of esophagus, stomach and duodenum at the animals was evaluated after the first and second stage of the experiment.

Results of the Study and their Discussion

At the morphological research after the first stage of experiment was discovered an irregular thickness of the gingival epithelium, presence of the dyskeratosis symptoms, incomplete keratinization. In the epithelium swelling of cells, formation of a pericellular edema, focuses of the hydropic degeneration and destruction of epithelial cells, formation of bubbles in a border of the thorny and keratosic layer were visualized (Fig.1).

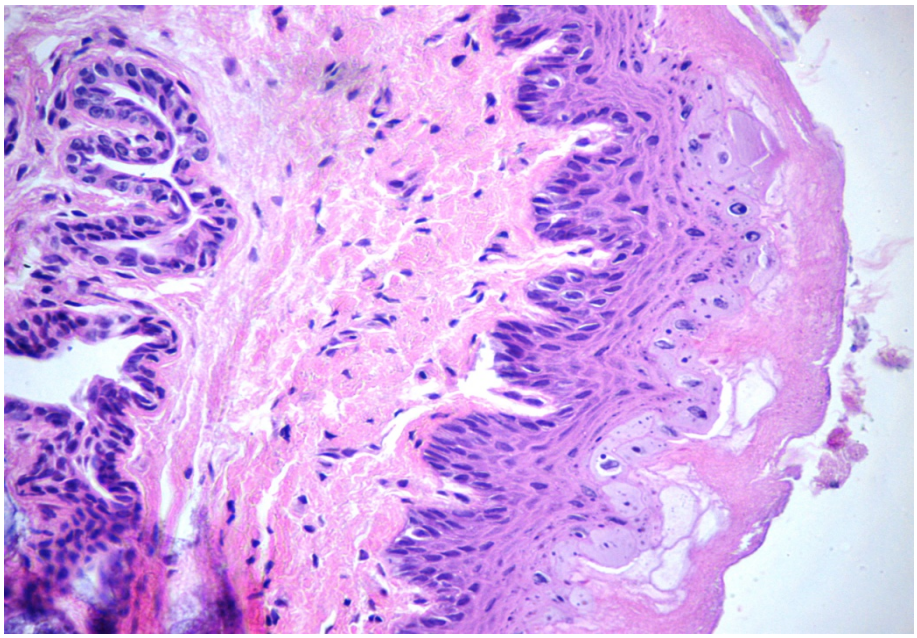

Fig. 1. Formation of the bubbles in a border of thorny and keratosic layer of the rats' gingiva in the experimental group. Staining – with hematoxylin and eosin. Magnification x 200.

Basal layer of an epithelium sometimes was represented by two rows of cells, which indicated about activation of proliferative processes in the gingiva (Fig. 2). Mitotic activity in the basal and parabasal layer of epithelium was intensive. In the gingival lamina propria the collagen fibers were thickened and hardened, phenomena of fibrosis and obstruction of blood capillaries was found.

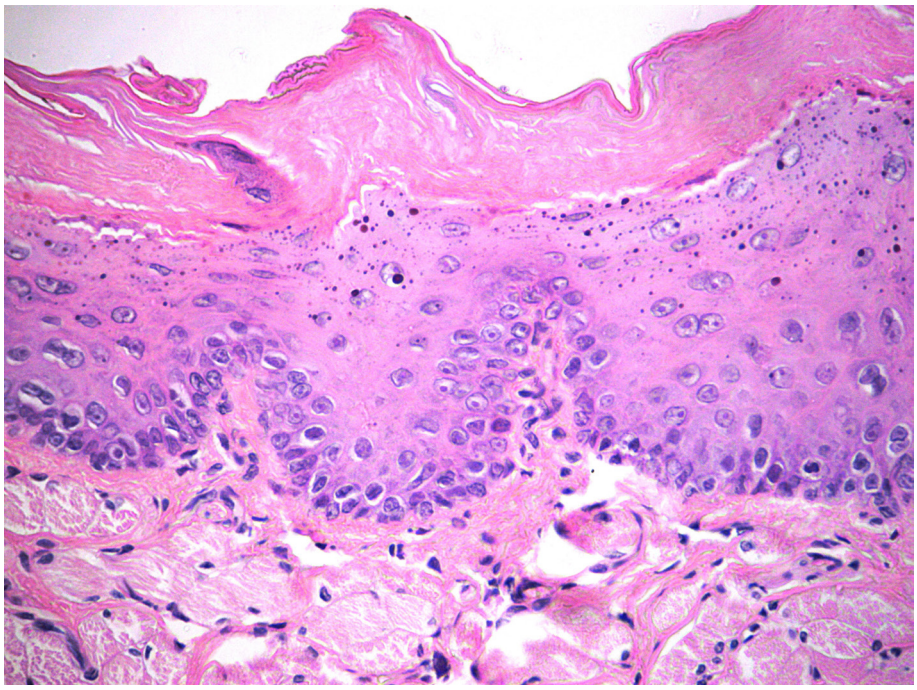

Fig. 2. Basal layer of the gingival cells at rats in the experimental group. Staining – with hematoxylin and eosin. Magnification x 400.

After second stage of the experiment was shown reduced thickness of the epithelium, closely connected with decreasing of the thorny and keratosic layer as a result of the epithelium desquamation. In a thorny layer visualized cells with symptoms of karyolysis and karyopyknosis. Basal layer of cells has an uneven thickness, sometimes manifested phenomena of mild dysplasia. Cells of the basal layer have a cylindrical shape. Collagen fibers in the gingival lamina propria were thickened and hardened. Focuses of the light plasmatic impregnation were revealed (Fig. 3, 4).

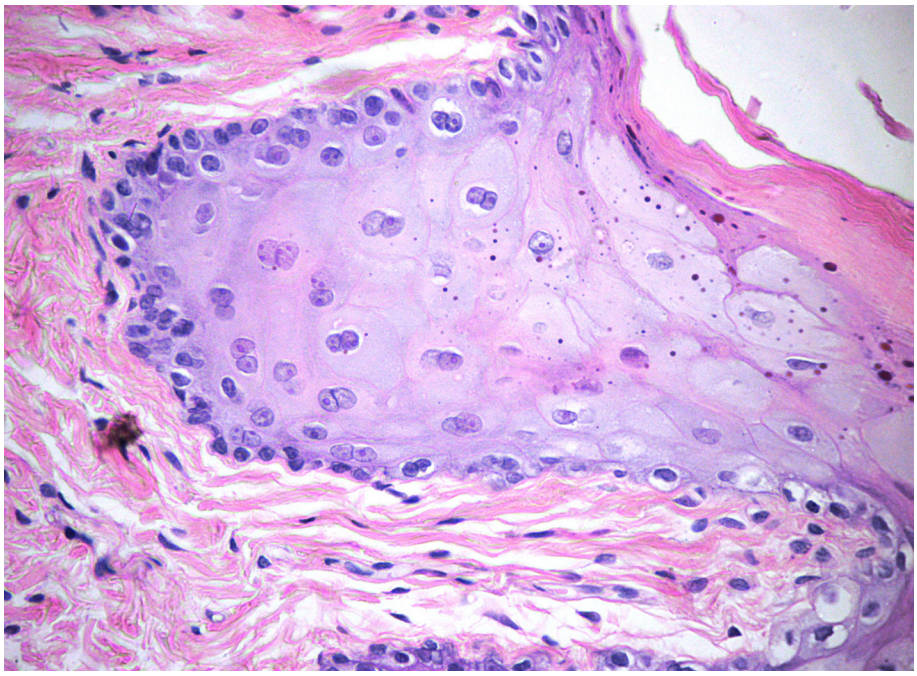

Fig. 3. Fibrosis of a lamina propria of the rats' gingiva in the experimental group at the lower molars area. Staining – with hematoxylin and eosin. Magnification x 400.

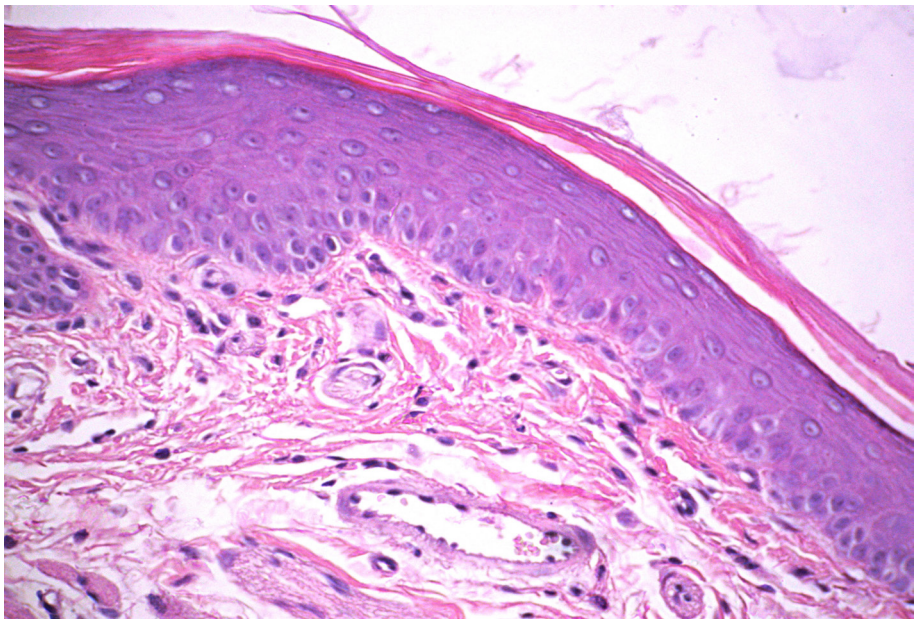

Fig. 4. The rats' gingiva in the experimental group. Plasmatic impregnation of the lamina propria. Staining – with hematoxylin and eosin. Magnification x 200.

In the gingival lamina propria were diffusely located single lymphocytes and single plasma cells. Increasing amount of mast cells demonstrated about early shifts of the inflammatory response.

After the first stage of experiment in the esophagus mucosa were observed effects of hyper– and parakeratosis. Layer of cell keratinous was sharply and irregularly thickened, in some places was on 1/3 from the epithelium width (Fig. 5). A granular layer has been thinned; the basal layer in some places was represented by two rows of cells, which indicated about infringement processes of differentiation in the epithelial layer. In the esophagus lamina propria visualized phenomena of fibrosis of a papillary layer. Capillaries were determined in the deeper layers of a connective-tissue plate.

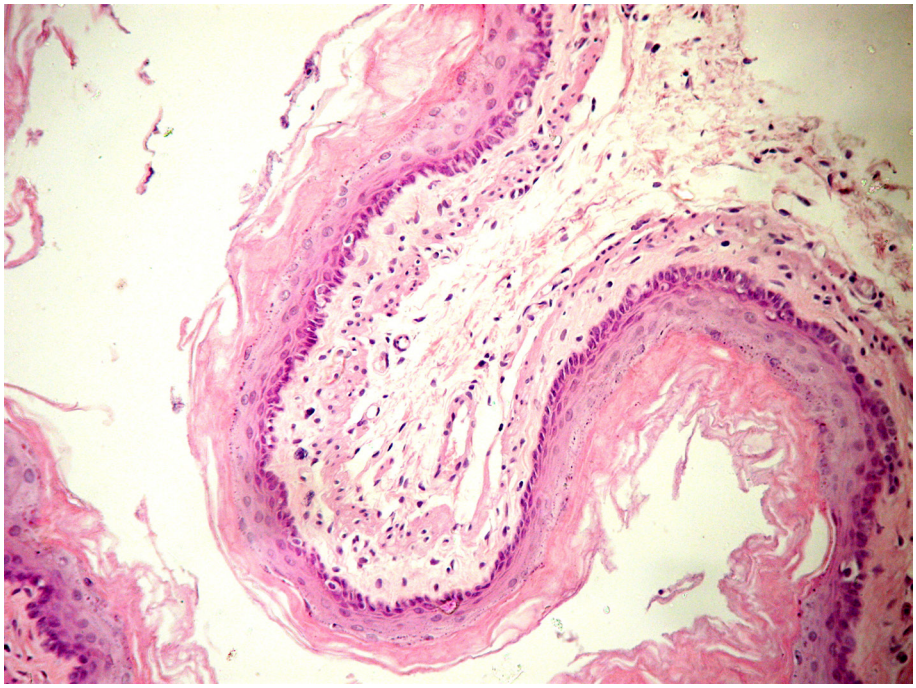

Fig. 5. Hyperkeratosis of the rats' esophagus in the experimental group. Staining – with hematoxylin and eosin. Magnification x 200.

After the first stage of experiment, macroscopic investigation of a gastric mucosa demonstrated single erosion. Mucous membrane of a stomach is not visually changed. Microscopic examination of the gastric mucosa had been shown superficial defects on the epithelium (Fig. 6). In the glandular part was determined moderate lymphocytic infiltration of the stroma with a mixture of eosinophils. Glandular cells were increased in a size. Gap of the glands was dilated.

In a duodenum mucosa the macro– and microscopic changes were absent.

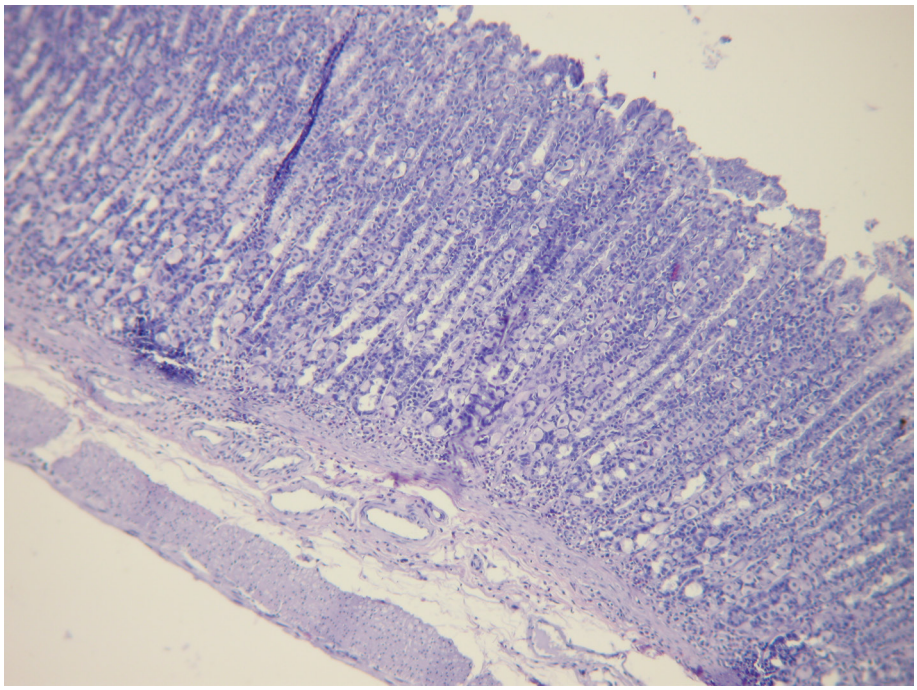

Fig. 6. Erosion of the rats' gastric mucosa in the experimental group. Staining – with hematoxylin and eosin. Magnification x 100.

After the second stage of experiment while macroscopic examination of the stomach was carried out, multiple erosions of the mucous membrane on a background of hyperemia had been shown. Microscopic examination of the gastric mucosa demonstrated superficial defects of the epithelium. Gap of the glands was enlarged, glandular cells were increased. In the cells of the surface epithelium of a stomach was revealed a severe parakeratosis. Stratification of the epithelial layers was disturbed. Vacuolization of the basal and thorny layers in a stomach epithelium was detected, which had been shown the infringement of intracellular contacts in a germinative area (Fig. 7, 8).

In a border of the epidermal and glandular parts of the stomach was a defined phenomenon of fibrosis in the stroma and the dense lymphocyte infiltration, mixed with eosinophils (Fig.9).

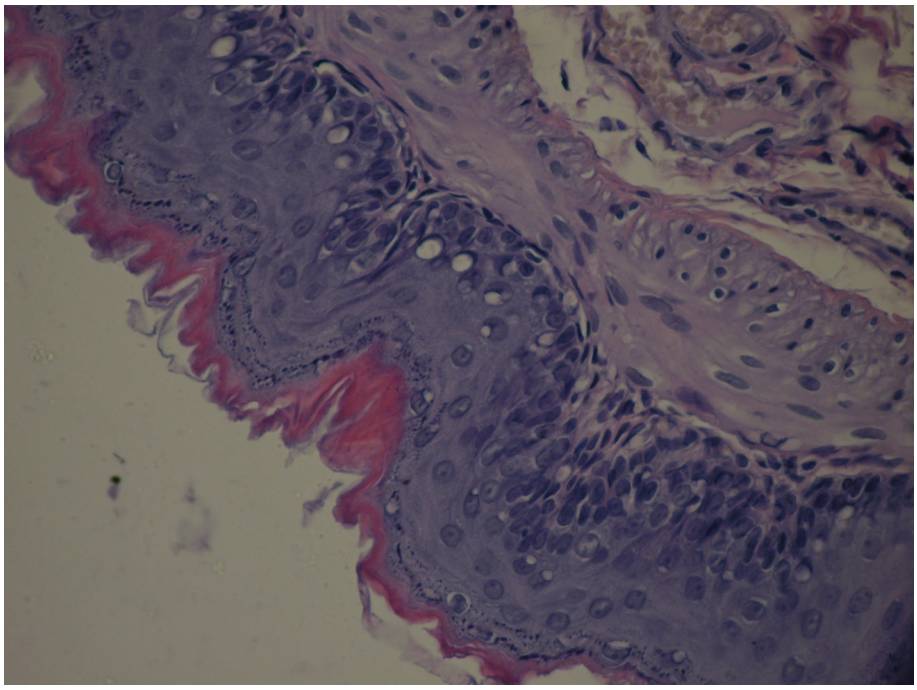

Fig. 7. Vacuolization of the basal and thorny layers of epithelium in the esophageal part of rats' stomach from experimental group. Staining – with hematoxylin and eosin. Magnification x 400.

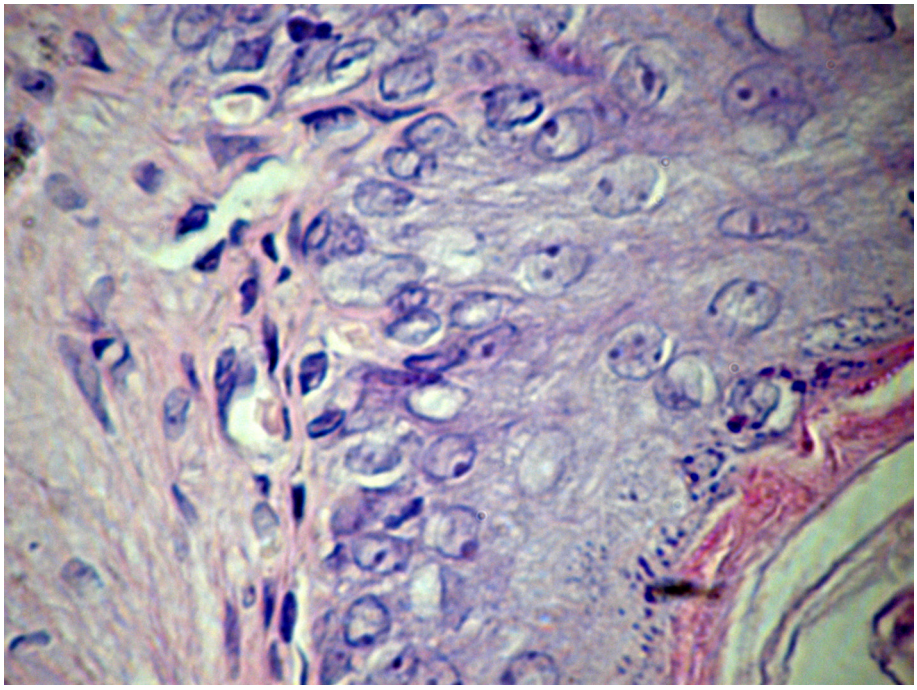

Fig. 8. Basal layer of epithelium cells in the oesophageal part of rats' stomach from experimental group. Staining – with hematoxylin and eosin. Magnification x 1000.

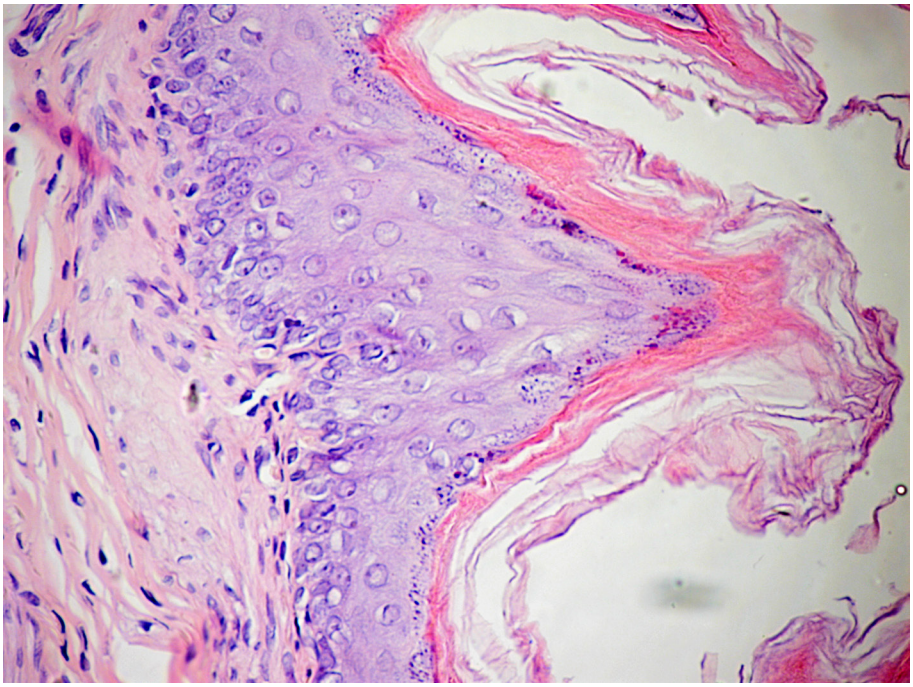

Fig. 9. Fibrosis of a lamina propria in the esophageal part of the stomach mucosa at the rats from experimental group. Staining – with hematoxylin and eosin.Magnification x 400.

In duodenum the glandular cells were in a state of apoptosis. In the proximal parts of mucosa had been revealed – desquamation of a glandular epithelium, light superficial erosions in the proximal villi (Fig. 10), which were covered with a large amount of mucus. In a stroma was determined lymphohistiocytosis infiltration (Fig. 11).

In the submucosal layer visualized sharply plethoric vessels in a mucosa – stasis in the capillaries, indicating about disruption of the microcirculation (Fig. 12).

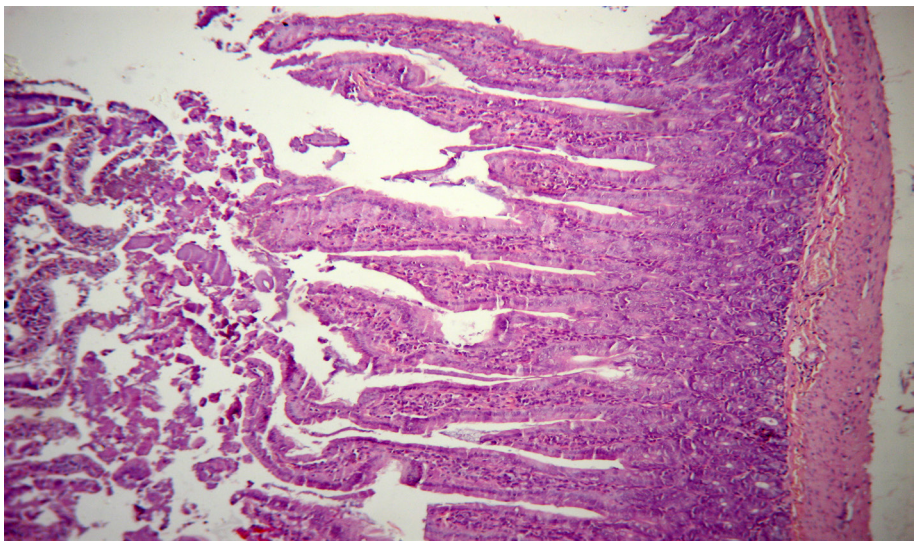

Fig. 10. Erosion of a duodenum mucous membrane at the rats from experimental group. Staining – with hematoxylin and eosin. Magnification x 100.

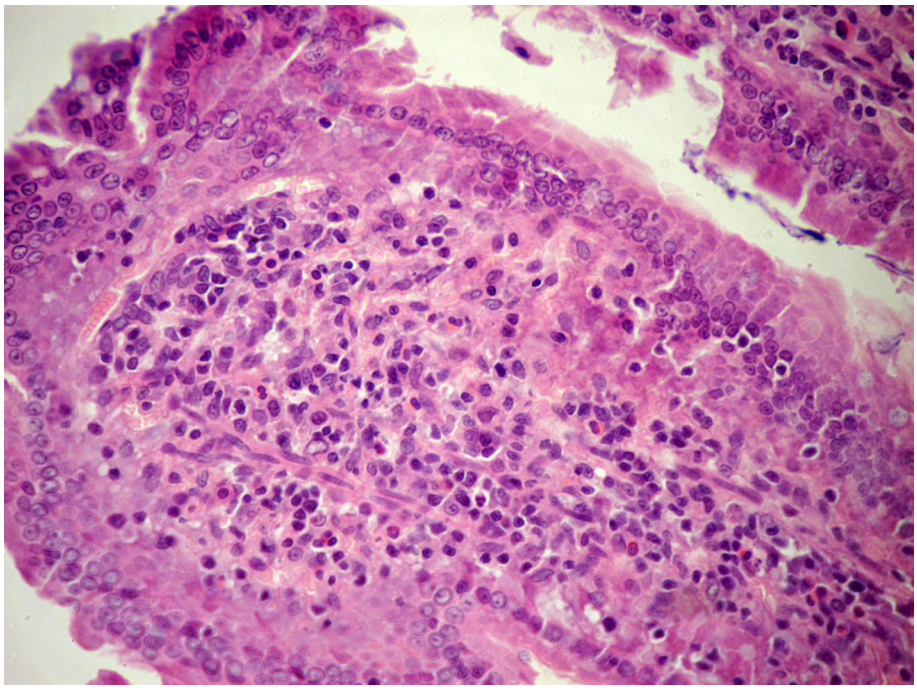

Fig. 11. Lymphohistioplasmocytosis infiltration in a duodenum mucous membrane at the rats from experimental group. Staining – with hematoxylin and eosin. Magnification x 400.

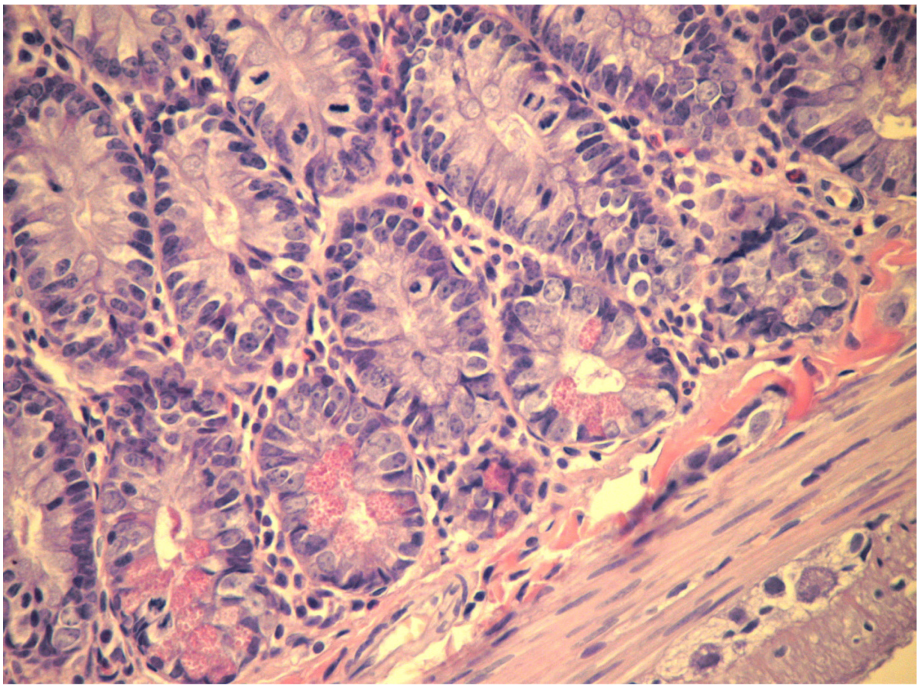

Fig. 12. Mucosa of the duodenum at the rats from experimental group. Plethora of vessels and capillaries. Staining – with hematoxylin and eosin. Magnification x 200.

Discussion

Bile acids are endogenous factors, acting aggressively on the mucous membrane of a stomach, carried out to the damage of lipid layer in the membranes of epithelial cells and destruction of mucus [17]. Character and severity of damage depends on the pH in the stomach contents, as well as a degree of hydroxylation of the bile acids [14]. Histological examination of the mucous, covering stomach of children with DGR, had been shown focuses of a foveolar hyperplasia, disorders of microcirculation [18]. The typical endoscope changes at the DGR were erythema and erosion of a gastric mucosa [19]. Reflux of a bile could cause an inflammatory and erosive changes

in the esophageal mucosa [13, 20–21], but changes in the oral cavity at the DGR case were not described.

Due to high variability mechanisms of diseases in the upper part of digestive tract, scientists have suggested many ways of their reproduction (intragastric administer of corrosive substances, violation of a blood supply in the stomach wall, using legations of vessels, stress effect on the central nervous system [11, 12, 22–24].

In a majority of clinical cases, DGR frequently occurs after the surgical interventions (in 52.6 % of cases – after suturing the duodenal ulcer, in 15.5 % of cases – after cholecystectomy). The great number of experimental methods includes techniques, which requires a surgical intervention [17, 25–26].

However, diseases of the upper digestive tract mostly occur in the childhood and associated with impaired of a motor-evacuation function [27], which allows applying introgastric way of administration a bile solution for the physiological reproduction of DGR on immature animals.

Experimental reproduction of DGR was performed in 28 immature rats of Wistar line, by intragastric administration 50% of medical bile solution during 40 days. At the morphological study was found significant structural changes in the gingival tissues, mucous membrane of an esophagus, stomach and duodenum. In the epithelial layer of gingiva and esophagus was observed a numerous infringements in the processes of cells differentiation, in a stroma – phenomenon of fibrosis in the papillary layer. Capillaries were determined in a deeper layer of the connective-tissue plate.

In a gastric mucosa was shown the multiple erosions, gaps of glands were enlarged. Glandular cells were increased. It was found mild vacuolization of the basal and thorny layers of epithelium in the stomach. The given changes indicated about infrigement of intercellular contacts in the germinative zone. In the stroma was determined a phenomena of fibrosis and compact lymphocytic infiltration, mixed with eosinophils.

There was determined desquamation of a glandular epithelium, superficial erosions of the villi, lymphohistioplasmocytosis infiltration of the stroma in the duodenum. In a submucosal layer was disturbed microcirculation.

Conclusions

Experimental reproduction of duodeno-gastric reflux demonstrates significant structural changes in the gingival tissues, mucous membrane of an esophagus, stomach and duodenum. In the epithelial layer of gingiva and esophagus was observed infringement of cells differentiation. In the stroma was shown fibrosis of a papillary layer, microcirculation disorders. In a gastric mucosa was found the multiple erosions, glandular cells were increased. In a stomach epithelium was demonstrated vacuolization. In a stroma were revealed fibrosis and the lymphocyte - eosinophils infiltration. In the duodenum was determined desquamation of a glandular epithelium. On a background of microvascular disorders, in the stroma was observed infiltration with lymphocytes, histiocytes and plasmocytes.

The conducted experiment demonstrates a role of functional disorders in the pathogenesis of combined pathology of the upper gastrointestinal tract.

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