Virchows Archiv A Pathol Anat (1989) 414:429–437

Virchows Archiv A
Pathological Anatomy and Histopathology

Polyethylene glycol effect on the oxygenated and hypoxic isolated perfused rat kidney

J. Kopolovic[1], M. Brezis[2], K. Spokes[3], and S. Rosen[3]

[1] Department of Pathology, The Edith Wolfson Hospital, Holon, Israel
[2] Department of Medicine, Hebrew University Hadassah Medical School, Jerusalem, Israel
[3] Departments of Pathology and Medicine, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, the Harvard-Thorndike Laboratory of Beth Israel Hospital, Boston, MA, USA

Summary. Polyethylene glycol protects against O_2 deprivation after clamping of the renal artery or norepinephrine infusion and in hypoxic primary cell culture. Isolated perfused kidneys under hypoxic conditions develop morphological alterations in all segments of the proximal tubule and medullary thick ascending limb. In an attempt to ameliorate the effect of hypoxia, rat kidneys were perfused for 90 min with regularly oxygenated (95% O_2 + 5% CO_2) or hypoxic perfusate (95% N_2 + CO_2) supplemented with 8–12% polyethylene glycol (MW ~8000). In oxygenated and hypoxic kidneys, polyethylene glycol produced similar changes in S_1–S_2 segments consisting of reduction of cell thickness and organelle compaction with internalization of brush border into the tubulo-vesicular system. In the S_3 segment, the cellular volume loss was more limited; the brush border was transformed to membranous whorls and the cytoplasm contained large, irregular, clear zones. Mitochondrial swelling was pronounced in the hypoxic proximal tubules. Polyethylene glycol quantitatively increased and emphasized the damage in the medullary thick ascending limb. Inclusion of 10^{-2} M ouabain preserved the medullary thick ascending limb from hypoxic injury and polyethylene glycol had no effect on this undamaged epithelium. Thus, polyethylene glycol affects renal tubules on the basis of their known water permeability and does not protect against but rather worsens hypoxic injury in the medullary thick ascending limb.

Key words: Polyethylene glycol – Perfused kidney – Hypoxia – Proximal tubules – Distal tubules

Offprint requests to: S. Rosen, Department of Pathology, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215, USA

Introduction

The effect of hypoxia on the isolated perfused kidney is well established (Brezis et al. 1984; Shanley et al. 1986). In those kidneys in which the perfusate is gassed with 95% O_2, and no O_2 carrier, such as hemoglobin, is included, the changes are limited to the inner zone of the outer medulla. In this region, medullary thick ascending limb (mTAL) injury occurs and is most severe in areas removed from the vasa recta and cortex (Brezis et al. 1984). A more extensive form of hypoxic change occurs when the perfusate is gassed with 95% N_2. This injury involves the proximal nephron, as well, causing mitochondrial swelling and brush border disorganization in the convoluted segments (S_1, S_2) and edema and fragmentation in the straight portion (S_3) (Shanley et al. 1986). Studies have shown that polyethylene glycol (PEG) protects against O_2 deprivation both in the ischemic situation (clamping of the renal artery and norepinephrine infusion) and in hypoxic primary cell cultures (Frega et al. 1979; Burke et al. 1983; Kreisberg et al. 1980). PEG acts evidently in these circumstances as an impermeant solute., i.e., a substance because of size or charge and absence of carrier or other transporting mechanisms does not cross cellular plasma membranes and thus exerts oncotic pressure. In an attempt to ameliorate the effect of hypoxia in the isolated perfused kidney preparation, PEG was added to the perfusate.

This study will show that hyperoncotic impermeant solutes such as PEG do not protect the cells of the isolated perfused kidney under conditions of mild or severe hypoxia. However, there was striking heterogeneity of cellular response to this agent that correlated with known water permeability of individual nephron segments.

Materials and methods

Male Sprague-Dawley rats weighing 370–470 g were used for all experiments. The rats were fed standard rat chow and allowed free access to water. Perfusion of the right kidney was performed according to the technique described by Ross et al. (1973). The animals were anesthetized intraperitoneally with Inactin 100 mg/kg. Mannitol, 200 mg and heparin, 1000 U were injected into the femoral vein. The peritoneal cavity was opened and a PE-10 catheter placed in the right ureter. A glass arterial cannula was inserted into the superior mesenteric artery and threaded across the aorta into the right renal artery. Perfusion was started while the cannula was still in the mesenteric artery, thereby avoiding any ischemia to the kidney. The kidney was then placed in a constant temperature cabinet where the temperature was kept at 37° C. Perfusion medium was recirculated continuously with a pulsatile flow at a pressure of 85 mmHg at the tip of cannula. The rate of flow of the perfusate was measured with a Brooks flowmeter installed in a series in the arterial line. The perfusion medium consisted of a Krebs-Ringer Henseleit solution containing (in millimolar): Na, 143; Cl, 103; K, 4.5; bicarbonate, 24; Ca, 2.5; Mg, 1.2; phosphate, 1.2; pH of 7.4, bovine serum albumin at a concentration of 6.7 g/dl; glucose at 5 mM was added in all experiments. PEG was obtained from Sigma (St. Louis, MO).

Experimental groups

Group A ($N=6$) Kidneys were perfused for 90 min with regularly oxygenated medium (95% O_2/5% CO_2).

Group B ($N=5$) Kidneys were perfused for 90 min with regularly oxygenated (95% O_2/5% CO_2); perfusion medium supplemented with 8%–12% PEG (MW 8000).

Group C ($N=5$) Kidneys were perfused with regular perfusion medium that was gassed with 95% N_2/5% CO_2.

Group D ($N=4$) Kidneys were perfused for 90 min with medium, gassed with 95% N_2/5% CO_2 and supplemented with 8% PEG.

Group E ($N=4$) Kidneys were perfused for 90 min with medium gassed with 95% N_2/5% CO_2 and the perfusion medium was supplemented with both 8% PEG and ouabain 10^{-2} M.

Morphologic techniques. Following perfusion, each kidney was fixed via the perfusion circuit with 1.25% glutaraldehyde in 0.1 molar phosphate buffer (pH 7.4). One to 2 mm-thick sections were removed from the central portion of the kidney and 4 × 4 mm sections containing the entire width of the cortex and outer medulla were taken from that slice, post-fixed in 2% osmium tetroxide, dehydrated and embedded in an araldite-Epon 812 mixture. Evaluation was done by light microscopy and one-micron sections with selected blocks were examined by electron microscopy. Quantitative analysis of mTAL was done as previously indicated (Brezis et al. 1984). Briefly, the damage to mTAL in the inner stripe of the outer medulla was evaluated in three regions: outer, mid, and deep. A percentage score was used to indicate the fraction of tubules involved with minimal to mild (chromatin margination, minor degrees of mitochondrial swelling), moderate (blatant mitochondrial swelling with limited nuclear pyknosis), or severe (blatant mitochondrial swelling with extensive nuclear pyknosis and cell fragmentation).

Results

Group A (oxygenated kidneys): The kidneys perfused with regular perfusion medium (95% O_2/5% CO_2) showed no damage in S_1–S_2 segments of proximal tubules. The S_3 segments showed changes (cell swelling and fragmentation) only focally in the areas underlying the medullary rays as previously described (Shanley et al. 1986). The mTALs displayed the characteristic changes of hypoxic cell injury (Brezis et al. 1984). The quantitative analysis of mTALs (Fig. 1) shows the typical limited involvement of the superficial inner stripe.

Group B (oxygenated kidneys supplemented with PEG): The S_1–S_2 segment was characterized by an overall reduction in cell thickness and by extensive brush border loss (Fig. 2). The brush border which remained had a variable orientation giving an irregular contour to the cellular surface. Immediately beneath the brush border area was a zone of small vesicles which occupied 1/4–1/3 of cell height. Scattered larger vesicles were also noted that occupied much of the entire cellular width.

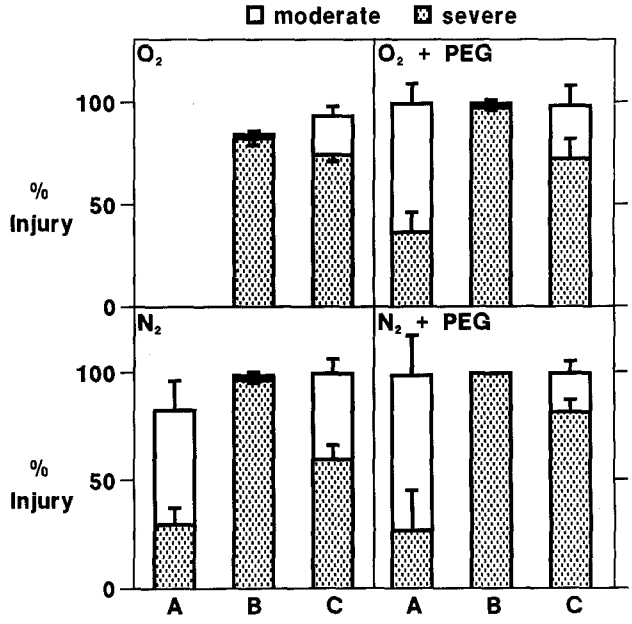


Fig. 1. The effect of PEG on mTAL injury. When the isolated perfused kidney is gassed with 95% O_2, selective mTAL necrosis occurs which is confined to mid and deep inner stripe (A = superficial, B = mid, C = deep inner stripe); if N_2 is substituted for O_2, the injury becomes more extensive. The severe damage (*top panel*) in mTAL (*zones A* and *B*) was significantly more extensive when PEG was added to the oxygenated perfusion medium ($p<0.01$). Under hypoxic conditions (*lower panel, zone C*), the moderate and severe damage was significantly more extensive when PEG was added ($p<0.05$, $p<0.005$, respectively)

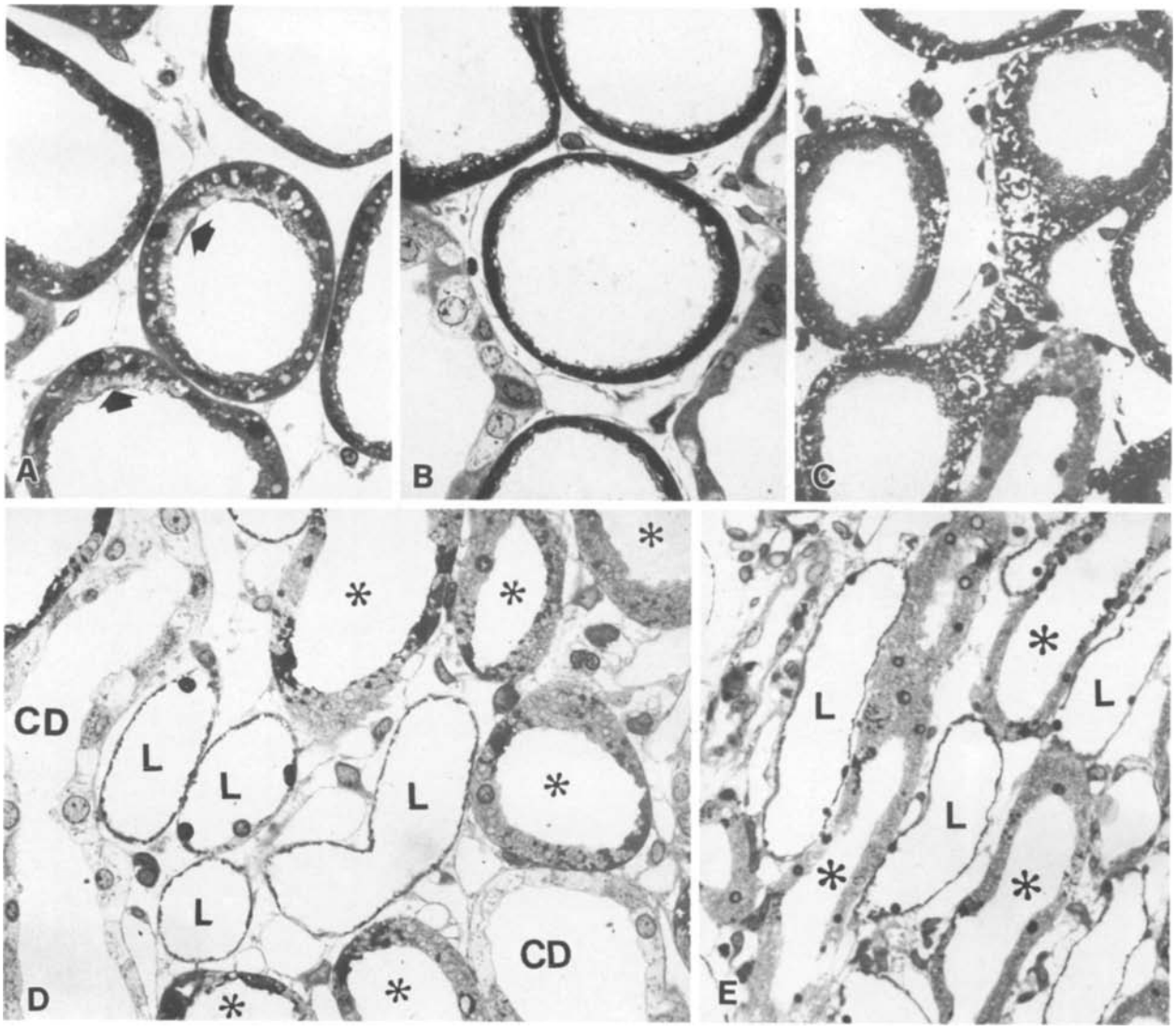


Fig. 2A–E. Oxygenated and hypoxic kidney supplemented with 8% PEG. The appearances are similar whether oxygenated (**A, C, D**) or hypoxic (**B, E**). The epithelium of the convoluted tubules (S_1, S_2) (**A** and **B**) shows reduction in thickness, increased density, and loss of brush border. Focally (*arrows*) a superficial zone of fine vacuoles can be appreciated. The brush border in (S_3) (**C**) is better maintained but very distorted. Zones of cytoplasmic clearing are apparent in the en face section of the tubule in the center. Sections from the inner stripe of the outer medulla (**D** and **E**) show unremarkable collecting ducts (*CD*). The cytoplasm of the upper portion of the descending limbs of the long loops (*L*) is fragmented and condensed; the nuclei show chromatin margination and pyknosis. Zones of cytoplasmic compaction/fragmentation and intact epithelium with high amplitude mitochondria swelling are noted in the mTAL (*). × 570, × 590, × 570, × 360, × 320 (reduced to 89%)

In the S_3 segment, epithelium (Fig. 2) was also attenuated, but the brush border was better maintained, albeit very abnormal. In this segment epithelial volume loss was much more limited but the luminal surface was irregular and the whole brush border zone was transformed to small vacuoles. There appeared to be a loss of cytoplasmic sap, such that large irregular clear zones were noted. The remaining cytoplasmic elements formed a reticular pattern. Cells immediately adjacent to vessels showed a largely normal configuration that appeared related to fixative rehydration. When PEG was included in the fixative, this "apparent restoration of form" was very limited.

Changes in the mTAL were noted primarily in the inner stripe of the medulla (Fig. 2). These were similar to those previously described: a sequence of mitochondria swelling and chromatin margination which progressed to cell fragmentation. These changes were most conspicuous in ar-

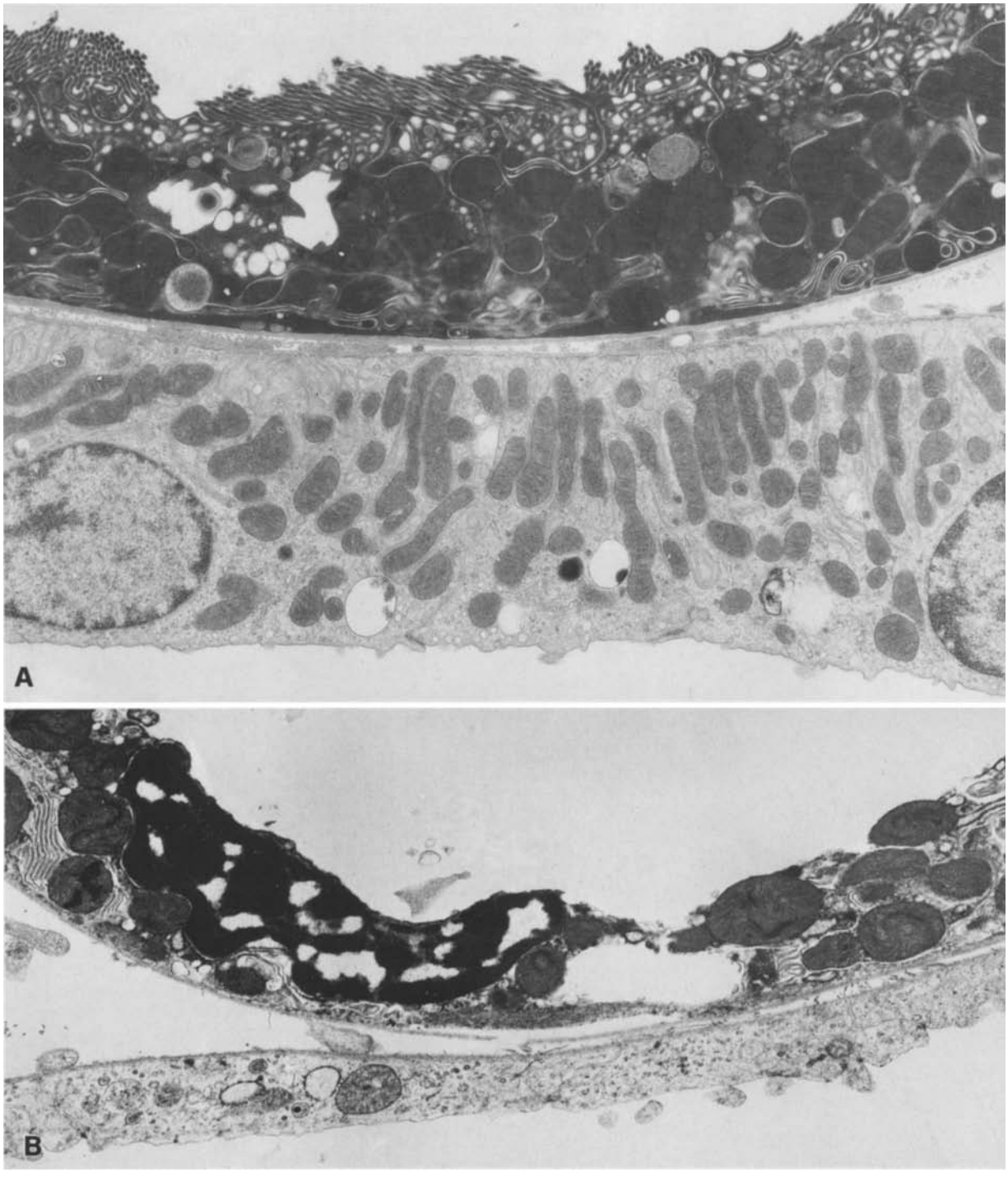


Fig. 3. Oxygenated kidney supplemented with 8% PEG. Fine structural studies of the proximal convoluted tubules (*upper*, **A**) show condensation of organelles, a spherical conformation to the mitochondria, and a superficial zone of small vesicles. Both brush border and basal membranes appear reduced and are poorly oriented with respect to the luminal membranes and basal lamina. In contrast (*lower*, **A**) the adjacent distal tubular segment is unremarkable. The medullary thick ascending limb (*upper*, **B**) shows loss of lumen membranes and portions of the cell body. The nucleus is markedly condensed; mitochondria are swollen and opaque. The adjacent thin limb (*lower*, **B**) is relatively intact. ×10500, ×10200

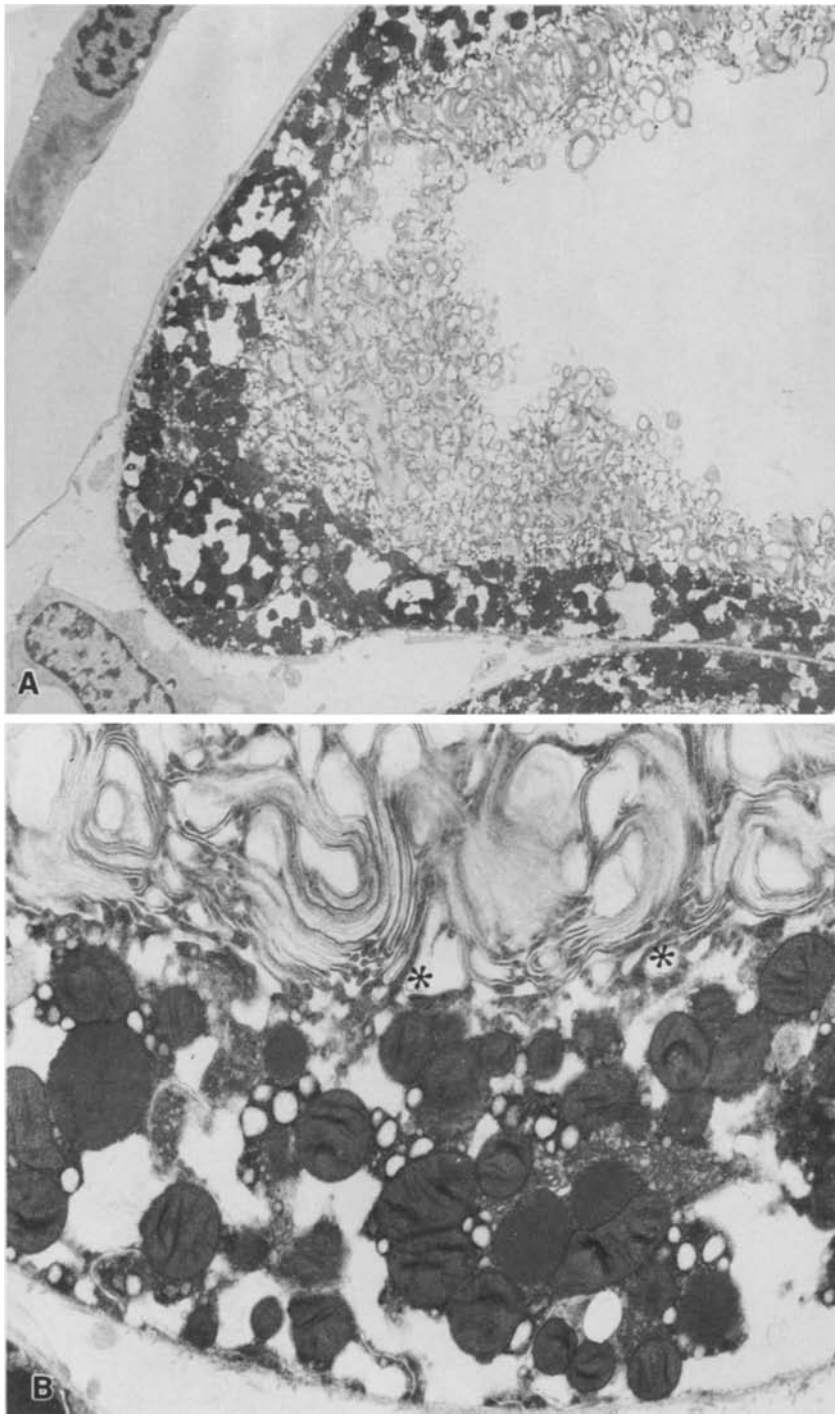
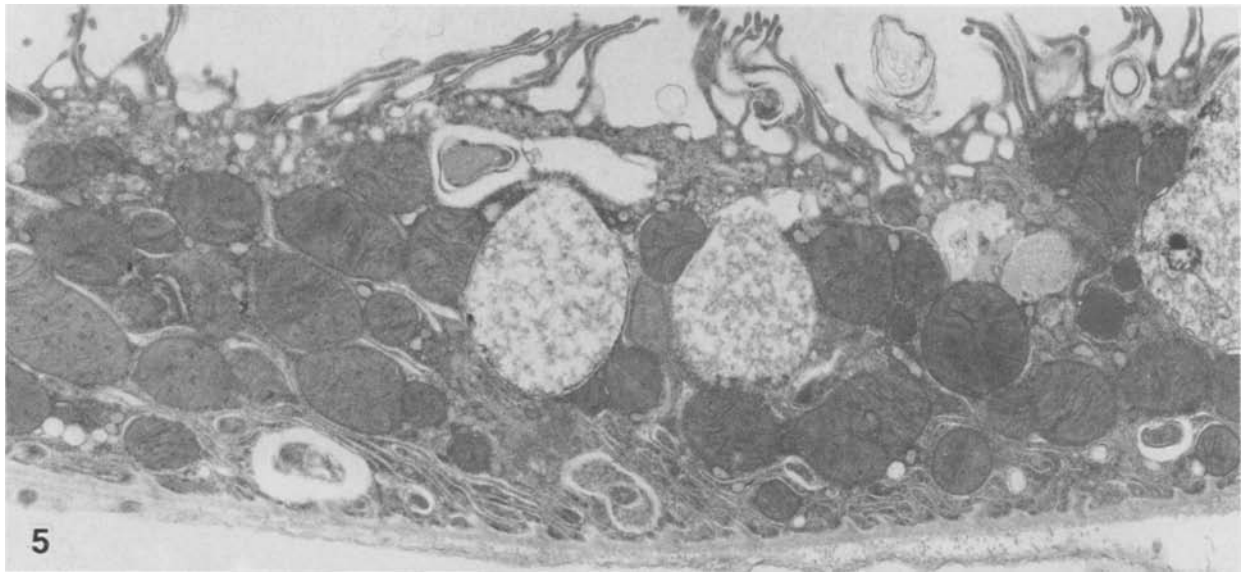


Fig. 4. Oxygenated kidney supplemented with 8% PEG. This low power electron micrograph (**A**) of S_3 shows diffuse transformation of the brush border to membranous whorls. Irregular electron lucent zones are noted in both the cell body and nucleus. An adjacent interstitial cell (*lower left*) and distal nephron segment (*upper left*) are unremarkable. At higher power (**B**) traces of intact brush border elements can be identified (*). Small vesicles are scattered throughout the condensed cytoplasmic sap; mitochondria are enlarged, and contain aggregated cristae. ×4000, ×15000

eas remote from the vascular supply (vasa recta) and cortex. However, in zones of fragmentation the remaining cellular elements showed marked condensation. In addition, the thin descending limbs of the long loop of Henle showed cytoplasmic condensation and lumenal border irregularity. The quantitation of mTAL damage is summarized in Fig. 1. It is apparent that perfusion with PEG with normal oxygenation induces more severe and extensive injury as compared to controls.

Fine structural studies of the S_1–S_2 segments revealed irregular brush border loss, shortening, and angulation of the microvilli (Fig. 3). The brush border plasma membrane appeared to be continuous with a copious tubulo-vesicular system that comprised 1/4–1/3 of the cellular thickness. The



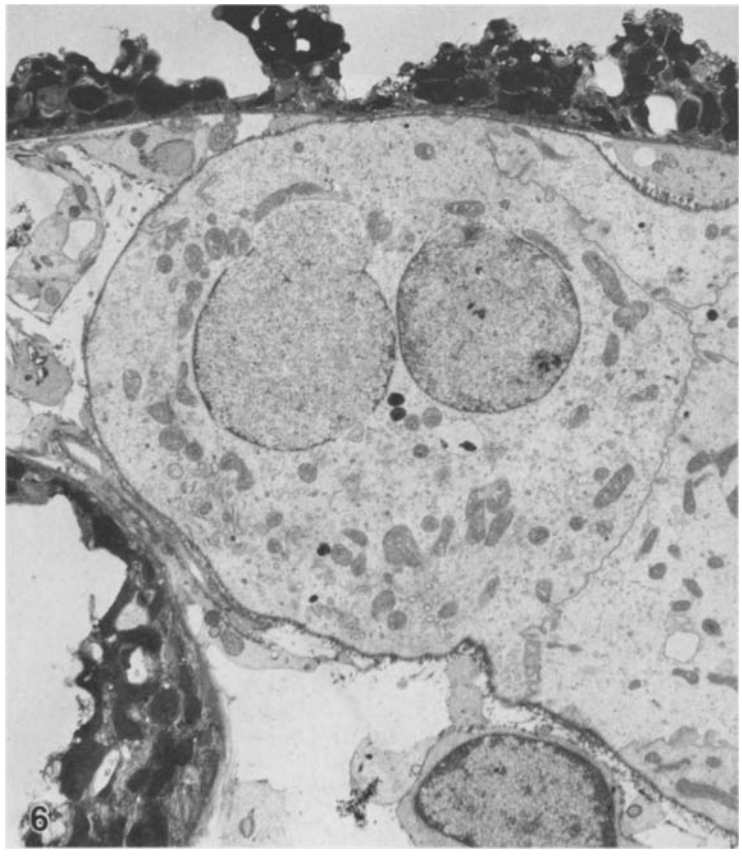


Fig. 5. Hypoxic kidney supplemented with 8% PEG. The fine structure of the proximal convoluted tubules (S_1, S_2) is largely similar to those of the oxygenated perfused kidney supplemented with PEG (see Fig. 3), i.e., disarray and reduction of brush border and basal membranes. Mitochondrial swelling is more evident and small electron densities can be noted (*left*). × 14000

Fig. 6. Hypoxic kidney supplemented with 8% PEG. The mTALs (*upper and lower left*) show fragmentation and marked cytoplasmic condensation as in Fig. 3. The adjacent collecting duct and interstitial cell (*lower right*) are unremarkable. × 11200

mitochondria were ovoid and enlarged, lacking the usual perpendicular orientation, and with cristae disordered and focally closely opposed. The basal membrane system did not have a perpendicular orientation, was diminished in extent, and focally expanded to form large vacuoles. Smaller vacuoles were noted dispersed among the cytoplasm sap. The endoplasmic reticulum and ribosomal elements were condensed, as was the nucleoplasm.

The brush border of S_3 could no longer be defined as such being transformed into membranous whorls (Fig. 4). Within the latter, segments of the original microvillous network could be identified. Large irregular non-membrane-bound, electron-lu-

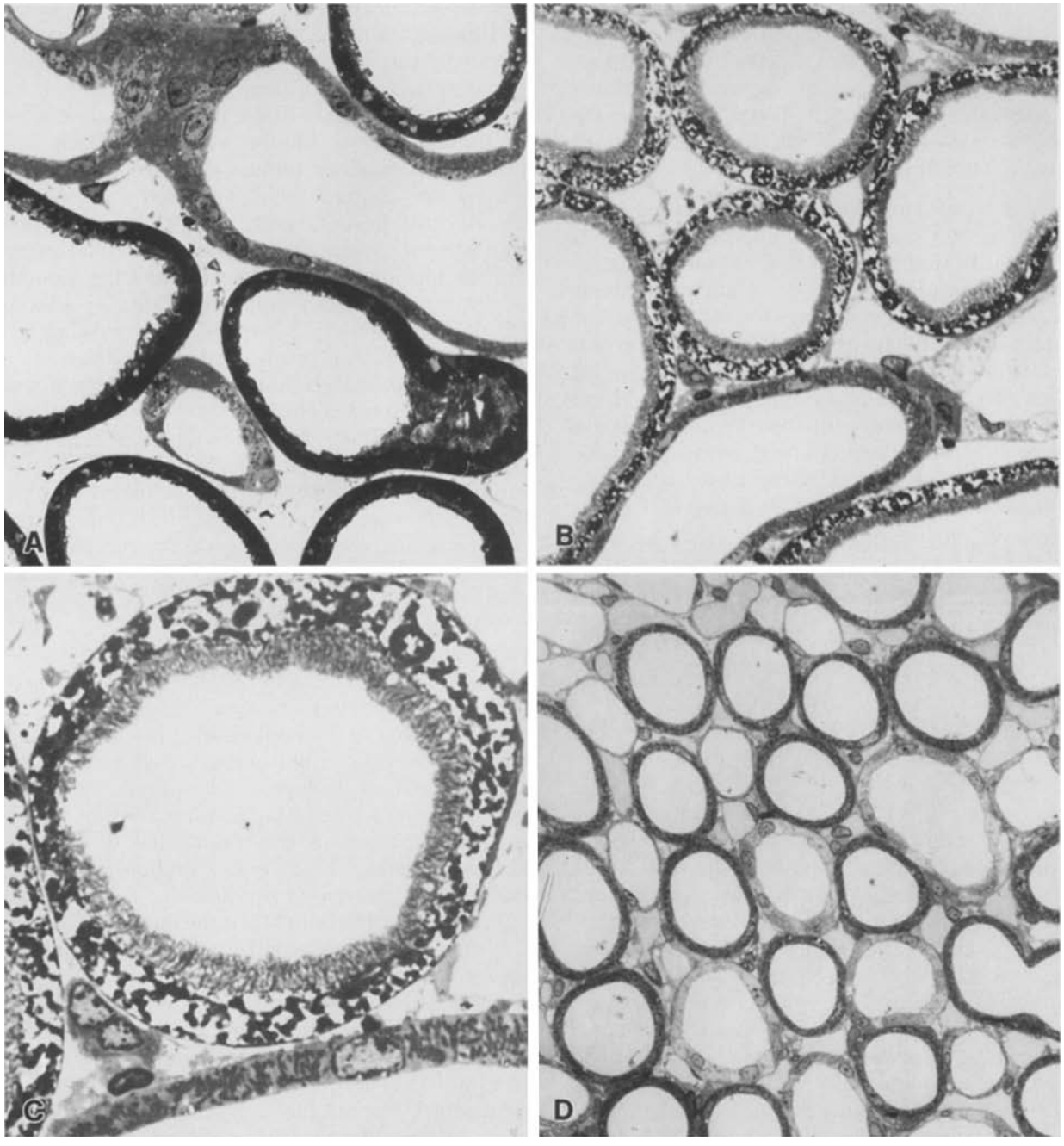


Fig. 7A–D. Hypoxic kidney supplemented with 8% PEG and 10^{-2} ouabain. The appearance of the convoluted proximal tubules S_1, S_2 (**A**) and pars recta S_3 (**B**) and (**C**) is similar to conditions without ouabain included in the perfusate. The medullary thick ascending limbs (**D**) are unremarkable. ×640, ×640, ×1600, ×250

cent zones were present intermingled with condensed cytoplasmic sap that included small vesicles of endoplasmic reticulum origin and mitochondria. These mitochondria contained multifocal zones of closely approximated cristae. Interstitial cells, endothelium, and distal nephron segments were basically unremarkable except for the mTAL. Focal mTAL cell fragmentation was present (Fig. 3) which was as previously described: extensive plasma membrane interruption, mitochondrial swelling with small densities and conspicuous nuclear chromatin condensation. The cytoplasm contained closely opposed membranous fragments forming whorls.

Group C (hypoxic kidneys): The kidneys perfused under hypoxic conditions showed changes as previously described (Brezis et al. 1984; Shanley et al. 1986), e.g. the S_1–S_2 segments showed either brush border clubbing and mitochondrial swelling or extensive formation of apical vesicles; mTAL injury became more extensive.

Group D (hypoxic kidneys supplemented with PEG): The light microscopic appearance (Fig. 2) was similar to that in group B. Quantitative analysis of mTALs showed nearly complete involvement by moderate or severe injury (Fig. 1). The extent of injury was not statistically different from group B. Electron microscopic studies of the proximal tubules (Fig. 5) revealed findings similar to those noted in group B except for that the mitochondria swelling was more marked and associated with small electron densities. Electron microscopic studies of the mTAL were similar to group B (Fig. 6).

Group E (hypoxic kidneys supplemented with PEG and ouabain): The proximal tubular changes (S_1, S_2, S_3) resembled those in groups B and D (Fig. 7). However the mTALs were preserved.

Discussion

In the early 70's, Flores et al. (1972) but forth the hypothesis that cell swelling was the primary determinant factor that did not allow recovery after prolonged ischemia. They showed that after renal artery obstruction, cellular elements were swollen and the available vascular space was limited. These changes and their functional consequence were corrected by the administration of hypertonic mannitol, but were unaffected by equivalent isotonic saline, extracellular fluid volume expansion. These findings were confirmed by others using the norepinephrine model of renal ischemia (Burke et al. 1983). The latter studies comparing hypertonic saline (permeant solute) and isotonic mannitol and PEG (impermeant solutes) concluded that the impermeant nature of the solute rather than its tonicity or concentration predicted best recovery from ischemic acute renal failure. Kreisberg et al. (1980) showed that anoxic effects of primary cultures of renal tubular epithelium could be mitigated if cell swelling was prevented by 8% PEG. Thus, these studies suggested that prevention of cell swelling per se was a primary determinant in cellular recovery from ischemia rather than unavailability of blood because of compromise of vascular pathways. The present study extends the work of Kreisberg et al. (1980) to the situation of the isolated perfused kidney under conditions of mild and severe hypoxia. The effect of an impermeant solute in this situation was to create combined changes in the proximal tubule and mTAL of both hyperoncotic effects and O_2 deprivation.

The extraordinary findings in this study related to the differential tubular effects of PEG. The proximal convoluted tubules underwent a series of remarkable changes which included an apparent loss of brush border membrane with incorporation into the tubulovesicular system. The basal membranes appeared reduced as well, and the amount of cytoplasmic space appeared diffusely diminished. Alterations in S_3 were equally striking but very different. The brush border membrane mass was better preserved but its basic structure was markedly altered. Cytoplasmic sap was removed in a more irregular fashion, such that large electron-lucent zones appeared. Thus the convoluted tubular response was a diffuse volume loss with membrane internalization, while the cells of the pars recta maintained their general structure without membrane internalization, but with marked distortion of cell sap cytoarchitecture suggesting a more rigid cellular structure than S_1, S_2. Both oxygenated and hypoxic kidneys showed similar changes, but mitochondrial swelling was pronounced in the latter situation.

In contrast to the remarkable proximal tubular changes, the distal tubules responded quite differently. In cells with hypoxic alterations, PEG emphasized injury by causing condensation of the remaining elements in the fragmented tubules. If, however, distal tubular injury (mainly medullary mTAL) was prevented by ouabain, PEG had no effect on the epithelium. Thus the uninjured mTAL cell did not respond to the effect of this impermeant solute.

The morphologic response to hyperoncotic impermeant solutes is consonant with the known permeability of the nephron segments (Kneffer and Burg 1983). The proximal tubule is highly water permeable, responsible for absorbing large volumes of ultrafiltrate. This volume flow can be apparenty accounted for by a small osmotic gradient in the lateral interspaces. On the other hand, distal epithelium is relatively water-tight and transport is dependent on pump activity. The proximal tubule is also responsive to the alteration of the peritubular hyperoncotic pressures both directly and indirectly (Agerup and Persson 1982).

The dramatic PEG effects on the convoluted proximal tubule suggests that cytoskeleton gel state, membrane interrelationships are such that marked shape alterations can occur. It is particularly interesting that part of the compensation for

volume loss is the internalization of the brush border via the tubulo-vesicular apical system. In contrast, S_3 appears a more rigid cell, i.e., the brush border is deformed, but not internalized; the cytoplasmic substance is not diffusely contracted, but irregularly condensed forming a reticular pattern of electron-lucent zones. The relative normalcy of other nephron segments and endothelium is remarkable in the face of hyperoncotic forces. When injury had increased membrane permeability in mTAL, the hyperoncotic pressure was capable of altering cellular constituents. When ouabain prevents cell injury, the mTAL epithelium becomes resistant to these changes.

In conclusion, this study has failed to show that an impermeant solute, PEG, protects against hypoxic injury in the situation of the isolated perfused kidney. However, this substance produces marked cellular alterations that appear to relate to the known water permeability of a given nephron segment.

Acknowledgments. This work was supported by a grant from the Fund for Basic Research administered by the Israeli Academy of Sciences and by U.S.P.H.S. Grant No. AM18078. The authors are grateful to Drs. F.H. Epstein and P. Silva for their advice and criticism.

References

Agerup B, Persson AE (1982) Modulation of proximal tubular hydraulic conductivity by peritubular capillary oncotic pressure. Acta Physiol Scand 115:335–359

Brezis M, Rosen S, Silva P, Epstein FH (1984) Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. J Clin Invest 43:182–189

Burke TJ, Arnold PE, Schrier RW (1983) Prevention of ischemic acute renal failure. Am J Physiol 244:F646–F649

Flores J, Dibona DR, Beck CH, Leaf A (1972) The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J Clin Invest 51:118–126

Frega NS, DiBona DR, Leaf A (1979) The protection of renal fucntion from ischemic injury in rat. Pflügers Arch 381:159–164

Kneffer M, Burg M (1983) Organization of nephron function. Am J Physiol 244:F579–589

Kreisberg JI, Mills JW, Jarrell JA, Rabito CA, Leaf A (1980) Protection of cultured renal tubular epithelial cell from anoxic cell swelling and cell death. Proc Natl Acad Sci (USA) 77:5445–5447

Ross BD, Epstein FH, Leaf A (1973) Sodium reabsorption in the perfused rat kidney. Am J Physiol 225:1165–1171

Shanley PF, Brezis M, Spokes K, Silva P, Epstein FH, Rosen S (1986) Hypoxic injury in proximal tubules of the isolated perfused kidney. Kidney Int 29:1033–1037

Received June 16, 1988 / Accepted December 19, 1988