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Renal Lesions in Ischaemic Kidneys Infused with Haemoglobin: An Electron Microscopic Study

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Summary. Male Wistar rats were used to study changes in ultrastructure of proximal renal tubular cells after an haemoglobin (Hb) load with or without temporary renal ischaemia. An i.v. injection of stroma-free Hb was given either 30 min before, immediately after, or 2 h after ischaemia. Appropriate control groups were used for comparison. Electron microscopic examination was performed on kidneys removed 48 h after treatment. Hb transport and degradation was markedly delayed by renal ischaemia. Hetero-ambi- and autolysosomes were found after Hb load, and the number of lysosomes was higher in animals with ischaemia than in sham-operated controls given Hb. Renal cellular damage was more pronounced in rats given Hb either 30 min before or 2 h after ischaemia than in those subjected to either Hb load or ischaemia alone. The findings show deleterious effects of haemoglobinuria in conjunction with renal ischaemia.

Key words: Kidney damage, Haemoglobinuria, Renal ischaemia, Shock kidney, Acute renal failure, Rats.

Introduction

The increased incidence of acute renal failure following haemolysis led early investigators to assume that chromoproteins damage the renal parenchyma directly [1, 2, 6, 7, 23, 28]. Later studies showed that purified haemoglobin (Hb) is tolerated without damage to healthy kidneys and that renal failure after haemolysis is caused either by shock and reduced renal blood supply or by other erythrocyte components [3, 9, 11–14, 19–21, 33, 34]. Consequently, experimental studies were carried out with stroma-free Hb added as oxygen carrier in infusion solutions in order to avoid ischaemic damage to tissues during inadequate circulation [15, 24, 25, 29, 31, 32]. Since these solutions may eventually be given to patients suffering from circulatory shock and because there is still reason to believe that chromoproteins do lead to renal damage under certain conditions [17, 18, 32], it is of interest to know whether large quantities of stroma-free Hb influence kidneys damaged by ischaemia. Previously, we reported results of functional and light microscopic studied on this topic [27]. In this paper we present our findings from electron microscopic investigations carried out on ischaemic kidney loaded with stroma-free Hb.

Material and Methods

Twenty-four male Wistar rats weighing 160–190 g were divided into eight groups of three animals. The experimental procedures were carried out as described previously [27]. Renal ischaemia was induced in some groups by bilateral clamping of the renal hilum for 60 min. An intravenous infusion (2 ml) of 16.4% stroma-free, isotonic, glucose-free human Hb (200 mg/100 g body weight) was administered to some groups either 30 min before, immediately after, or 2 h after renal clamping.

The experimental groups were:

Group 1: Control animals received an infusion of 2 ml isotonic saline immediately after sham bilateral clamping of the renal hilum.

Group 2: Control animals received no infusion and underwent bilateral clamping of the renal hilum.

Group 3. Received an infusion of Hb 30 min before bilateral clamping of the renal hilum.

Group 4. Received an infusion of Hb immediately after bilateral clamping of the renal hilum.

Group 5. Received an infusion of Hb 2 h after bilateral clamping of the renal hilum.

Group 6. Received an infusion of Hb 30 min before sham bilateral clamping of the renal hilum.

Group 7. Received an infusion of Hb immediately after sham bilateral clamping of the renal hilum.

Group 8: Received an infusion of Hb 2 h after sham bilateral clamping of the renal hilum.

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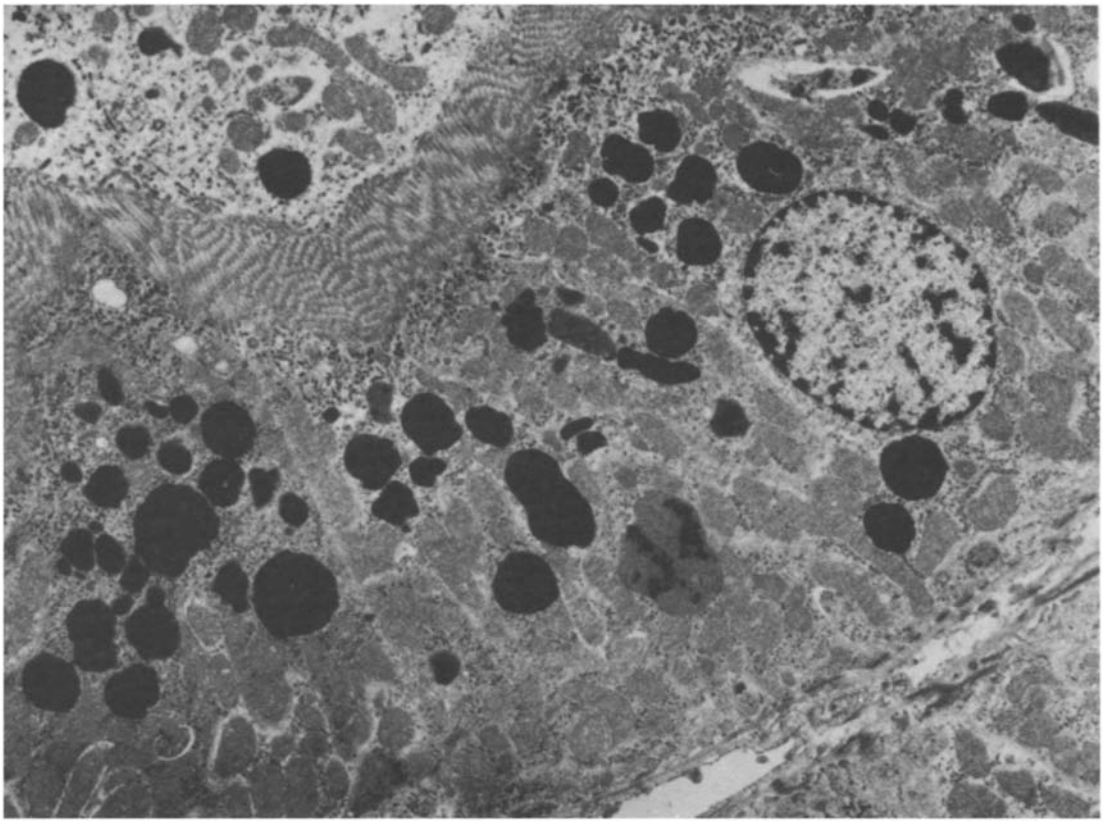

Fig. 1. Renal proximal tubular cells of a rat given an i.v. injection of 200 mg stroma-free haemoglobin per 100 g body weight immediately after 1 h ischaemia. Sample taken after 48 h. Note homogeneous electron-dense phagosomes increasing in size in apicalbasal direction which suggests that haemoglobin transport and degradation is in an early stage. Uranylacetate (x 6,300)

The left kidney was removed from each rat 48 h after operation. Thin tissue lammella was fixed in 4% buffered formaldehyde, pieces of tissue of approximately 1.5 mm length were excised from five different areas of the renal cortex, refixed in 2% OsO_4 solution, and embedded in Araldite following the procedure of Glauert et al. (1956). After producing toluidin-blue coloured semi-thin sections for an overall view, areas suitable for the electron microscopic examination were trimmed. The 60–100 nm thick pieces were developed for 45 min saturated uranyl acetate solution followed by 5 min with lead citrate solution after the method of Reynolds (1963). Electron microscopy was carried out at a magnification of 1,000–40,000 on a Siemens Elmiscope 102.

Results

Group 1

Kidneys from this group looked almost normal in the light microscope, although mitochondrial swelling of mixed type (Thoenes 1964) as well as solitary necrotic tubular epithelium with hydropic swollen cytoplasm appeared. An abrupt breaking off of the brush border and emptying of the cytoplasm contents in the narrow tubule lumen was also observed, but the cytoplasm contained only a very few autolysosomes with residues of mitochondria.

Group 2

Tubular cell damage of all grades of severity was observed in kidneys in this group. The damage consisted mainly of hydropic swollen cells with low, broken brush borders, occasional formation of apical vesicles, widening of the endoplasmic reticulum into tract-like areas, loosening of the basal labyrinth and swelling of the matrix-, crista- and mixed-types of mitochondria. A moderate quantity of tubular epithelial necrosis, mostly of the hydropic type, was seen in this group. The majority of phagosomes present were autophagosomes with a few heterophagosomes that had absorbed electron-dense-material.

Group 3

Kidneys in this group contained electron-dense material of a partly homogenous, partly cloudy nature stored in the phagosomes of the proximal tubular epithelium. In spite of extensive cell damage, many pinocytotic vesicles were present that emptied their electron-dense contents in apical vesicles. The electron-dense phagosomes were orientated in an apical-basal direction. The number of ambiphagosomes present was similar to that of simple heterophagosomes. The endo-

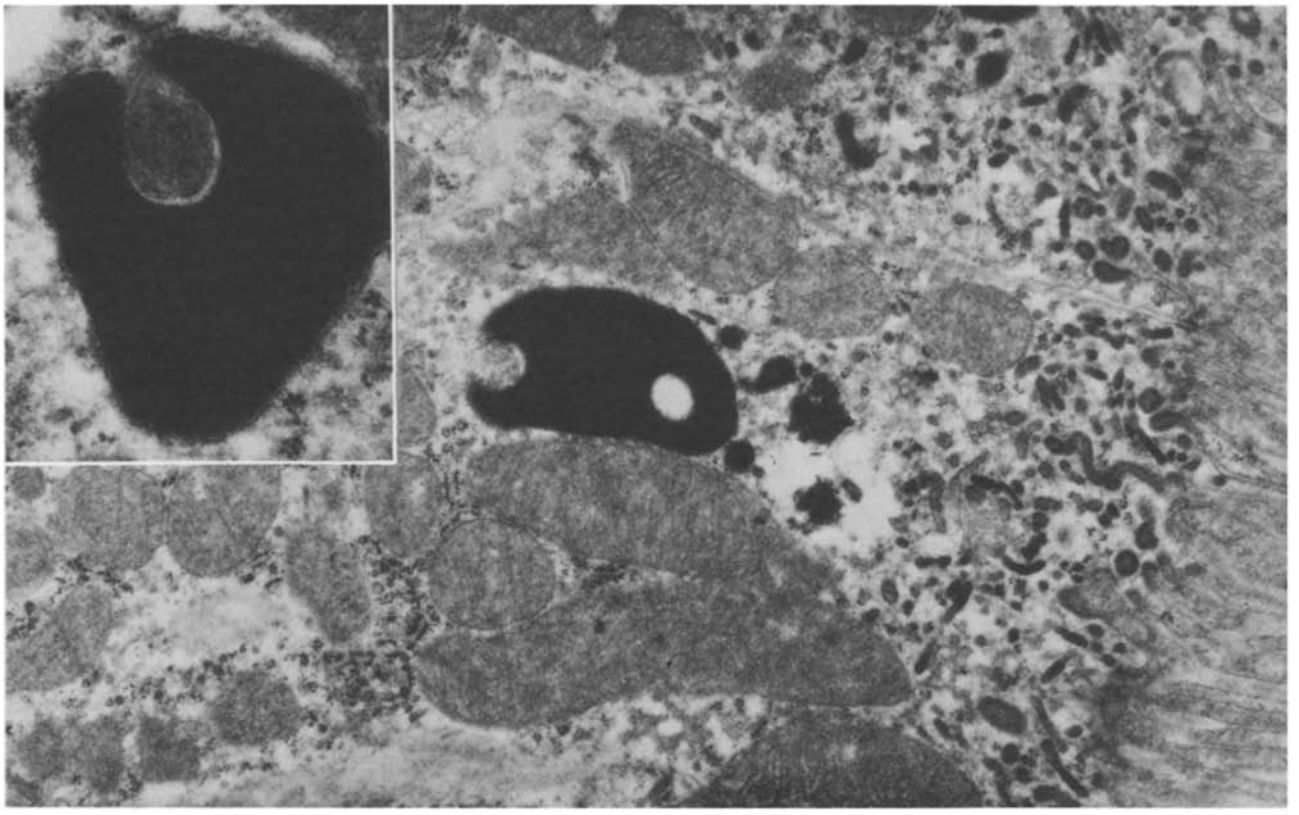

Fig. 2. Renal proximal tubular cell in rat of same experimental groups as in Fig. 1. Note apical vesicles at the base of the brush border (*right margin*) and a large heterophagosome taking up endogenous material, probably a mitochondrion (*inset*). Uranylacetate (x 21,000)

plasmic reticulum was increased greatly and dispersed evenly. The basal labyrinth was generally missing or widely dispersed. A large number of hydropic tubular epithelial necroses were present, often throughout the tubule. The remainder of the brush border was thin and the wide lamina contained isolated nuclei, organelles and phagosomes with electron-dense material.

Group 4

The epithelium of proximal tubules in this group had absorbed a large quantity of electron-dense material which was localised mainly in the apical and intermediary areas of the proximal tubular cells (Fig. 1). Apical cell areas contained small vesicles strung out and becoming larger in the direction of the cell base. The main organelles that stored Hb were phagosomes; their contents were more or less electron-dense and were located primarily in the apical cell region. In all reabsorbing proximal convoluted tubules, many pinocytotic vesicles filled with electron-dense material were seen detached from the base of the brush border and combined to apical vesicles (Fig. 2). The small vesicles in the apical cell areas contained mostly homogeneous electron-dense material whereas the larger vesicles had a cloudy structure. The phagosomes that were filled with heterologous material absorbed supplementary autologous material, for instance, mitochondria, and thus became ambiphagosomes. Well-defined ambiphagosomes larger than phagosomes and with granulated- and filament-formed contents were found in the basal direction. Larger phagolysosomes contained granulated material which, after examination with Ericsson's method, showed the presence of haemosiderin. Generally, Hb reabsorption and degradation seemed less advanced in this group than in group 3 and necroses were less frequent in this group than in group 3. Isolated anhydropic necroses were found in tubules. However, judging from the position and form of the electron-dense droplets seen in the group, transport processes were just beginning. The cell residues in the wide lamina consisted of endoplasmic reticulum, autolysosomes, phagosomes, ambiphagosomes and only relatively slightly damaged mitochondria.

Group 5

Ambilysosomes with clear signs of heterophagia were found in this group. In many cases, ambilysosomes, telolysosomes and residual bodies (Maunsbach 1969) with fine granulated material (Fig. 3) were found even in the apical areas. Their structure, when examined after the method of Ericsson (1964), showed hemosiderin and ferritin. In addition, bar-formed crystals were seen in phagolysosomes in hydropic swollen cells. In contrast to groups 3 and 4, electron-dense heterophagosomes were not present in this group. However, Golgi complexes around large ambiphagosomes were ap-

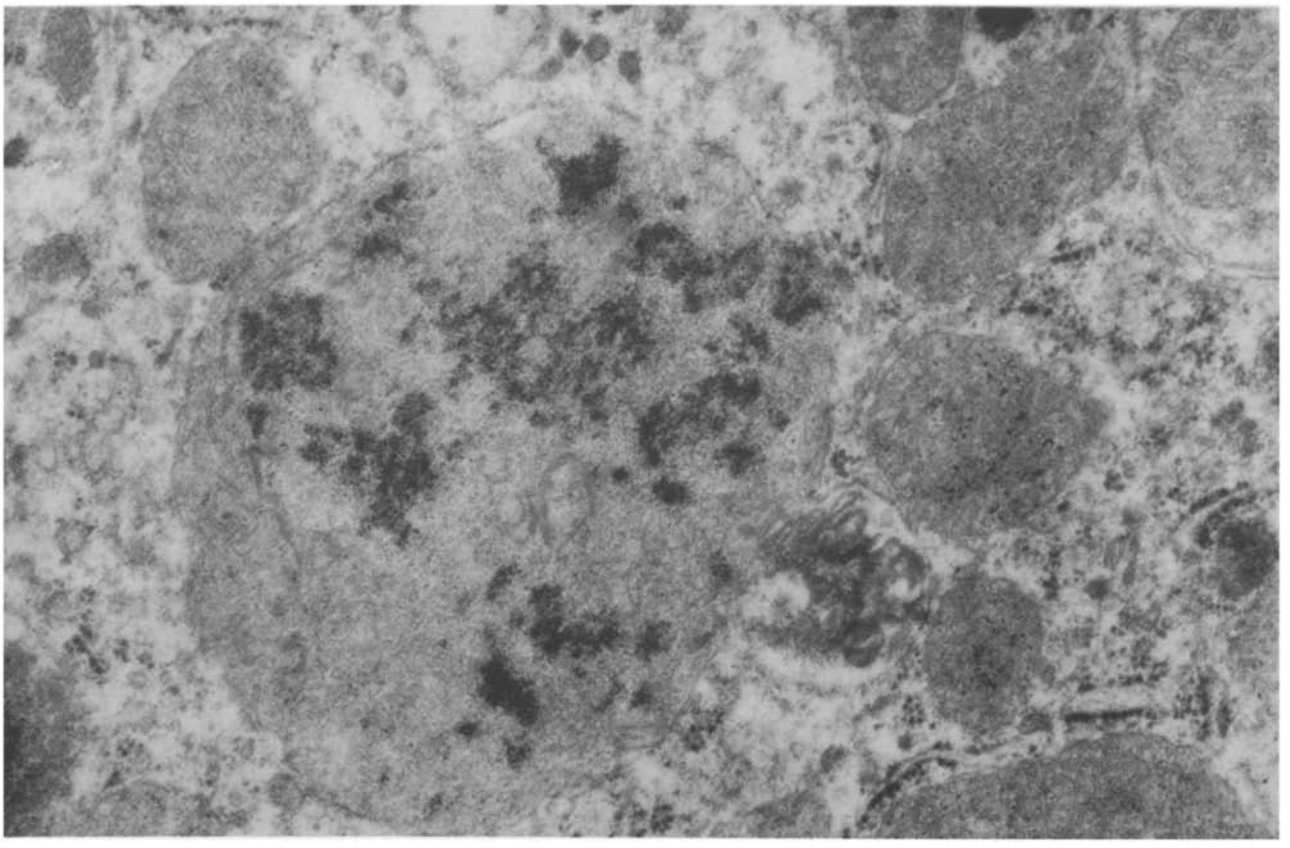

Fig. 3. Proximal tubular cell of rat kidney 48 h after 1h renal ischaemia and i.v. injection of 200 mg stroma-free haemoglobin per 100 g body weight given 2 h after termination of ischaemia. Note ambi-and telolysosomes of considerable size. Uranylacetate (x 42,000)

parent in this group as in group 3. Fat globules were found in the basal cell areas and large numbers of cells with hydropic swelling and necroses were evident, although they were not as clearly defined as in group 3. This group showed occasional anhydropic necroses with the wide lumina containing a few discharged cells or cell contents. On the whole, Hb reabsorption was well advanced in this group, as shown by the absence of homogeneous electron-dense heterophagic vesicles.

Groups 6, 7 and 8 (SHb)

The findings were approximately the same in all three of these groups. Phagosomes were relatively rare, and the few lysosomes present were mainly telolysosomes with a fine granular content. There were many hydropic swollen cells which, in most cases, contained residues of Hb. Many myelin figures were recognisable in Group 7, and heavily damaged cells with transparent cytoplasm and few organelles often contained ambilysosomes and telolysosomes with ruptured membraned and fine granular contents (Figs. 4 and 5). In spite of extensive cell damage, however, the mitochondria remaining in SHb groups were not altered markedly and the endoplasmic reticulum was increased only in cells containing telolysosomes. The brush borders were much higher in groups 6, 7 and 8 than in groups 3, 4 and 5, while the proximal convoluted tubule lumina were narrower.

Discussion

Previous studies show Hb degradation to be relatively rapid in normal kidneys, being completed within 8 h after Hb infusion in most instances [5, 12]. The present findings show, in contrast, the Hb degradation is still going on 48 h after infusion in kidneys made ischaemic around the time of the Hb load. Ultrastructural changes in ischaemic kidneys infused with Hb consisted mainly in ambi- and heterophagosomes, and electron-dense droplets in phagosomes, while non-ischaemic kidneys treated with Hb contained only fine granulated siderin- and ferritin-like material as well as telolysomes or residual bodies 48 h after treatment. The greatest delay in Hb degradation in the present study was seen in animals that received Hb immediately after renal ischaemia, while administration of Hb either 30 min before or 2 h after ischaemia caused less of a delay in Hb catabolism. It is of interest to note that the stage of Hb degradation observed after 48 h in rats given Hb either 30 min before or 2 h after renal ischaemia resembled that seen 2 h after Hb administration to otherwise untreated rats [5, 12]. Thus, the present findings show that removal of Hb from kidney is markedly delayed when haemoglobinuria occurs in association with renal ischaemia. It is likely that the retardation in Hb degradation in ischaemic kidneys is due to an energy deficit that persists until regenerative proces-

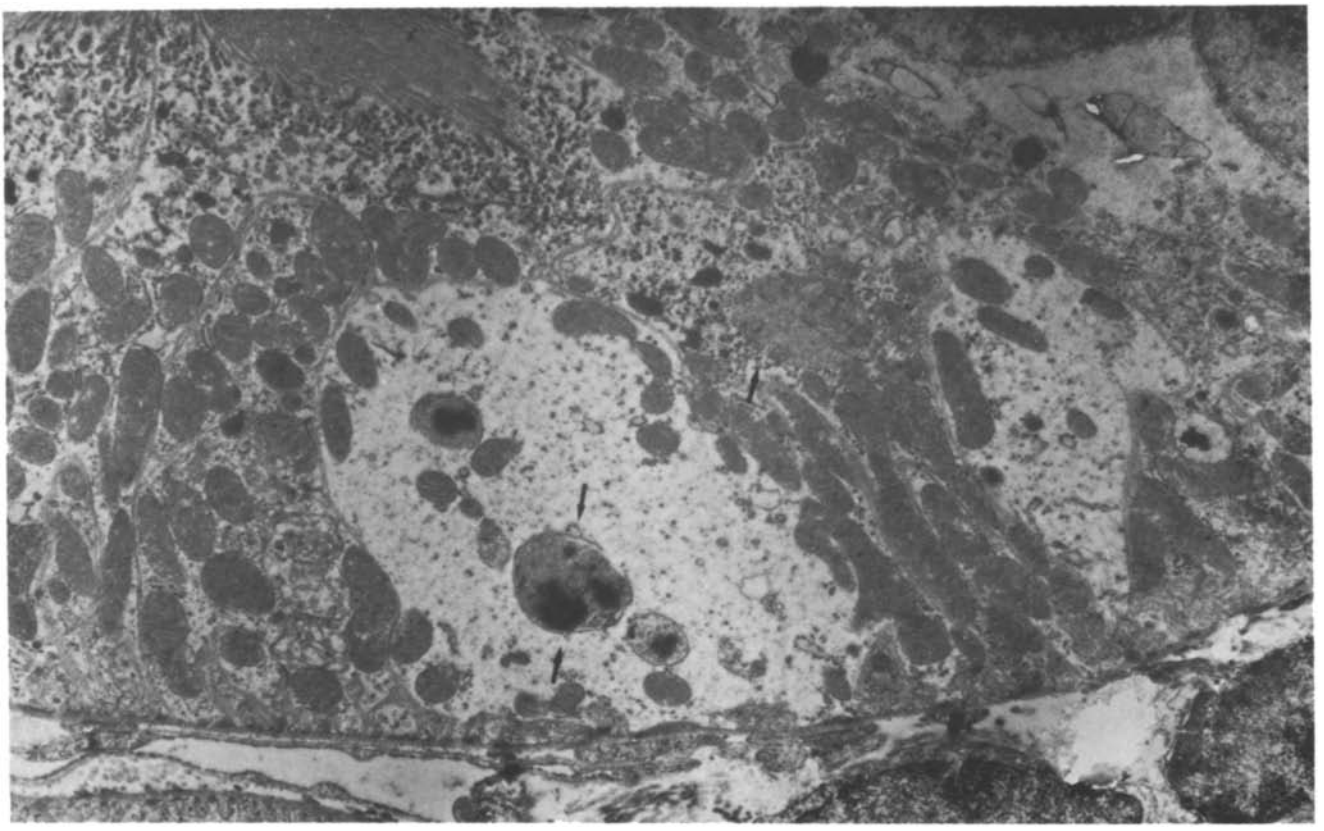

Fig. 4. Sham operated rats injected with 200 mg stroma-free haemoglobin per 100 g body weight. Note hydropic cells containing telelysosomes (→) and less changed cells containing autolysosomes (→). Uranylacetate (x 6,300)

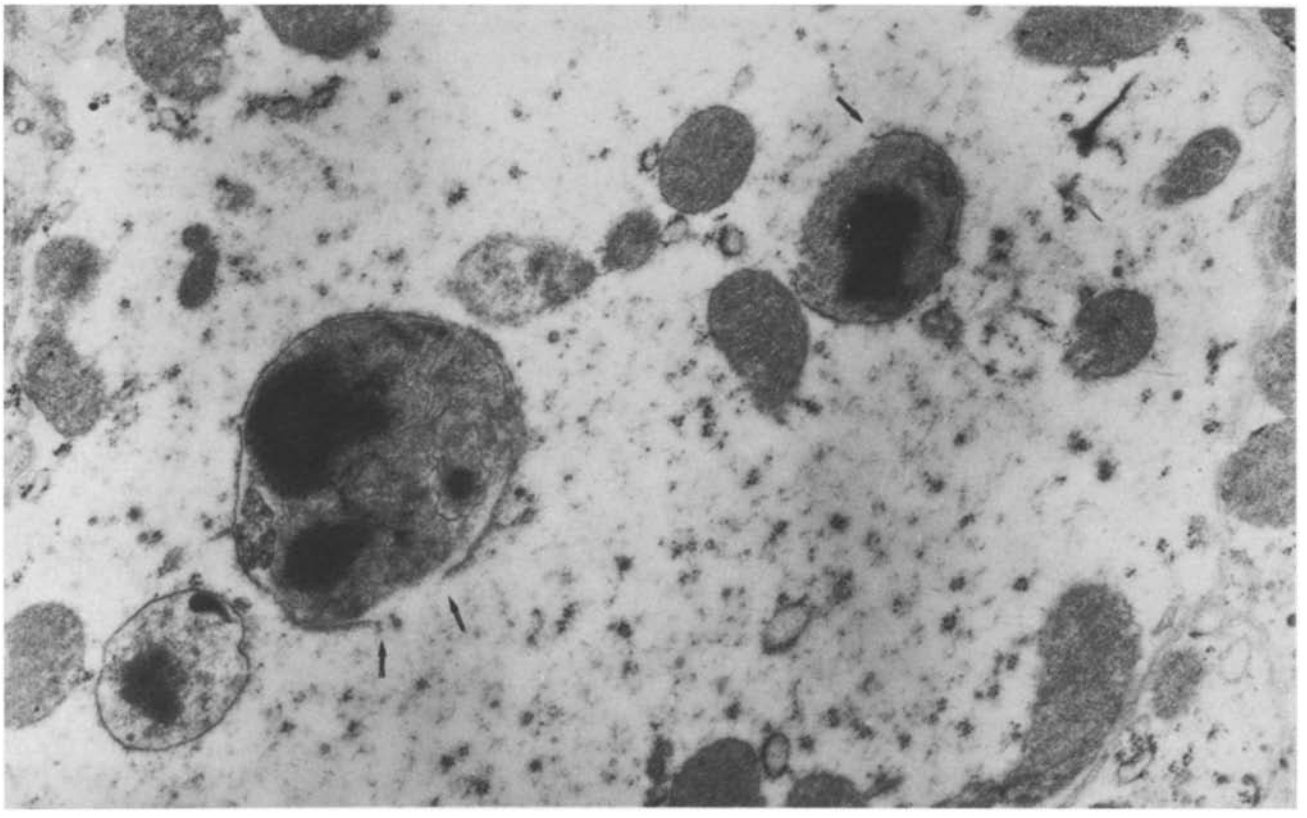

Fig. 5. Hydropic renal tubular cell of rat 48 h after i.v. injection of 200 mg stroma-free haemoglobin per 100 g body weight 30 min prior to sham ischaemia. Note lysosome loaded with haemoglobin or haemoglobin-degradation products, and ruptured lysosomal membrane with lysosomal contents in contat with cytoplasma (*arrow*). Uranylactate (x21,000)

ses provide sufficient energy for intracellular metabolism systems to operate properly.

The present findings show the Hb load to alter the size of lysosomes, in that the diameter of phagosomes and phagolysosomes were four times larger than mitochondria in rats infused with Hb, while lysosomes were rarely larger than mitochondria in groups given no Hb. It is also of interest that telolysosomes with ruptured membrane were observed solely in the hydropic swollen epithelium of the proximal convoluted tubule in sham operated groups given Hb. While we are unable to explain this finding fully, it is noteworthy that previous studies suggest lysosome expansion with disruption of membrane to be due to a disproportion between the amount of material present for degradation and the capacity of lysosomal enzymes [4, 10]. Perhaps inadequate cell metabolism after ischaemia disrupts membrane structure and increases the vulnerability of the cell to breakage. Thus, renal damage produced by a large dose of Hb, as seen in the present and previous studies [17, 18, 22, 32] may reflect mainly effects of overloading intracellular metabolic systems. Since the worst lesions were found in the present study in kidneys from rats that underwent renal ischaemia and Hb load, the results support the view that Hb absorption by the tubular epithelium makes these cells more vulnerable to damage by ischaemia.

Thoenes (1964) concluded, on the basis of electron microscopic studies in rats, the reabsorption and transport of fluids is improbable after one hour of ischaemia, albeit at a reduced rate. Evidently, regenerative events occur within hours after ischaemia and provide the kidney with sufficient energy for some active transport processes [8].

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