EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Ultrastructural and Biosynthetic Characteristics of Glomerular Endotheliocytes and Periglomerular Arterioles during Systemic Lupus Erythematosus

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We studied ultrastructural characteristics of renal cortical cells in patients with systemic lupus erythematosus. Endotheliocytes in periglomerular arterioles underwent primary and most pronounced changes. Examination of glomerulocytes revealed early alterations in endotheliocytes, compensatory proliferation of mesangial cells, overproduction of the mesangial matrix, and metaplasia of podocytes. Biosynthetic reactions reflected the structural and functional heterogeneity of endotheliocytes associated with their damages and regeneration.

Key Words: *systemic lupus erythematosus; nephropathy; antiphospholipid syndrome; endothelium; electron microscopy; in vitro autoradiography*

Renal syndrome during systemic lupus erythematosus (SLE) is associated with autoimmune processes [4,6, 14]. However, the pathogenetic mechanisms of this disease remain unclear. Generalized autoimmune reactions to various tissue components [10] contrast with organ-specific autoimmune processes resulting in the production of pathogenic autoantibodies against one epitope.

Renal damages during SLE are related to high sensitivity of immunocompetent cells to basal membrane antigens [4]. Genetically determined polyclonal hyperactivity of B cells and abnormal autoregulation of T cells contribute to the development of SLE [6]. Previous studies revealed deficiency in the fas/APO-1 ligand system, whose activation leads to apoptosis in lymphocytes [7]. There are no data on genetic disturbances or impairment of APO-1/fas receptor expression in humans. Apoptosis leads to the appearance of nucleosomes, which are expressed and released from apoptotic cells together with other antigens playing an important role in the pathogenesis of SLE [6]. It was reported that the imbalance between apoptotic processes in renal glomerular and tubular cells is an important pathogenetic factor of glomerulo-, tubulointestial, and lupus nephrites [15].

Lupus anticoagulant and antibodies against native and denatured DNA are the main markers of antiphospholipid syndrome (APS). SLE is frequently accompanied by APS. Moreover, antiphospholipid and antiendothelial antibodies play and important role in clinical manifestations of this syndrome [8,16]. These data indicate that studies of structural changes in endotheliocytes are of considerable importance [3].

Here we performed ultrastructural and autoradiographic analyses of endotheliocytes in renal bioptates from patients with SLE.

MATERIALS AND METHODS

We performed clinical and pathomorphological examinations of 8 patients (6 women and 2 men, below

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40 years) with chronic SLE. The patients received prolonged hormonal therapy (prednisolone) and courses of cytostatic treatment (cyclophosphamide). SLE was accompanied by renal syndrome: chronic glomerulonephritis (hypertensive and edematous-and-hypertensive types) and chronic kidney failure. SLE was verified by clinical, cytological, and serological markers. The main markers included a positive LE-phenomenon and the presence of antibodies against native and denatured DNA. Transcutaneous biopsy of the left kidney was performed using puncture needles (Baxter).

For light microscopy, the bioptates were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin by the van Gieson's method combined with Perls reaction. Elastic fibers were stained with Weigert's resorcin-fuchsin. Periodic acid-Schiff reaction was performed. Congo red and gentian violet were used for histological staining of amyloid. Fragments for electron microscopy were fixed in 4% paraformaldehyde, postfixed with 1% OsO_4, treated by routine methods, and embedded into Epon-Araldite. Semithin sections were stained with azure II and Schiff reagent. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-1010 electron microscope.

Metabolic and proliferative activities of cells in renal bioptates were *in vitro* estimated by autoradiography [1] using tritium-labeled DNA (^{3}H-thymidine) and RNA (^{3}H-uridine) precursors. Quantitative analysis of autoradiographs included calculation of labeled cells and estimation of labeling density.

RESULTS

Pathomorphological examination of bioptates revealed insignificant focal proliferation of mesangial cells or total fibrosis of most glomeruli (Fig. 1, *a*, *b*). Pathomorphological changes in the kidneys typical of SLE were found in patients with severe disease. We revealed diffuse thickening of glomerular basal membranes looking like "wire loops", pronounced alteration and desquamation of endotheliocytes with signs of karyorrhexis, lobular composition of glomerular vessels, fibrinoid necrosis of individual capillary loops, and the presence of hyaline thrombi in capillaries. Focal or diffuse proliferation of mesangial cells and enlargement of the mesangial matrix were found. Changes in the glomerular capsular endothelium included the formation of synechiae and epithelial or fibroepithelial crescent-shaped structures.

Examination of convoluted tubules revealed dystrophy, atrophy, necrobiosis and, sometimes, fibrinoid necrosis of epitheliocytes. Necrotic changes in the tubular epithelium were accompanied by thickening and fibrinoid infiltration of tubular basal membranes, which looked like "wire loops". In the interstitium we revealed focal sclerotic changes, hemodynamic disturbances, periglomerular and perivascular infiltrates, formation of large follicle-like lymphoid aggregates, and individual plasma cells and neutrophils. Long-lasting SLE was characterized by fibroplasia and focal cortical atrophy: glomerulosclerosis of various degrees (fibrous changes in the mesangium or total glomerular fibrosis), diffuse interstitial sclerosis, and myoelastofibrosis of arteries and arterioles. Intimal hyperplasia of periglomerular arterioles was accompanied by vasoocclusive damages due to proliferation of endotheliocytes and luminal obliteration (Fig. 1, *c*).

Electron microscopy revealed various ultrastructural changes in renal cortical cells of the glomerular, tubulointerstitial, and vascular layers. Most pronounced alterative changes were found in endotheliocytes of glomeruli and periglomerular arterioles independently on the duration and severity of SLE.

Atrophy and degeneration of glomerular endotheliocytes were manifested in karyopyknosis, increased density of the cytoplasmic matrix, reduction and alteration of cytoplasmic organelles, and impaired fenestration of the endothelium. In some regions the glomerular basal membrane was visually intact, while luminal outgrowths of peripheral processes formed arcades. The glomerular basal membrane was thickened (Fig. 2, *a*) and characterized by irregular electron density, interposition of the mesangial matrix and cell processes and, more rarely, pronounced osmiophilic depositions. Mesangial cells displayed high functional activity only in the initial stage of SLE (polymorphic nuclei and nucleoli, numerous membrane organelles, and free ribosomes). Signs of karyopyknosis and degenerative changes in cytoplasmic organelles were revealed at later stages of the disease.

In patients with long-lasting SLE, the most pronounced structural and spatial changes in the glomerular filter were associated with impaired cytoarchitectonics of podocytes (urine compartment). We found signs of cell metaplasia. Podocytes lost the trabecular organization typical of differentiated cells and gained ultrastructural signs of dedifferentiation (structural and functional characteristics of the embryonic phenotype). Fusion of cytopodia, reduction of cytopedicles, enlargement of perikaryon, and accumulation of fibrillar structures, vacuoles, and polymorphic vesicles in the cytoplasm of podocytes indicated functional dislocation of the primary urine formation. The compensatory reaction included substitution of cytopodium-mediated urine passage for the pinocytotic pathway of filtration, which was confirmed by the presence of cellular central structures in the perinuclear zone of podocytes. Individual podocytes underwent villous transformation.

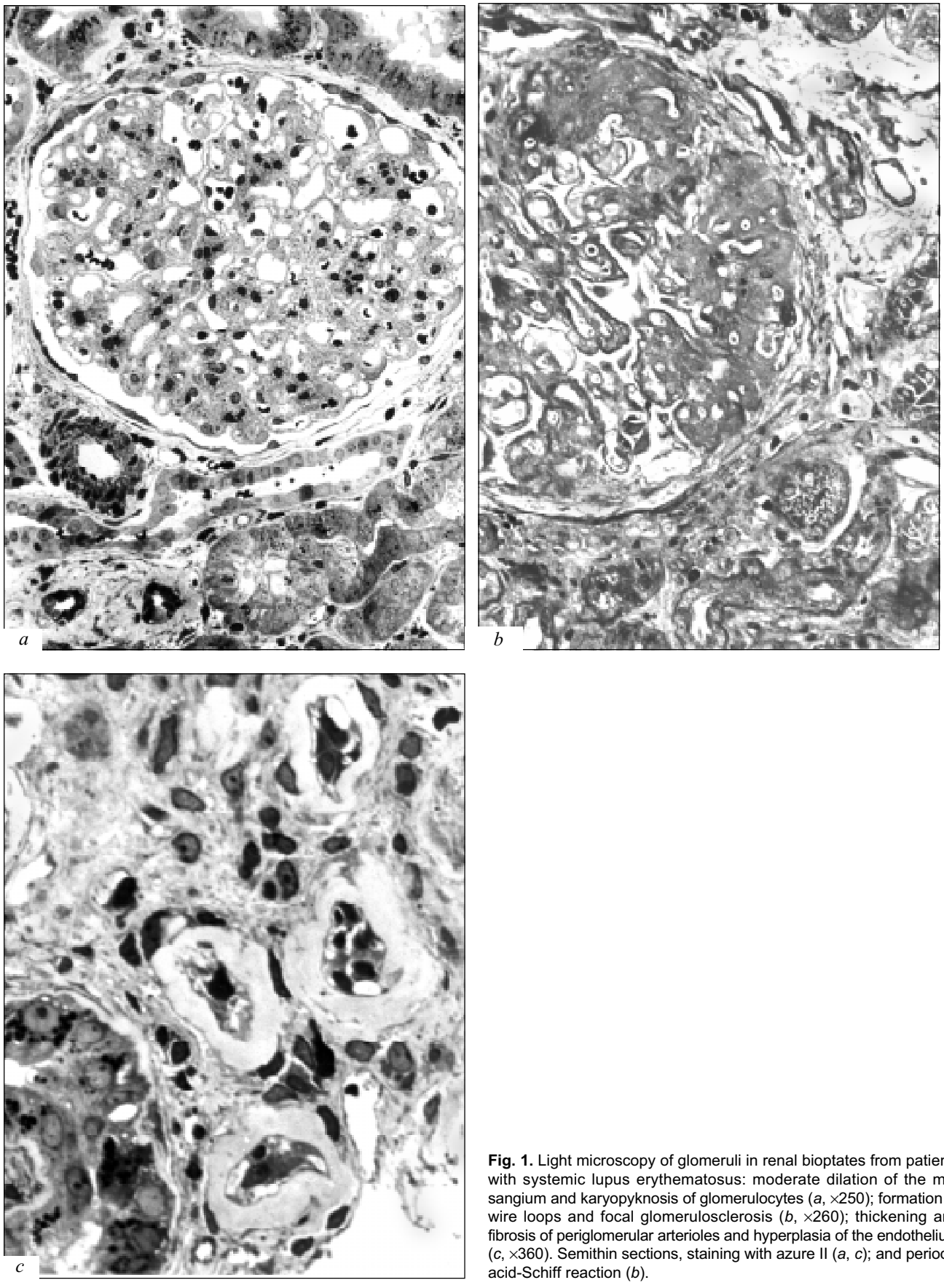

Fig. 1. Light microscopy of glomeruli in renal bioptates from patients with systemic lupus erythematosus: moderate dilation of the mesangium and karyopyknosis of glomerulocytes (*a*, ×250); formation of wire loops and focal glomerulosclerosis (*b*, ×260); thickening and fibrosis of periglomerular arterioles and hyperplasia of the endothelium (*c*, ×360). Semithin sections, staining with azure II (*a*, *c*); and periodic acid-Schiff reaction (*b*).

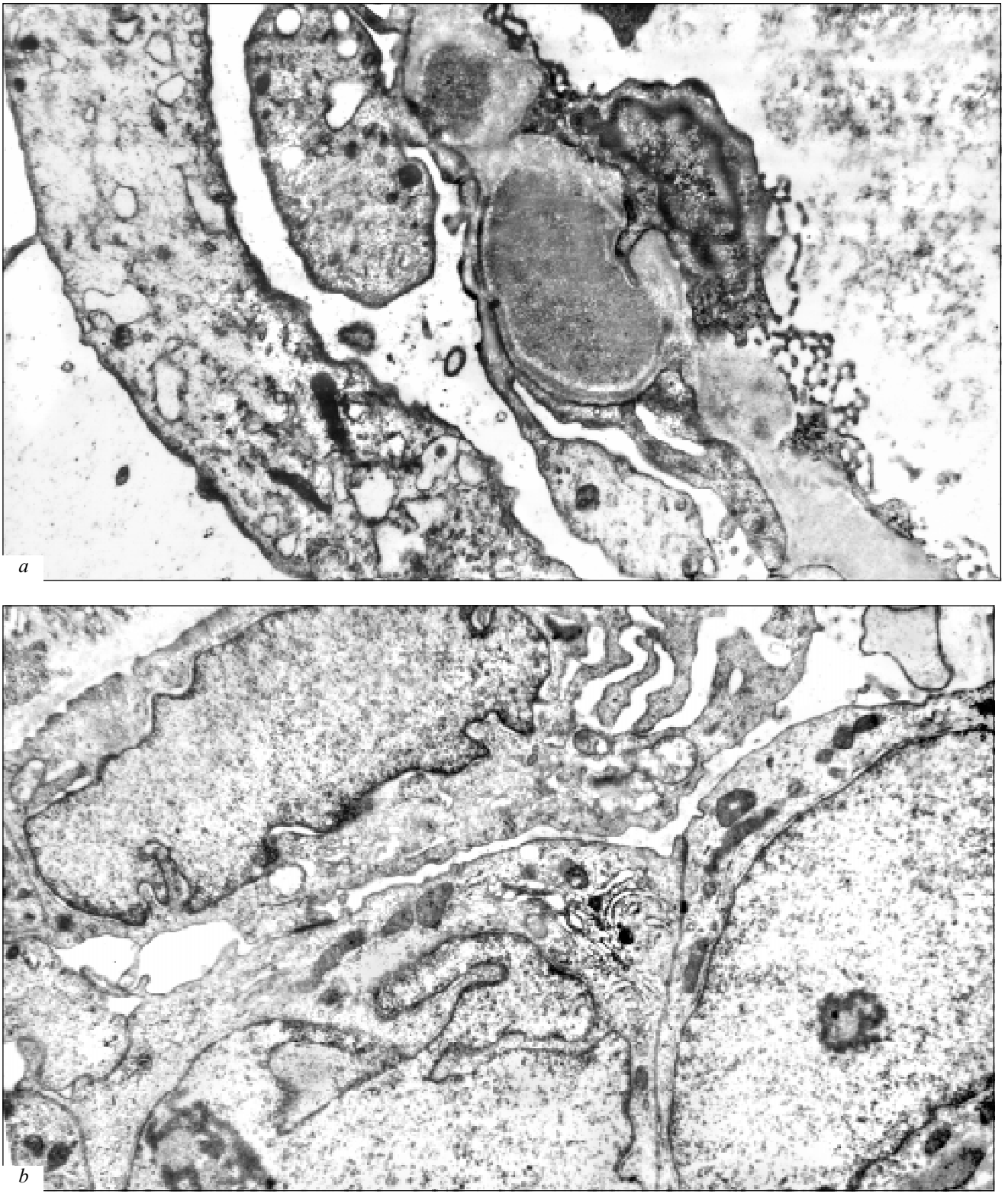

Fig. 2. Ultrastructural characteristics of endotheliocytes in microvessels of the renal cortical layer during systemic lupus erythematosus: pronounced and irregular thickening of the glomerular basal membrane, presence of depositions, high electron density of the cytoplasmic matrix in endotheliocytes, and fusion of podocytic cytopodia (*a*, ×8000); euchromaticity and polymorphism of nuclei, increase in cytoplasmic volume and count of cytoplasmic organelles in endotheliocytes of periglomerular arterioles (*b*, ×15,000).

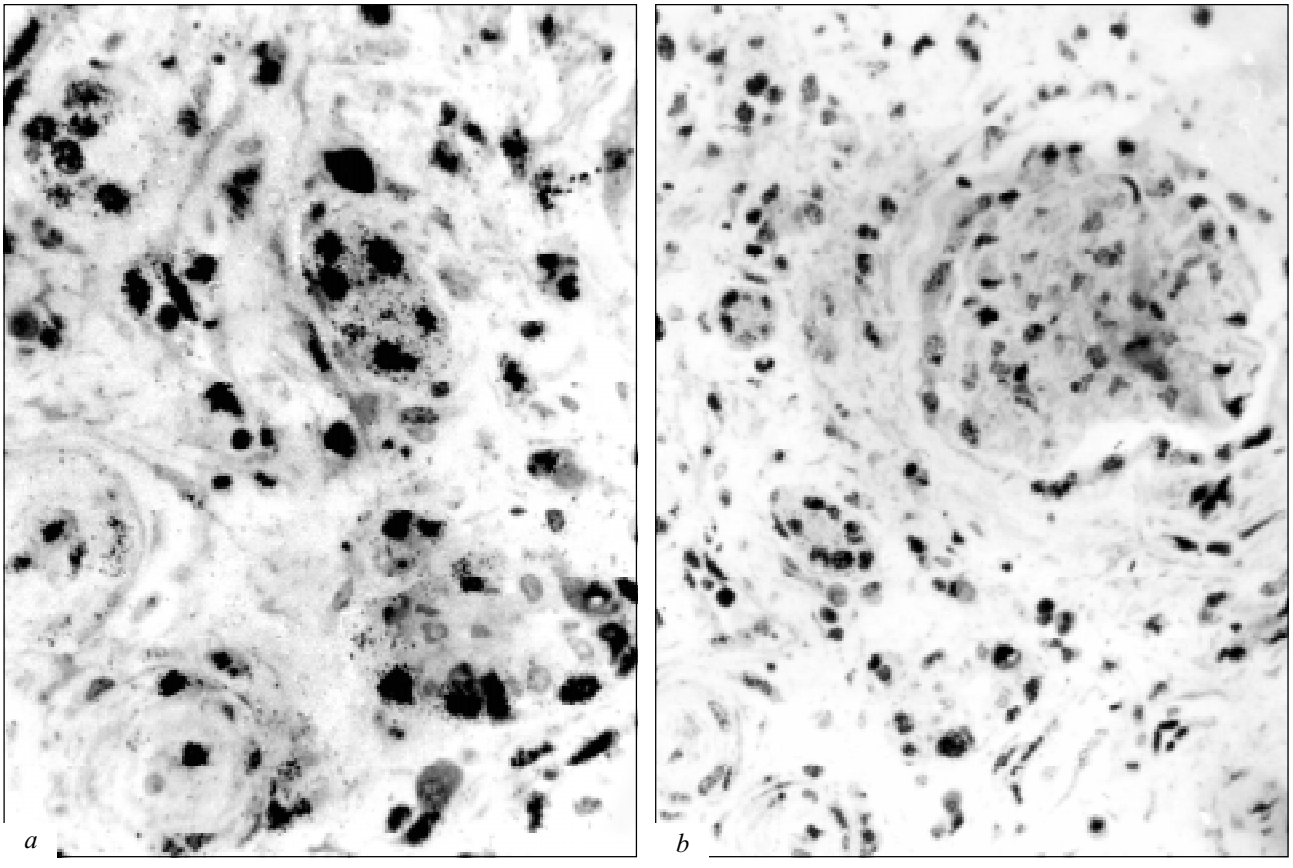

Fig. 3. Biosynthetic reactions of cell populations in renal bioptates from patients with systemic lupus erythematosus. Semithin sections, incubation with ^{3}H-uridine, azure II staining. Various labeling densities in cortical microvessels (*a*, ×350); low metabolic activity of glomerulocytes (*b*, ×250).

Independently on the duration and severity of SLE, we revealed 2 subpopulations of endotheliocytes in periglomerular arterioles and other interstitial microvessels. Some endotheliocytes underwent atrophy and degeneration, while others were hyperplastic and hypertrophic. This reflected a wavelike course of alterative and compensatory processes. Degenerative changes in endotheliocytes were manifested in a sharp increase in the content of nuclear heterochromatin, karyopyknosis, thinning of peripheral processes, flattening of the perikaryon, disappearance of luminal processes, suppression of pinocytotic activity, condensation of the cytoplasmic matrix, reduction and alteration of organelles, and desquamation of the endothelium. The endothelial basal membrane was irregularly thickened and stratified.

Hypertrophic endotheliocytes were oval in shape, did not form peripheral processes, and contained large polymorphic euchromatic nuclei and nucleoli, electron-dense cytoplasmic matrix with numerous pinocytotic vesicles, free ribosomes, polysomes, small mitochondria, granular cytoplasmic reticulum, and Golgi complex (Fig. 2, *b*). Swollen endotheliocytes obliterated the lumen of vessels. In some samples we revealed contacts between erythrocytes and other blood cells.

Thus, endotheliocytes in periglomerular arterioles underwent most pronounced ultrastructural changes. They were permanent and found even without significant morphological changes in glomeruli and tubules. Examination of glomerulocytes showed that early alterations in endotheliocytes were accompanied by compensatory proliferation of mesangial cells, overproduction of the mesangial matrix, and metaplasia of podocytes. These changes were directed to the recovery of glomerular filter. Our results indicate the formation of primary alterations in the endothelial compartment: cortical microcirculatory disturbances are followed by or coincide with damages to the glomerular compartment, which probably plays an important role in the pathogenesis of SLE.

Autoradiography showed that incorporation of labeled DNA and RNA precursors into renal cortical cells was different (Fig. 3). Incorporation of ^{3}H-uridine into endotheliocytes was low in microvessels with atrophic endothelium. Hypertrophic proliferating endotheliocytes contained a considerable amount of reduced silver grains. Metabolic activity of endotheliocytes in glomerular vessels correlated with the severity of structural changes and was relatively high at the initial stage of SLE. ^{3}H-thymidine label was found

only in individual cells, which was directly related to their proliferative activity.

SLE is a systemic disease accompanied by damages to various tissues and organs [4,6]. This is related to integral properties of the endothelial monolayer. This structure plays a central role in the pathogenesis of various diseases and, therefore, attracts much recent attention. It should be emphasized that pathological processes accompanied by alteration and apoptosis in endotheliocytes are characterized by the highest morbidity and mortality rates [13].

Most studies of the pathogenesis and prognosis of renal syndromes are devoted to evaluation of mechanisms underlying apoptosis in endotheliocytes and glomerular mesangial cells [2,5,12], search for markers of apoptotic death, and estimation of factors initiating or inhibiting this process. Our experiments revealed no pathomorphological markers of programmed death of endothelial and mesangial cells, which was related to long-term therapy of patients and development of medicinal pathomorphosis. Karyorrhexis, formation of hematoxylin bodies, pronounced ultrastructural changes, and LE-phenomenon were observed only in patients with severe SLE after the withdrawal of treatment.

The search for new pathogenetic approaches to the therapy of patients with SLE should be performed with due consideration of important general pathological role and contribution of APS and endotheliopathy into the pathogenesis and clinical manifestations of LE-nephropathy [9]. Thrombotic microangiopathy in patients with SLE leads to occlusion of the interstitial microcirculatory bed. A positive correlation was found between the presence of antiphospholipid antibodies and occlusion of periglomerular arterioles in patients with APS (histological and hematological criteria) [11]. The presence of large follicle-like lymphoid aggregates in renal bioptates from patients with SLE suggests a long-term persistence of infection (similarly to viral hepatitis C) and localization of the virus in endotheliocytes [4].

Our results indicate that the pathogenetic mechanisms of SLE include not only autoimmune reactions that determine the course of therapy, but also the phenomenon of apoptosis. Accelerated death and regeneration of endotheliocytes change vessel architectonics and, therefore, lead to obliteration of capillaries.

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