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Biosynthetic Reactions and Ultrastructure of Urothelial Cells in Chronic Cystitis and Cystopathies

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A new form of morphogenesis of pathological process, cystopathy, was distinguished on the basis endoscopy data and morphofunctional analysis of the urinary bladder in chronic cystitis. Cystopathy is characterized by predominance of diffuse degeneration and atrophy of the urothelium, stromal sclerosis, absence of inflammatory cell infiltration, and inhibition of biosynthetic reactions in urothelial cells (compared to chronic cystitis). Cystopathy results from regeneratory and plastic failure. Instability of the bladder epithelium can be a morphological marker of oncological risk.

Key Words: *chronic cystitis; cystopathy; urinary bladder mucosa biopsy; urothelial cells; electron microscopy; in vitro autoradiography*

High prevalence of cystitis and frequent chronization of this process attract much attention, especially in view of differential diagnosis of tumor and pretumor injuries [1,2,8,10,12]. Urinary bladder tumors are a heterogeneous pathology with blurred clinical picture. The most severe neoplasms develop in females, which is believed to be due to higher prevalence of cystitis [13]. Pathomorphological classification of chronic cystitis (CC) is based on the presence or absence of urothelium proliferation and dysplasia [9].

Urinary bladder mucosa, similarly to mucosa in other organs, contacts with exo- and endogenous substances concentrating them "at the output" [4]. The urothelium prevents penetration of aggressive urinary substances into the bladder wall; impairment of this barrier leads to the development of pathological processes, interpreted in the majority of cases as inflammation. The dynamics of alteration and regeneration of the urothelium after intravenous injection of protamine sulfate was studied experimentally: the toxin first affected the surface (umbrella-shaped) urothelial cells and then induced alteration and proliferation of intermediate cells [11].

Here we present the data of clinical endoscopic and complex pathomorphological study of chronic nontumor pathology of the urinary bladder and analyze variants of its morphogenesis.

MATERIALS AND METHODS

A total of 101 female patients aged 17-73 years with CC were examined. Complex examination included ultrasonic scanning of internal organs, radionuclide renography, scanning and excretory urography, cystography, cystoscopy, and biopsy of the urinary bladder mucosa. The presence and content of residual urine, volume and duration of bladder washing, and state of the ureteric orifice were evaluated during cystoscopy. Biopsy specimens were taken from ulcerated or

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altered sites, bladder neck, and right wall with a special biopsy forceps.

Biopsy specimens of the bladder mucosa were studied by light and electron microscopy and by autoradiography. Paraffin sections were stained with hematoxylin and eosin in combination with Pearls' reaction, Van Gieson staining with poststaining of elastic fibers by resorcin-fuchsin after Weigert, and periodic acid-Schiff reaction. Specimens for electron microscopy were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2-7.4). Semithin sections were stained with Schiff reagent and Azur II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

The intensity of biosynthetic reactions in cell populations of the bladder mucosa was evaluated by *in vitro* autoradiography. To this end the specimens were incubated separately with ^{3}H-uridine and ^{3}H-thymidine [8]. Fragments of bladder biopsy specimens (<1 mm^3) were put in flasks with medium 199 and incubated with tritium-labeled DNA and RNA precursors at 37°C for 1.5 h. The intensity of RNA and DNA synthesis was evaluated by incorporation of ^{3}H-uridine (200 μCi/ml, specific radioactivity 26.6 Ci/mM) and ^{3}H-thymidine (100 μCi/ml, specific radioactivity 48 Ci/mM). After incubation the samples were washed in Millonig phosphate buffer (pH 7.2-7.4), fixed in 4% paraformaldehyde, and treated routinely for electron microscopy. Semithin sections were coated with type M photoemulsion, exposed for 7 and 13 days at 4°C, and processed. The sections were stained with 1% Azur II. Labeling density and index in bladder mucosa cells were counted under a light microscope at ×600.

RESULTS

Clinically apparent CC was found in 49 of 101 patients: leukocyturia, growth of pathogenic microflora during bacteriological tests of the urine, and positive effect of antibiotic and uroseptic drugs. Cystoscopy revealed focal or diffuse hyperemia of the mucosa, which was combined with ulcers in 4 cases.

Transitional epithelium was preserved only in some regions of the bladder mucosa biopsy specimens from CC patients. The relief of the mucosa was changed, degenerative changes and atrophic foci alternated with foci of epitheliocyte hyperplasia (Fig. 1, *a*) and zones of stratified epithelium (14-18 layers and more) with acanthosis (Fig. 1, *b*). A characteristic structural feature of the bladder mucosa in CC is pronounced proliferative changes in the urothelium with foci of glandular and squamous-cell metaplasia (Fig. 1, *c*) and heterotopic growth. Numerous large Brunn nests were seen. Electron microscopy of the epithelium showed cells with normal ultrastructure and abnormal urothelial cells with signs of focal and total alteration. In foci of squamous-cell metaplasia urotheliocytes looked like polygonal cells with numerous processes, electron-dense cytoplasm, and minimum number of cytoplasmatic organelles (Fig. 1, *d*).

Vascularized connective tissue papillae were seen in the subepithelial stroma in zones of acanthosis. Plethoric capillaries, endotheliocyte hypertrophy, pronounced diffuse polymorphic infiltration with numerous neutrophils and active transepithelial leukodiapedesis were observed. Pronounced sclerosis of the lamina propria of different severity was seen.

Erosive changes in the mucosa were paralleled by pronounced stromal sclerosis, hemodynamic disorders, atypical vascularization due to angiomatously deformed vessels, focal perivascular cell infiltration with numerous heterogeneous mast cells. Activation of mast cells was pronounced and did not depend on the location and degree of mastocytosis. Proinflammatory and vasoactive cytokines produced by mast cells can be involved in the pathogenesis of interstitial cystitis [14].

Clinical and pathomorphological features not characteristic of CC were observed in 52 patients with similar complaints (frequent painful scanty urination, imperative vesical tenesmuses, painful bladder): the absence of inflammation (leukocyturia), sterile urine, and resistance to antibiotics and uroseptic drugs. In view of the absence of typical inflammatory and infectious changes the diagnosis of CC was transformed into cystalgia. Cystoscopy showed local moderate hyperemia of the trigone and bladder neck in 70% cases.

Light microscopy of biopsy specimens showed diffuse atrophy: transitional epithelium lost signs of morphological stratification, was thinned, and consisted of 1-2 layers of irregular cubic epitheliocytes. Urothelial cells had signs of degeneration, easily desquamated (Fig. 2, *a*), and sometimes only solitary basal cells remained on the basal membrane (Fig. 2, *b*). In 12 cases pronounced degeneration and atrophy of the urothelium were associated with its abnormal differentiation (squamous-cell metaplasia). Reduction of the microcirculatory bed with angiomatous transformation of individual capillaries and stromal sclerosis were observed in the lamina propria. A characteristic sign was polymorphism of endotheliocytes (combination of hypertrophic and atrophic cells). Inflammatory cell infiltration was absent, solitary lymphocytes, fibroblasts, and extremely rare mast cells were seen.

Ultrastructural organization of urothelial cells at sites of atrophy and degeneration was similar and characterized by sharp reduction of protein-synthesizing structures and destruction and vacuolation of mitochondria (Fig. 2, *c*). In the urothelium transformed into stratified squamous epithelium the cells contained degenerating

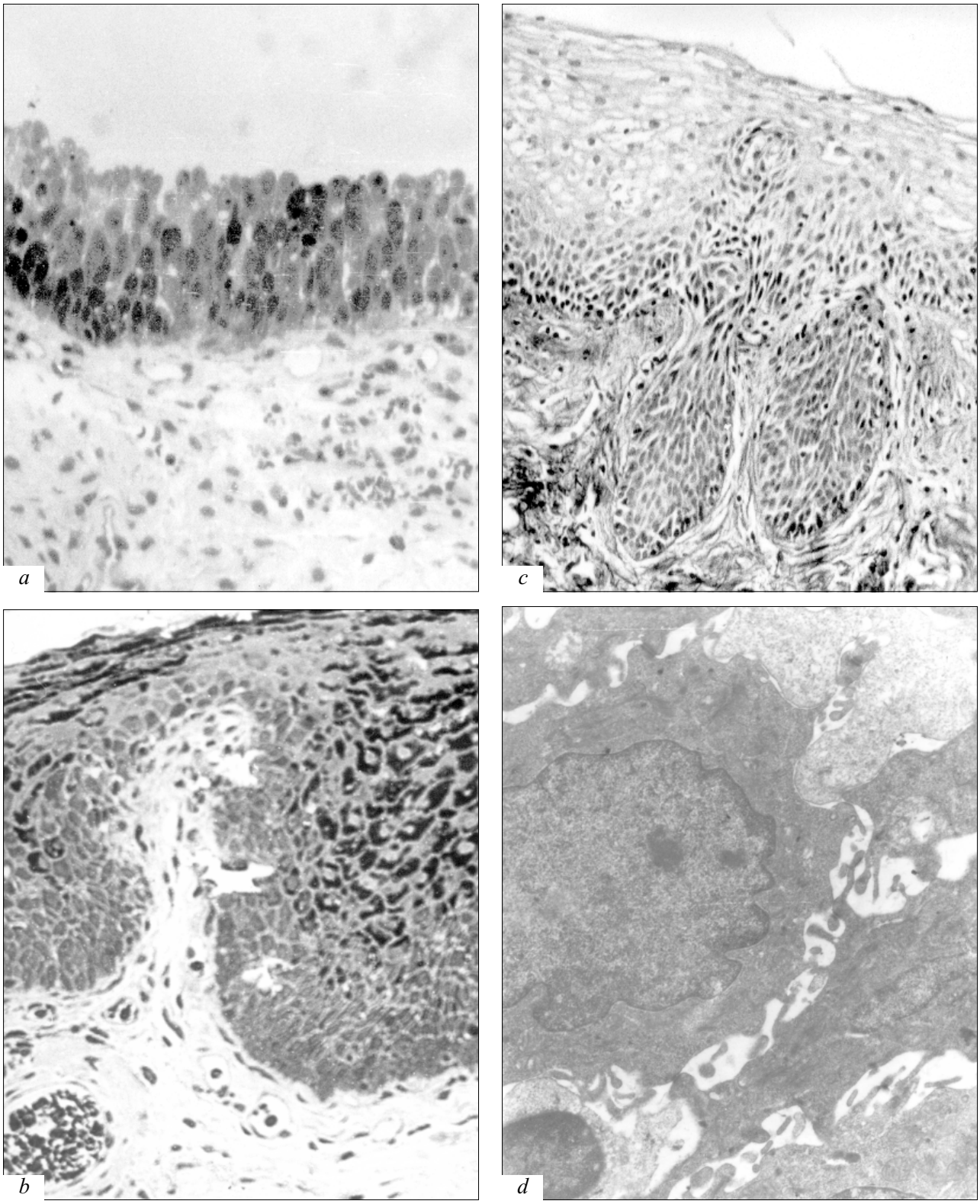

Fig. 1. Pathomorphology of bladder mucosa biopsy specimens in chronic cystitis. *a*) urothelial proliferation, inflammatory infiltration of the stroma. Hematoxylin and eosin staining, ×200; *b*) squamous-cell metaplasia of the urothelium. Periodic acid-Schiff reaction, ×600; *c*) hyperplasia and squamous-cell metaplasia, sclerosis of the stroma. Van Gieson staining, ×300; *d*) squamous-cell metaplasia of urothelium. Electronogram, ×400.

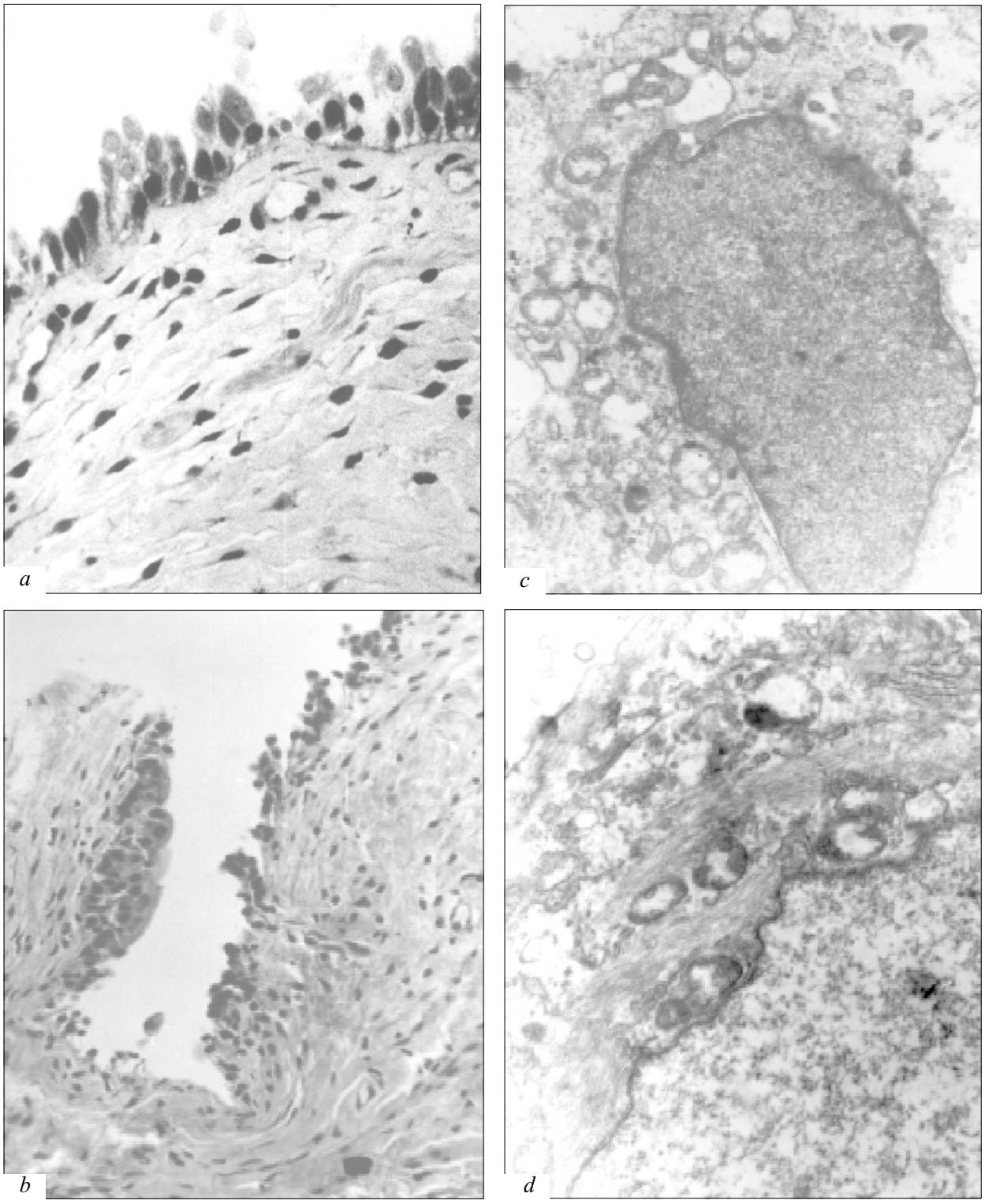

Fig. 2. Pathomorphology of bladder mucosa in cystopathy. *a*, *b*) atrophy and desquamation of the urothelium, absence of inflammatory infiltration. Semithin section, Azur II staining, ×600 (*a*); hematoxylin and eosin staining, ×200 (*b*); *c*) degeneration of cytoplasmic organelles in urotheliocyte, ×6000; *d*) microfilaments in the perinuclear zone of urotheliocyte. Electronogram, ×10,000.

mitochondria, solitary profiles of granular endoplasmic reticulum and numerous microfilaments (Fig. 2, *d*).

Autoradiography of these specimens (Fig. 3) revealed a decrease in both metabolic (^{3}H-uridine label index <70% and extremely low density of silver granules) and proliferative activities of the urothelium (^{3}H-thymidine label 2.8-3.9%) compared to specimens from patients with CC. In CC the protein-synthesizing

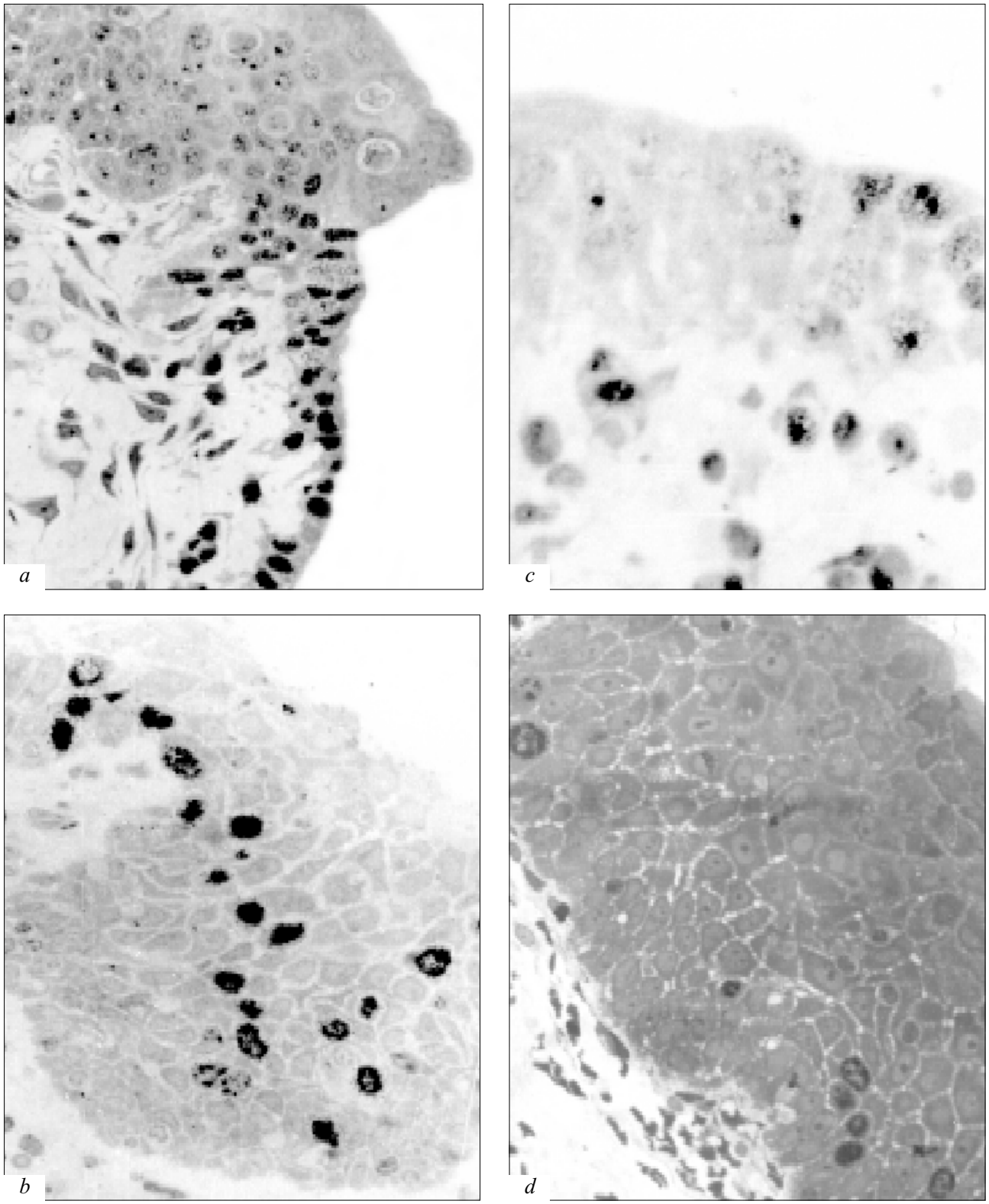

Fig. 3. Biosynthetic reactions in urothelial cells in chronic cystitis (*a*, *b*) and cystopathy (*c*, *d*). Semithin sections, Azur II staining. *a*) intensive RNA synthesis, mosaic structure of urothelium (alternation of acanthosis and atrophy), ×350; *b*) high labeling index and density in transitional epithelium, ×450; *c*) low intensity of RNA synthesis, ×600; *d*) solitary radioautographs in urothelium, ×450; *a*, *c*) incubation with ^{3}H-uridine; *b*, *d*) with ^{3}H-thymidine.

and proliferative activities of the urothelium were higher (^{3}H-uridine and ^{3}H-thymidine label indexes were 87-93% and 15-23%, respectively), the highest label density was observed in hyperplasia foci. The positive correlation between the intensity of biosynthetic reactions in epitheliocytes and stromal cells, primarily endotheliocytes, reflects the phenomenon of morphogenetic interactions between epithelial and stromal cells.

Chronic cystitis can be considered as a heterogenetic syndrome with little studied morphogenesis variants [3]. We distinguished 2 forms of morphogenesis of chronic nontumor process in the bladder wall: CC (clinical and pathomorphological markers of inflammation) and cystopathy. Cystopathy is characterized by predominance of diffuse degeneration and atrophy of the urothelium, stromal sclerosis, absence of inflammatory cell infiltration, and lower intensity of biosynthetic processes in cell populations compared to CC. This suggests that cystopathy results from regenerative plastic failure, morphologically presented as pronounced urothelial atrophy resultant from long-term exposure to adverse factors and subsequent impairment of the regeneration and differentiation processes. Distinguishing of these two morphogenetic variants substantiates principally different approaches to the therapy of chronic cystic diseases.

Mosaicism of the epithelial layer (urothelium), i.e. alternation of foci of atrophy, hyperplasia, metaplasia, and dysplasia (phenomenon of "instability") of different severity, is observed in both variants of chronic cystic disease; this was previously described for bronchial and skin diseases [5,6] and was referred as a marker of oncological risk. Presumably, the prevalence of this phenomenon underlies the multifocal nature of tumor growth in the bladder mucosa. It is of the utmost importance, because, despite comprehensive study of a variety of clinical, morphological, immunological, and other parameters, no universal prognostic sign determining the development and course of tumor process in the urinary bladder was proposed yet.

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