Ultrastructural Changes in Colorectal Mucosa after Chronic Contact with Urine

A. B. Stroganov[1], E. I. Yakovleva[2], V. A. Atduev[2,3], M. V. Rakhcheeva[2], V. V. Shutov[4], Ch. S. Zakirov[1], and I. A. Samsonov[1]

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Ultrastructural changes in the colorectal mucosa in response to chronic contact with the urine were demonstrated in outbred albino rat experiments. Oral correction with slightly alkaline sodium hydrocarbonate solution reduced the destructive effect of the urine on rat colorectal mucosa.

Key Words: *colorectal mucosa; electron microscopy*

Several variants of the urine diversion into the large intestine or its isolated segment are widely used in clinical practice. The mechanism of intestinal wall adaptation to chronic contact with the urine remains unclear. It was found that these operations could be followed by disorders of different severity in the blood acid base and electrolyte balance because of absorption of urine elements by the intestinal epithelium and mucus release with different intensity [3,6,10,11]. In addition, opinions on the type and terms of changes in the colorectal mucosa after chronic contact with the urine vary. The muscular, vascular, and nervous system of the intestinal wall is retained during the early period after intestinal plasty of the urinary bladder. Solitary dividing epitheliocytes virtually without microvilli, numerous necrotic epitheliocytes, and a moderate number of goblet cells with conglomeration of mucous granules are detected in the mucosa during this period. The degenerative processes progress 5-6 years after sigmocystoplasty, presenting by restructuring of all components of the mucosa with interstitial fibrosis, smooth muscle cell and nerve fibril atrophy, reduction of vascularization, and epithelial lining degeneration.

Despite long contact with the urine, no metaplasia of the intestinal epithelium into transitional urothelium takes place. However, the development of malignant tumors in the mucosa exposed to the urine was registered in some cases, 15 (0.3-54) years after the intervention, on average [2,9]. Tumor relapses most often develop in zones of the uretero-reservoir anastomoses. The pathogenesis of these tumors is poorly studied. It is assumed that the development of these tumors results from combined carcinogenic effect on the mucosal cells in the anastomosis zones [9]. Other authors think that cell mutation during healing in the presence of carcinogenic substances is responsible for the development of epithelial tumors in the anastomosis zone, as well as other factors, such as bacteriuria, use of different suturing material, *etc.* [5]. Contact with the urine potentiates adaptive reaction of the intestinal epithelium because of uncommon novel conditions of its functioning; the absorption capacity of the colorectal epithelium does not lead to coarse disorders of the homeostasis [3,4,7]. However, the mechanisms of adaptation of mucosa cell in the large intestine, used for urine diversion, remain little studied.

We studied morphofunctional adaptation changes in the large intestinal mucosa after chronic contact

[1]Institute of Federal Safety Service of Russia, Nizhny Novgorod; [2]Nizhny Novgorod State Medical Academy; [3]Volga Regional Medical Center, Federal Biomedical Agency of Russia, Nizhny Novgorod; [4]N. A. Semashko Regional Clinical Hospital, Moscow, Russia. ***Address for correspondence:*** andrey_stroganov@rambler.ru. A. B. Stroganov

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with the urine in experimental rats at the ultrastructural level.

MATERIALS AND METHODS

We created a biological model on small laboratory animals (albino rats; innovation No. 1957 of March, 4, 2008) simulating situation after diversion of the urine into the sigmorectal compartment by the Mainz pouch II method. After this operation, the urine flows through the ureters into a newly formed intestinal reservoir and after 3-5 h is evacuated during defecation.

No surgical intervention was needed for creation of a long contact of the urine with the intestinal sigmorectal mucosa in rats.

Experiments were carried out with consideration for "Regulations for Studies on Laboratory Animals" on 60 outbred laboratory male albino rats (209.0±2.6 g). The animals were divided into 4 groups, 15 per group: 1) intact controls; 2) controls receiving saline rectally; 3) rectal injection of the urine; and 4) rectal injection of the urine in parallel with permanent oral correction with slightly alkaline sodium hydrocarbonate solution. The urine or saline (1.0 ml) was injected rectally twice a day with a special device (microirrigator; innovation No. 1955 of March 4, 2008). The injected urine was preliminary tested for acid ionic composition: mean circadian levels of potassium 8.45±0.01 g/liter, sodium 83.03±0.01 mg/liter, calcium 3.3±0.02 g/liter, and acidity 6.1±0.19. The maximum duration of observation was 5 months.

The preparations for the analysis were collected routinely. The colorectal wall was dissected circularly 3 cm proximally from the anus after collection of a biopsy specimen (1.0×1.0×1.3 mm) for electron microscopy from the edge contralateral to the mesentery. Biopsy specimens were fixed in 2.5% glutaraldehyde solution and postfixed in 1% osmic acid, dehydrated in ascending alcohols, and embedded in epon 812 and araldite mixture [1]. Ultrathin sections were sliced on an Ultracut ultratome (Reichert-jung) and examined under a Morgagni 268D electron microscope (FEI). The preparations for optic microscopy were made by the standard methods, stained with hematoxylin and eosin and by van Gieson method. Morphometry was performed using AnalySIS software. The results were statistically processed using Stat*Ex*-2004.2 and Excel software.

RESULTS

The histoarchitectonics of all layers was preserved in all groups after 1, 3, and 5 months of contact with the urine. The time course of changes in the intestinal wall in groups 3 and 4 was presented by mucosal hypertrophy during the early period (just 1 month after exposure to the urine), transforming into stable atrophy later (after 3 and 5 months). Optic microscopy showed atrophic processes: thinning of the colorectal mucosa, lesser depth and thickness of crypts (these changes were more pronounced in group 3). In none groups, metaplasia of the intestinal epithelium into a multilamellar squamous epithelium was observed after 5-month contact with the urine.

Electron microscopy showed that microvilli on the apical surface of epitheliocytes were distributed evenly in control groups 1 and 2, their matrix containing fibrillar structures passing into the cell cytoplasm. Ultrastructure of the mucosa was characterized by the presence of numerous tissue basophils containing granules in cryptic epithelium. The nuclei were compact, round or oval. Some tissue basophils were in the state of partial degranulation (Fig. 1, *a*, *b*). The length

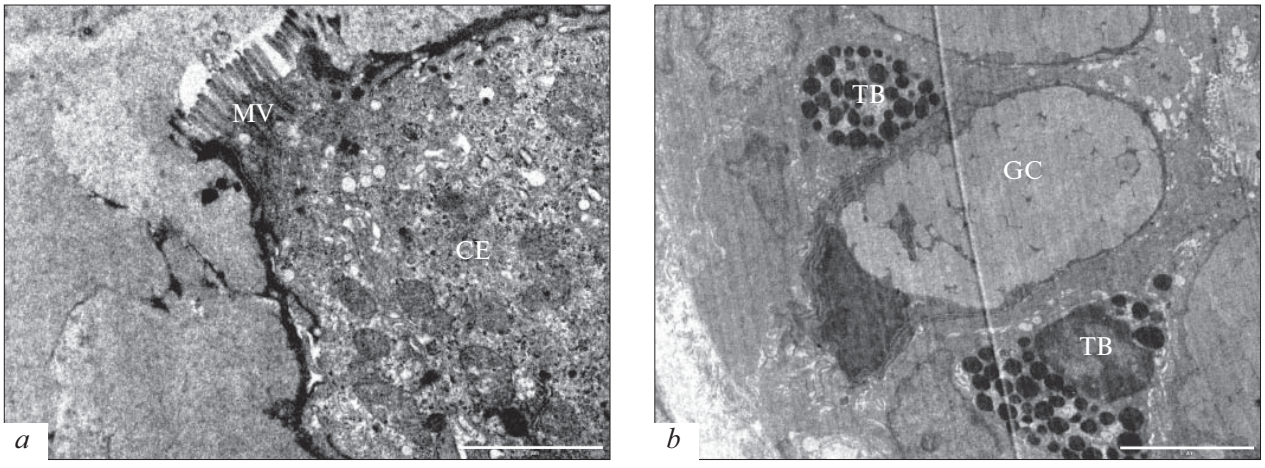

Fig. 1. Ultrastructure of colorectal mucosa in control groups. *a*) cryptic lumen and apical part of a columnar epitheliocyte with microvilli, mitochondria, and secretory vacuoles, ×11,000; *b*) tissue basophil, ×5600. Here and in Figs. 2, 3: MV: microvilli; GC: goblet cell; CE: columnar epitheliocyte; TB: tissue basophil; ES: eosinophil; MtC: mast cell; MC: mitochondria; SV: secretory vacuoles.

of columnar epitheliocyte microvilli was 727.52±15.56 nm.

After 5-month contact with the urine the following ultrastructural changes in the mucosa were detected in group 3: alteration of the surface epithelium (cell desquamation and necrosis) and goblet cells hypersecretion (Fig. 2, *a*, *b*). The mucosa in the majority of crypts was degenerative.

The content and size of secretory vacuoles in the columnar epitheliocytes increased; vacuoles fused and formed large vacuoles. The cell ultrastructure also changed: Golgi complex was reduced, cisterns of the granular endoplasmic reticulum were fragmented, their diameters unevenly dilated; necrotic epitheliocytes were seen (Fig. 2, *d*). Tight junctions in the apical part of columnar epitheliocytes were impaired in some cases. Detritus was seen in the lumen of some glands, in some cases with fragments of microvilli (Fig. 2, *a*). Crypt lumens were often filled by unevenly distributed secretion of goblet cells in the state of hypersecretion (stimulation of the Golgi complex and increased diameters of the granular endoplasmic reticulum cisterns). The granules fused in some cells, and dilated goblet cells compressed the columnar epitheliocytes. Proliferation of poorly differentiated cells was observed. Dilated cell-cell spaces were seen in the basal parts of the crypts. The counts of tissue basophils significantly decreased compared to the control, and they were in a state of partial or complete degranulation. A considerable number of lymphocytes were detected in the epithelium. Accumulation of lymphoid cells was seen in the lymph capillaries of the lamina propria. The lamina propria of the mucosa was characterized by microcirculatory disorders presenting by changed status of the blood and erythrocyte aggregation, swelling of endothelial nucleus, formation of endothelial cell protrusions. Pronounced infiltration of eosinophils (Fig. 2, *c*), presence of macrophages, mast cells, monocytes, fibroblasts, and necrotic mass (Fig. 2, *d*) were seen. Reduction of the microvilli and significant shortening of their height to 572.24±28.28 nm in comparison with the control groups (727.52±15.56 nm; t=4.81, p<0.05) were noted. Ultrastructural changes in the mucosa were also observed in group 4, but cell

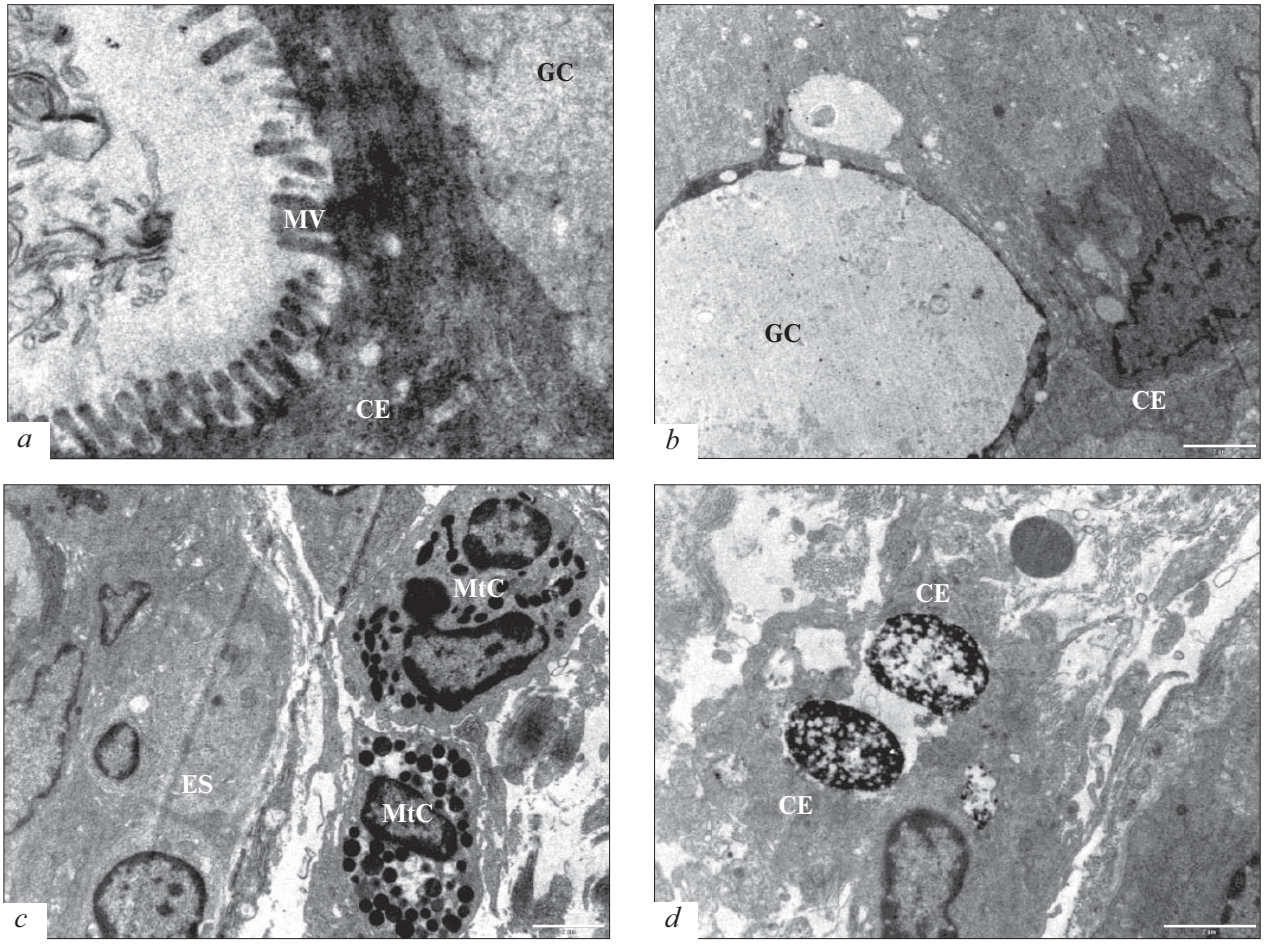

Fig. 2. Ultrastructure of colorectal mucosa in experimental group 3. *a*) reduction of columnar epitheliocyte microvilli and detritus in cryptic lumen, ×17,000; *b*) goblet cells, 5600; *c*) eosinophil and mast cell, 5600; *d*) necrosis of columnar epitheliocytes and an erythrocyte outside the vessel, ×7100.

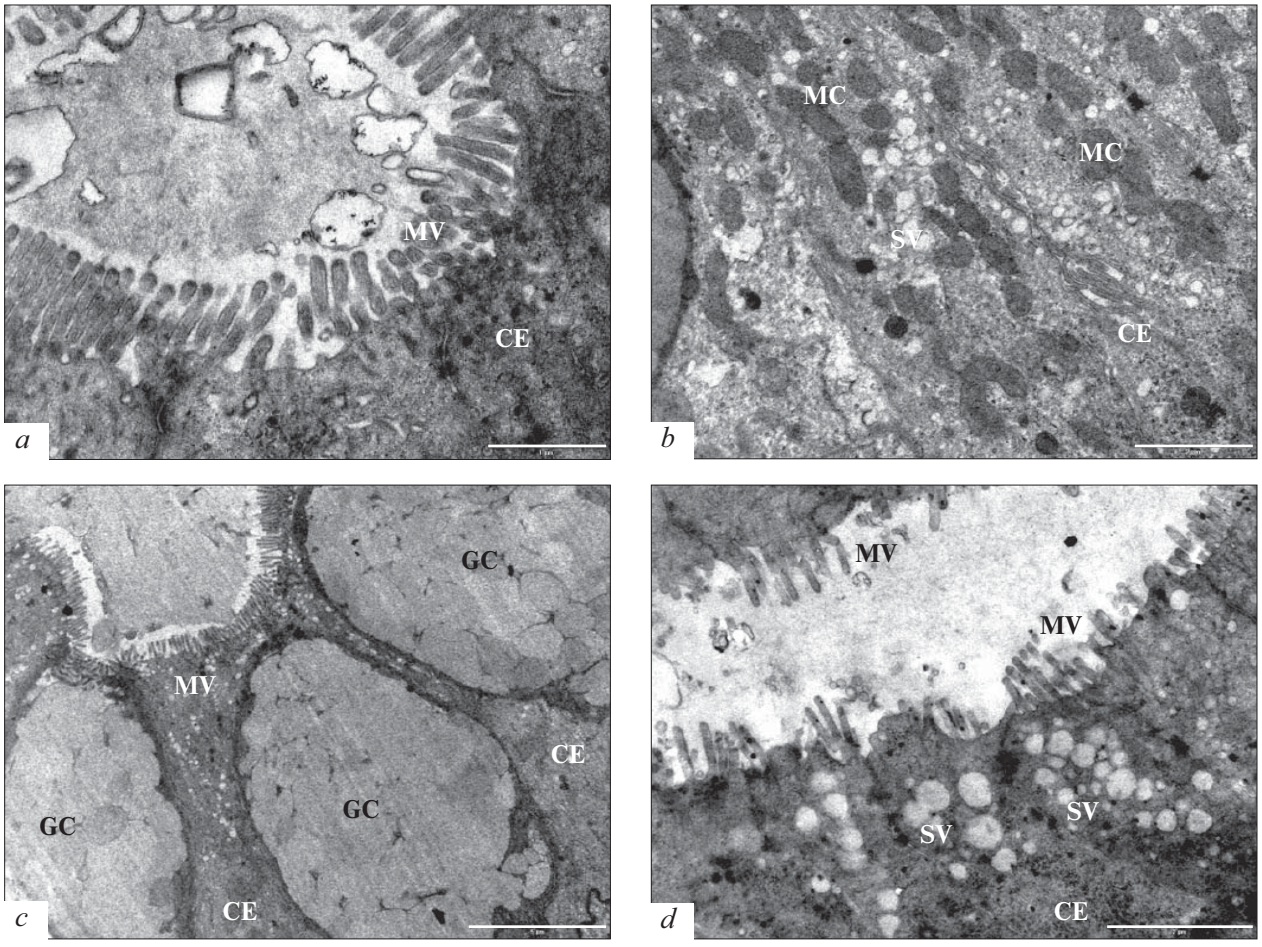

Fig. 3. Ultrastructure of colorectal mucosa in experimental group 4. *a*) microvilli with intact structure; goblet cell mucus and columnar epitheliocyte secretion in the glandular lumen, ×18,000; *b*) fragment of columnar epitheliocyte: secretory vacuoles and numerous mitochondria with intact structure, ×8900; *c*) cryptic lumen filled by mucus; goblet cells, columnar epitheliocytes with microvilli, ×4400; *d*) cryptic lumen with some mucus, reduction of the columnar epitheliocyte microvilli, ×11,000.

structure remained in fact intact in the majority of cases. Columnar epitheliocytes in the greater part of crypts had oval nuclei with euchromatin, great number of secretory vacuoles, and numerous mitochondria with compact matrix. Tight juncions in the apical part of cells were retained. The ultrastructure of columnar epitheliocyte microvilli (Fig. 3, *a*) and their height (766.68±23.4 nm) virtually did not differ from those in the control groups (t=1.07, p=0.337).

Increased formation of secretory vacuoles fusing into large vacuoles in the apical part of cells was detected in the columnar epitheliocytes (Fig. 3, *b*). A similar picture was observed in control groups. Goblet cells had normal ultrastructure (Fig. 3, *c*) and active nuclei with well-discernible nucleolus and well-developed granular intracellular reticulum. Lymphocytes were often seen in the basal part of the crypts between epitheliocytes. Cells with large granules were numerous: tissue basophils, the majority of them partially or completely degranulated. Endocrinocytes were detected in solitary cases. Proliferation of poorly differentiated cells was detected in the basal parts of the crypts. Fibroblasts, macrophages, solitary eosinophils were detected in the mucosal lamina propria, the blood was present in the capillaries. It is noteworthy that more severe disorders in the ultrastructure of columnar epitheliocytes were detected in some crypts. The microvilli in these sites were reduced and detritus was detected in the lumen of some crypts (Fig. 3, *d*). Goblet cells were filled with granules, however, the secretion was disordered in many cases.

Long-term contact with the urine was the factor triggering the development of these morphofunctional changes in cells of the colorectal mucosa. This led to changes in the ultrastructure of the columnar epitheliocyte microvilli and reduction of their length by 1.3 times (p<0.05) in comparison with the control. Adaptive hypersecretion of the mucus by goblet cells should be regarded as the defense reaction of the intestinal epithelium to irritating agents of the urine and more acid medium than is normal for the intestinal lumen. The efficiency of various substances (N-acetyl-

cysteine, aspirin, and ranitidine) expected to reduce mucus production and urine viscosity is not proven [8]. Understanding of the mechanism of action of the irritating urinary agents on goblet cells will promote more effective drug correction of the function of these cells, reducing their capacity to mucus production. This will appreciably improve patients' quality of life after diversion of the urine into the large intestine.

REFERENCES

1. *Microscopic Techniques. A Manual*, Eds. D. S. Sarkisov and Yu. L. Perov [in Russian], Moscow (1996).
2. M. Austen and T. Kälble, *J. Urol.*, **172**, No. 3, 831-838 (2004).
3. E. W. Gerharz, A. Roosen, and W. Mansson, *Eur. Urol.*, **3**, 156-168 (2005).
4. R. E. Hautmann, *J. Urol.*, **169**, No. 3, 834-842 (2003).
5. M. J. Malone, J. K. Izes, and L. J. Hurley, *Urol. Clin. North. Am.*, **24**, No. 4, 723-728 (1997).
6. R. D. Mills and U. E. Studer, *J. Urol.*, **161**, No. 4, 1057-1066 (1999).
7. U. Nagele, Karl-Dietrich Sievert, A. S. Merseburger, *et al.*, *Eur. Urol.*, **3**, 129-137 (2005).
8. J. N'Dow, C. N. Robson, J. N. Matthews, *et al.*, *J. Urol.*, **165**, No. 5, 1433-1440 (2001).
9. R. Pickard, *World J. Urol.*, **22**, No. 3, 227-234 (2004).
10. M. Racioppi, A. D'Addessi, A. Fanasca, *et al.*, *Urology*, **54**, No. 4, 629-635 (1999).
11. B. P. Schrier, M. P. Laguna, F. van der Pal, *et al.*, *Eur. Urol.*, **47**, No. 5, 679-685 (2005).