Cell Reorganization in Gastric Transplant during Esophagoplasty

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We studied peculiarities of cell reorganization of the gastric transplant during the delayed period after esophagoplasty for benign pathologies of the esophagus. Polymorphism of adaptive and pathological structural rearrangements in the mucosa of the gastric tube was observed. Degenerative and atrophic changes in epithelial structures, hypertrophy of smooth muscular components, and sclerotic reorganization of the stroma were predominating morphological phenomena. Heterogeneity of ultrastructural modifications of the epithelium reflected high secretory activity of foveal mucocytes in combination with degenerative changes in cytoplasmic organelles and impaired secretory function of fundic gland cells. Hypertrophied cells with minor ultrastructural changes, leiomyocytes with signs of biosynthesis, and degenerated smooth muscle fibers were revealed in the population of smooth muscle cells.

Key Words: *esophagoplasty; gastric tube; morphology; ultrastructure*

Reconstructive technologies are now actively used for correction of malignant and benign pathologies of the esophagus. The number of esophagoplastic surgeries increases from year to year. Esophagoplasty with the stomach can be performed from either the whole stomach, or via formation of a gastric tube from the greater curvature with iso- or antiperistaltic orientation. Numerous studies showed that artificial esophagus formed from the gastric tube exhibits the best functional properties and therefore is a preferable method of esophagoplasty [8,13].

The transplant then undergoes structural reorganization, which is determined by the type of reconstructive intervention, morphofunctional peculiarities of the donor organ, abnormal topography of digestive tract organs, and antiphysiological conditions of functioning. The incidence of pathological stages of the artificial esophagus after different replacement surgeries varies in a wide range and constitutes 23-26% [6].

The advances in studies of pathological states of the artificial esophagus are related to the development of a concept on the priority of the morphological substrate. In most studies, macroscopic visualization was used for evaluation of the morphofunctional state of the gastric tube [1,8,12,16]. Reorganization of the transplant at the tissue and cellular levels was studied in only few studies and the results obtained are scanty, contradictory, and fragmentary. The development of inflammatory changes, ischemia, atrophy, fibrosis, ulcers, and neoplastic transformation was reported [4,9, 10,14,15].

Little is known about structural mechanisms underlying these disturbances, which determine the results of esophagoplasty and affect the function of the artificial esophagus. The least studied aspect is ultrastructural modification of cell populations in the gastric tube [5], which is of principal importance for understanding of transplant restructuring induced by its transposition and influence of negative factors under novel conditions. It should be emphasized that pathological processes developing in the transplant with time are closely related to the realization of its adaptive potential. The accumulated data are insuffi-

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cient for making a concept on regularities of structural organization of the gastric transplant after esophagoplasty.

Here we studied the peculiarities of cell organization in the gastric transplant mucosa in the delayed period after esophagoplasty.

MATERIALS AND METHODS

We performed complex morphological examination of the artificial esophagus in 19 men and 27 women (age 20-63 years) after esophagoplasty for benign pathologies of the esophagus. Reconstructive intervention in all cases consisted of extirpation of the damaged esophagus with simultaneous plasty with isoperistaltic gastric tube. For evaluation of the morphofunctional state of artificial esophagus after esophagoplasty (1 month — 8 years after surgery), clinical and morphological monitoring was performed. Mucosa biopsy specimens from visually altered sites of the transplant and esophagogastric anastomosis zone were obtained during endoscopic examination of the gastric tube.

For light microscopy, the mucosa samples were fixed in 10% neutral formalin and processed routinely. Paraffin sections were stained with hematoxylin and eosin in combination with Pearls reaction, after van Gieson with poststaining with resorcin-fuchsin for visualization of elastic fibers, PAS reaction was performed, and Giemsa staining was performed for identification of *H. pylori*. Paraffin sections were examined under a Leica DM 4000B universal microscope, photographed by Leica DFC 320 camera, and processed using Leica QWin software.

For electron microscopy, the tissue fragments were fixed in 4% paraformaldehyde on Millonig buffer (pH 7.2-7.4), postfixed in 1% OsO_4, and embedded in epon and araldite. Semithin sections were stained with 1% azure II and Schiff reagent. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1400 (Jeol) electron microscope at accelerating voltage of 80 kV. Electronograms were prepared using a Veleta camera and iTEM software.

RESULTS

Endoscopic examination showed that the gastric tube had elastic walls with solitary longitudinal folds, smooth glance surface somewhere coated with mucus (Fig. 1, *a*). In case of stenosis of the esophagogastric anastomosis, the degree of narrowing in different patients varied from minor to pronounced, which was often associated with local cicatricial changes in this zone (Fig. 1, *b*). Visually, mosaic erythematous changes (69.6%) or foci of atrophy (28.3%) were found in the transplant mucosa; in some cases, bile reflux into the transplant and symptoms of pylorospasm were observed. No signs of hypotonia and deformities of the gastric tube were revealed.

Light microscopy of transplant biopsy specimens showed extensive atrophic and sclerotic reorganization of the mucosa (76.1%). A peculiarity of these changes was predominant reduction of the glandular layer against the background of preserved architectonics of foveal structures; the latter were deep and screw-shaped. The connective tissue component was characterized by lymphoplasmocytic infiltration of varying intensity, hypertrophy of the smooth muscle components, and enhanced collagen formation in the lamina propria and submucosa.

The cylindrical epithelium lining the mucosa demonstrated high secretory activity, abundant mucoid accumulation was seen in the cytoplasmic matrix of epithelial cells (Fig. 2, *a*). Hypersecretion of the foveal epithelium is probably a compensatory reaction aimed

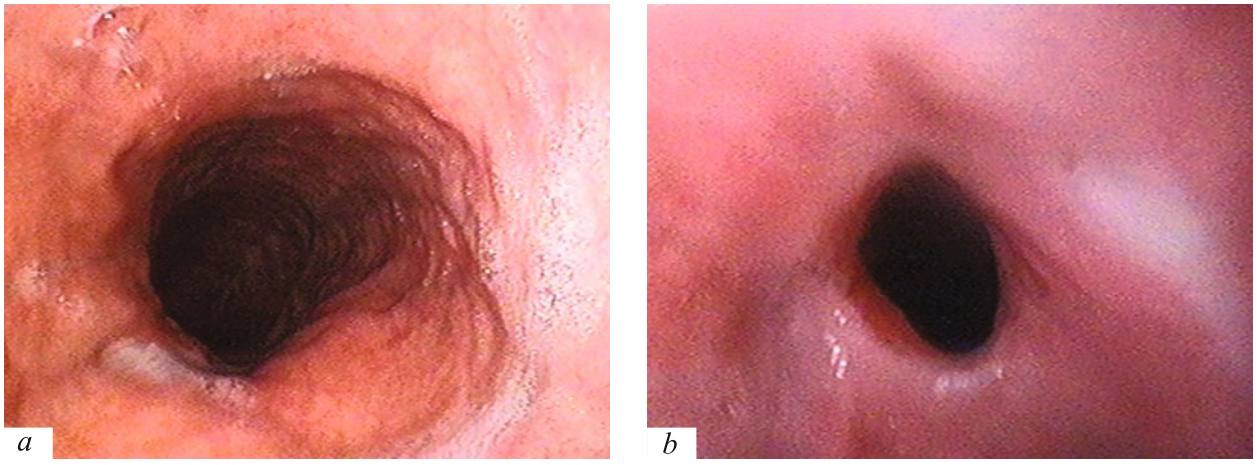

Fig. 1. Artificial esophagus formed from the gastric tube. *a*) elastic mucosa with typical relief; *b*) stenosis of esophagogastric anastomosis, cicatricial changes in the mucosa.

at strengthening of the cytoprotective potential of the transplant mucosa under antiphysiological conditions.

In some specimens, degenerative changes of surface epithelium were noted; they manifested in focal disintegration of epithelial cells, blurred cell contours, and pronounced vacuolation of the cytoplasm in some epitheliocytes (Fig. 2, *b*). The height of the cell layer somewhere tended to decrease, the content of PAS-positive secretion in mucocytes decreased, and small desquamation foci appeared. An important role in these changes is played by bile reflux associated with long-term contact of the epithelium with aggressive components of the refluxate, often in combination with other damaging factors of the luminal content [6.11].

Bacterioscopy detected *H. pylori* in 37% artificial esophagus biopsy specimens. Bacterial contamination of the gastric tube was realized on the fundic gastric mucosa, but no cell differentiation disorders with transformation into intestinal metaplasia or dysplasia were found. At the same time, the degree of degeneration of the epithelium and glands increased. Ultrastructure of mucosytes was characterized by reduced secretory compartment of the cytoplasm and heterogeneity of mucoid granules. In the stroma, hyperplasia of lymphoid follicles, accumulation of inflammatory elements in the infiltration, and enlargement of transepithelial leukodiapedesis foci were noted. It can be hypothesized that the persistence of *H. pylori* in the gastric tube can lead to depression of cytoprotective mechanisms and promote the development of mucosa atrophy [2].

In samples from the esophagogastric anastomosis zone, a tendency to hyperplasia of the squamous epithelium of the esophagus with signs of moderate acanthosis was observed. In some specimens, a considerable part of epithelial cells underwent vacuolar degeneration, intercellular spaces were somewhere widened and contained solitary intraepithelial lymphocytes.

Analysis of the structure of fundic glands in the gastric tube revealed signs of atrophy of varying severity in 66.7% specimens. Light microscopy revealed a decrease in the number and shortening of gland profiles against the background of thickening of intersti-

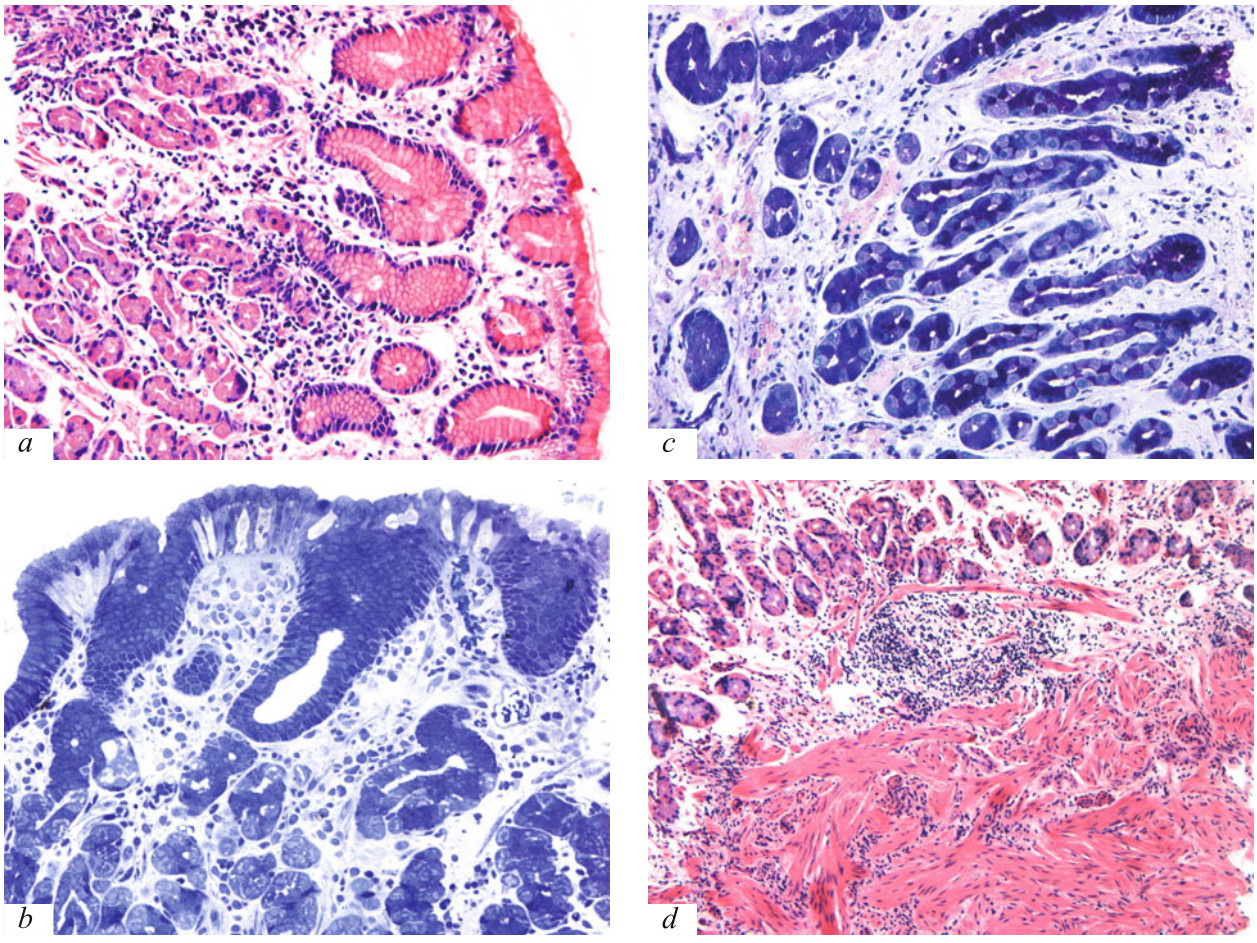

Fig. 2. Reorganization of the gastric transplant mucosa after esophagoplasty. *a*) hypersecretion of the surface epithelium, atrophy of fundic glands; *b*) degeneration of surface epithelium and glands, lymphoplasmocytic infiltration of the stroma; *c*) atrophy and pyloric metaplasia of glandular epithelium; *d*) hypertrophy of mucosae lamina muscularis. *a*,*d*) hematoxylin and eosin staining; *b*) semithin section, azure II staining; *c*) PAS reaction, ×200.

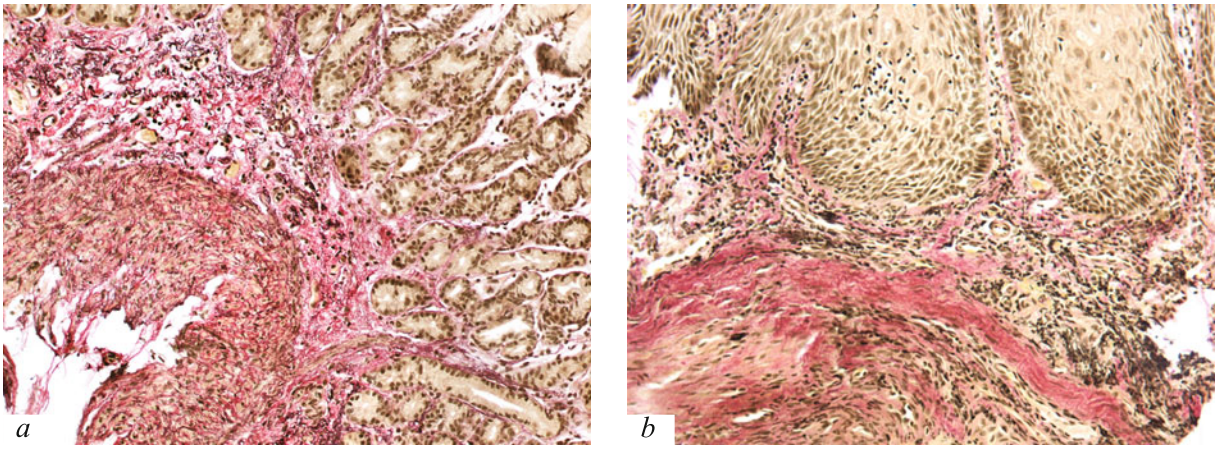

Fig. 3. Sclerotic reorganization of artificial esophagus mucosa formed from the gastric tube. *a*) gastric transplant; *b*) esophagogastric anastomosis. van Gieson staining, ×200.

tial layers; some acini underwent cystic deformation. Degenerative chief, parietal, and accessory glandular cells constitute epithelial lining of the secretory compartments (Fig. 2, *c*). The phenomenon of polarization of glands (substitution of highly specified glandular cells by hyperplastic accessory cells) was found in 42.9% specimens.

Heterogeneity of the cell reaction of the connective tissue stroma in the gastric tube mucosa was revealed. Pronounced focal lymphoplasmocytic infiltration of the foveal layer was found in some specimens, while in others diffuse spreading of the infiltration containing lymphoid and plasma cells, solitary macrophages, and mast cells was observed. In some preparations, the admixture of eosinophilic and neutrophilic leukocytes and foci of edema and uneven capillary plethora were detected.

In most specimens, visible lamina muscularis mucosa was thickened and sclerotic; it was formed by large leiomyocytes and some smooth muscle cells penetrated the lamina propria (Fig. 2, *d*). Signs of intensive collagen production with hyperelastosis and perivascular sclerosis foci were found. Sometimes, a fibrous connective tissue layer appeared between the lamina muscularis mucosa and glandular layer (Fig. 3, *a*). Sclerotic reorganization of the subepithelial stroma was also observed in specimens from the esophagogastric anastomosis zone (Fig. 3, *b*).

Sclerotic reorganization of the gastric tube mucosa is closely related to microcirculatory disturbances and local tissue ischemia [7], which are most crucial during functional strain in the esophagogastric anastomosis zone. Of particular importance for the gastric transplant is the phenomenon of regenerative hypertrophy of smooth muscle cells induced at the stage of its formation, which can lead to considerable enlargement of smooth muscle cells in the mucosa and can be accompanied by the formation of collagen and elastic fibrils [3].

Ultrastructural analysis of biopsy specimens from the gastric transplant revealed changes in intracellular organization of the epithelium largely determined by modulation of its secretory function. Ultrastructural modifications of epithelial cells in the foveal layer determined to the realization of the mucosa cytoprotective potential differed from those in glandular cells responsible for specific gastric secretion.

Mucocytes of the surface epithelium were characterized by polar distribution of cytoplasmic organelles and demonstrated signs of high secretory activity. Numerous mucoid granules little varying by their electron density were concentrated in cell cytoplasm (Fig. 4, *a*). In degenerated epithelial cells, widened cytoplasmic network channels and swelled mitochondria were detected. Against the background of marked alteration, dilatation and fragmentation of membrane organelles and partial lysis of mitochondria were observed, the number of mucoid granules was reduced (Fig. 4, *b*).

Electron microscopy revealed heterogeneity of ultrastructural modification in the epithelium of fundic glands reflecting peculiarities of intracellular organization and secretory function of glandular cells. Accessory cells were characterized by marked polarity and abundance of secretory granules in the apical cytoplasm. In the population of chief cells, cells with typical ultrastructure, high content of zymogen granules, large elements of the Golgi complex, and high density of cisterns of the granular cytoplasmic network were detected. At the same time, chief cells with signs of disturbed secretion containing only few zymogen granules in the cytoplasm were found; their cytoplasm contained also fine granular secretory vesicles, solitary lipid vesicles, and large autophagosomes (Fig. 4, *c*).

Most parietal cells had some signs of functionally immature glandular cells: poorly developed intracellular channel elements, low tubulovesicular activity,

osmiophilic lamellae in obliterated lumens. The cytoplasmic matrix contained numerous mitochondria with dense matrix and poorly discernible cristae; large vacuoles and multivesicular bodies were often seen (Fig. 4, *d*).

The stroma of the gastric transplant mucosa was characterized by excessive growth of collagen fibrils; fibroblasts with well developed biosynthesis organelles were found (Fig. 5, *a*). In the population of smooth muscle cells, hypertrophic leiomyocytes with little changed ultrastructure, hyperplasia of dense bodies, and cytoplasm evenly saturated with myofilaments were observed (Fig. 5, *b*). Some leiomyocytes were characterized by reduction of myofilaments, but contained well developed elements of the Golgi complex, clusters of large mitochondria, vesicles and vacuoles (Fig. 5, *c*). Sometimes, degenerated smooth muscle cells with irregular shrunk contours and osmiophilic poorly differentiated cytoplasm were seen between thickened collagen bundles (Fig. 5, *d*).

Thus, our study showed that the gastric tube transplant underwent considerable cellular reorganization. The main morphological characteristics of the mucosa are degeneration and hypersecretion of the surface epithelium, atrophy of fundic glands, hypertrophy of smooth muscle components, lymphoplasmocytic infiltration, and sclerosis of the stroma. Cell populations of the gastric transplant are characterized by heterogeneity of ultrastructural modifications. Ultrastructure of the epithelium reflects high cytoprotective function of foveal layer mucocytes, disordered secretion of the acid and peptides by glandular cells, degenerative changes in cytoplasmic organelles of epithelial cells. Hypertrophied cells with minor ultrastructural changes predominated in the population of smooth muscle cells, leiomyocytes with signs of intracellular biosynthesis were also found, degenerated smooth muscle fibers were revealed in zones of pronounced sclerosis.

Cell reorganization of the gastric tube mucosa represents a complex of adaptive and pathological reactions, which can be determined by both reconstructive intervention and novel conditions of transplant functioning implying the influence of some aggressive and pathogenic factors. Among a variety of structural

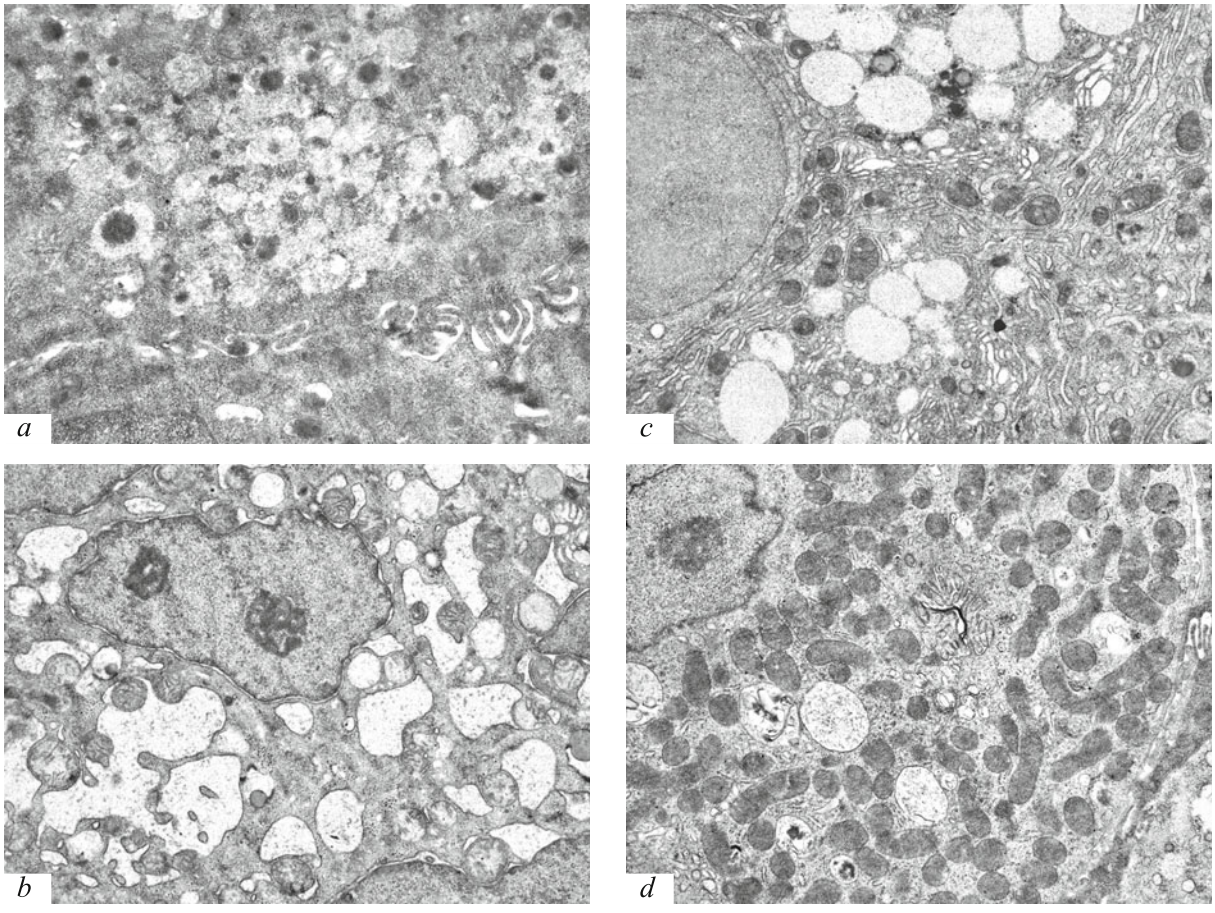

Fig. 4. Ultrastructural modifications of gastric transplant epithelium. *a*) abundant secretory granules in mucocyte cytoplasm, dilatation of intracellular spaces, ×8000; *b*) vacuolation of cytoplasmic network, swelling and lysis of mitochondria in epithelial cell, ×5000; *c*) solitary zymogen granules and large autophagosomes in chief cell cytoplasm, ×8000; *d*) large vacuoles, residual bodies, and osmiophilic lamellae in channel lumen of a parietal cell, ×10,000.

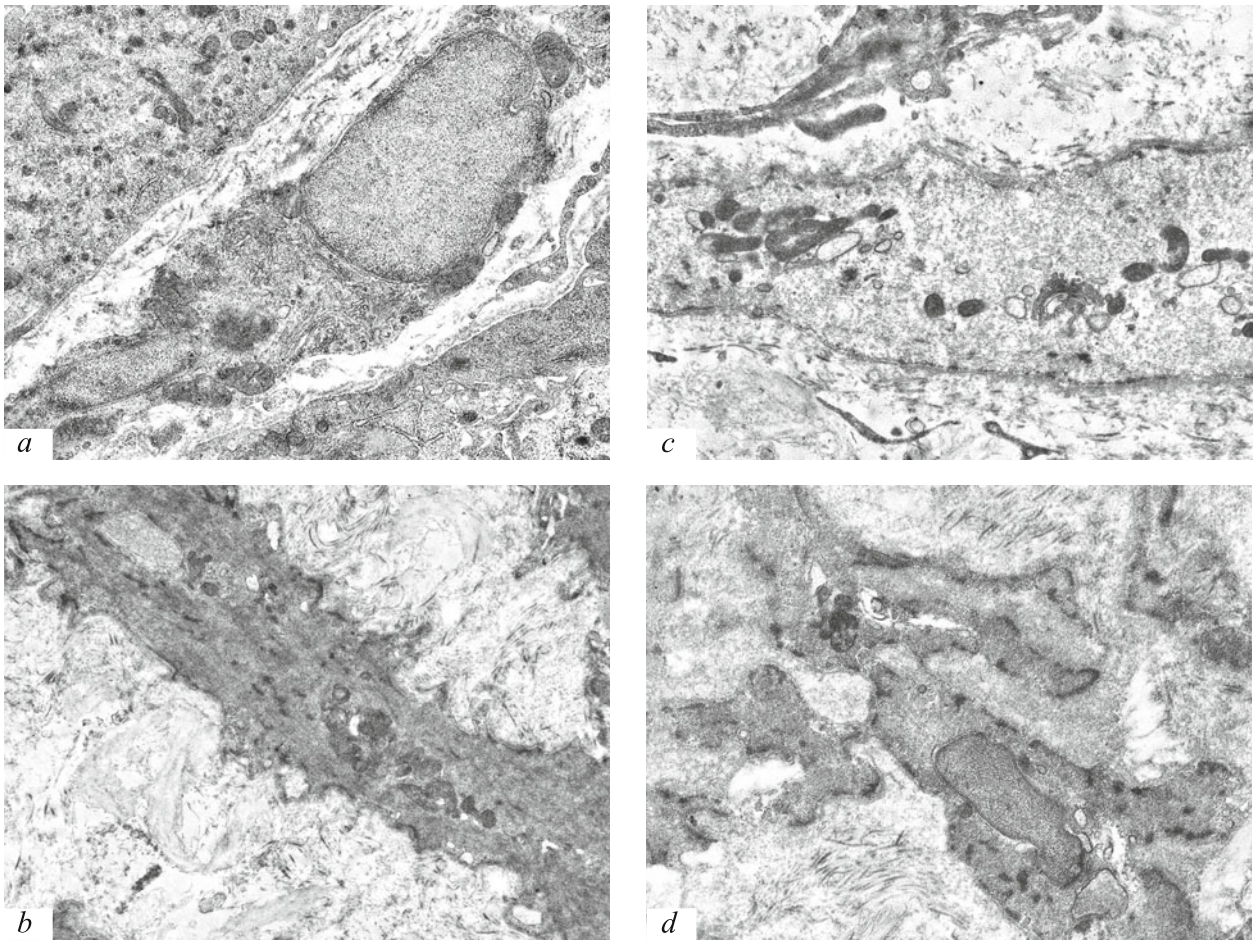

Fig. 5. Ultrastructural modifications of connective tissue cells of the gastric transplant. *a*) fibroblast-like cells with well developed biosynthetic organelles, ×8000; *b*) hyperplasia of dense bodies and a group of polymorphous mitochondria in leiomyocyte cytoplasm, ×8000; *c*) large Golgi complex, clusters of mitochondria and vacuoles, reduction of myofilaments in leiomyocyte cytoplasm, ×10,000; *d*) leiomyocytes with signs of degeneration are surrounded by collagen fiber bundles, ×8000.

modifications detected in biopsy specimens of the gastric transplant, the most significant morphological phenomena associated with morphofunctional disturbances of the artificial esophagus during the delayed period after esophagoplasty are degenerative changes in the epithelium and sclerotic reorganization of the connective tissue compartment of the gastric tube mucosa.

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