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Acute morphological changes in canine kidneys after exposure to extracorporeal shock waves

A light and electron microscopic study

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Summary. The acute effects of extracorporeal shock waves on renal morphology were studied by light and electron microscopy in 14 dogs. One kidney received an average clinical number of exposures, the nonexposed, contralateral kidney serving as control. The original Dornier HM—3 generator was used in 3 animals, the modified version in 11. Intravascular radiographic contrast medium was administered in five animals. Damage was observed in all exposed kidneys, none in the contralateral control kidney. The effects were characterized by renal and perirenal hemorrhage and edema, parenchymal hemorrhagic foci with tissue destruction, often extending from cortex to medulla. In the nearby regions there was endothelial cell damage in arteries, veins and glomerular capillaries. Breaks in the wall of these vessels were detected with platelet plug formations and thrombi. In glomeruli, breaks of Bowman's capsule and epithelial cell damage with loss of foot processes were observed. A wide range of tubular cell damage was demonstrated, ranging from vacuolization to complete necrosis. Tubular lumina were filled with red cells, indicating renal origin of hematuria. The tissue damage was less pronounced in kidneys exposed to the modified lithotriptor than to the original. No difference in the quantity or quality of damage was detected whether radiographic contrast medium was administered or not.

Key words: Extracorporeal shock waves – tissue damage – light and electron microscopy – animal experiment

Clinical series from all over the world indicate extracorporeal shock wave lithotripsy (ESWL) to be effective and safe. Little is known, however, about acute and long-term effects on renal morphology and function. Although Chaussy [7] in his early works reported very small morphological changes in the kidney after ESWL, there is accumulating evidence that shock waves impose damage to renal parenchyma which may be of functional importance. Magnetic resonance imaging [3] detects renal injury in up to 85% of patients treated by ESWL, and previous animal experiments [1, 4, 6, 8, 11, 15–18] demonstrate several renal morphological changes following exposure to extracorporeal shock waves.

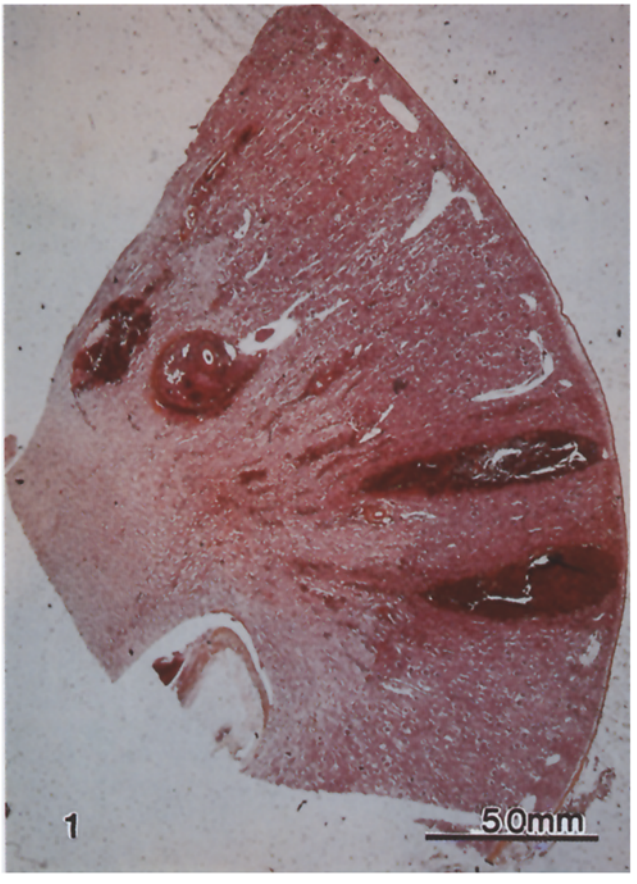
There is a need to characterize the effects of extracorporeal shock waves on the kidney at the cellular level. In a controlled canine model we have investigated the acute functional (submitted for publication) and morphological changes in renal parenchyma induced by shock waves generated by both the original and the modified [10] Dornier HM-3 lithotriptor. As knowledge of ultrastructural changes is scarce, these findings are emphasized. Any additional adverse effects due to the presence of a systemically administered radiographic contrast agent were looked for.

Materials and methods

Animals

Fourteen female English Setter dogs weighing from 16 to 21 kg were used. General anesthesia was induced with pentobarbital 25 mg/kg body weight and maintained by small, repetitive doses. Peroral tracheal intubation was performed and spontaneous respiration maintained throughout the procedure. Ringer acetate infusion was administered via a peripheral paw vein at the rate of 5 ml per minute. For clearance studies a priming dose and sustaining infusion of 5% Inulin (Laevosan International AG, Zürich, Switzerland) and 20% aminohippurate sodium (PAH, Merck Sharp & Dohme, West Point, PA 19486, USA) were administered intravenously in order to keep plasma concentrations within 20–30 mg/100 ml and 2–4 mg/100 ml, respectively. Rectal temperature was registered at regular intervals, as was arterial blood pressure via a percutaneously placed transfemoral 5-F aortic catheter connected to a transducer and a printer.

A low midline mini-laparotomy was performed and a 7 F ureteric catheter inserted into the lower ureter on each side for selective urine collection. The ureteric catheters were secured by ligatures and the abdomen closed. Bilateral, selective renal vein catheters were placed via a percutaneous transfemoral approach. Fluoroscopy without administration of contrast medium and acid-base measurements on blood samples from aorta, vena cava and both renal veins assured their correct positioning, central in both renal veins.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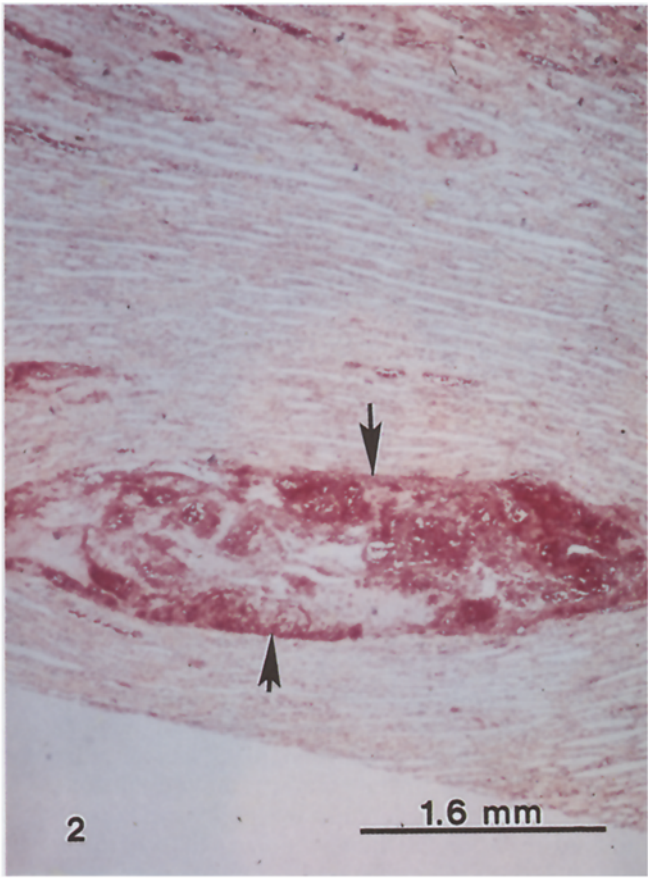
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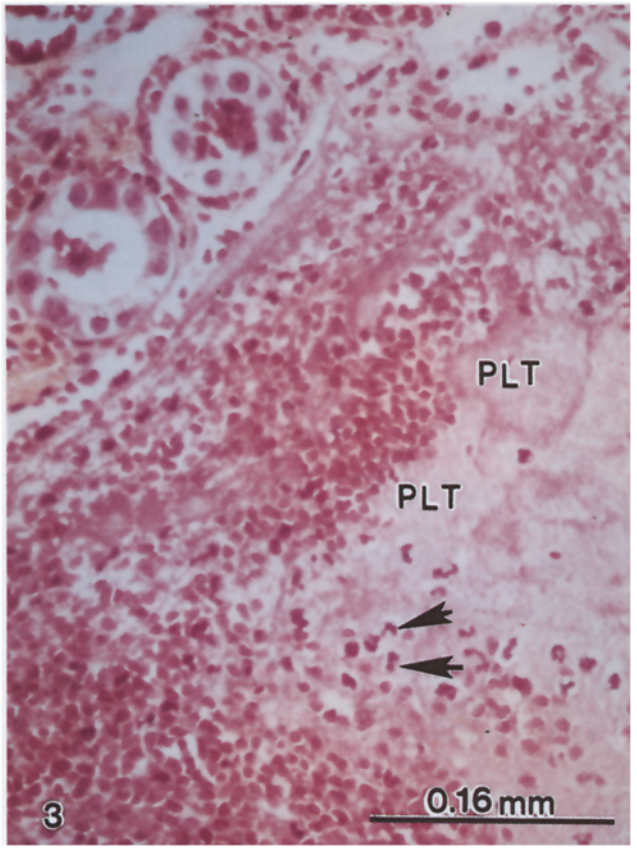
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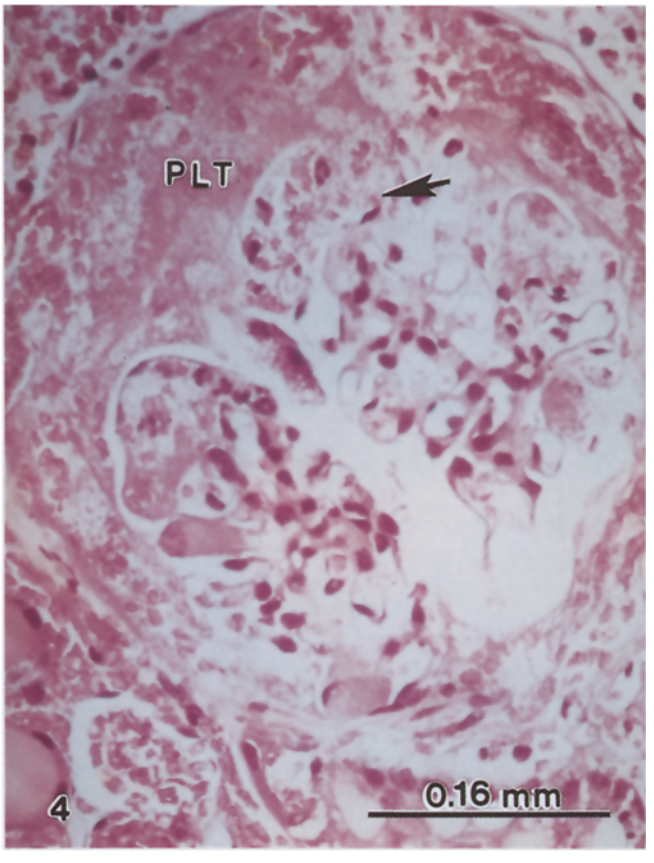
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Fig. 1. Kidney section demonstrating pronounced parenchymal damage with hemorrhagic foci located both in cortex and medulla, H&E

Fig. 2. Wedge-shaped focus in the medulla. Note the sharp border of the lesion (*arrows*). H&E

Fig. 3. Platelet masses (*PLT*), leukocytes (*arrows*) and fibrin within a hemorrhagic focus. H&E

Fig. 4. Glomerulus with capillary damage (*arrow*) and deposition of thrombus material and masses of red cells, platelets (*PLT*) and fibrin in the subcapsular urinary space. H&E

Shock wave procedure

The Dornier HM-3 lithotriptor was used in 3 dogs and the modified version in 11. The animals were secured in the supine position by strapping the padded paws to surrounding structures of the gantry. Immersed in the degassed water bath at 37°C, all animals were subjected to shock waves aimed at one kidney, the untreated side serving as control. The animals were not shaved.

The waves were focused on the renal pelvis, visualized by a very flexible guidewire, 0.89 mm in diameter, momentarily inserted via the ureteric occlusion catheter to curl up in the pelvis. In nine dogs the relations of the guidewire (renal pelvis) to the tip of the renal vein catheter and surrounding bony landmarks were carefully noted and the guidewire removed. Thus correct focusing was continually assured by repeated fluoroscopy without the use of contrast media. In five dogs additional radiographic contrast medium in the pelvis assured the positioning. Between 1 and 1.5 h before shock wave exposure, a bolus of 25 ml iohexol (Omnipaque, Nycomed, Oslo), 300 mg I per milliliter, was administered by a Medirad Mark II contrast medium injection pump via the aortic catheter. The flow rate was 18 ml per second. Four identical repetitive injections were administered at hourly intervals after shock wave exposure for further provocation.

The dogs ($n = 3$) shocked by the Dornier HM-3 machine received 1,500 impulses at 18–20 kV, an average number of impulses in clinical work. Those subjected to the modified machine ($n = 11$) received 2,000 impulses at the same voltage range, this also representing a medium therapeutical dose in patients. The impulses were manually triggered at a frequency of about 70 per minute.

Bilateral nephrectomy was performed 4–5 h after the application of shock waves, and thereafter the animals were killed by a lethal dose of pentobarbital.

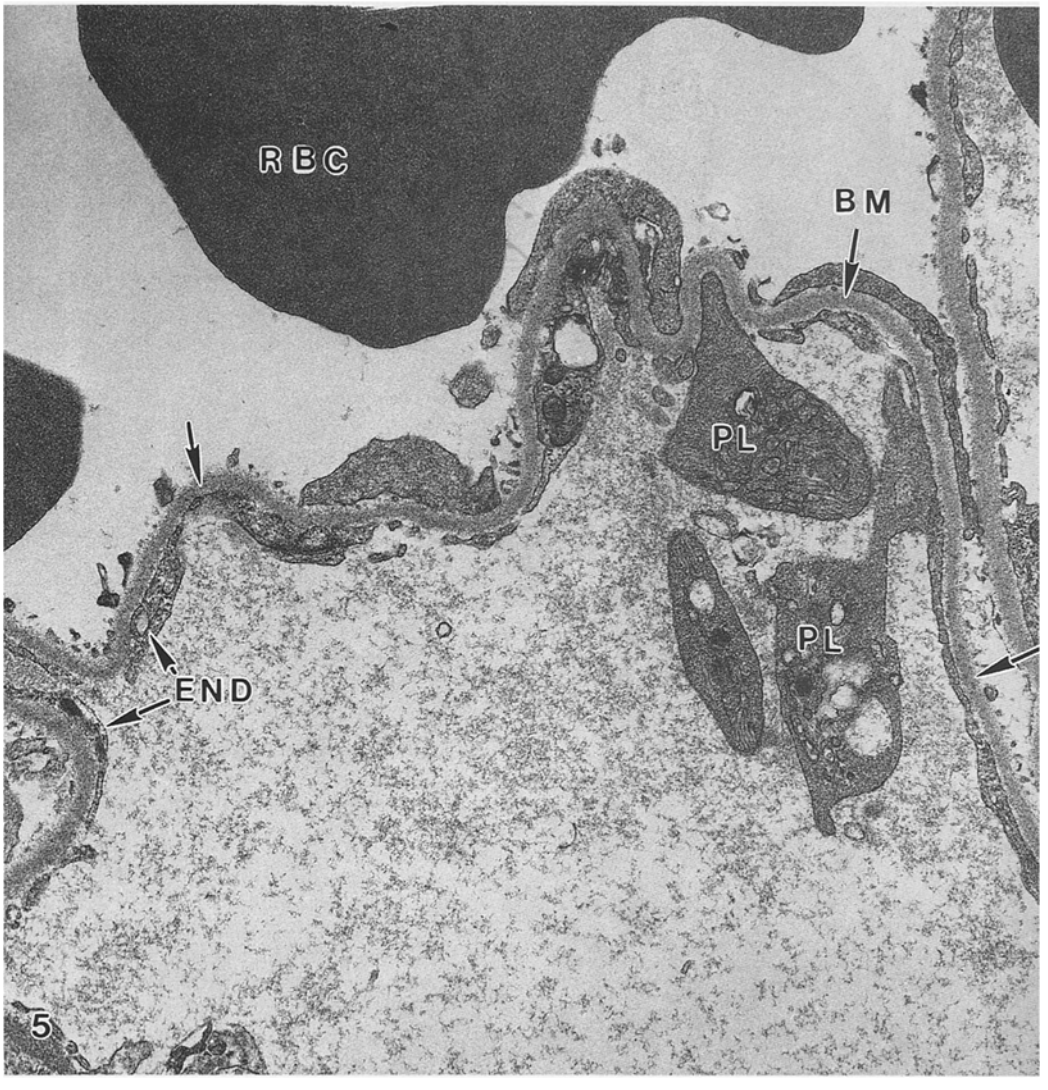


Fig. 5. Electron micrograph demonstrating glomerular capillaries with loss of foot processes (*arrows*) and foci of endothelial (*END*) damage with two platelets (*PL*) attatched to the basement membrane (*BM*). Note red blood cells (*RBC*) in the urinary space

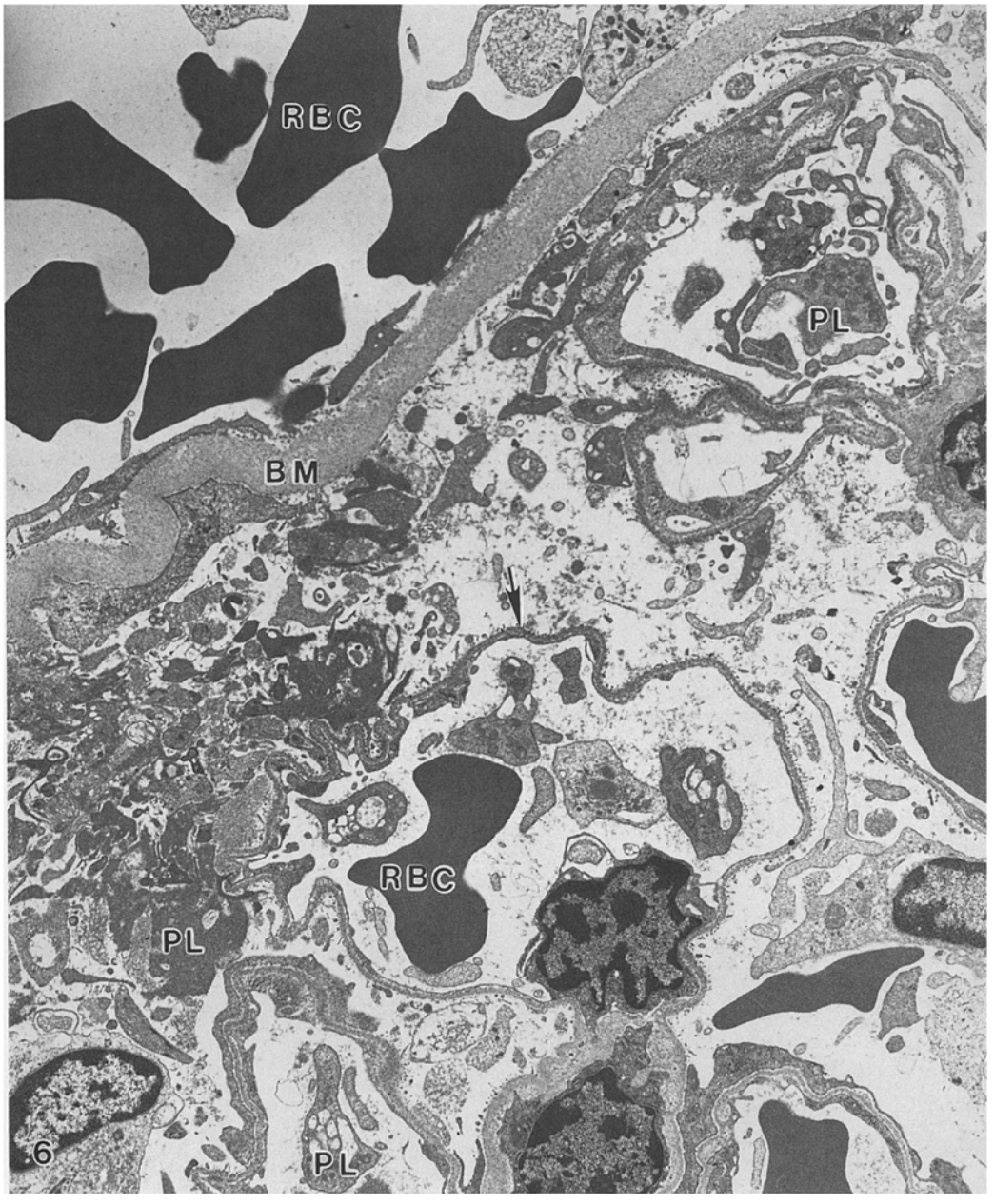


Fig. 6. Glomerular capillaries with luminal accumulation of platelets (*PL*) which also are aggregated in the urinary space. *BM*, blasement membrane of Bowman's capsule; *RBC*, red blood cells

Table 1. Weight difference a between the exposed and the contralateral, nonexposed kidney in eight dogs

Dogs (*n*)	Weight difference in grams (%)
3	18.9 (22.5)
4	10.0 (12.8)
5	19.5 (26.2)
6	15.0 (25.0)
7	23.5 (29.5)
8	6.0 (12.6)
9	10.0 (23.5)
11	9.6 (19.5)
Mean	14.1 (21.5)

[a] All exposed kidneys were heaver

Preparation for light and electron microscopy

At room temperature the tissue specimens were immediately transferred to the fixative. Immersion fixation only was used in all except three animals, in which perfusion fixation was carried out first. The vascular beds in these kidneys were flushed with Ringer solution administered by a Medirad Mark II injection pump at a flow rate of 5 ml per second before application of the glutaraldehyde perfusion fixative. Thereafter the specimens were fixed as described for immersion fixation.

For light microscopy (LM) the specimens were fixed in 4% buffered formaldehyde or in a mixture of glutaraldehyde and paraformaldehyde [14]. For electron microscopy (EM) 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer was used, with additional fixation in 1% osmium tetroxide in 0.1 *M* phosphate buffer. Specimens were also fixed in McDowell's fixative.

The specimens prepared for LM were embedded in paraffin and stained with hematoxilin-eosin (H&E). The EM specimens were

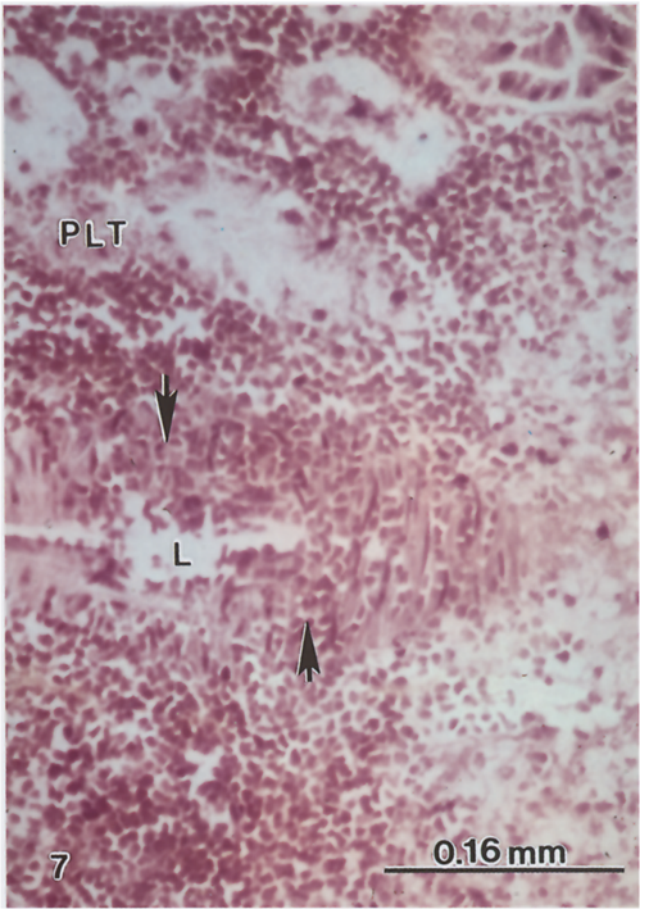


Fig. 7. Artery with partial destruction of the vessel wall and red cells escaping (*arrows*). *L*, vessel lumen; *PLT*, platelet mass. H&E

embedded in Spurr's epoxy resin. The ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Jeol 100B or 1,200EX electron microscope.

Results

The character of changes observed were very similar in all animals. No gross injury was observed to neighboring organs or to the nonexposed, contralateral kidney or its perirenal tissues. LM and EM detected no pathology in the nonexposed kidneys except for tubular cell vacvolization, which was a constant finding in both kidneys.

The degree of renal damage appeared more pronounced using the original Dornier HM-3 generator than with the modified version. Morphometry was not performed, but neither LM nor EM disclosed obvious differences between kidneys subjected or not subjected to radiographic contrast medium.

Macroscopic changes

At laparotomy about 200 ml blood-stained fluid was present in the peritoneal cavity. On incising Gerota's fascia, perirenal edema and blood-stained fluid was found. The perirenal fat tissue showed characteristic changes with edema and hemorrhage.

The exposed kidney appeared swollen and edematous and was larger than the contralateral organ. In eight animals the weight of both kidneys was registered, and in all cases the shocked kidney ws the heavier (Table 1). Capsular and subcapsular hemorrhages were present in the blast path of the shock waves. On the cut surface well-circumscribed, often wedge-shaped hemorrhages were seen, most frequent in the cortex and the transitional zone, but also occasionally in the medulla. The hemorrhagic lesions appeared smaller and more sharply demarcated from macroscopically normal parenchyma in the kidneys shocked by the modified lithotriptor.

Observations by light and electron microscopy

LM confirmed the presence of hemorrhagic foci in the parenchyma. The number, shape and size of the foci varied, but the bleedings could be large (Fig. 1). Although small hemorrhagic foci were more frequent, some foci were wedge shaped, ranging from the cortex to the medulla. Most extensive foci were observed in the kidneys subjected to the original Dornier HM-3 lithotriptor. Even in the absence of bleeding venous and capillary packing of red cells was seen in some areas.

The border between the mass of red cells and the renal tissue usually was sharply defined (Fig. 2). In the center of the hemorrhagic foci there was complete tissue destruction. More peripherally, however, fragments of renal tissue could be identified. These fragments consisted mainly of tubular tissue, but glomerular and vessel wall fragments were also present. The renal tissue adjacent to the foci showed signs of compression. Large aggregates of platelets, fibrin and leukocytes, in particular neutrophil granulocytes were present, especially in the larger foci (Fig. 3).

Glomeruli. The glomeruli near the lesions demonstrated various degrees of damage. Occasionally total destruction was observed by LM. Rupture of glomerular capillaries and masses filling the subcapsular urinary space were noted (Fig. 4).

Ultrastructurally, there were small defects in the capillary endothelial cells. Single platelets adhered to the capillary basement membrane (Fig. 5). Blood platelets accumulated in the capillary lumina (Fig. 6), and small platelet plugs were observed at sites of capillary wall rupture. Changes in the glomerular capillaries were focal and not generally distributed in well-preserved areas.

Fibrin and platelet masses were located in the urinary space. There was an occasional loss of foot processes, and the visceral epithelial cells often showed signs of damage (Figs. 5, 6). Breaks in Bowman's capsule were frequently detected. The periglomerular space was often occupied by a mantle of red cells, and fibrin and platelet masses were present.

Arteries, veins and peritubular capillaries. Damage to the arterial wall was a frequent finding (Fig. 7). EM detected focal damage and loss of endothelial cells (Fig. 8), with a few platelets attached to the subendothelial structures. Larger thrombi were present. Arteries were observed

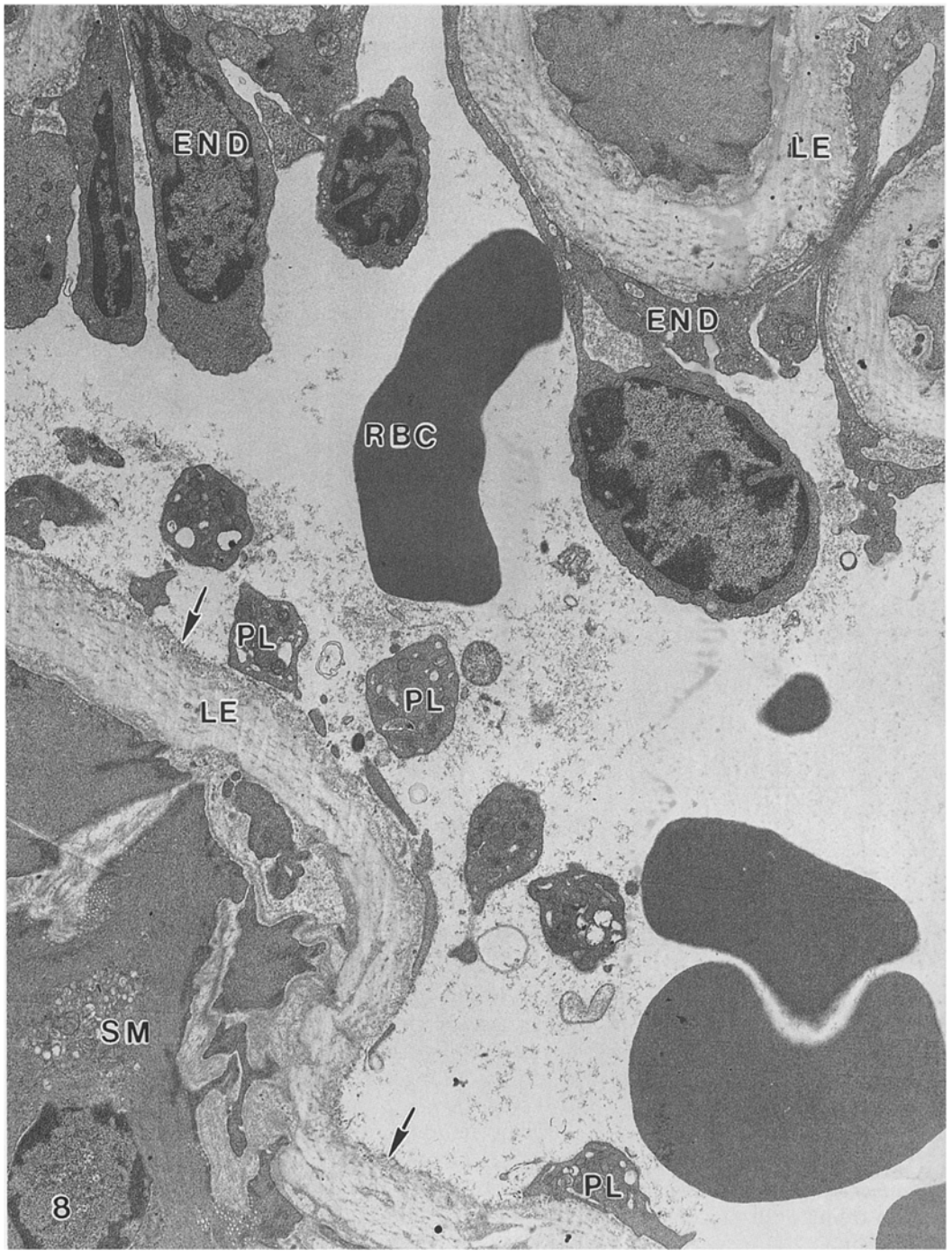


Fig. 8. Detail of artery with focal destruction of endothelial cells (*arrows*) and blood platelets (*PL*) adhering to exposed subendothelial tissue. *End*, endothelial cells; *LE*, lamina elastica interna; *SM*, smooth muscle cells

within the hemorrhagic foci or surrounded by a mantle of red cells, even without obvious change in the vessel wall.

Ruptures of both veins and capillaries were observed, often with adherance of platelet plugs (Fig. 9). The endothelial cells in these regions were swollen, and swelling of mitochondria indicated signs of damage. The platelet plugs were associated with intravascular thrombi.

Packing of red cells in capillaries and veins with single red cells passing through the vessel wall was a characteristic finding in some areas (Fig. 10). These areas were not sharply defined by LM and not associated with foci of tissue destruction.

In the perfusion-fixed specimens the observations were essentially the same as after immersion fixation only, except for the lack of red cells in the vessel luminae.

Tubules. The fragments of tubular tissue present in the hemorrhagic foci presented marked alterations of the tubular cells with areas of complete destruction (Fig. 11). Near the hemorrhagic foci the tubuli were filled with red blood cells (Fig. 12). The epithelial cells showed a wide range of change, from slight vacuolization to complete necrosis (Figs. 13, 14). Ultrastructurally damaged tubular cells presented marked mitochondrial changes. Swelling

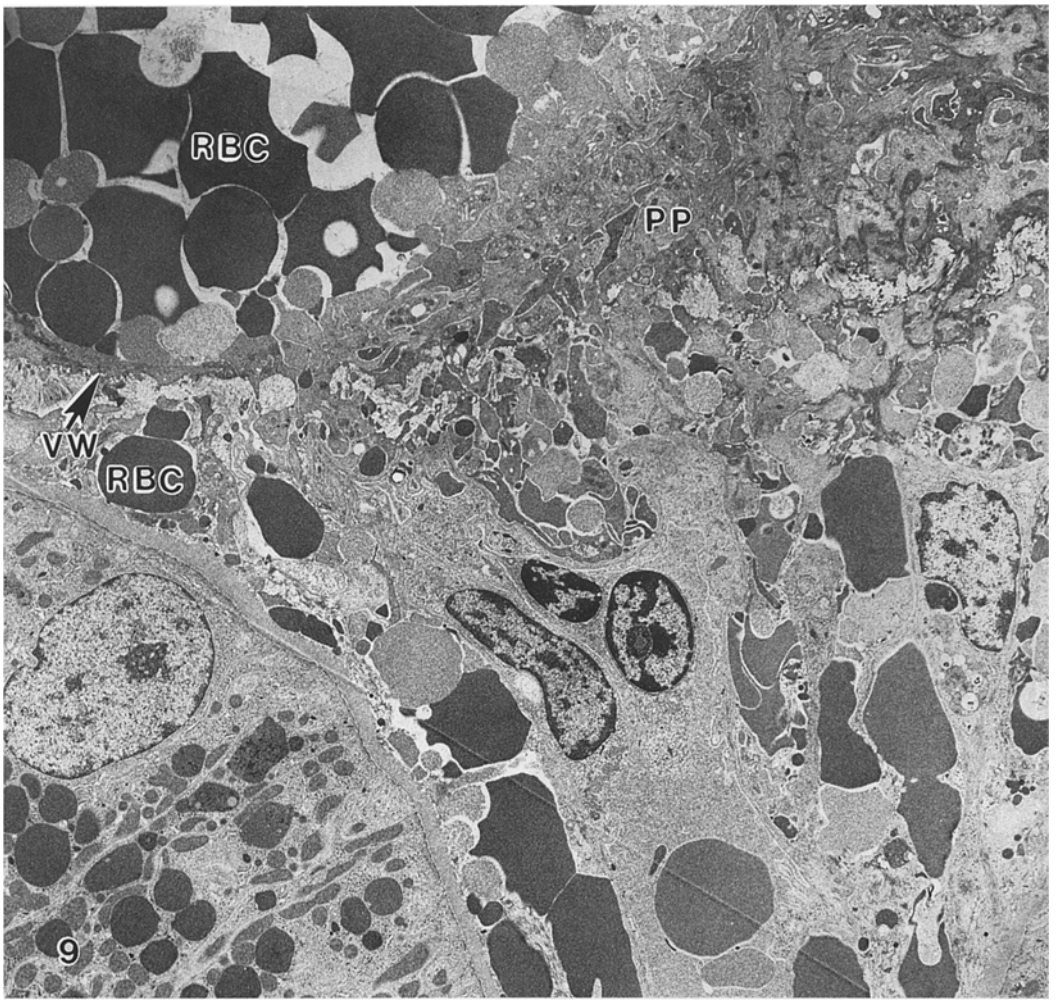


Fig. 9. Rupture of vein with formation of platelet plug (*PP*). *RBC*, red blood cells; *VW*, vessel wall

and rupture of cristae with accumulation of electron-dense granules was detected (Figs. 13, 14), and myelinlike structures were noted. Similar findings were made in both perfusion, and immersion-fixed specimens. In these proximal tubular cells there was loss of microvilli. In better preserved cells the loss of microvilli was less marked, and in some tubuli there was increased vacuolization only. Tubular cell vacuolization, however, was a constant finding even in the contralateral, nonexposed control kidneys, whether subjected to radiographic contrast medium or not.

Interstitial tissue. Changes in the interstitial tissue were characterized by edema and accumulation of red cells and leukocytes. Platelet fibrin plugs were present in the vicinity of vessels and glomeruli.

Discussion

Edema (Table 1), focal renal tissue destruction and hemorrhage occurred in all exposed kidneys. The characteristic lesions seen both at LM and EM were essentially the same in all animals, independent of the method of fixation. The degree of damage was reduced when using the modified compared to the original Dornier HM-3 lithotriptor, confirming the findings in pig kidneys by Müschter et al. [15].

The vascular endothelial cell damage, erythrodiapedesis and breaks observed in the capillary wall suggest increased vessel permeability to be the cause of the edema, which may cause the interference with renal hemodynamics in the acute phase [13].

Macroscopic hematuria occurs in the majority of patients after ESWL, as was the case in all animals in the present study. Our findings agree with other reports [1, 6, 11, 16, 17] that this is caused by renal damage imposed by the generated shock waves and not by fragmented calculi traumatizing the urothelium. We confirm the presence of linear hemorrhages extending from the renal cortex to the medulla [1, 17] which may cause linear scar formation. Their clinical importance is still unknown.

Small veins are reported to be particularly susceptible to injury by shock waves [16, 17, 18]. Our findings show that bleeding is related to damage of arteries and capillaries as well as veins. The presence of the platelet masses suggests the release of platelet constituents such as adenine nucleotides and serotonin in the acute phase after shock wave exposure. The capillary sludging and leakage of red cells through the damaged capillary wall may also

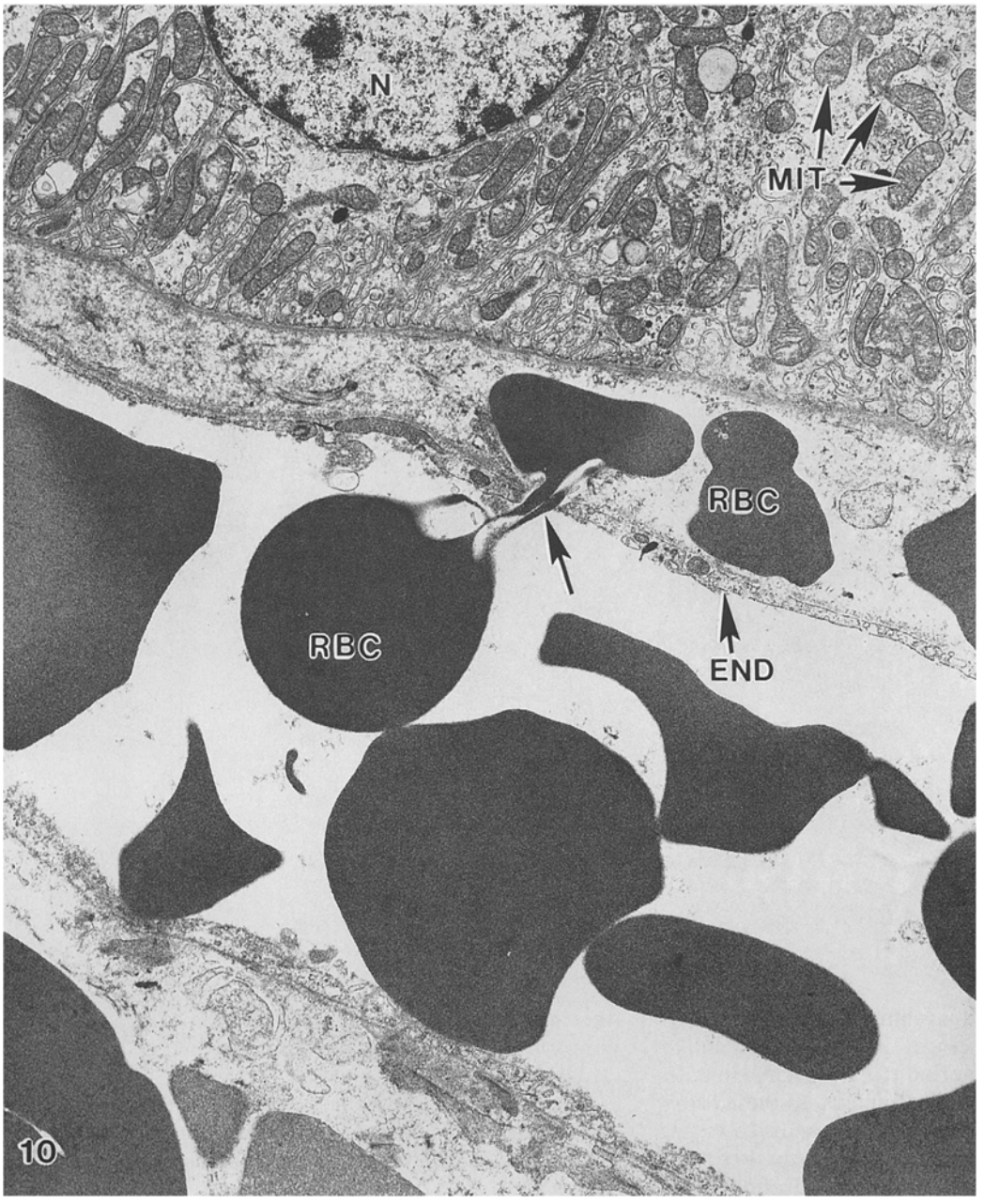


Fig. 10. Peritubular capillary with a red blood cell (*RBC*) penetrating the wall (*arrow*) and red cells in the edematous interstitial space. *END*, endothelium; *MIT*, rather well preserved tubular cell with numerous mitochondria; *N*, nucleus

contribute considerably to the accumulation of red blood cells in the interstitial tissue.

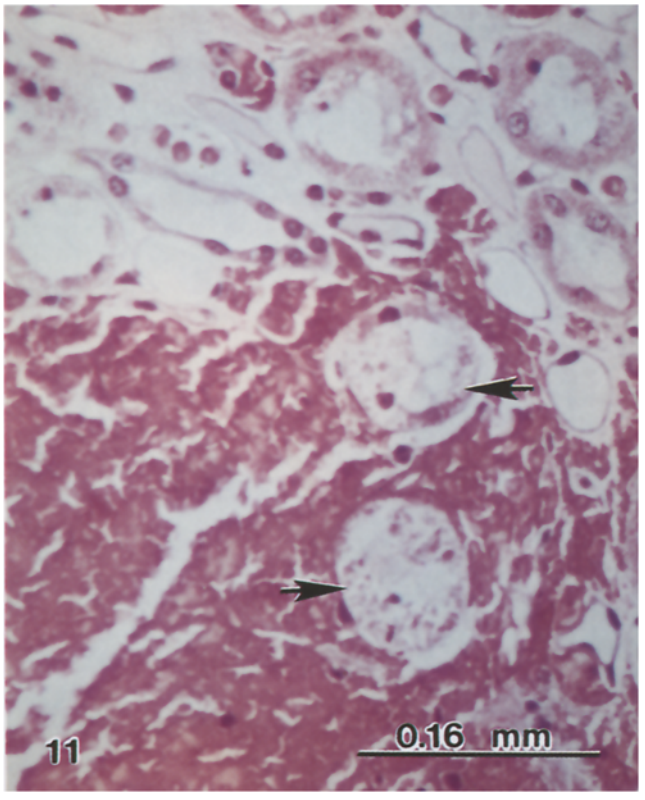
The hemostatic mechanisms at the capillary level both in the glomeruli and in the peritubular vessels were operating by formation of platelet plugs.

The damage of endothelial cells in the arteries appeared to be focal and associated with deposition of platelet material. Whether such focal thrombus formation leads to focal intima fibrosis and thereby influencing long-term renal function is not clear.

The glomerular damage in the areas near foci of tissue destruction involved both endothelial and epithelial cells of the capillaries as well as the Bowman's capsule. These changes may be part of the mechanism behind the transient proteinuria which occurs after ESWL treatment [9, 19]. Extensive glomerular lesions may explain the decrease in renal plasma flow [13] and glomerular filtration rate [5] in the acute phase.

The possibility of ESWL causing hypertension by the Page kidney mechanism in some patients is offered by several authors [13]. In our EM studies we unsuccessfully looked for any alterations in the juxtaglomerular apparatus, and a possible hypertensive effect of the ultrastructural lesions detected in arteries and glomeruli with their associated vessels is only speculation.

The mitochondrial changes in many tubular cells were pronounced and clearly indicate interference with intracellular metabolism. The mitochondrial alterations, however, were not present in areas with well-preserved tissue. Our findings therefore do not support any theory of generalized damage to mitochondria or cellular organells such as lysosomal granules [14]. The areas of marked



tubular damage in some kidneys were extensive, probably accounting for the transient changes in tubular function in the acute phase following exposure to the shock waves [2].

Vacuolization of tubular cells may signify the start of tubular cell necrosis. This pathological alteration has previously been observed following shock wave exposure [11, 18] and also occurs after injection of radiographic contrast media alone [12]. We detected this phenomenon in both kidneys, the degree appearing to be equal whether provoked by contrast media or not. This sign is therefore probably non-specific and may be related to immersion of the animals or intravenous infusions.

The lesions from shock wave exposure rapidly induce an inflammatory, reparative process, visualized by the presence of neutrophil granulocytes.

Apart from renal tissue edema, the acute lesions demonstrated in the present study, involving glomeruli, vessels and the tubular apparatus, were localized in distinct foci. The major areas of the kidney were spared, and scarring of the localized foci is unlikely to result in alterations of kidney function which are of clinical significance.

Fig. 11. Periphery of hemorrhagic focus with isolated tubuli showing marked structural changes (*arrows*). H&E

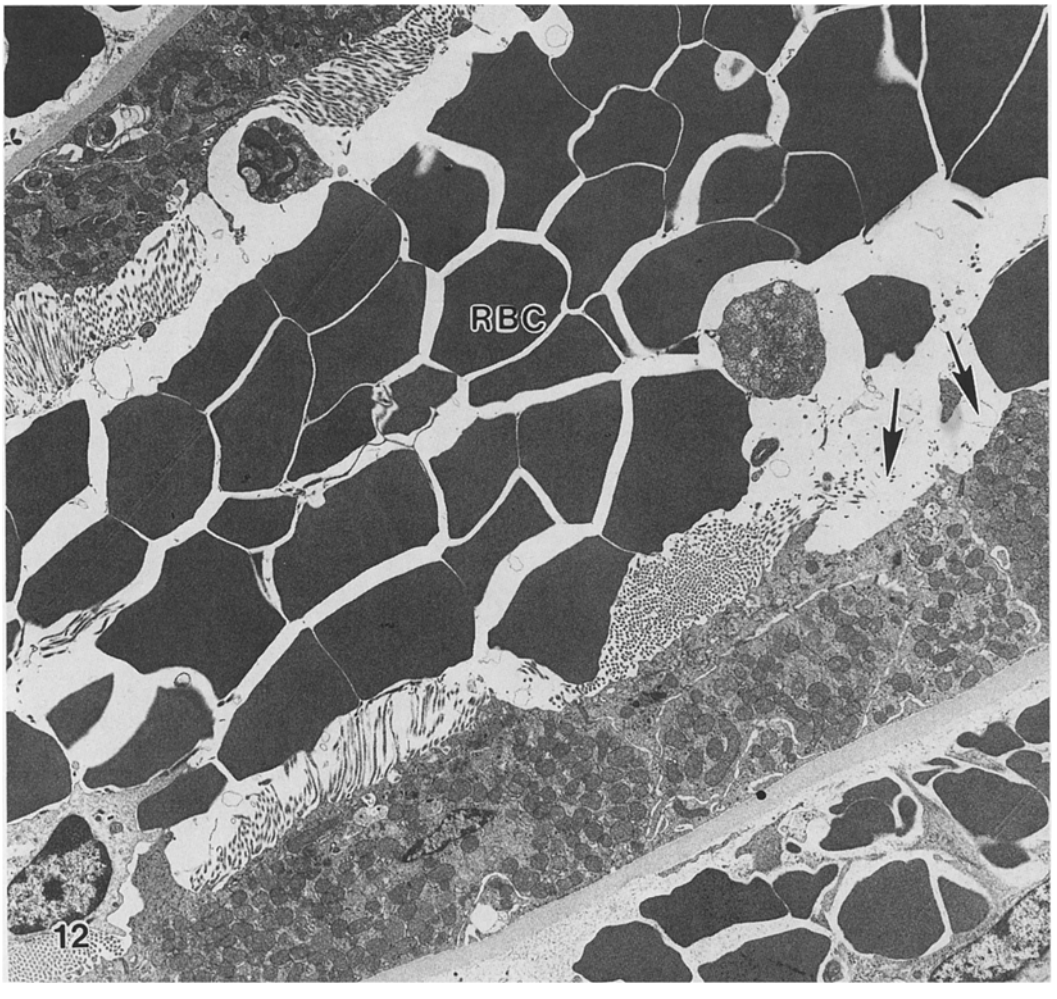


Fig. 12. Presence of red blood cells (*RBC*) within the tubular lumen. Well-preserved tubular cells but slight damage of microvilli (*arrows*)

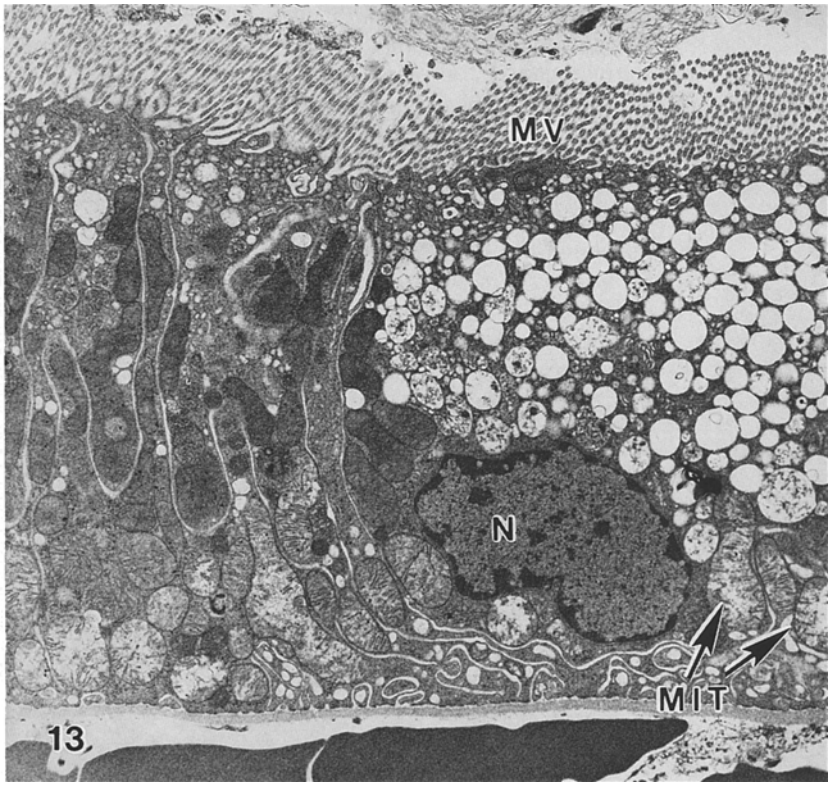


Fig. 13. Marked vacuolization and moderate mitochondrial alterations of proximal tubular cell. *MIT*, mitochondria; *N*, nucleus; *MV*, microvilli

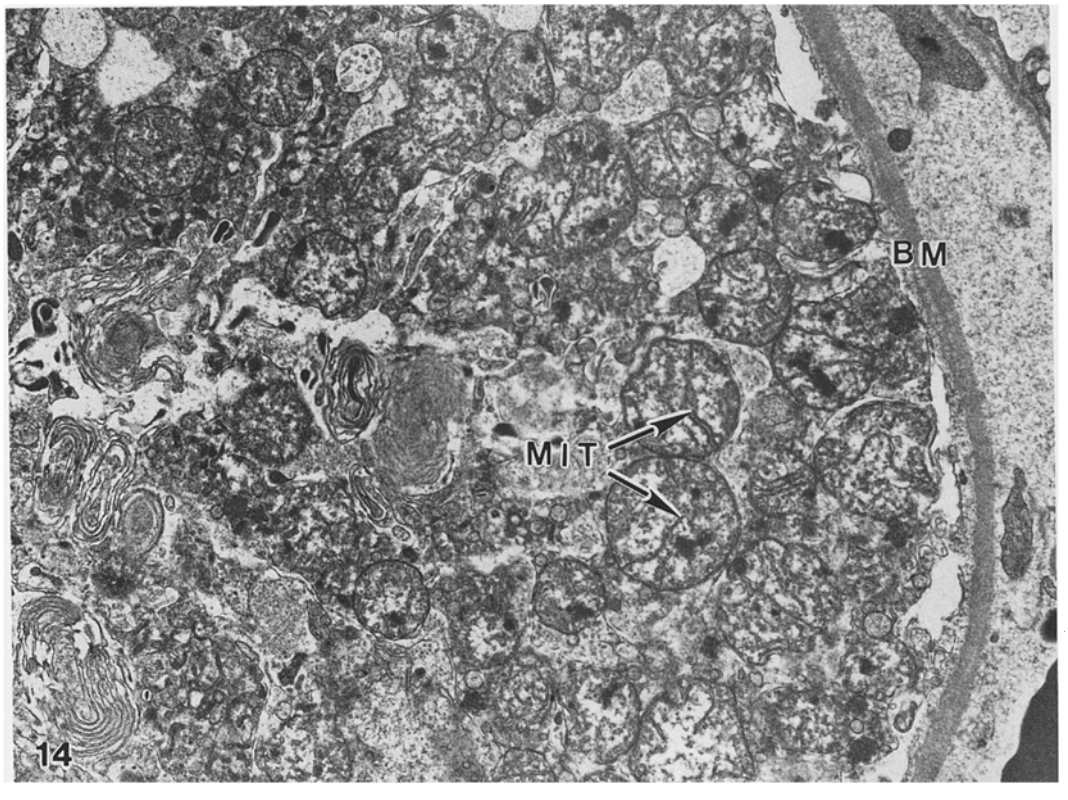


Fig. 14. Markedly altered mitochondria (*MIT*) with swelling, loss of matrix, breaks of cristae and numerous electron dense granules. *BM*, basement membrane

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