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Ultrastructural changes of the renal cortex after septic shock in rats

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Summary. The purpose of this study was to observe the ultrastructural changes of the renal cortex that occur in septic shock.

Twenty male Wistar rats were used. They were divided into 4 groups of five animals each one. A septic shock was caused by intraperitoneal injection of 5×10^6 living E.Coli, in a dose of 2ml/100gr B.W., in four rats of each group. The fifth rat of each group was used as control, and was injected by an equal volume of normal saline. A small segment from the right kidney was excised from every rat of each group, respectively 1, 2, 4 and 6 hours after the onset of the shock.

Morphologically, alterations of the endothelium of the peritubular and glomerular capillaries were found. The endothelial layer was thick with many cytoplasmic folds. In the glomeruli, the basal lamina as well as the overlying epithelial podocytes and their foot processes were swollen. The urinary space contained erythrocytes and cell debris. Lesions of the proximal and distal tubule epithelium were also observed. The basal infoldings of the cell membrane disappeared in many epithelial cells of the proximal tubule and the brush border showed varying degrees of disruption. Within the tubular lumen much cellular debris was found. The epithelium of the distal tubule was thinner in some portions and in others it was swollen. The lesions were focal during the first hour after the onset of shock, while at the fourth and sixth hours they were most extensive.

In conclusion, the ultrastructural findings after septic shock were non-specific and indicated cellular injury.

Key words: E.Coli, Septic shock, Renal cortex ultrastructure

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Introduction

This experimental study has been performed in order to examine the ultrastructure of the renal cortex after the onset of a septic shock, and to relate the lesions with the corresponding functional disturbance of the organ.

The word shock has been used for at least a century to describe a progressive but gradual collapse of vital organ functions (McLean, 1985). The failure of the circulatory system to deliver the chemical substances necessary for cellular survival and to remove the waste products of cellular metabolism, is the common denominator in shock (Abboud, 1979).

Septic shock was first clearly described only in 1951 by Waisbren. Trump (1975) suggested that anoxia and ischemia were probably the «key mechanism» of cellular damage in human shock and he also could not exclude direct membrane damage effects, especially in septic shock. The renal participation in septic shock is manifested with a prolonged, progressive decrease in outer cortical flow, leading to ischemia, which is due in the increased vascular resistance (Thompson, 1975a; Lucas 1976).

Materials and methods

Twenty Wistar rats weighing 250-280gr were used, divided into four groups. An intraperitoneal injection of 5×10^6 living E.Coli organisms (NCTC 10418) per ml normal saline in a dose of 2ml/100 gr B.W. was determined by prior experiments as causing death in 8.8 $\pm$1.2 hours. Four rats of each group received an intraperitoneal injection of E.Coli suspension and one control animal of each group was injected with an equal volume of normal saline.

The experimental and control animals were anaesthetized (Pentothal Sodium, 1mg/100gr B.W.) and, after laparotomy performance, a small segment of renal cortex from the right kidney was excised respectively 1, 2, 4 and 6 hours after the onset of

septicaemia from each group. All animals were then sacrificed by high doses of benzodiazepine intracardially.

Small pieces of tissue were fixed in buffered solution of 3% glutaraldehyde for 2 hours. Subsequently they were postfixed in 2% osmium tetroxide-buffered solution for 90min. After washing with buffered solution the specimens were dehydrated through graded series of alcohols and embedded in EPON. Semithin sections, 1μm thick, were stained with toluidine blue and surveyed by optical microscopy. Ultrathin sections were cut with a Reichert-Jung ultramicrotome, stained with uranyl acetate and lead citrate solutions and examined in a JEOL TEM 100cx electron microscope, in 80KV.

Results

Our study showed alterations of the endothelium of the peritubular and glomerular capillaries. The capillary lumens were packed with erythrocytes and the endothelium of the peritubular capillaries was swollen in many portions (Figs. 1, 2). The endothelial layer of the glomerular capillaries showed many cytoplasmic protrusions which penetrated into the glomerular basement membrane (Figs. 3, 4). In other portions cytoplasmic processes of the mesangial cells could be seen projecting into the capillary lumen, covered by endothelial cytoplasm (Fig. 5). Cytoplasmic junctions were also observed between the mesangial and endothelial cells (Fig. 6), as well as focal disappearance of the lumen of the glomerular capillaries.

At the renal corpuscles the glomerular basement membrane was thicker in some portions, containing collagen fibres, microvacuoles and granules (Figs. 3, 7). The epithelial cells of the visceral layer of Bowman's capsule showed morphological alterations as well. In many of them the major processes were flattened, while the cytoplasm of others contained myelin like figures (Fig. 4). The cell membrane was broken in some podocytes and their organelles filled the urinary space which also contained many erythrocytes (Fig. 8).

Fig. 1. A peritubular capillary. Its lumen is packed with erythrocytes. The endothelium is slightly swollen.
Px = proximal convoluted tubule. × 16,000

Histological lesions of the proximal and distal tubule epithelium were also observed. The epithelium of the proximal convoluted tubules showed more extensive disorganization. The basal infoldings of the cell membrane had disappeared in many epithelial cells. Focal thickening of the basal cytoplasmic membrane was also observed in some of them (Fig. 9). Other epithelial cells showed more extensive disorganization of their cytoplasm and in places the membranes of the cell and the nucleus were broken. (Fig. 10). The brush border was intact in many proximal tubules. In some others there was a loss of the brush border. The microvilli were shorter and within the lumen of the tubules damaged mitochondria, many disrupted microvilli and cell debris were observed (Fig. 11). The epithelium of the distal tubule was thinner while in others it was swollen and their cytoplasm presented extensive disorganization (Fig. 2).

The histological lesions that were observed, did not include all the renal corpuscles and tubules and they were not the same in all cells. They were focal during the first hour after the onset of shock, while at the fourth and sixth hour they were more extensive.

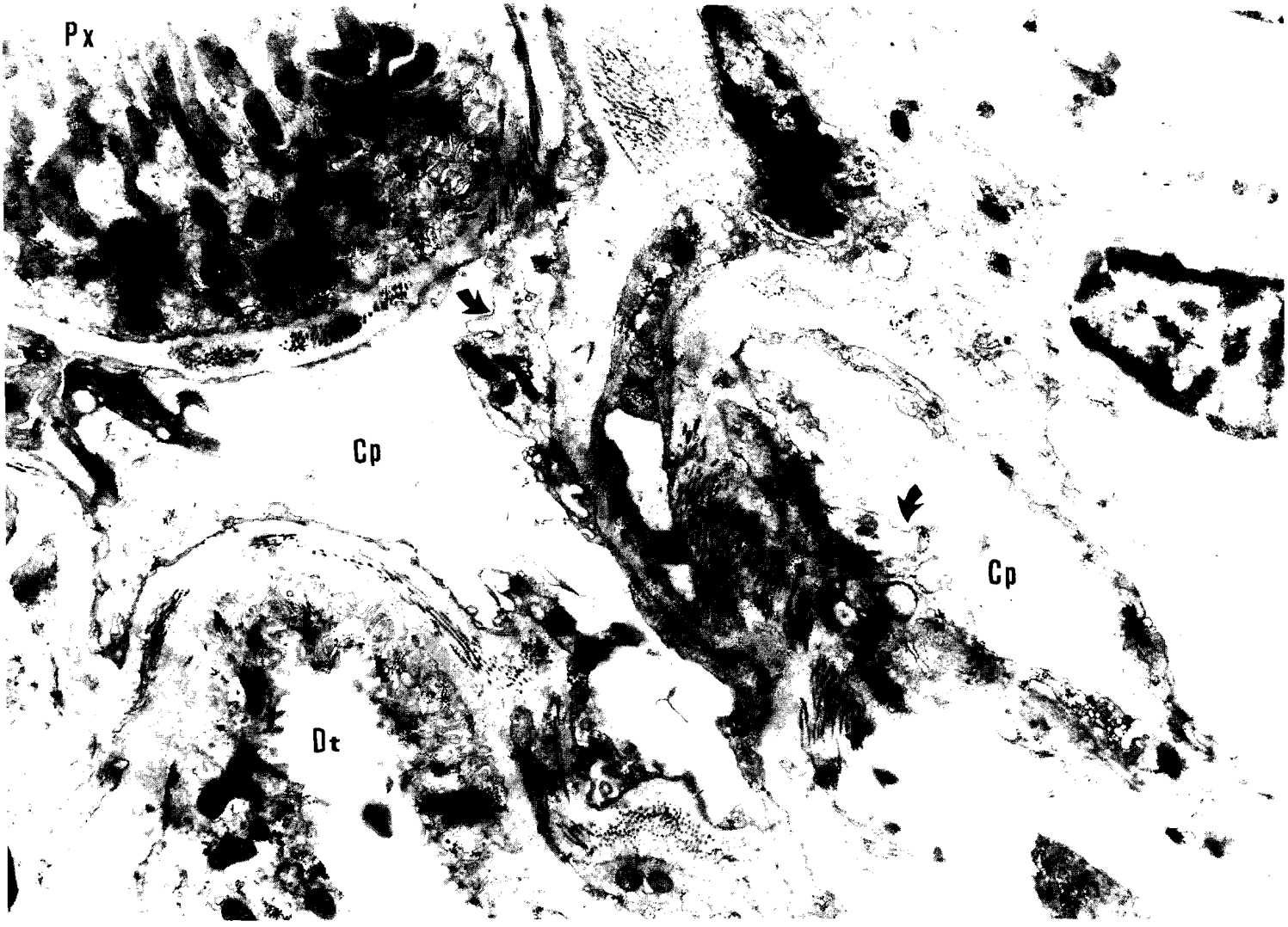


Fig. 2. Part of a proximal and distal tubule and peritubular capillaries, whose endothelium is swollen (←—)
Px = proximal tubule Dt = distal tubule with thinner epithelium. cp = capillary. × 9,000

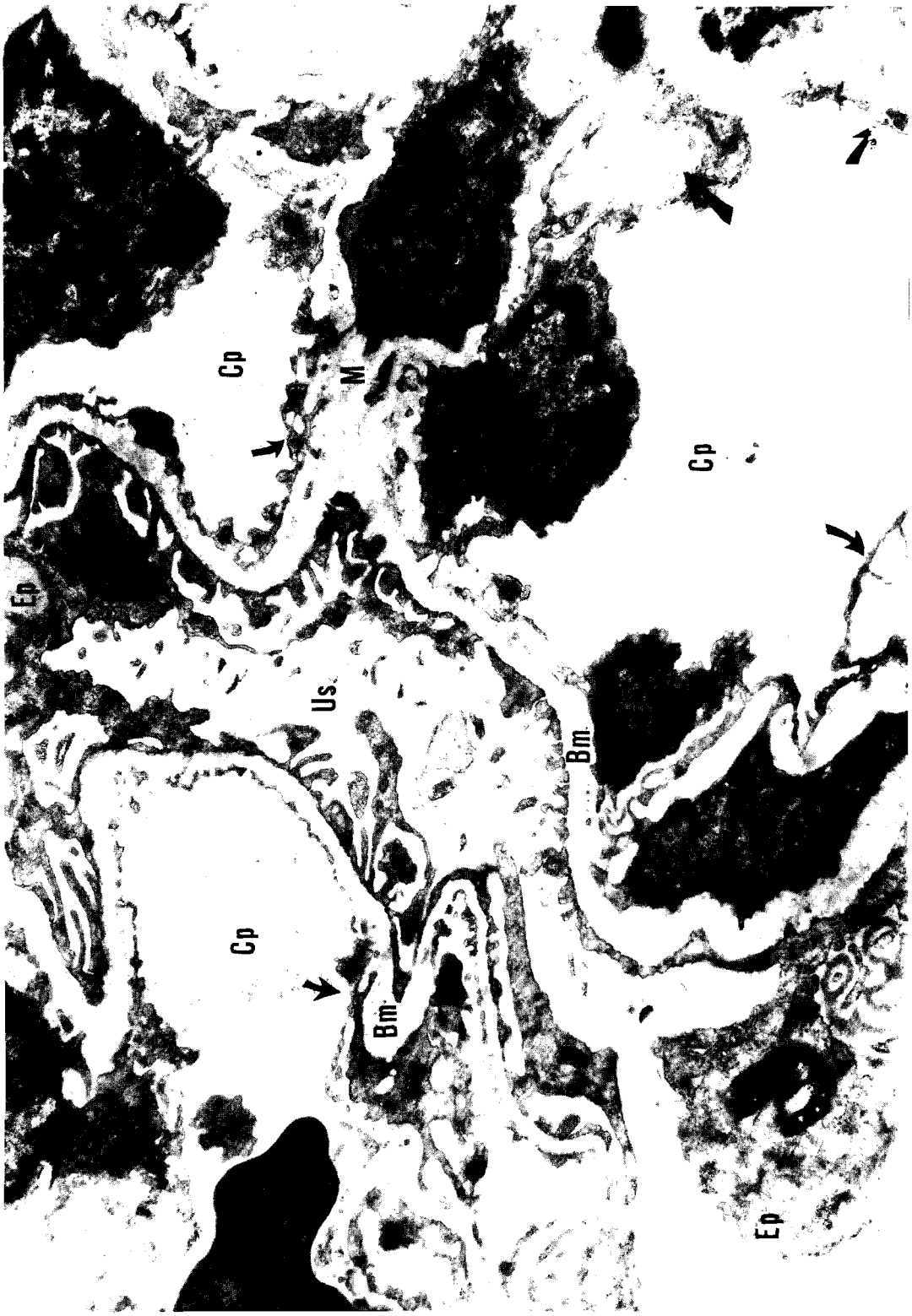


Fig. 3. Part of a renal corpuscle. The endothelial layer shows many cytoplasmic protrutions (◄—) that penetrate into the glomerular basement membrane (Bm) which contains collagen fibres. Us = urinary space. cp = capillary. Ep = podocyte. M = mesangion. × 10,500

Fig. 4. Part of a renal corpuscle. The podocyte (Ep) contains a myelin-like figure. Very flattened major process between the head arrows as well as cytoplasmic protrusions (←—) from the endothelial layer, which penetrate into the glomerular basement membrane (Bm) can be seen. cp = capillary, Us = Urinary space, Fp = foot process. x 17,000

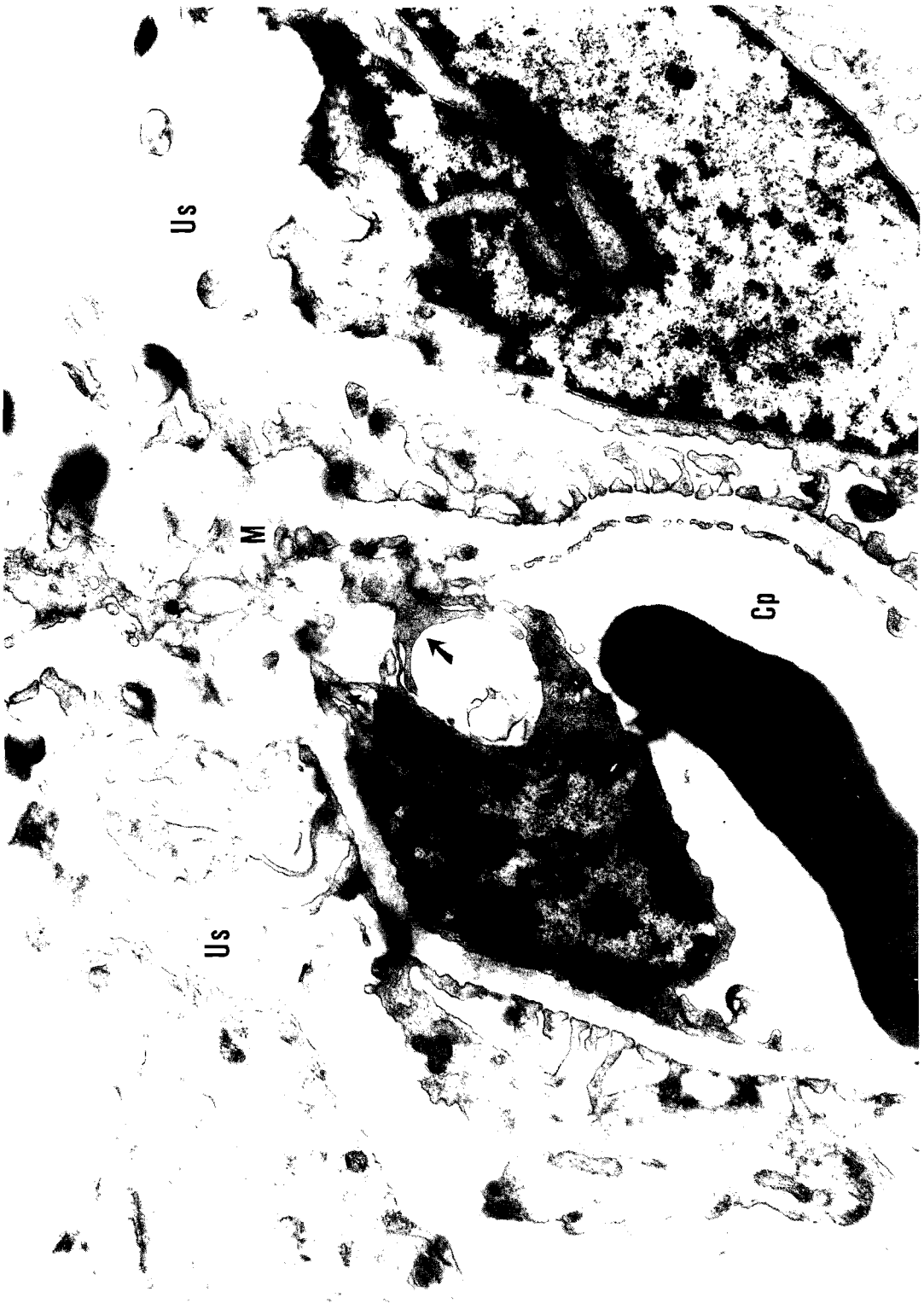


Fig. 5. Part of a renal corpuscle. Cytoplasmic processes (←—) of the mesangial cells can be seen projecting into the capillary lumen, covered by endothelial cytoplasm. cp = capillary, M = mesangion, Us = urinary space. × 17,000

Fig. 6. Part of a renal corpuscle. Cytoplasmic junctions (◂—) between the mesangial cells can be seen. A cytoplasmic process of a mesangial cell projects into the capillary lumen (head arrow). cp = capillary. Us = urinary space. M = mesangion. × 16,000

Fig. 7. Part of a renal corpuscle. The glomerular basement membrane (Bm) is thicker and contains microvacuoles. cp = capillary, Ep = podocyte, Fp = foot process. × 30,000

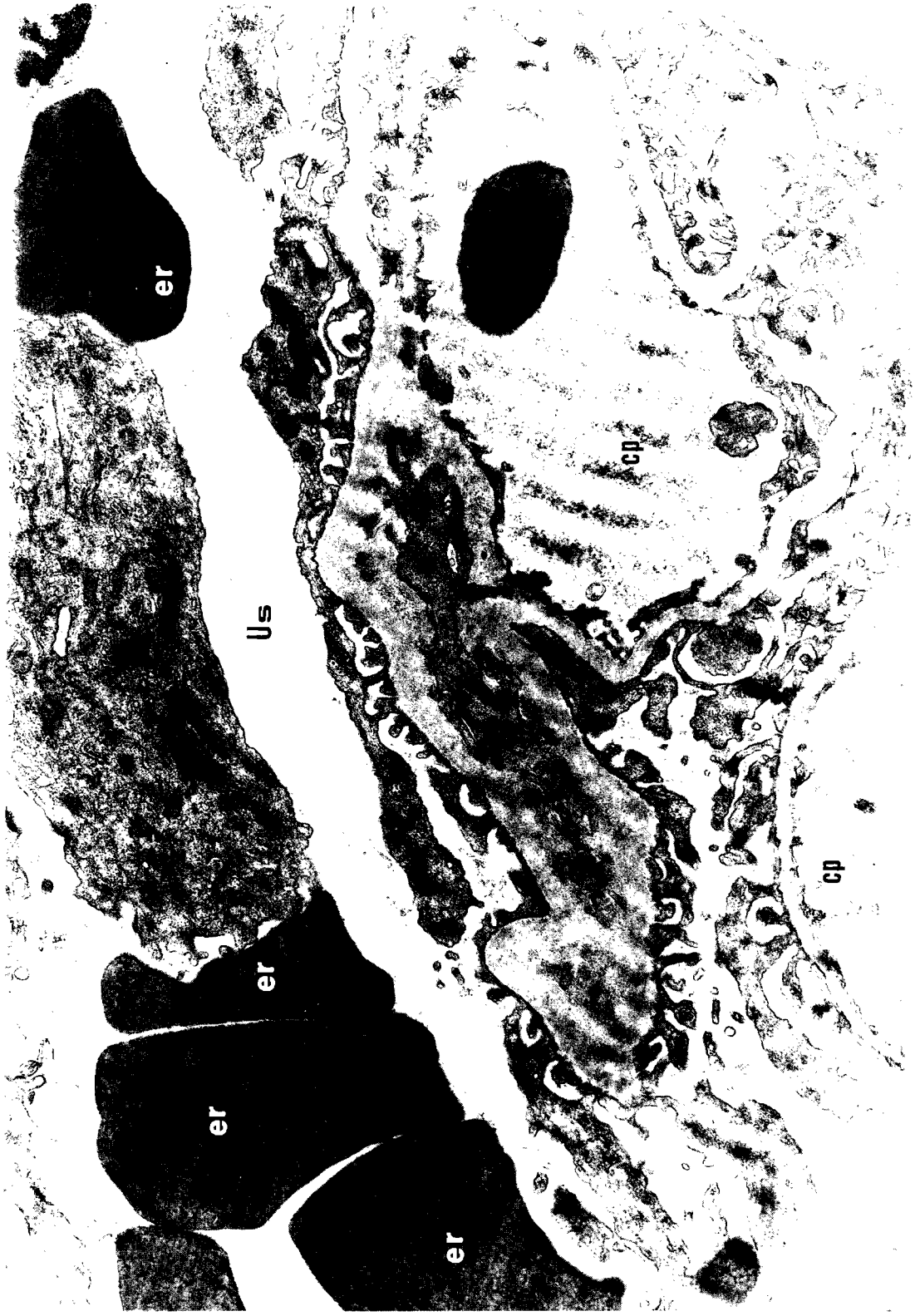
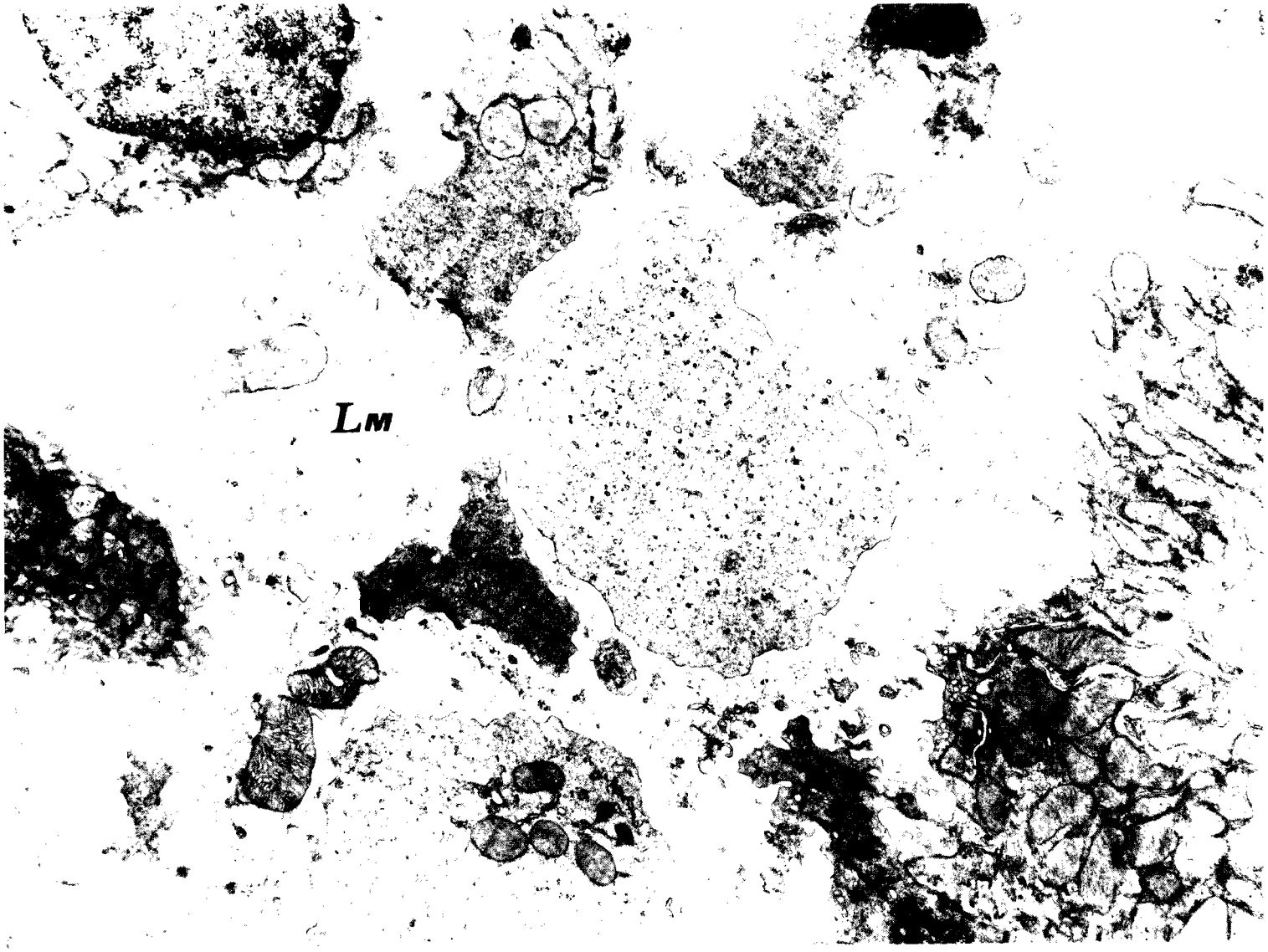


Fig. 8. Part of a renal corpuscle. The urinary space (Us) contains erythrocytes (er). cp = capillary. × 11,500

Fig. 9. Part of a proximal tubule (Px). The basal infoldings (←—) of the cell membrane have disappeared. Focal thickening of the basal cytoplasmic membrane (head arrows) can be seen. bm = basement membrane. x 14,000

Fig. 10. Part of a proximal tubule. The basal interdigitations reveal a severe decrease. (◂—). The cytoplasm shows extensive disorganization and the nuclear membrane is broken N = nucleus, Bm = basement membrane. x 15,000

Fig. 11. Many disrupted microvilli, damaged mitochondria and cell debris are observed within the lumen of a proximal tubule. Lu = lumen. × 12,000



Discussion

Shock is an acute syndrome of cardiovascular failure, in which many vital organs -such as heart, liver, kidney, pancreas, skeletal muscle- are nourished inadequately (Thompson, 1957b). Historically, sepsis has been characterized as a hypodynamic state with increased total peripheral resistance and decreased cardiac output. The kidneys, traditionally, shared in this response with increased renal vascular resistance and decreased renal blood flow, especially outer cortical flow, leading to ischemia (Lucas, 1976).

In our series, the endothelial cells of the peritubular capillaries were somewhat swollen, while the endothelial cells of the glomerular capillaries exhibited cytoplasmic processes which covered or were covered by cytoplasmic processes of the mesangial cells, projecting through gaps of the endothelium. Similar changes have been described in rats which developed renal failure after injection of glucerin (Susuki and Mostofi, 1970) or temporary renal ischemia (Cook, 1965). Dalgaard and Pedersen (1961) observed 5 oliguric patients and could find no glomerular abnormality, while Olsen and Skjoldborg (1967) described such projections of mesangial cytoplasm into the glomerular

capillaries in a few patients with oliguric renal failure and Rosenberg et al. (1971) found cytoplasmic remnants filling the glomerular capillaries. They appeared to arise from extrusion of mesangial cell cytoplasm and this material seemed to obstruct the capillaries. In our series, no material was found to obstruct the glomerular capillaries, while others described ectatic capillaries containing fibrin strands, occasional bacteria and degenerated poly-platelet elements (McKay et al., 1967; DePalma et al., 1967; Coalson et al., 1978).

The glomerular basement membrane of the renal corpuscles was thickened in many portions, containing microvacuoles, granules and collagen fibres. Such alterations had been described after focal glomerulonephritis due to streptococcus or staphylococcus (Zollinger, 1978).

Foot processes of the glomerular epithelium were flattened, producing a significant reduction of the filtration slits on the glomerular capillary surface. Similar changes were found in ischemic kidney in the rabbit. This alteration increased with increasing duration of ischemia, as well as in renal biopsies from poorly functioning kidneys in easy post-transplant period, there being a positive correlation between capillary surface covered by podocytes and the serum creatinine levels (Racusen and Solez, 1984).

Some podocytes contained myelin-like inclusions, while others had their cytoplasmic membranes broken and the urinary space was filled with fragments of epithelial cytoplasm and erythrocytes. Some other investigations found only mild edema of the podocytes and the foot processes (DePalma et al., 1967; Coalson et al., 1978).

Alterations of the proximal and distal tubule epithelia were also observed. The basal cytoplasmic membrane showed focal thickening, the «attachment bodies», as referred to by Jones (1984) in acute renal failure patients. Perhaps, these «attachment bodies» were in correlation with the severe decrease of the basal interdigitations. Some epithelial cells showed more extensive disorganization of their cytoplasm, accompanied by cell or nuclear membrane disruption, alterations that are considered irreversible. The brush border was intact in many proximal tubules, while in others brush border losses were described and the microvilli were projected into the lumen of the tubules. These findings were in agreement with the findings of Coalson et al. (1978).

The epithelium of the distal tubules was swollen, while in other portions was thinner than the normal one. The cytoplasm in some of them showed extensive disorganization.

All alterations observed were extended only in some renal corpuscles and tubules, their number depending upon the time intervals after septicaemia induction.

In spite of many investigations that have been done, we have not yet made clear if the histological lesions of the kidney are responsible for the functional insufficiency of the organ, as Solez and Finckh (1984) have also concluded in most recent investigations.

It is probable that the necrotic lesions –perhaps because of their focal appearance- do not participate directly on the disturbance of the functional parameters. When the necrosis reaches the point beyond return, it is possible then, to correlate it with the functional insufficiency of the organ.

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