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MORPHOLOGICAL ASSESSMENT OF STRUCTURAL CHANGES IN ARTERIES AND ARTERIOLES OF THE KIDNEY IN EXPERIMENTAL RENAL ISCHEMIA

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The effect of experimental ischemia of varied duration on the structural organization of the renal parenchyma has been studied by several investigators [2, 4, 7, 9, 11]. Previous publications [5, 6] have contained descriptions of ultrastructural changes in the glomerular capillaries of the kidney and certain parts of the nephron in ischemia with or without restoration of the circulation it it.

In the present investigation structural changes in the walls of the intramural arteries and glomerular arterioles of the kidney, which characteristically give a marked spastic response [1], were studied during ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 84 adult Wistar albino rats with 7 animals used at each time. Animals of group 1 were killed 30 min and 1, 2, and 3 h after ligation of the left renal artery and vein, animals of group 2 were killed 3 and 30 days after restoration of the circulation through the artery. At the same time the right kidney was removed in order to bring the experimental conditions close to those of clinical transplantation of the kidney. The control group consisted of 10 animals. Pieces of kidney were fixed, dehydrated, and embedded in paraffin wax blocks. Sections 5-6 μ thick were stained with hematoxylin and eosin, with Weigert's fuchselin, and counterstained by Van Gieson's method. Material for electron-microscopic study was fixed in 1% OsO_4 solution and, after dehydration, embedded in a mixture of Epon and Araldite. Sections from the blocks were studied with the IEMB-100B and JEM-100B electron microscope with an accelerating voltage of 75 kV. For an objective analysis of changes in the parameters of the vessel wall, the vessels were subjected to morphometry, followed by statistical analysis of variance of the numerical data.

EXPERIMENTAL RESULTS

In the first 30 min after occlusion of the renal artery and vein of the left kidney appreciable changes were observed in the endothelial cells in the wall of the interlobar arteries (they swelled and projected into the lumen of the vessel). The inner elastic membrane (IEM) had clearly distinguishable coils. The smooth-muscle cells (SMC) in the media followed a circular course and their nuclei remained elongated in shape. The outer elastic membrane contained shallow indentations (Fig. 1a). In the smaller arteries (arcuate, interlobular)

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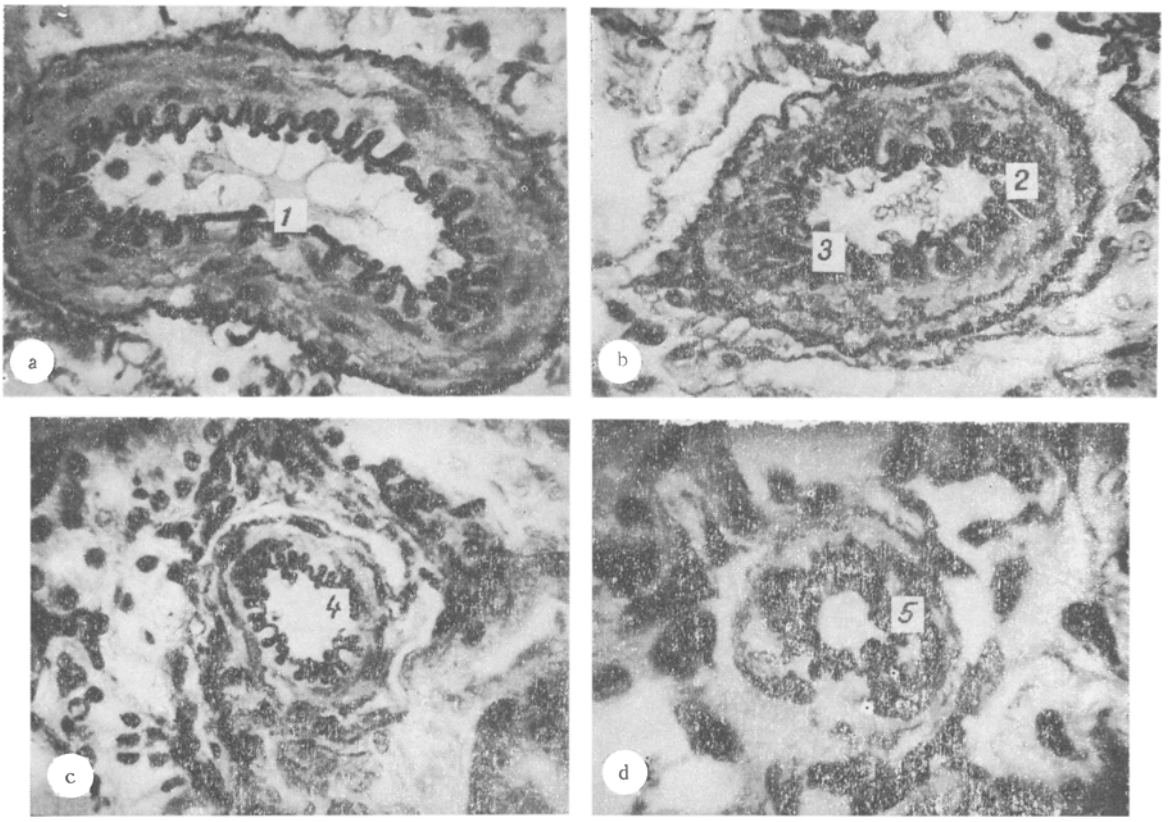

Fig. 1. Structural changes in walls of renal artery of a rat after 30 min of ischemia due to ligation of the vascular pedicle. a) Interlobar artery: 1) IEM with indentations of unequal depth, 300 ×; b) arcuate artery: 2) swollen endothelial cells, 3) irregular folding of IEM, 300 ×; c) interlobular artery; 4) nuclei of endothelial cells at spices of folds. IEM, 400 ×; d) afferent arteriole: 5) swollen nuclei of endothelial cells, 1350 ×. Stained with fuchselin and picrofuchsine.

the IEM showed irregular folding with deep invaginations and evaginations (Fig. 1b, c). The lumen of the afferent and efferent glomerular arterioles was irregular in shape. In certain areas the folding of IEM became irregular. The endothelial cells were swollen and their nuclei located on the apices of the folds, irregularly (Fig. 1d). Because of swelling of the endothelium the lumen of the vessels was narrowed. Statistical analysis of the numerical data reflecting variation in size of the lumen showed an increase in the thickness of the wall ($P < 0.001$) in the afferent arterioles, but the diameter of the lumen of the vessels was reduced. This was attributable to edema of the structural components of their wall, and also to the spastic response of the vessels to the fall of pressure resulting from ligation of the renal artery.

At subsequent times during the experiments (1, 2, and 3 h) more marked swelling of the endothelial cells was observed, and as the duration of ischemia lengthened, they became detached and lay freely in the lumen of the vessel. Folding of the IEM was no longer uniform, and the membrane itself was stretched. SMC in the media were vacuolated. The outer elastic membrane had indistinct boundaries. As a result of prolonged hypoxia the tone of the arterial vessels was reduced and their walls relaxed.

In experiments in which the circulation in the kidney was restored and nephrectomy of the opposite kidney was performed, the structural elements of the walls of the larger (interlobar) arteries in the early stages of ischemia (30 min) with recirculation showed no marked changes on the 3rd day. In some places the endotheliocytes were swollen and the coils of the IEM were stretched. In the arcuate and interlobular arteries the IEM was greatly stretched and split, and the vessel wall itself was thinner and showed aneurysmal evaginations.

Endothelial cells in the afferent and efferent arteriloes in the early stages (3 days) after 60 min of ischemia projected sharply into the lumen of the arterioles, which was not

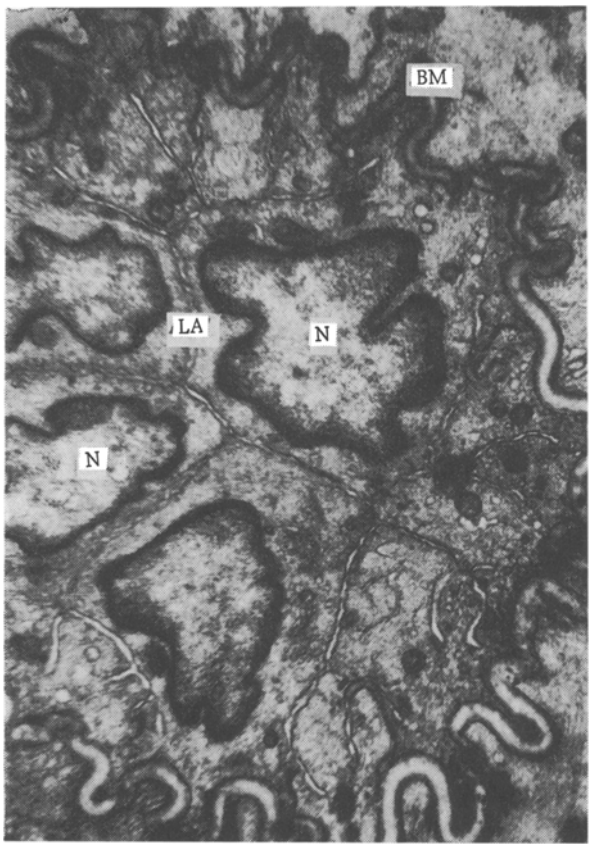

Fig. 2. Afferent glomerular arteriole of residual kidney 3 days after 60 min of ischemia. Lumen of arteriole (LA) completely closed. Nuclei (N) of endotheliocytes are curiously shaped. Basement membrane (BM) festooned. 6000 ×.

significantly narrowed compared with normal. In the afferent arteriole, the lumen of which was not completely closed (3.45 ± 0.10 μ compared with 7.61 ± 0.26 μ in the control), the cytoplasm of the endotheliocytes had low electron density and contained numerous small and large vesicles. The nuclei were swollen, elongated in shape, and oriented radially relative to the lumen of the vessel. The outlines of the nuclear membrane were smooth. Chromatin granules were distributed irregularly in the nucleoplasm, and condensed in places. In the glomerular arterioles with a completely closed lumen, the endothelial cells were closely apposed. Their nuclei were curiously shaped, with invaginations of the nuclear membrane. Chromatin material was in a state of marginal aggregation (Fig. 2). The cytoplasm of the endotheliocytes was of average electron density. The mitochondria varied in their morphological state: some were small in size, with an electron-dense matrix, whereas others were enlarged, with a translucent matrix and solitary cristae. The number of secondary lysosomes was increased. The cisterns of the endoplasmic reticulum were dilated. The vesicular components predominated in the Golgi complex. The outlines of the luminal plasmalemma of the endothelial cells were blurred in places, and their intergrity was disturbed. Large vacuoles with clearly demarcated membranes and with contents of low electron density were seen in the sarcoplasm of the SMC. Many myofilaments were oriented toward the basement membrane. Osmiophilic granules were rare in the juxtaglomerular cells of the afferent glomerular arterioles on the 3rd and 7th days after ischemia for 60 min. Large cisterns appeared between the granules. The mitochondria were swollen.

When a longer period of ischemia (2 and 3 h) was followed by restoration of the circulation on the 3rd day, endothelial cells in the interlobar vessels, in a state of destruction, were arranged in groups in the lumen of the vessel. The IEM was totally absent in some places (Fig. 3a). The muscular coat, because of dystrophic changes in the SMC, was homogenized.

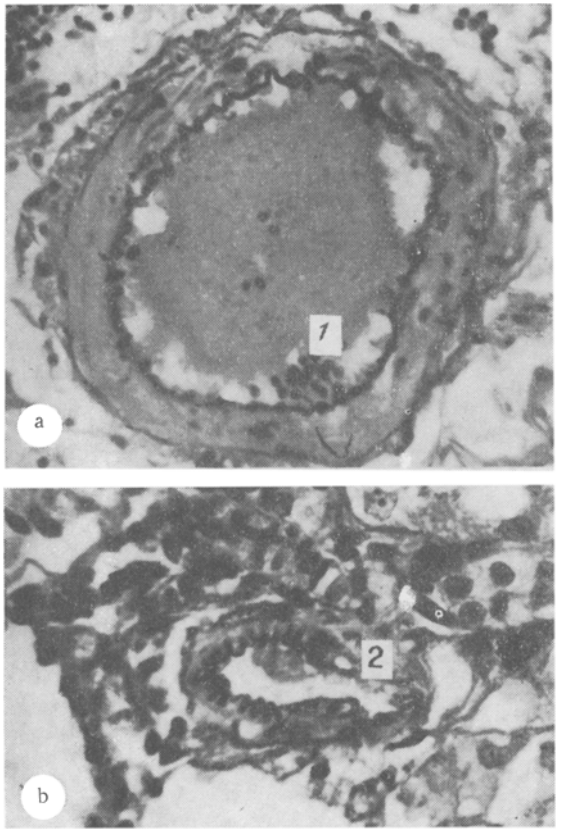

Fig. 3. Structural changes in arterial wall 3 days after ischemia of left kidney for 2 h. a) Interlobar artery: 1) endothelial cells destroyed, with nuclei in lumen of vessel, 300 ×; b) interlobular artery: 2) disturbance of integrity of IEM with formation of aneurysmal evaginations of vessel wall. Stained with fuchselin-picrofuchsine, 600 ×.

Because of the great decrease in thickness of their walls the interlobular arteries formed evaginations of microaneurysmal type (Fig. 3b). Restoration of the circulation in the kidney on the 30th day after transient ischemia (30 min) led to complete restoration of the histological structure and ultrastructure of the walls of the arteries and glomerular arterioles. After ischemia for 60 min, destructive changes in the endothelium and IEM were observed in individual arteries. After ischemia of the kidney for 2-3 h all the experimental animals died in the course of 1 week as a result of severe impairment of the renal circulation.

These experiments demonstrated that acute ischemia of the kidney caused by occlusion of its vascular pedicle for 30-60 min, without subsequent restoration of the circulation, leads to comparatively mild microultrastructural changes in the walls of the intramural arteries and arterioles. With an increase in the duration of ischemia (2 and 3 h) these changes increased appreciably, the endothelial cells of the vessels are detached, the fibers of the IEM become unwound, and the muscle cells of the media swell.

Three days after temporary ischemia of the residual kidney for 30-60 min (the other kidney was removed), disturbance of the integrity of the endotheliocytes, stretching of the IEM, and a decrease in thickness of its walls were observed in the small vessels. With a passage of time (30 days after temporary ischemia) the structural components in the walls of the intramural arteries and arterioles were completely restored. Prolonged but temporary ischemia (2 and 3 h) of the kidney; followed by restoration of the circulation in it, led to substantial changes in the structural components of the vessels. Elements of the muscular-elastic skeleton of the arterial walls and, in particular, IEM, which is subjected to the greatest functional hemodynamic load, lost their inherent plasticity, elasticity, and tone, and underwent destructive changes [10]. In the postischemic period the lumen of most afferent and

efferent glomerular arterioles remained greatly constricted. This was due to their prolonged reactive spasm and corresponding disturbances of the structure of the vascular membranes with injury to endothelial and muscle cells [4]. This last phenomenon caused considerable slowing or complete cessation of the microcirculation in the glomerular and peritubular capillaries [1]. The gross disturbacne of the regional circulation at the level of the interlobular arteries with total spasm of the afferent arterioles caused reduction of the cortical circulation in the postischemic period, with shunting of blood along the juxtamedullary circle of the renal circulation [3, 12]. In this way secondary ischemia of the kidney arises and causes irreversible changes in its tissue structures [1].

Experimental renal ischemia leads to appreciable morphological changes in the structural components of the walls of the intramural arteries. They increased in severity particularly in the small resistive vessels during subsequent restoration of the circulation, in connection with the increase in hemodynamic load. During recirculation after renal ischemia for 2-3 h irreversible changes take place in the structural components of the walls of the renal vessels.

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