Ultrastructural and Biosynthetic Changes in Epitheliocytes of Renal Tubules during the Development of Chronic Renal Failure

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Structural and functional studies of changes in the tubular compartment during progressive renal dysfunction showed that the major types of damage to tubular cells include dystrophy, degeneration, and atrophy. These changes were associated with inhibition of biosynthetic processes in the tubular epithelium, progressive interstitial fibrosis, and disturbances in the structure and function of peritubular microvessels.

Key Words: *renal failure; renal tubular epithelium; nephrobiopsy; ultrastructure; in vitro autoradiography*

Tubular damage during glomerular dysfunction is usually accompanied by interstitial fibrosis. Study of progressive renal dysfunction focused on the progression of this disorder [7,9]. The reduction of tubules under conditions of progressive fibrosis is of considerable interest. Morphological studies found a direct correlation between the severity of interstitial fibrosis and degree of renal dysfunction [8]. The extensively used morphological variants of glomerular damage are not accompanied by structural differentiation of tubular damage (except for acute renal failure) [4-7].

Here we performed ultrastructural and autoradiographic studies of the tubulointerstitial compartment of the kidney during renal dysfunction of different severity.

MATERIALS AND METHODS

We performed pathomorphological study of nephrobiopsy specimens from 106 patients (61 men and 45 women, 14-71 years) with a clinical diagnosis of chronic glomerulonephritis (n=97) or chronic tubulointerstitial nephritis (n=9). The patients were divided into 3 groups depending on functional activity of the kidneys estimated by nitrogen excretion, osmo-, and ionoregulatory functions of the kidneys [1]. Group 1 included 44 patients with normal renal function. Group 2 included 25 patients with transitory or borderline decrease in renal function. Group 3 included 37 patients with permanent renal dysfunction and chronic renal failure. Transcutaneous puncture biopsy of the lower renal pole in all patients was performed using Tru-Cut disposable needles (Baxter).

Nephrobiopsy specimens were examined under light and electron microscopes [3]. Metabolic and proliferative activity of cells in nephrobiopsy specimens was *in vitro* studied by autoradiography using tritium-labeled DNA and RNA precursors (^{3}H-thymidine and ^{3}H-uridine, respectively) [2]. The severity of interstitial sclerosis was estimated by a semiquantitative method and classified as follows: minimal interstitial fibrosis (degree I) and moderate (degree II) or severe diffuse interstitial sclerosis (degree III).

RESULTS

Examination of nephrobiopsy specimens from most patients of group 1 revealed moderate changes in ne-

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phron tubules. Dimorphism of the proximal and distal compartment was preserved and associated with dimorphism of epitheliocytes in each tubule. Heterogeneity of cells in the proximal tubules (PT) was related to dystrophic changes of different severity and variations in functional activity of cells. These cells differed in the height, content of granular inclusions, and severity of focal destruction in the brush border. Sometimes we revealed necrobiosis of epitheliocytes. The distal tubules (DT) underwent less pronounced changes. Desquamated epitheliocytes, fresh and leached erythrocytes, and protein or erythrocytic cylinders were present in the lumen of DT in patients with nephrotic syndrome. Hemosiderin granules were found in the cytoplasm of individual epitheliocytes in patients with persistent hematuria.

Changes in the tubular compartment were more pronounced in group 2 patients. We revealed severe dystrophy of epitheliocytes. Atrophy and desquamation of tubular cells were especially pronounced in the distal nephron. It should be emphasized that dystrophy and atrophy concerned both renal compartments in 50% patients. The lumen of DT was obliterated with heterogeneous cylinders. These changes were accompanied by cystic deformation and necrobiosis of tubular cells.

Considerable pathological changes in the tubular compartment of group 3 patients were manifested in total atrophy of the epithelium, loss of proximal and distal dimorphism, necrobiosis, desquamation of the epithelium, and obliteration of the lumen with cylinders. We revealed necrosis of individual tubules. The decrease in the numerical density of tubules was accompanied by widening of the interstitium.

Electron microscopy of nephrobiopsy specimens focused on cells in PT and DT. The structure of proximal tubular cells in nephrobiopsy specimens from group 1 patients reflected high functional activity of these cells (despite polymorphism of epitheliocytes). Ultrastructural characteristics of the apical pole in cells with distinct brush border, numerous heterogeneous vesicles, and small mitochondria with dense matrix provided intensive endocytosis and further formation of secondary lysosomes (Fig. 1, *a*). Most cells had well-formed basal folds, adjacent mitochondria, and free ribosomes and polysomes located perinuclearly. Several PT were lined with a flattened epithelium and had reduced number of mitochondria. The cytolemma was smoothed. The cytoplasm included numerous large polymorphic deposits of lipofuscin, which attested to degenerative changes.

DT epitheliocytes had transparent matrix and reduced number of mitochondria (Fig. 1, *b*). Nephrotic syndrome was manifested in disorganization of the basal cytoplasmic network and mitochondria and depletion of the cytoplasm. These changes were accompanied by thickening of the basal membrane and formation of a wide zone with peritubular collagen fibrils.

Nephrobiopsy specimens from group 1 patients differed in the compensatory and adaptive epithelial response of PT. Most tubules were lined with high prismatic epithelium and had narrow lumen. The cytoplasm of epitheliocytes was readily stained with histological dyes. Therefore, the cytoplasm appeared as an irregular structure under light microscope. Azurophilic heterogeneous dense granules were revealed in semithin sections of epitheliocytes. We visualized the corrugated basal cytolemma with lacunas and deep interdigitations of the lateral plasmalemma. Most epitheliocytes of PT were ultrastructurally characterized by pronounced hyperplasia of mitochondria that nearly completely occupied the cytoplasm (phenomenon of high density under light microscope), severe corrugation of the basal cytolemma, hypertrophy of the brush border, presence of numerous pinocytotic vesicles filled with the protein substrate, and formation of large electronically dense vacuoles. These changes reflected a strain in the reabsorption function of PT epitheliocytes.

Progression of renal dysfunction was accompanied by flattening of PT epitheliocytes, focal destruction of cells in the brush border, destruction of the outer and inner membrane in mitochondria (Fig. 1, *c*), and formation of polymorphic vacuoles with an electronically dense or heterogeneous osmiophilic content.

The distal compartment was more engaged in the pathological process compared to the proximal compartment. Convoluted DT were lined with flattened epithelium and had widened lumen. Electron density of the cytoplasmic matrix varied within the same tubule. The apical and basal plasmalemma had simple shape. Cytoplasmic organelles were reduced in the protein-synthesizing compartment. The epitheliocytes undergoing necrobiosis differed in karyorrhexis, pronounced consolidation of the cytoplasmic matrix, focal vacuolation of the cytoplasm, and destruction of intracellular organelles (Fig. 1, *d*).

Interstitial changes in nephrobiopsy specimens from group 1 patients were manifested in mild periglomerular sclerosis and presence of a few small focuses of fibrosis. They were predominantly localized in the zone of tubular atrophy. Interstitial fibrosis was not found in 45% biopsy specimens. Study of nephrobiopsy specimens from group 2 patients revealed diffuse interstitial fibrosis, which was especially pronounced in the perivascular and periglomerular zone. The severity of fibrosis varied and corresponded to degrees I, II, and III in 18, 62, and 20% biopsy specimens, respectively. The severity of interstitial fibrosis

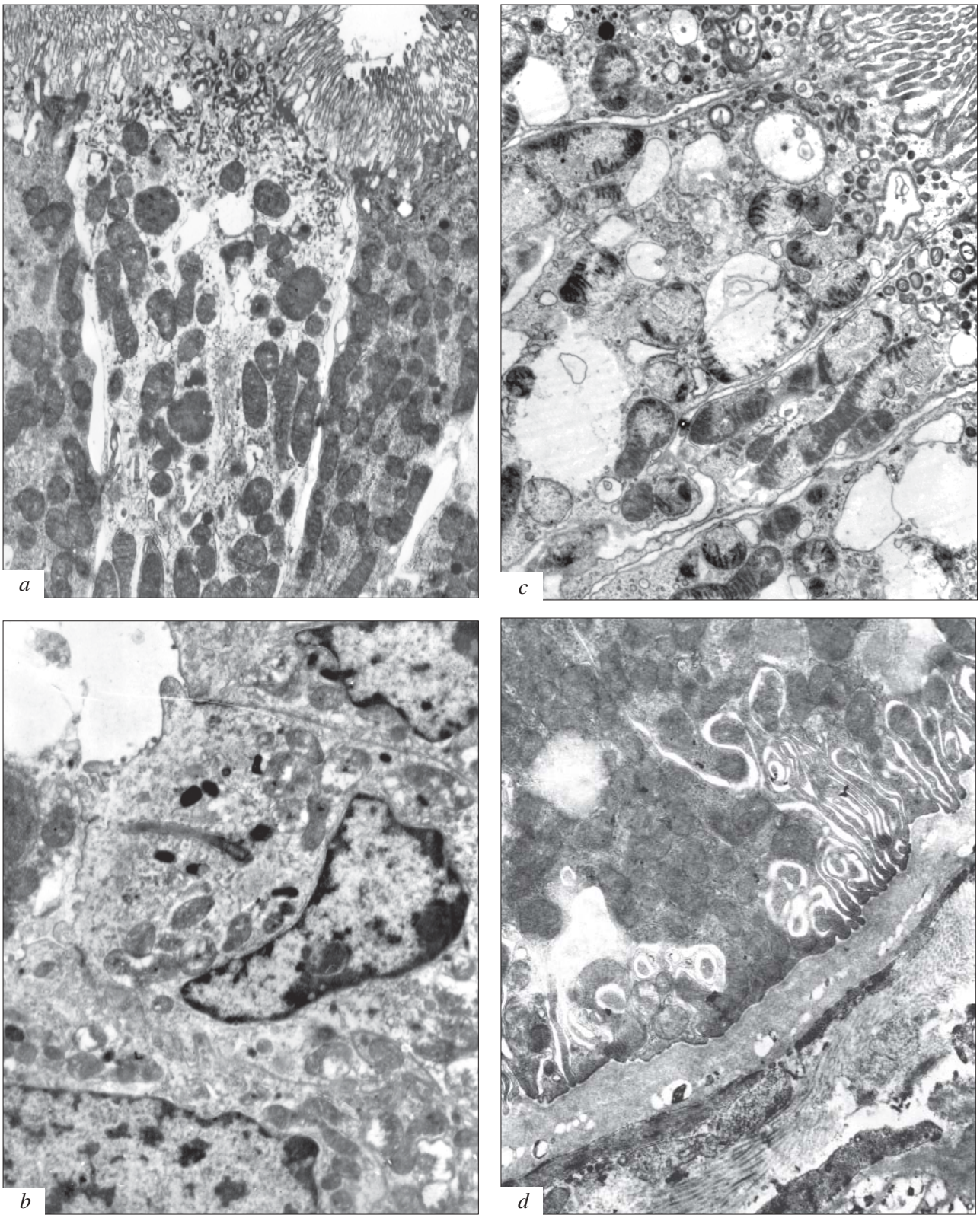

Fig. 1. Ultrastructural changes in epitheliocytes of the proximal (*a, c*) and distal tubules (*b, d*) in nephrobiopsy specimens under normal activity (*a, b*) and dysfunction of the kidneys (*c, d*). Regular brush border, numerous endocytotic vesicles and mitochondria (×4000, *a*); reduction and focal destruction of mitochondria (×4000, *b*); vacuolation of the cytoplasm and destruction of mitochondria (×6000, *c*); degeneration of cytoplasmic organelles, peritubular collagen fibrils (×6000, *d*).

was highest in nephrobiopsy specimens from group 3 patients. Diffuse coarse-fibrous fibrosis of the stroma was most pronounced in the peritubular and perivascular zone (degree II-III fibrosis).

Nephrobiopsy specimens from patients of various groups were rarely infiltrated with cells. Small mononuclear infiltrates that mainly consisted of lymphocytes were rarely found in the stroma. Study of pathological changes in the interstitium revealed several phenomena. First, the severity of fibrosis in the stroma did not depend on the type and degree of glomerular dysfunction. And second, the development of focal interstitial sclerosis was usually associated with atrophy of the tubular epithelium.

Vascular pathology was mainly associated with arterial hypertension and structural manifestations of this syndrome (hyperelastosis of arterioles and small arteries, myoelastosis, and myoelastofibrosis). Vascular remodeling in nephrobiopsy specimens from group 1 patients was manifested in hyperelastosis and, more rarely, in myoelastosis. Signs for profound reconstruction of the vascular wall were revealed in nephrobiopsy specimens from group 2 patients. They included myoelastofibrosis associated with dystrophy of endotheliocytes. Fibrosis of the vascular wall (myoelastofibrosis) was a characteristic sign in nephrobiopsy specimens from group 3 patients. The duration and severity of arterial hypertension depended on the degree of structural reconstruction in arterial vessels. Postglomerular capillaries in biopsy specimens from group 1 patients retained normal structure. We revealed only the increase in functional activity of endothelial associates (large number of pinocytotic vesicles, ribosomes, and pseudovilli on the luminal surface). As differentiated from group 1 patients, group 2 patients were characterized by the development of pericapillary fibrosis and structural-and-functional reconstruction of the endothelium. Some endotheliocytes differed in high functional activity due to hyperplasia of cytoplasmic organelles, while others underwent degenerative and dystrophic changes (reduction and destruction of cytoplasmic organelles, including mitochondria). It should be emphasized that dystrophy of the endothelium was accompanied by thickening of

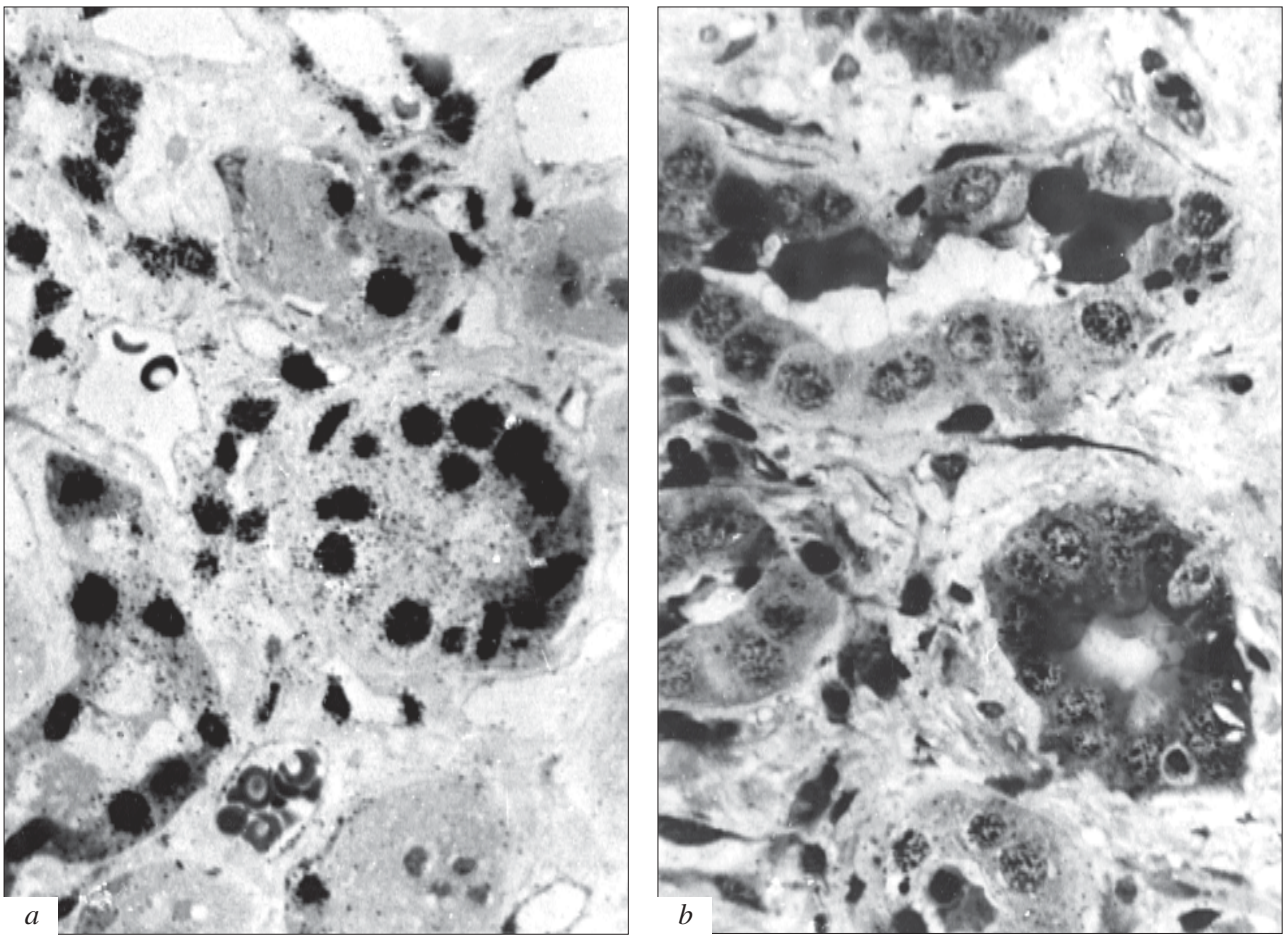

Fig. 2. Biosynthetic processes in cells of the tubulointerstitial compartment in nephrobiopsy specimens. Incubation with ^{3}H-uridine. Semithin sections, azure II staining. High index and density of labeling with ^{3}H-uridine for tubular cells and endotheliocytes in peritubular capillaries (×800, *a*); low-intensity synthesis of RNA in tubular cells, degeneration and desquamation of the tubular epithelium (×600, *b*).

the basal membrane in capillaries and development of pericapillary fibrosis, which resulted in significant changes in transcapillary metabolism. Progressive changes in capillaries of biopsy specimens from group 3 were followed by fibrosis of the wall, degeneration of the endothelium, and obliteration of the lumen (in several specimens).

An autoradiographic study revealed additional signs for structural and functional changes in the tubular epithelium and stromal cells. Tubular cells in nephrobiopsy specimens from group 1 patients were characterized by high index of ^{3}H-uridine labeling (95-100%) and high labeling density. Endotheliocytes in vascular capillaries exhibited a simultaneous response; 85-90% cells contained ^{3}H-uridine (Fig. 2, *a*). Study of ^{3}H-thymidine incorporation showed that only individual tubular cells synthesize DNA. We examined tubular cells in nephrobiopsy specimens from group 2 patients. Biosynthetic and proliferative activity of epitheliocytes in these patients was lower than in group 1 patients. The index of labeling with ^{3}H-uridine in group 2 patients was 41-60%. The labeling density was quite low (Fig. 2, *b*). The ^{3}H-uridine label was absent.

Plastic supply to functional activity of tubular cells progressively decreased in nephrobiopsy specimens from group 3 patients. The index of labeling with an RNA precursor varied from 5 to 40%; the labeling density was extremely low. The index of labeling with ^{3}H-thymidine was 0.1-1%. In group 3 patients the index of endotheliocyte labeling with ^{3}H-uridine was lower than in group 1 patients. In these patients incorporation of ^{3}H-uridine into fibroblasts was noted, which reflected activation of collagen synthesis. Changes in biosynthetic processes in tubular cells reflected progressive damage to cells (from dystrophy to degeneration and atrophy). Degenerative changes in endotheliocytes were not compensated by regenerative reaction. It increased the intensity of collagen synthesis and contributed to dysfunction of the tubular compartment.

Our results show that progressive renal dysfunction is associated with the inhibition of biosynthetic processes in tubular cells and endotheliocytes due to the increase in collagen synthesis in fibroblasts.

REFERENCES

1. N. A. Mukhin, I. E. Tareeva, and E. M. Shilov, *Diagnostics and Therapy of Kidney Diseases* [in Russian], Moscow (2002).
2. L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, S. V. Aidagulova, *et al.*, *Byull. Eksp. Biol. Med.*, **128**, No. 11, 591-596 (1999).
3. N. L. Tov, L. M. Nepomnyashchikh, S. V. Aidagulova, and A. A. Onishchenko, *Ibid.*, **138**, No. 12, 702-707 (2004).
4. B. I. Shulutko, *Diseases of the Liver and Kidneys* [in Russian], St. Petersburg (1993).
5. S. Klahr and J. J. Morrissey, *Kidney Int.*, **57**, No. 75, S7-S14 (2000).
6. W. Kriz, I. Hartmann, H. Hosser, *et al.*, *J. Am. Soc. Nephrol.*, **12**, 496-506 (2001).
7. T. W. Meyer, *Kidney Int.*, **63**, 774-787 (2003).
8. R. Rasch, J. R. Nyengaard, N. Marcussen, and T. W. Meyer, *Ibid.*, **62**, 496-506 (2002).
9. J. Yang and Y. Liu, *J. Am. Soc. Nephrol.*, **13**, 96-107 (2002).