Ultrastructural Study of Cell Populations in the Gastric Transplant after Esophagoplasty

G. A. Lapii, L. M. Nepomnyashchikh, I. E. Sudovykh, A. V. Kutepov, and R. V. Govorkov

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Ultrastructural characteristics of cell populations in the gastric tube mucosa were studied after plastic surgery for benign esophageal disease. The foveolar epithelium mainly consisted of secretory active mucocytes with small changes in cytoplasmic organelles. Functionally immature parietal cells and gland cells in the epithelium of fundal glands were characterized by atrophy and degeneration. Ultrastructural features of the epithelial compartment attest to adaptive cellular hypersecretion of the mucoid against the background of impaired acid production and enzyme secretion. The stromal compartment was characterized by low pinocytotic activity of endothelial cells in blood vessels and polymorphism of smooth muscle cells. Hypertrophic leiomyocytes were shown to dominate in the population of these cells. Some cells had signs of biosynthesis and reduction of filaments. Single leiomyocytes were degenerated. The observed ultrastructural modification of epithelial and connective cells can be interpreted as structural adaptation of the gastric transplant.

Key Words: *esophagoplasty; gastric tube; epithelium; leiomyocytes; ultrastructure*

The introduction of various methods for reconstructive esophagoplasty necessitates complex study of implant reconstruction, which determines normal function of the graft under new physiological conditions. Extirpation of the esophagus and plastic surgery with the isoperistaltic gastric tube are preferred among a variety of methods for reconstruction in benign esophageal disease [10-12]. Studying the delayed consequences of esophagogastroplasty revealed a high risk for the development and progression of pathological changes (despite high functional activity of the gastric tube) [4,5,8,13].

The majority of morphofunctional studies with artificial esophagus were performed by means of macroscopic visualization [6,7,9,15]. Little attention was paid to the progression of tissue and cellular modifications in the gastric transplant [1,3,14]. Our previous studies showed that a variety of structural modifications in the chronic transplant reflect not only the adaptive capacity, but also abnormal changes in the graft (atrophy of the mucous membrane) [2]. Little is known about ultrastructural mechanisms of these transformations. Therefore, they require a detailed study. The process of intracellular reorganization is a key stage in structural adaptation of the transplant. This process is directed toward the maintenance of normal functions under new conditions that often suggest the influence of adverse factors.

Here we studied ultrastructural characteristics of cell populations in the gastric transplant during the delayed period after esophagoplasty.

MATERIALS AND METHODS

Ultrastructural study of the mucosa in an artificial esophagus (from the gastric tube) was performed in 12 men and 11 women (27-60 years). Esophagoplasty

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. G. A. Lapii

was conducted for benign diseases (cicatricial stricture of the esophagus due to chemical burn, 12 patients; grade III-IV cardiac achalasia, 10 patients; and peptic stricture in esophageal reflux disease, 1 patient). Fibroesophagoscopy and targeted excisional biopsy were performed 1-7 months after reconstruction. Electron microscopy of 34 samples of the mucous membrane from the gastric transplant was performed.

Tissue fragments (1 mm^3) were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, routinely processed, and embedded into a mixture of Epon 812 and araldite M. Semithin (1 μ) and ultrathin sections were prepared on a LKB-III microtome. Semithin sections were stained (in a dropwise manner) with 1% azure II. PAS reaction was carried out. The sections were repeatedly stained with azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate (Reynolds method) and examined under a JEM-1400 electron microscope (Jeol, accelerating potential 80 kV). The images were photographed using a Veleta digital camera (iTEM software, Olympus).

RESULTS

Electron microscopy of biopsy specimens from the gastric transplant mucosa was performed in delayed period after esophagoplasty. Changes in the intracellular organization of the epithelium were mainly related to secretory dysfunction. Ultrastructural modification of epitheliocytes in the foveolar layer (determined for the protective capacity of the mucous membrane) differed from that of gland cells in the glandular layer (determined for the specific gastric secretion). Considerable heterogeneity of these changes was shown to contribute to the mosaic pattern of ultrastructural signs. It was particularly pronounced in biopsy specimens with severe atrophy of the mucous membrane.

Monomorphic secretory cells with high functional activity were visualized in the surface foveolar epithelium of all samples. The mucocytes were characterized by a cylindrical configuration and polar distribution of cytoplasmic organelles. The apical plasmalemma of cells was often smoothed due to reduction of newly formed short microvilli. The intercellular space in the basal epithelium was slightly dilated due to edema.

The cytoplasmic matrix of mucocytes contained numerous and closely located granules of mucoid. The granules varied in electron density and were often conglomerated. Narrow profiles of the rough cytoplasmic reticulum, elements of the Golgi complex, and round mitochondria with short cristae were seen in the granule-free perinuclear and subnuclear space. Some cells of the epithelial layer were altered and differed in the widening and fragmentation of membrane organelles, mitochondrial swelling, partial lysis of the matrix, disorganization of cristae, and low number of secretory granules.

Significant heterogeneity of ultrastructural modifications was observed in the epithelial population of fundal glands in the gastric transplant. These signs reflected specific structural features of cells, differences in secretory function, and atrophic and degenerative changes in cytoplasmic organelles. Independently on the degree of mucosal atrophy, the majority of accessory gland cells were characterized by polarity of intracellular organization. It was manifested in the basal location of oval nuclei and presence of a considerable number of secretory granules in the apical part of the cytoplasm (Fig. 1, *a*). Polymorphic mucoid granules were found in the secretory compartment of cells. Optically dense loci were revealed in these granules. Small amounts of a flaky substrate were found in cisterns of the cytoplasmic reticulum.

Examination of chief cells showed that some cells of the glands retain normal ultrastructure. They were characterized by medium content of electron-transparent zymogenic granules, large elements of the Golgi complex, considerable number of cisterns of the rough cytoplasmic reticulum, and mitochondria located between them. A considerable number of chief cells had the signs of reduced secretion. The cytoplasm of these cells included a small number of zymogenic granules (despite the well-developed organelles of biosynthesis). Individual lipid vesicles, fine-grained secretory vesicles, and polymorphic lysosomes were also identified in the cell cytoplasm (Fig. 1, *b*).

Parietal cells had signs of partially differentiated and functionally immature gland cells. Electron microscopy revealed poorly-developed intracellular tubule structures and low tubulovesicular activity. Few vesicles were found in the pericanalicular region of the cytoplasm. Osmiophilic lamellae were often revealed in the obliterated lumen (Fig. 1, *c*). A considerable number of mitochondria with dense matrix and hardly distinguishable narrow cristae were seen in the cytoplasmic matrix of parietal cells, large vacuoles and multivesicular bodies were often present. Small areas of local cytoplasmic lysis were found in some cells.

Single endocrine cells were present in the epithelium of fundal glands. These signs reflected the incretory secretion in the mucous membrane of the gastric transplant. The ultrastructure of endocrine cells was characterized by the presence of well-developed elements of the Golgi complex. They were located in the supranuclear compartment of the cytoplasm. A considerable number of electron-dense small secretory granules were accumulated in the basal compartment (Fig. 1, *d*). Endocrine cells were often in contact with parietal cells. These features illustrate close relationship between these cells in the intercellular regulatory

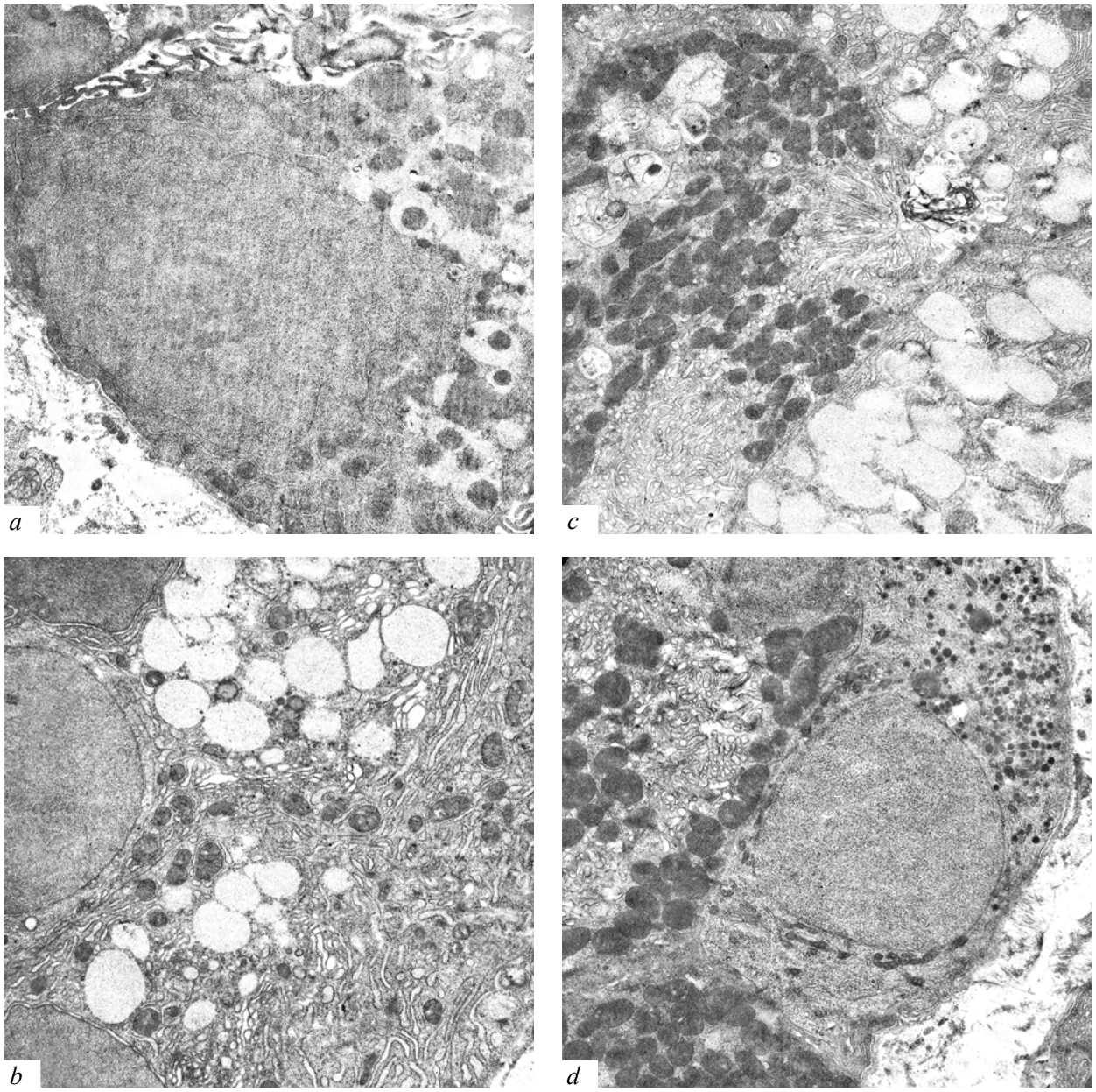

Fig. 1. Ultrastructural characteristics of the epithelium in the gastric transplant after esophagoplasty. Accessory cell with heterogeneous secretory granules; dilation of the intercellular spaces (*a*, ×8000). Chief cells with the reduced number of zymogenic granules and large phagolysosomes (*b*, ×8000). Parietal cell with residual bodies; myelin-like lamellae in the lumen (*c*, ×10,000). Endocrine cell with secretory granules (in the basal compartment); junctions with parietal cells (*d*, ×10,000).

interaction. This interaction is of particular importance under conditions of abnormal innervation due to mobilization of the transplant.

Signs of collagen production were revealed in the proper mucous membrane. Numerous collagen fibrils were shown to penetrate the stroma in various directions. Small groups of connective tissue cells were identified between these fibrils (Fig. 2, *a*). The visualized microvessels had widened walls. The majority of endothelial cells were characterized by low micropinocytotic activity. Several mitochondria with focal clearing of the matrix, lysosomes, and single microtubules were found in the perinuclear region of the cytoplasm. The profile of the luminal and basal plasmalemma was formed by small folds and invaginations (Fig. 2, *b*).

A specific feature of the stromal compartment was an increase in the number of leiomyocytes, which probably resulted from regenerative hypertrophy of smooth muscle cells in the mucous membrane of the transplant due to reconstructive treatment. It should be emphasized that the population of smooth muscle cells was polymorphic. We revealed a considerable number

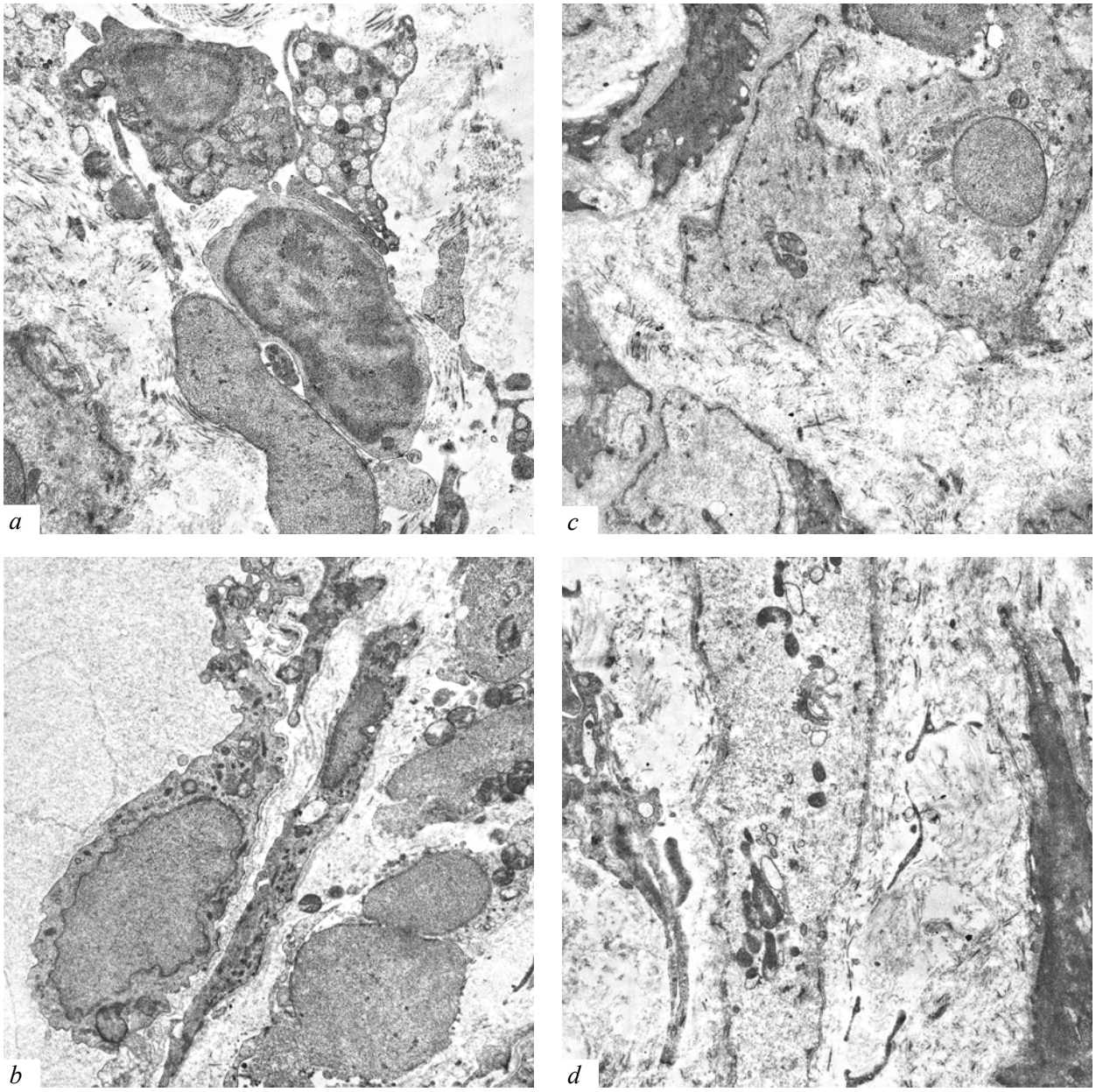

Fig. 2. Ultrastructural characteristics of connective tissue cells in the gastric transplant after esophagoplasty. Cluster of connective tissue cells and surrounding collagen fibrils (*a*, ×8000). Fragment of the microvessel; swollen mitochondria and polymorphic lysosomes in the endotheliocyte; cluster of hypertrophic leiomyocytes in the perivascular region (*b*, ×8000). Leiomyocytes with a considerable number of filaments, hyperplasia of dense bodies, and well-developed Golgi complex (*c*, ×8000). Leiomyocytes with a large Golgi complex, clusters of mitochondria and vacuoles, and reduction of filaments (*d*, ×10,000).

of hypertrophic leiomyocytes with a filament-enriched cytoplasm, high content of mitochondria, and hyperplasia of dense bodies (underlying the cytolemma; Fig. 2, *c*).

The other leiomyocytes were characterized by well-developed elements of the Golgi complex, clusters of large mitochondria, vesicles, and vacuoles, and reduced number of filaments. Hence, the cytoplasmic matrix was locally or diffusely lightened (Fig. 2, *d*). Degenerated smooth muscle cells were rarely found. They were characterized by wrinkled shape and poorly-structured osmiophilic cytoplasm. Thick bundles of collagen fibers were positioned around these cells. Progressive sclerosis in the mucosa of the gastric tube probably contributes to atrophy and degeneration of hypertrophic leiomyocytes.

Our results indicate that esophagoplasty is followed by ultrastructural changes in epithelial and stromal cells of the mucous membrane of the gastric transplant. The increased formation of cellular mucoid,

disturbances in acid secretion, and abnormalities of enzyme production are the major signs of intracellular reorganization of the epithelial compartment. Mucocytes of high secretory activity were shown to prevail in the foveolar epithelium. Cytoplasmic organelles are preserved in these cells. The functionally immature parietal cells and gland cells in the glandular epithelium are characterized by atrophy and degeneration. An excess growth of collagen fibrils, low pinocytotic activity of endotheliocytes in blood capillaries, and increased number of smooth muscle cells are typical of the connective tissue compartment. Hypertrophic leiomyocytes were shown to dominate among smooth muscle cells. Some cells have the signs of biosynthesis and reduction of filaments. Single leiomyocytes are degenerated.

Ultrastructural modification of the gastric transplant is related to the adaptive and compensatory response. This response is directed toward an increase in the adaptive capacity of the mucous membrane due to foveolar secretion [3]. The intracellular features of the glandular epithelium and transformation of stromal cells are mainly related to reconstructive treatment. It is associated with gastric tissue damage and partial injury to the nervous and microcirculatory pathways [4,5]. The inappropriate degree of these changes can play a pathological role, which modulates functional activity of an artificial esophagus.

REFERENCES

1. L. N. Inshakov, I. G. Matsevich, L. M. Asanina, *et al*., *Vestn. Khir*., No. 2, 13-16 (1999).
2. G. A. Lapii, L. M. Nepomnyashchikh, I. E. Sudovykh, and A. V. Kutepov, *Byull. Eksp. Biol. Med*., **149**, No. 5, 584-588 (2010).
3. G. A. Lapii, Yu. V. Chikinev, I. E. Sudovykh, *et al*., *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, **134**, No. 6, 119-124 (2008).
4. F. A. Chernousov, D. V. Ruchkin, and A. V. Tavadov, *Khirurgiya*, No. 12, 60-64 (2008).
5. J. S. Donington, *Thorac. Surg. Clin*., **16**, No. 1, 53-62 (2006).
6. J. H. Fu, *Zhonghua Wei Chang Wai Ke Za Zhi*, **11**, No. 1, 8-10 (2008).
7. N. M. Gupta and R. Gupta, *Ann. Surg*., **239**, No. 3, 359-363 (2004).
8. E. J. Hazebroek, F. W. Hazebroek, S. Leibman, and G. S. Smith, *Pediatr. Surg. Int*., **24**, No. 7, 869-871 (2008).
9. H. K. Kim, Y. H. Choi, J. H. Shim, *et al*., *World J. Surg*., **32**, No. 9, 2010-2014 (2008).
10. H. Makuuchi, *Nippon Geka Gakkai Zasshi*, **109**, No. 5, 256-260 (2008).
11. A. Matsuki, T. Kanda, S. Kosugi, *et al*., *Surg. Today*, **39**, No. 3, 261-264 (2009).
12. M. Nakajima, H. Kato, T. Miyazaki, *et al*., *Hepatogastroenterology*, **54**, No. 73, 104-110 (2007).
13. A. R. Ruiz-Elizalde, M. J. Haley, J. C. Fisher, *et al*., *J. Pediatr. Surg*., **44**, No. 7, 1440-1442 (2009).
14. M. Samuel, D. M. Burge, and I. E. Moore, *ANZ J. Surg*., **71**, No. 1, 56-61 (2001).
15. V. A. Williams, T. J. Watson, S. Zhovtis, *et al*., *Surg. Endosc*., **22**, No. 6, 1470-1476 (2008).