ULTRASTRUCTURAL CHANGES IN COMPONENTS OF THE GLOMERULAR CAPILLARY FILTRATION BARRIER OF THE RESIDUAL KIDNEY FOLLOWING TEMPORARY ISCHEMIA

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During transplantation the kidney is unavoidably subjected to the influence of various harmful factors which have a significant effect on the outcome of the operation and on the functional state of the grafted organ. One such factor, most hazardous for the viability of the transplanted kidney, is ischemia. Many investigators have described morphological disturbances of the structural organization of the nephrons and capillaries of the kidney when its circulation is disturbed [1-4, 6, 8] and during its subsequent restoration [5, 9-11]. There have been only a few investigations into the effect of temporary circulatory hypoxia on the residual kidney exposed to an increased functional load, as is the case when the kidney is transplanted [7, 12].

With this in mind, in the investigation described below the character of the submicroscopic changes arising in components of the filtration barrier of the residual kidney was investigated at different stages of function after temporary ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 40 adult sexually mature Wistar rats. The control group consisted of five animals. A soft clamp was applied to the vascular pedicle of the left kidney (the right kidney was removed) for 30 min, 1 h, and 2 h, followed by recirculation for 3, 7, 14, 30, 60, 180, and 360 days. Pieces from the middle part of the renal cortex were fixed in 1% OsO_4 solution in phosphate buffer, pH 7.4. They were then washed to remove excess of fixative with buffer, dehydrated in alcohols of increasing strength, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut from blocks on Tesla BS 490A and LKB III ultramicrotomes, stained with lead citrate, and examined in the field of vision of the UÉMF-100B and JEM-100B electron microscopes.

EXPERIMENTAL RESULTS

In the early stages (3rd and 7th days) after 30 min of ischemia in the residual kidney the cytoplasm of the peripheral part of the endotheliocytes of the glomerular capillaries became almost 1.5 times thicker (90.27 ± 3.31 nm compared with 56.12 ± 1.56 nm in the control). Numerous discrete cytoplasmic "islets" with many vesicles and vacuoles appeared in it, and the diameter of the pores and fenestrae increased (from 55.01 ± 1.44 nm in the control to 62.37 ± 1.31 nm). The nuclei of the endotheliocytes became larger and their nucleoplasm had low electron density. Chromatin granules underwent regrouping and were in a state of marginal aggregation. The tubules and cisterns of this rough endoplasmic reticulum were dilated. The Golgi complex consisted of flattened sacs and round vesicles. The mitochondria in the perinuclear zone were somewhat swollen, their matrix translucent, and the number of cristae was reduced. The basement membrane of the glomerular capillaries was widened in places (250.49 ± 4.15 nm compared with 166.76 ± 2.62 nm in the control). The epithelial cells of the glomeruli (podocytes) contained many vacuoles. Their nuclei were irregular in shape, the nucleolemma had evaginations, and the nucleoplasm had low electron density. Profiles of the Golgi complex and rough endoplasmic reticulum of the podocytes were widened. Many mitochondria were enlarged.

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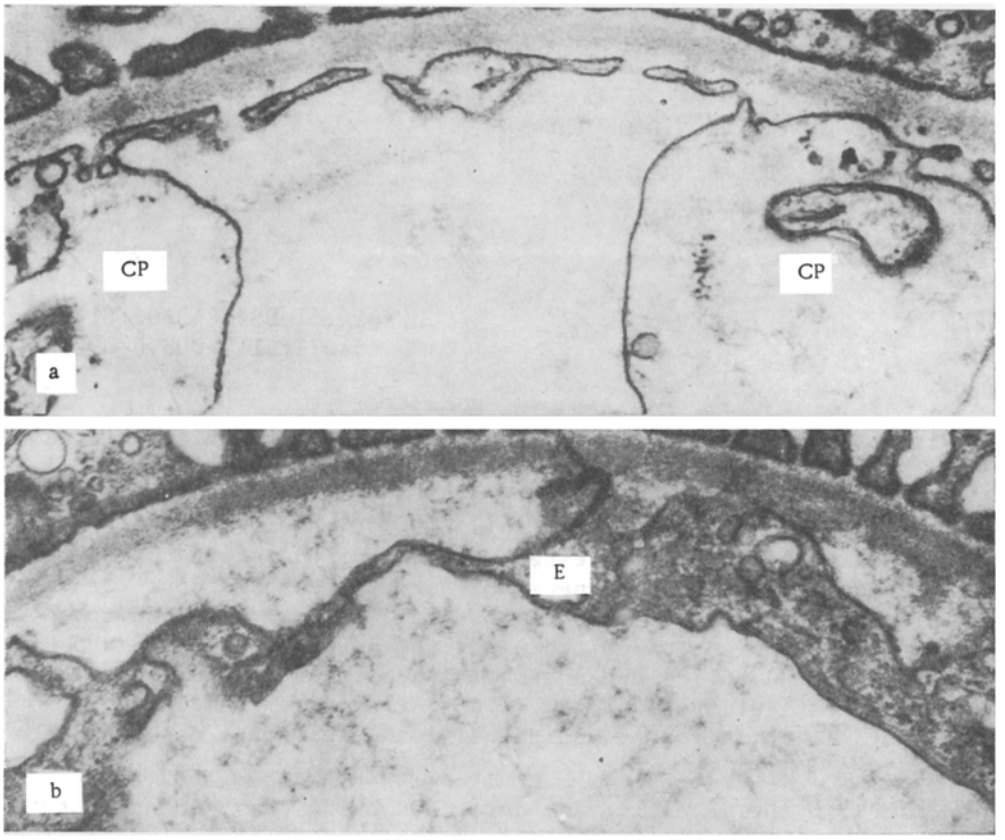

Fig. 1. State of endothelial cells of glomerular capillaries of residual kidney 3 (a) and 7 (b) days after ischemia for 60 min. Edema of cytoplasmic processes (CP) and detachment of flattened part of endotheliocytes (E). 14,000 ×.

The cytopodia were a little longer and thinner than normal, and the filtration spaces between them were widened. The mesangial cells were enlarged and their processes showed a tendency to advance toward the lumen of adjacent glomerular capillaries. The ultrastructural manifestations of circulatory hypoxia described above in the residual kidney became less marked 14 and 30 days after 30 minutes of ischemia. Signs of hypertrophy and hyperplasia of the bioenergic and biosynthetic organelles were observed in structural components of the filtration barrier, a reactive response to the process of compensatory hypertrophy developing in the residual kidney. These changes became more marked after 180 and 360 days.

Toward the end of the 3rd and 7th days after 60 minutes of ischemia of the residual kidney, cytoplasm of the endotheliocytes of the glomerular capillaries was swollen, with an electron-transparent hyaloplasm, and contained many micropinocytotic vesicles and vacuoles. Its peripheral part was thickened to 95.98 ± 1.22 nm and the diameter of its pores and fenestrae was increased to 65.33 ± 3.01 nm. The cytoplasmic processes were distinctly edematous and projected far into the lumen of the glomerular capillaries (Fig. 1a). In some areas the flattened peripheral part of the endothelial cells was detached from the basement membrane, with the formation of subendothelial spaces (Fig. 1b). The nuclei were enlarged and chromatin granules were distributed in large masses. Polymorphic vacuoles with pale contents could be seen in the perinuclear zone. The basement membrane of the glomerular capillaries was considerably thickened (277.51 ± 5.88 nm) and its relief disturbed. Podocyte nuclei had smooth outlines and contained aggregations of chromatin. The perinuclear space was ill-defined. The Golgi complex consisted mainly of large vesicles. The outer membranes of the cisterns of the rough endoplasmic reticulum were thin and free from granules. The mitochondria were swollen, with a translucent matrix and destruction of their cristae. Large, closed profiles of varied electron density, resembling protein drops, surrounded by membranes, appeared in the cytoplasm of the podocytes (Fig. 2). The cytopodia of the podocytes were lengthened (440.39 ± 15.01 nm compared with 359.23 ± 9.21 nm in the control) and were deformed in places. The filtration spaces were widened to 65.04 ± 2.01 nm compared with the control (35.92 ± 1.12 nm). In some

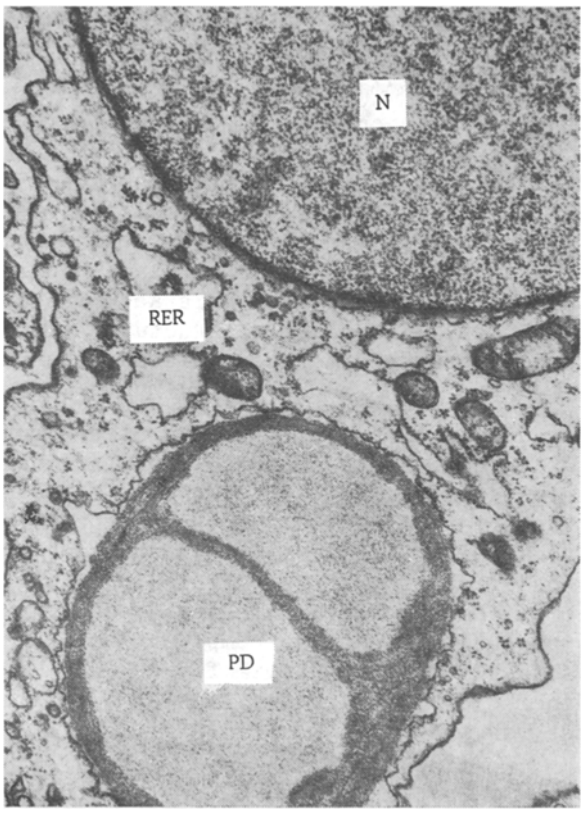

Fig. 2. Ultrastructure of podocytes of glomerular capillaries 7 days after ischemia of residual kidney for 60 min. Enlargement of nuclei (N), widening of cisterns of rough endoplasmic reticulum (RER), and appearance of protein drops (PD) in cytoplasm. 8000 ×.

glomerular capillaries the foot processes were vacuolated, and in some places they were joined together into a single band. Damage to the structural components of the glomerular filter imposed a state of functional stress on the mesangial cells and their processes. After 3 and 7 days the mesangiocyte nuclei became polymorphic, with numerous invaginations and evaginations of the karyolemma and peripheral condensation of chromatin. The dictyosomes of the Golgi complex were transformed into vacuoles and vesicles. The rough endoplasmic reticulum consisted of dilated cisterns and tubules. The number of hypertrophied processes of mesangiocytes was increased. Their pear-shaped peripheral parts exerted pressure on the foci of endothelial proliferation, reducing their thickness, and projected into the lumen of the glomerular capillaries.

After 14 and 30 days of the experiment the thickness of the peripheral part of the endothelial cells was still increased. The diameter of the pores and fenestrae was 67.78 ± 2.48 nm. The nuclei were enlarged, their contours smoothed, and granules of chromatin substance were dispersed. In the perinuclear zone cisterns of the rough endoplasmic reticulum, and vesicles and sacs of the Golgi complex were dilated. The mitochondria were enlarged, their matrix translucent, and the orientation of their cristae disturbed. The thickness of the basement membrane of the glomerular capillaries was a little less than at the previous time (220.04 ± 4.41 nm). The podocyte nuclei were curiously shaped and chromatin granules were located along the inner nuclear membrane. The rough endoplasmic reticulum consisted of numerous cisterns and tubules. A few independent Golgi complexes with the typical, and slightly hypertrophied, triad of components, appeared in the region of the perikaryon. The number of small mitochondria with an electron-dense matrix was increased. The podocyte cytopodia were deformed in places. Some processes of mesangial cells attained a considerable size and projected freely into the capillary lumen. On the 180th and 360th days after 60 minutes of ischemia of the residual kidney, besides persistent morphological manifestations of postischemic

damage to the ultrastructure, in most glomerular capillaries signs of hypertrophy and hyperplasia of the intracellular organelles could be seen in the endothelial, epithelial, and mesangial cells.

The severest destructive changes in components of the filtration barrier of the residual kidney were observed after 2 hours of ischemia. For instance, after 3 and 7 days the cytoplasm in the endotheliocytes of the glomerular capillaries became swollen and the thickness of its peripheral part reached 109.81 ± 4.11 nm. The nuclei were enlarged, with a reduced number of granular elements in the karyoplasm. Cisterns of the endoplasmic reticulum and elements of the Golgi complex were flattened and their outlines poorly defined. Mitochondria were swollen with a translucent matrix and reduced cristae. The cytoplasm in the podocytes was rich in large and small vacuoles. Cytoplasmic structures also were in a state of functional overstrain. The cytopodia were deformed and relations between them and the filtration spaces disturbed.

These investigations showed that temporary ischemia of the residual kidney leads to marked ultrastructural changes in components of the glomerular capillaries, which depend directly on the duration of disturbance of the blood flow, in the early stages of its compensatory hypertrophy [5]. After temporary ischemia for 30 min the hypoxic phenomena had no significant damaging effect on them and the process of compensatory hypertrophy developed in structures of the glomerular capillaries just as in the kidney whose circulation was undisturbed. A longer disturbance of the circulation in the residual kidney, followed by recirculation, led to marked ultrastructural changes in components of the filtration barrier, which were manifested particularly clearly after ischemia for 2 hours. Under these circumstances destructive processes predominated over reparative. In damaged areas, the intensity of compensatory reparative processes was weak.

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