# MORPHOLOGY AND PATHOMORPHOLOGY

# Structure of Gastric Wall in Wistar Rats in Health and after Experimental Traumatic Brain Injury

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The structure of gastric wall was studied by histological methods in Wistar rats in health and after skull trephination and insertion of a needle into the brain. Experimental brain injury led to the development of destructive changes in the gastric wall (in the lymphoid structures located between the gland bottoms and muscle plate of the mucosa). Changes in the structure of cardiac glands and desquamation of the epithelium were detected. Microcirculation was disordered, signs of inflammation appeared. The counts of medium-sized lymphocytes and plasma cells increased in the layer of lymphoid cells and in the submucosa.

**Key Words:** *stomach; traumatic brain injury*

The gastric mucosa (GM) is often exposed to various factors because of direct contact with the environment (food). This explains its well-developed lymphoid system [4,5], sufficiently well studied in various compartments of human stomach [2,3]. Structural elements of the gastric wall in subjects of different age are less studied [1,7], as well as their reactions to injuries of various kinds [8], for example, brain injuries [6].

We studied the gastric wall of rats with experimental traumatic brain injury.

## MATERIALS AND METHODS

The study was carried out on 15 adult 4-5-month-old male Wistar rats (250-300 g). The rats ($n$=10) were narcotized with chloral hydrate (400 mg/kg, intraperitoneally), after which trephination of the skull was carried out in the left caudate nucleus area. A hole (0.5 mm) was made in the skull and the brain tissue was injured by insertion of a G22 injection needle to a depth of 3 mm (no blood was injected through the needle). Control group consisted of 5 intact rats. The animals were decapitated on days 1, 3, and 7 after experimental treatment (these periods were chosen because of the development of changes in the gastric wall and reactions of cells in the GM). Fragments of the gastric wall collected in the cardiac compartment were fixed in 10% neutral formalin and embedded in paraffin. The sections (4-5 μ) were stained with hematoxylin and eosin, azur II and eosin, and by the methods of van Gieson and Mallory. Cell composition of the lymphoid tissue in the GM lamina propria was studied in two areas: GM folds and between them in a section area of 880 $\mu^2$. The data were statistically processed using SPSS software ($t$ test for paired variables, ANOVA). The animals were handled in accordance with the Order of the Ministry of Higher Education of the USSR No. 742 of November 13, 1984 "On Regulations of Studies on Experimental Animals".

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## RESULTS

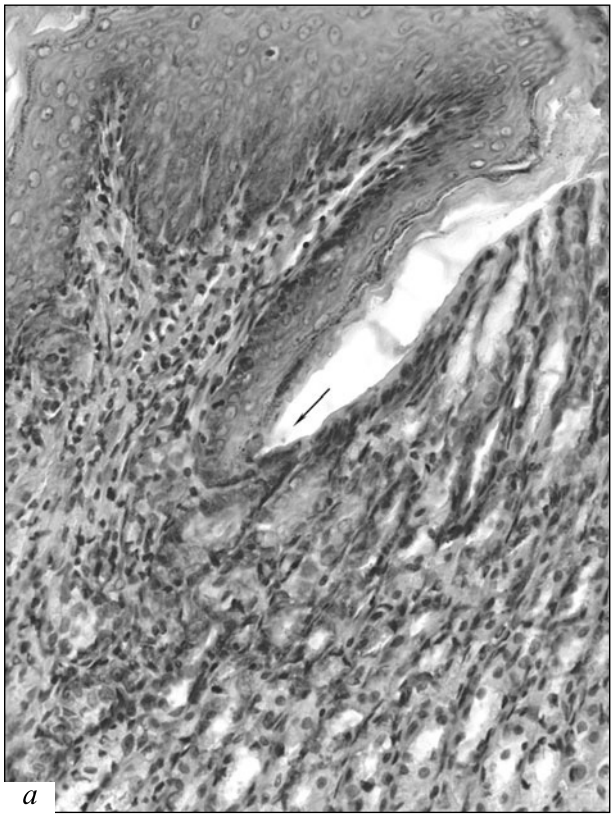
The gastric wall of intact animals had typical structure. Stratified squamous epithelium in sites of forming folds near the inlet was thinned significantly, transforming directly into columnar epithelium in the bottom (Fig. 1, *a*). The gastric surface had deep fossae (up to one-fourth of the GM lamina propria thickness). The cardiac glands were located near the transition of stratified epithelium into single layer one. The connective tissue between the glands was poorly expressed. Accumulation of lymphoid cells was seen in the narrow space between the gland bottom and muscular plate; these cells were located along the walls of the entire stomach. The muscle plate was well developed, consisted of 2-3 layers of smooth muscle cells, and was a direct continuation of the esophageal muscle plate. The plate was much thicker in the gastric inlet and looked loose because of dissociation of bundles of smooth muscle cells.

Submucosa consisting of loose connective tissue had different thickness in different places depending on the presence of vascular bundles and their sizes. It was more compact between the folds, but thinner than in other places. In the folds, the submucosa was thick and impressed in a cone-like mode into the folds, forming its base together with the muscle plate. Blood vessels, lymph capillaries and vessels were located at the bases of these cones. The submucosa was looser in these regions and contained fatty cells.

The tunica muscularis consisted of two layers: the inner circular and outer longitudinal. The inner layer was 3-4 times thicker than the outer one (Fig. 1, *b*). The muscular layers in the gastric inlet were irregularly arranged; large bundles of smooth muscle cells surrounded by thick pads of loose connective tissue changed their direction as they fused with the layers of the tunica muscularis. The tunica muscularis was not separated into three layers.

The serosa was very thin and was presented by a single layer of mesothelial cells.

Lymphoid structures in the gastric wall of Wistar rats were presented by just a thin uneven layer of lymphoid cells located directly under the gland bottom. In the folds, the counts of cells in a section of a standard size (880 $\mu^2$) varied from 18.15 to 19.85 (19.00±0.85). The main portion of lymphoid cells were minor lymphocytes: 3.9-4.9 (4.4±0.5), 3.43-4.97 (4.20±0.77) between folds; the absolute count of these cells constituted about one-fourth of all lymphoid cells per section of 880 $\mu^2$. Medium-sized lymphocytes were rare (<1%). Eosinophils were found in the folds and between them in the gastric walls: 1.61-1.99 (1.80±0.19) cells per 880 $\mu^2$. These cells were often found not only in the lymphoid layer, but also between the glands. No plasma cells were found in the studied regions of



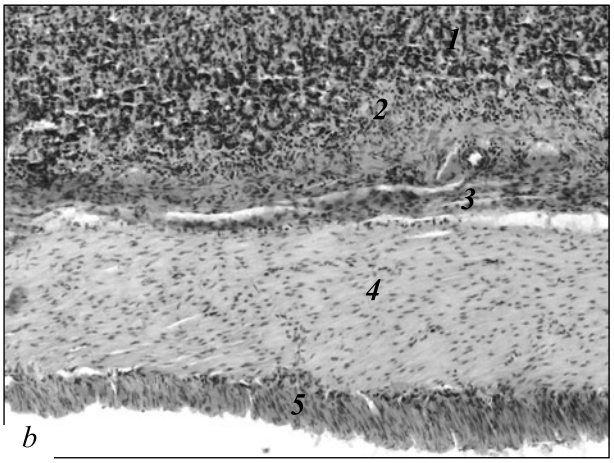




**Fig. 1.** The gastric cardiac compartment wall in an intact Wistar rat. *a*) transition of hornifying epithelium into columnar one (arrow); *b*) tunica muscularis of the gastric cardiac portion. *1*) lamina propria with glands; *2*) lymphoid cell layer; *3*) submucosa; *4*) inner circular layer of smooth muscle cells; *5*) outer longitudinal layer of tunica muscularis. Hematoxylin and eosin staining, ×320 (*a*), ×160 (*b*).

the fold. On the other hand, equal counts of stab and segmented neutrophils were found in the lymphoid cell layer. About 2% cells (0.40±0.22 per 880 $\mu^2$) were destroyed. All these cells were found among numerous stromal cells (fibroblasts, fibrocytes, reticulocytes) constituting 60% of total cell count. No blasts, large lymphocytes, proliferating cells, or macrophages were found in the lymphoid cell layer.

The density of cell distribution in the lymphoid layer was virtually the same as in the folds, their count being 20.0±0.85 per 880 $\mu^2$; the summary content of lymphocytes >25%. In addition, plasma cells (mainly plasmocytes) were found between the folds (2.06±0.28% or 0.80±0.18 cells per 880 $\mu^2$). Eosinophils between the folds were rarer than in the folds (about 5%). The content of neutrophilic leukocytes in the gastric wall folds was similar to those in other areas, but they were found irregularly. No poorly differentiated cells (blasts, large lymphocytes), proliferating cells, or macrophages were found in the lymphoid layer between the folds, similarly as in the folds.

Experimental traumatic brain injury in rats caused changes in the gastric walls. These changes consisted in stretching of the gastric wall, as a result of which the wall thinned, particularly tunica muscularis (Fig. 2), while GM folds were somewhat smoothed out. More intense desquamation of epitheliocytes was seen in the gastric lumen. The thickness of the glands somewhat decreased because of glandular cell shrinkage; the gland walls became more compact and the glandular lumens were stenosed. The spaces between the glands were enlarged, signs of edema were seen in connective tissue layers between them. Epitheliocytes in the mouth and neck of the glands were often short and oriented towards the lumen. The structure of glandular cell nuclei was unchanged. Glandular cells in the bottom of the glands looked clearer, with foamy cytoplasm. They tightly adhered to each other, as a result of which the lumen of the duct between the cells was closed. Glands in a state of destruction replaced by growing connective tissue were sometimes found. The level of the lymphoid cells between the glands did not change much, but the layer of lymphoid cells between the glands and muscle plate grew wider.

The submucosa was edematous. Rare compact elongated foci of lymphoid cells were located along the vessels.

The tunica muscularis was thin: one-fourth of the gastric wall thickness. The outer longitudinal layer of the membrane was particularly thinned.

The lymphoid cell reaction to experimental exposure depended on the region. In the fold, the total count of cells in a standard section area (880 $\mu^2$) was 19.85±0.85. Lymphocytes were the most numerous here, their sum reaching 27.29% (about one-third of all lymphoid cells), the greater part thereof were minor lymphocytes (20%). An appreciable count of plasma cells appeared in folds under conditions of our experiment; their level reached 11.1±1.2%, mature cells predominating among them (8.99±1.5%, or 1.8±0.31 cells/880 $\mu^2$). The level of plasmoblasts was significantly lower: 2.12±0.82% or 0.40±0.15 cells/880 $\mu^2$. The level of eosinophils in the folds decreased in comparison with that in intact animals and was 7.10±1.06% or 1.40±0.21 cells. An appreciable portion of the lymphoid layer cells were neutrophils (about 10%), approximately equal counts of stab and segmented forms. The level of destroyed lymphoid cells in the mucosal folds was 1.20±0.31 cells/880 $\mu^2$ or 5.95±1.47%. Macrophages were rare. No cells in mitosis were found in the lymphoid layer in the folds; large lymphocytes appeared (2.35±1.18%). More than 36% lymphoid cells in this region were stromal cells.

Traumatic brain injury in rats caused significant changes in the cellular composition of the lymphoid layer on day 7 of experiment. The density of cell distribution between the GM folds reduced in experimental rats to 15.80±0.61 cells/880 $\mu^2$. More than 22% of the cells there were lymphocytes, 15.10±3.09% of these (2.90±0.48 cells/880 $\mu^2$) were minor lymphocytes. The percentage of medium-sized lymphocytes between the GM folds was almost 3-fold less than of minor lymphocytes. The level of plasma cells in the GM between the folds reached 10%, 6.77±1.26% (1.20±0.24 cells) cells were mature and 4.21±1.61 (0.80±0.31 cells) were plasmoblasts. The level of neutrophils was almost the same. Mainly segmented cells were found (7.24±1.57% or 1.30±0.28 cells/880

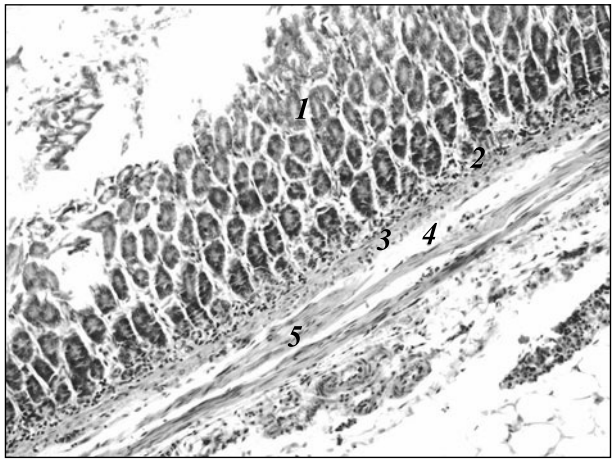



**Fig. 2.** Gastric cardiac wall of Wistar rats on day 7 after traumatic brain injury. Drastic thinning and dissociation of smooth muscle cell bundles in the tunica muscularis layers of the organ. *1*) mucosal lamina propria; *2*) lymphoid cell layer; *3*) mucosal smooth muscle plate; *4*) submucosa; *5*) tunica muscularis of the gastric wall. Hematoxylin and eosin staining, ×160.

$\mu^2$). Stab leukocytes were found 2-fold less frequently. The level of eosinophils in the lymphoid layer of the gastric mucosal lamina propria was 7.17±1.66% or 1.30±0.28 cells/880 $\mu^2$. The levels of destroyed cells were similar.

No macrophages, dividing or poorly differentiated cells (blasts and large lymphocytes) were found. Slight quantitative shifts (decrease) in the levels of minor lymphocytes were paralleled by an increase in the share of medium-sized lymphocytes: their level between the folds increased almost 8-fold. The increase in the level of medium-sized lymphocytes seemed to be due to appearance of numerous plasma cells (more than 11%), not found in intact animals. Quantitative changes in granulocytes were also found in experimental animals. In the folds the percentage of eosinophils decreased almost 2-fold and slightly between the folds; this was paralleled by the appearance of neutrophils in the folds and between them (10% of all cells). The level of lymphoid cells decreased 3-3.5 times in all the studied places of the gastric wall on day 7 after the injury. This led to reduction of cell density per standard section area, but caused no appearance of macrophages.

The submucosa of the gastric wall in experimental animals contained 13.90±1.63 cells/880 $\mu^2$. Of these, 24% were lymphocytes. Minor lymphocytes predominated (15.79±2.04% or 2.1±0.3 cells), while the content of medium-sized lymphocytes was 2-fold less. Numerous plasma cells were found in the submucosa: 9.59±2.87% (1.60±0.57 cells) of plasmocytes and 1.89±0.99% (0.40±0.21 cells) plasmoblasts.

Hence, our study showed that an experimental traumatic brain injury involved pronounced changes in the gastric wall. Poor tone of the wall was paralleled by intensification of destructive processes in the gastric mucosa and microcirculatory disorders, consisting in intense desquamation of the lining epithelium, degradation of lymphoid cells, restructuring and destruction of a part of the glands. Signs of inflammatory process appeared. The formation of numerous antibody-producing cells should be regarded as a response to these processes, caused by cell degradation, and presumably associated with disorders in the organ functioning.

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