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Phagocytosis of bacteria by proximal tubular epithelium in experimental pyelonephritis*

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Summary. Acute pyelonephritis was induced in rats by temporary unilateral ureteric obstruction and the intravenous injection of *Escherichia coli*. Animals were sacrificed 48 h after infection and changes in renal cortical tubules due to the presence of bacteria were studied. Bacteria appeared and multiplied in the tubular lumina and proximal tubular epithelial cells endocytosed the microorganisms in large numbers. Coalescence of phagosomes with lysosomes resulted in the surrounding of engulfed bacteria with acid phosphatase. However, the lysosomal apparatus of the cells did not eliminate *Escherichia coli* since the bacteria multiplied within phagosomes and destroyed the normal cell architecture. The peritubular interstitial inflammatory infiltrate caused ischemia of tubules, enhancing bacterial damage to the proximal tubules. The cytoplasm of the injured tubular cells was sometimes detached from the basement membrane. Cells of the distal tubules and collecting ducts did not show significant endocytosis or bacterial tubular damage.

Key words: Pyelonephritis – *Escherichia coli* – Phagocytosis – Proximal tubules – Acid phosphatase cytochemistry

Introduction

The proximal tubular cells of the mammalian kidney reabsorb colloidal particles as well as homologous and exogenous proteins from the luminal fluid by means of endocytosis. Apical tubular invaginations internalize the material, forming endocytic vacuoles which transport the proteins for degradation to the lysosomes (Trump 1961; Straus 1964; Miller and Palade 1964;

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Ericsson 1965; Graham and Karnovsky 1966; Tisher et al. 1966; Thoenes and Langer 1969; Christensen 1976; Bariéty et al. 1980). Cortical distal and collecting ducts are incapable of protein uptake from the tubular fluid (Bourdeau et al. 1972; Bariéty et al. 1980).

We have previously described the responses of the tubular epithelium to injury caused by tubulointerstitial inflammatory infiltration in experimental pyelonephritis (Iványi et al. 1983). We documented ultrastructurally that cellular casts originated through circumscribed necrosis of the tubular wall, apparently for the drainage of interstitial abscesses. Electronmicrographs revealed tubular cells containing membrane-bound vacuoles containing easily recognizable bacteria. Others have also observed engulfed organisms in the tubular epithelium in experimental pyelonephritis (Tan and Heptinstall 1969; Shimamura 1981) but systematic study of this phenomenon and of potential direct bacterial injury to the tubules was not considered. In the present work we have found evidence that proximal tubular cells are able to internalize not only colloidal particles and proteins but also infecting agents and that this leads to considerable tubular damage.

To determine the exact localization of *E. coli* in the cortical tubules, we used semithin sections and transmission electron microscopy (TEM). Additional scanning electron microscopy (SEM) studies were also carried out. With the aid of acid phosphatase (AcPase) cytochemistry we ensured that bacteria really appeared in phagolysosomes. After glutaraldehyde fixation, the characteristic nucleoid in peroxisomes is lacking (Ericsson and Trump 1966), and accordingly it is sometimes difficult to distinguish peroxisomes and lysosomes in electronmicrographs (Beard and Novikoff 1969). We therefore applied both AcPase and catalase ultrahistochemistry.

Materials and methods

Male Wistar rats weighing 250–350 g and *Escherichia coli* (O1:K1:F11) were used as described previously (Iványi et al. 1983). The left ureter was ligated for 48 h; 1.5 h after the ligature was applied, the rats received intravenously 7.5×10^7 bacteria suspended in 1 ml physiologic saline per 100 g body weight. A total of 13 animals showing abscesses in their left kidney were killed 48 h after bacterial infection.

Light-microscopy

Fragments of renal cortex ($5 \times 5 \times 1$ mm) from four animals were fixed by immersion in cold 4% neutral formalin for 6 h, and then embedded in paraffin. Sections (5 μm thick) were stained with hematoxylin-eosin, PAS and toluidine blue.

Ultrastructural procedures

In our experience it is very difficult to obtain adequate perfusion fixation in acute pyelonephritis. For this reason we chose two fixatives (A and B) for TEM.

A. The kidneys of three animals were fixed by retrograde perfusion through the abdominal aorta with Karnovsky's fixative (1965) for 10 min, followed by immersion of 1 mm^3 pieces of renal cortex in the same solution for 2 h at 4° C.

B. The kidneys of six animals were perfused in situ (as described in paragraph A.) for 10 min for TEM, SEM, AcPase and catalase ultrahistochemistry in Maunsbach's fixative (1966), followed by immersion fixation in the same solution for 2 h at 4° C. SEM and TEM embedding techniques were as described previously (Iványi et al. 1983). Semithin sections were

stained with methylene blue-basic fuchsin. Tubules devoid of inflammatory infiltration of their walls, containing bacteria in the lumen and in the cytoplasm, were selected. Light microscopically intact proximal tubules were compared ultrastructurally with proximal tubular segments showing phagocytic activity. Thin sections were stained with uranyl acetate and lead citrate. Other blocks of renal cortex were washed at 4° C in 0.1 M Na-cacodylate buffer (pH: 7.2–7.4) containing 7.5% sucrose for 2 h, and 50 μm-thick frozen sections were then cut on a freezing microtome. Incubation was performed at 37° C for AcPase, according to the method of Gömöri as modified by Barka and Anderson (1962) for 10–15 min. Slices were incubated for catalase in a 3,3′-diaminobenzidine medium (Novikoff et al. 1972) at 37° C for 60 min. Post-fixation for 1 h in 1% OsO_4 containing s-collidine was followed by washing with collidine buffer, dehydration in ethanol and embedding in Durcupan ACM (Fluka). Control reactions were made without substrate. Both stained (uranyl acetate and lead citrate) and unstained sections were prepared and examined with a TESLA BS 500 transmission electron-microscope operating at an accelerating voltage of 60 kV, and with a Philips 501/B scanning electron-microscope at 15 kV.

Results

Light-microscopic findings

By 48 h a completely developed acute suppurative pyelonephritis was detected with focal abscesses, heavy polymorphonuclear leukocytic and monocytic infiltration of the interstitium and tubular walls and patchy necrosis of the latter; many tubules contained cellular casts. Between abscesses a striking feature was the formation of bacterial colonies in the cortex (Fig. 1). Semithin sections revealed some tubules with tubular wall rupture around microabscesses and in their immediate vicinity many lumina of dilated proximal tubular segments were filled with hundreds of organisms, sometimes accompanied by a few inflammatory cells. In these areas bacteria were also found alone or in small groups within cytoplasmic vesicles of the apical, middle and basal regions of the tubular cells (Fig. 2). In advanced cases massive bacterial invasion accompanied the necrosis of the tubular epithelium, and the large numbers of E. coli colonies in the lumen were separated from the inflammed interstitium only by the intact but denuded basement membrane. Occasionally, bacilli were attached to the luminal surfaces of dilated distal or collecting tubules, without any signs of their incorporation within the epithelium.

Ultrastructural findings

Bacteria were loosely distributed in a fine amorphous, granular, slightly electron-dense, flocculent material filling the lumen of the proximal tubules. Where organisms were particularly numerous, this material was not always visible. Bacteria both in the tubular lumina and within tubular cells were surrounded by a clear "halo". Rarely, E. coli was found in large apical vesicles of distal tubular cells without any alteration of the cell structure (Fig. 3). Collecting ducts did not show endocytosis.

Accumulation of E. coli with no tubular damage (Fig. 4)

Microorganisms were situated at the base of the brush border, pushing the microvilli aside. Beneath them a generally smooth-surfaced, elongated

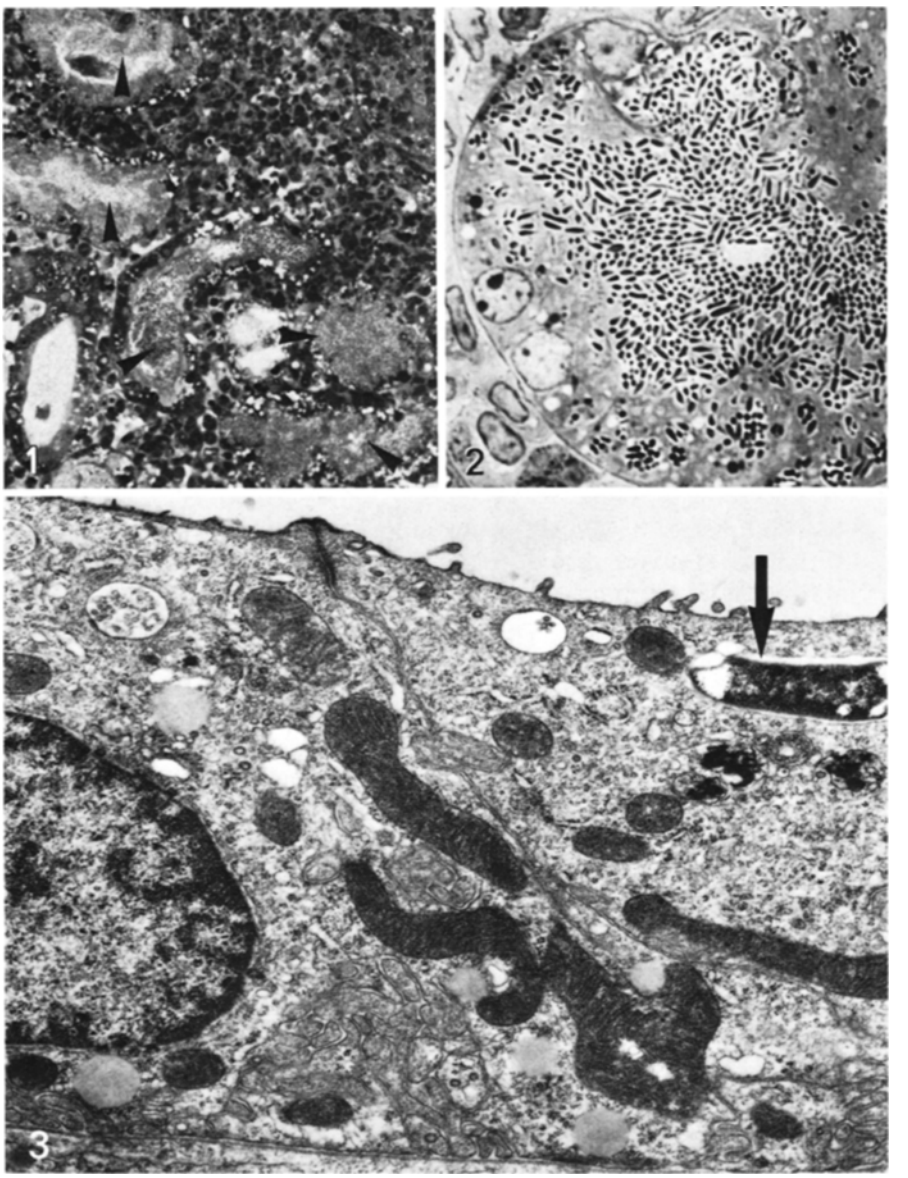


Fig. 1. 2-day-old acute pyelonephritis. The interstitium is broadened by acute inflammatory cells. Around an interstitial abscess the tubules contain fine granular material (*arrowheads*) which represent bacterial colonies (as shown at higher magnification in Fig. 2). Methylene blue-basic fuchsin, ×400

Fig. 2. The lumen of a tubule contains bacteria. They are also visible in small groups within tubular cells. Epithelial necrosis is not present. The peritubular interstitium is inflamed. Methylene blue-basic fuchsin, ×1,400

Fig. 3. Cells of a distal tubule. There is a bacterium within a large apical vesicle (*arrow*). ×15,000

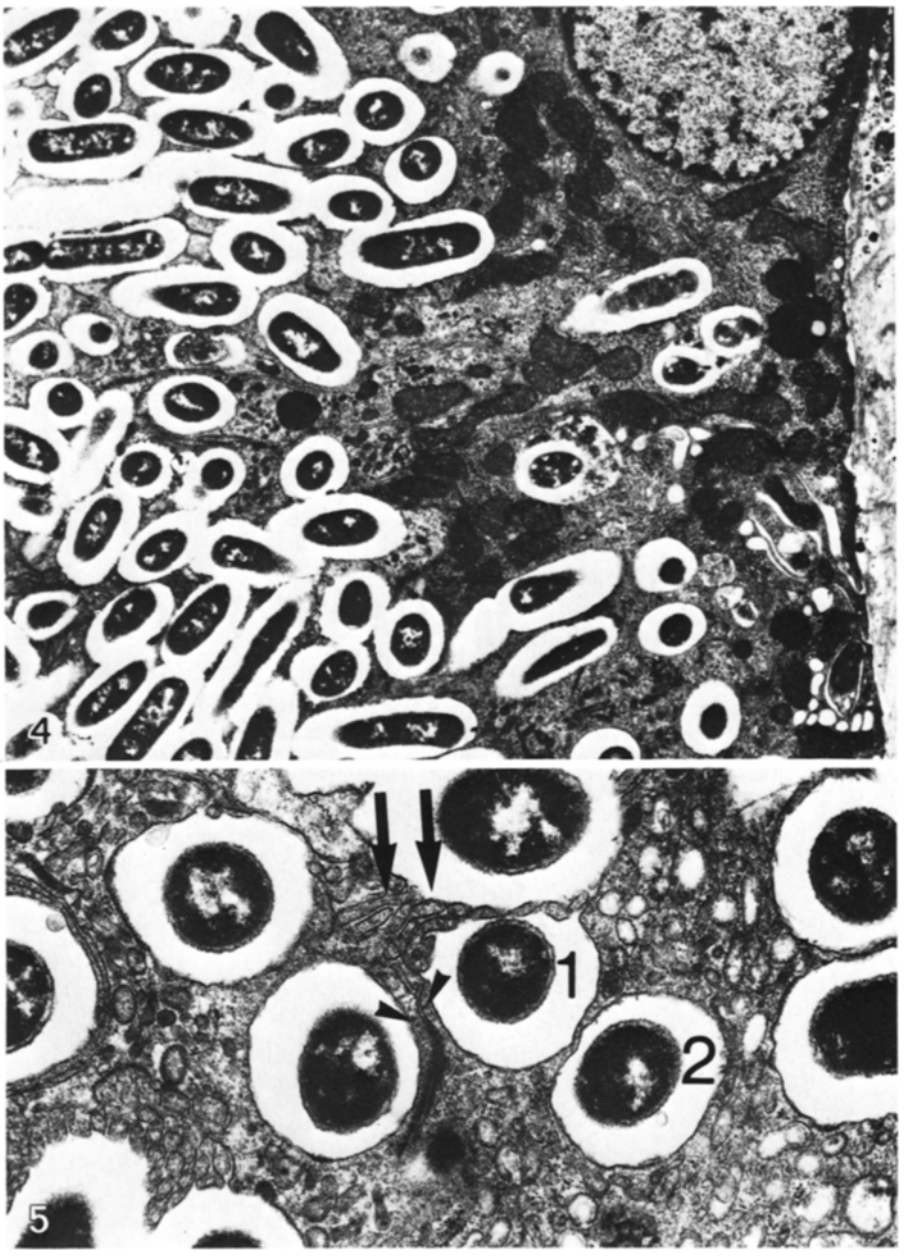


Fig. 4. Bacterial uptake without marked tubular damage. The luminal pole is at the left, and the basal pole at the right. Phagosomes with *E. coli* are distributed widely in the epithelial cytoplasm. Their number exceeds the number of lysosomes and phagolysosomes. × 10,500

Fig. 5. Steps of bacterial endocytosis. At number 1 the apical cell membrane forms a recess. Beneath it, at number 2, the bacterium is interiorized in a large apical vesicle. *Arrows* – microvilli, *arrowheads* – tight junction. × 24,000

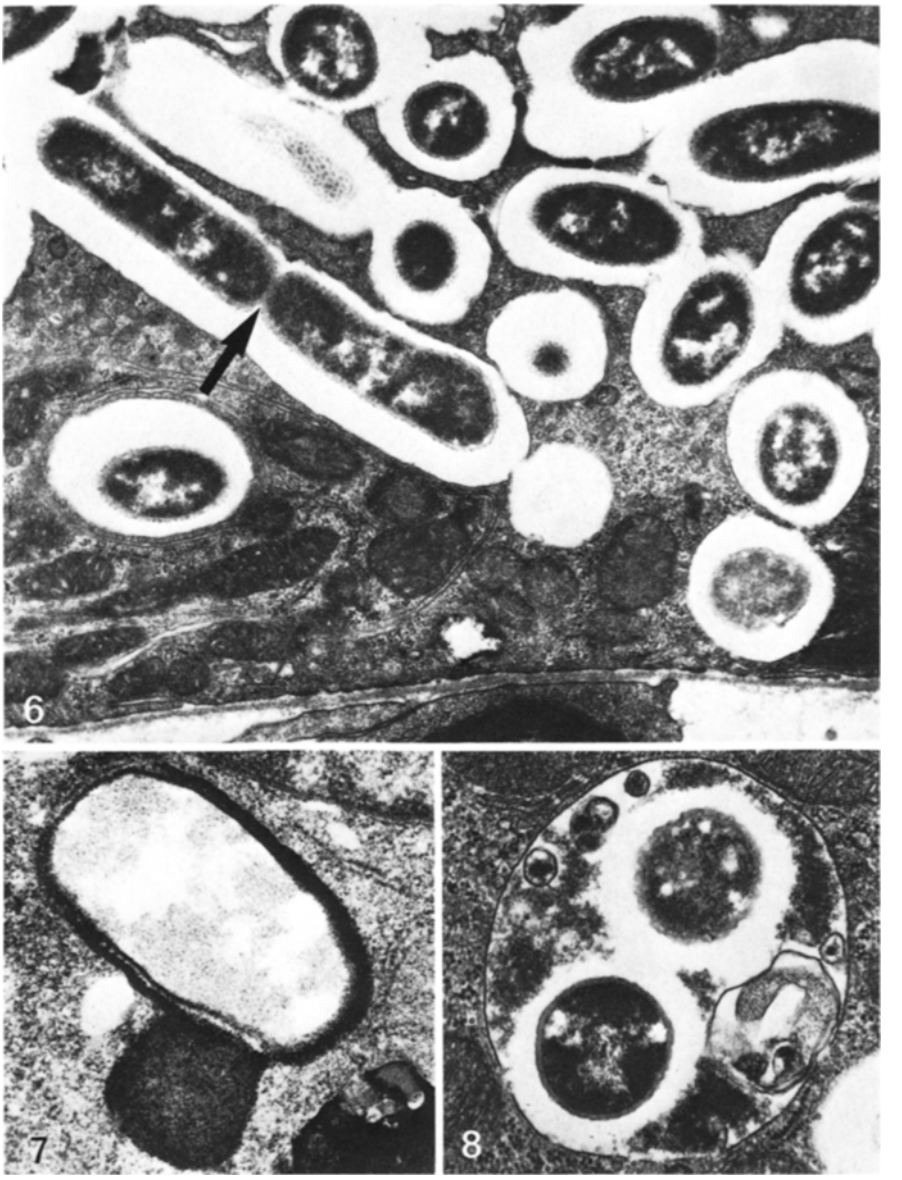
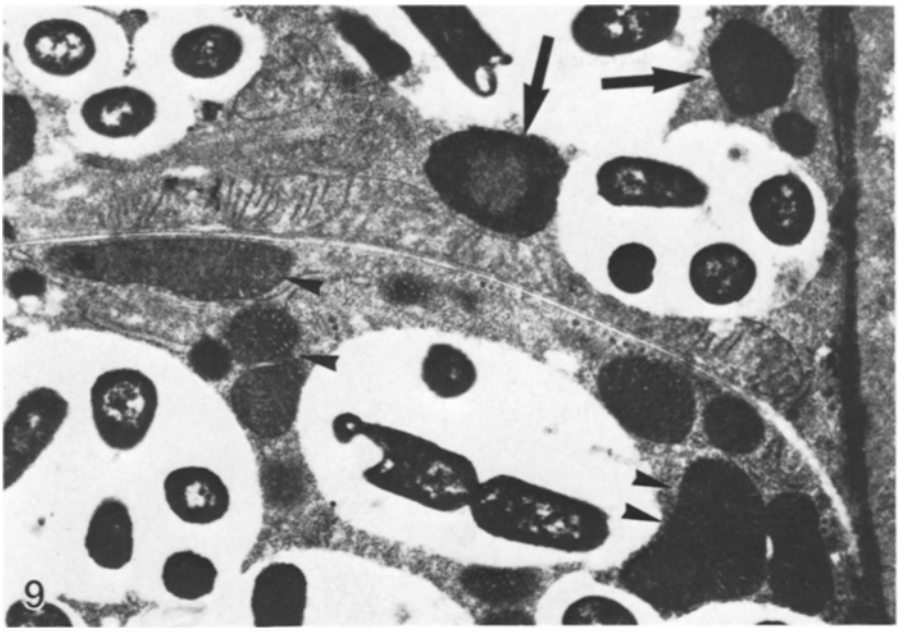


Fig. 6. Basal region of a proximal tubular cell. The merging phagosomes contain bacteria. The *arrow* points at just dividing microorganisms. × 17,500

Fig. 7. A lysosome fuses with a phagosome containing *E. coli*. The reaction product encircles the bacterium. Gömöri reaction for acid phosphatase, stained with uranyl acetate and lead citrate. × 26,500

Fig. 8. Bacteria within a lytic vacuole. × 26,400



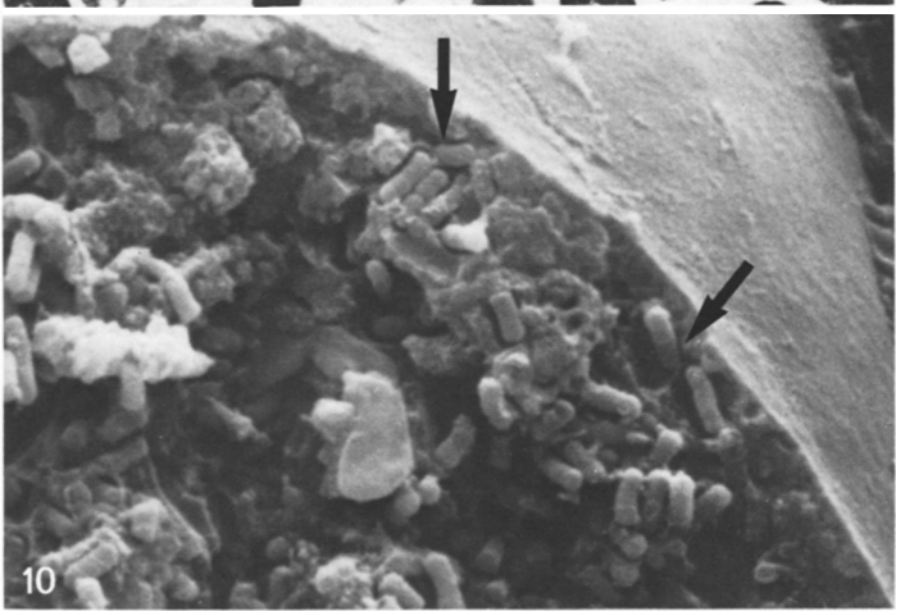


Fig. 9. Peroxisomes (*arrows*) around merging phagosomes. The mitochondria at the bottom cell have a dense matrix and moderately dilated intracristal spaces (*arrowheads*). The mitochondrium of the top cell seems to be unaltered. DAB reaction for catalase, stained with uranyl acetate and lead citrate. × 15,500

Fig. 10. Incorporated bacteria (*arrows*) in the tubular wall. There is no distinct border between the lumen and the epithelium. The outer surface of the tubular basement membrane can be seen. SEM, × 2,500

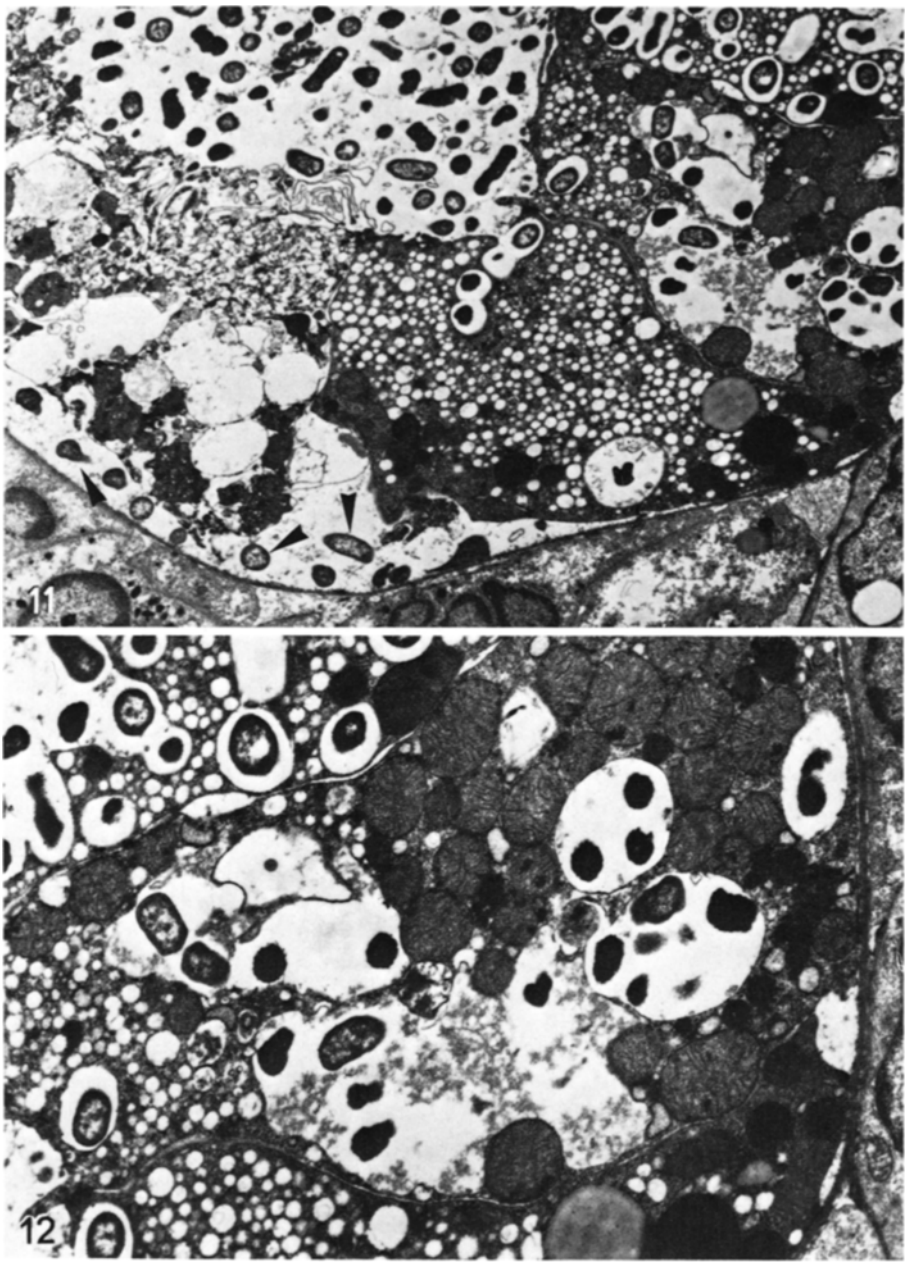


Fig. 11. Sublethal epithelial injury. The cytoplasm of the cells are extremely vacuolated. The cells are partially detached from the underlying unruptured tubular basement membrane, where some bacteria can be seen (*arrowheads*). × 5,500

Fig. 12. Right part of Fig. 11. Phagosomes with *E. coli*. Mitochondria have rounded shape, pale matrix and dense flocculent material within the inner compartment. × 10,200

recess in the apical cell membrane developed, sometimes showing small infoldings indicating the openings of small apical vesicles. Characteristic tubular invaginations were not encountered, but it seemed that these infoldings of the cell surface represented expanded tubular invaginations yielding enough room for bacteria to fit in (Fig. 5). In deeper regions most of the large apical vesicles were filled with isolated incorporated bacteria, while others had flocculent material similar to that seen in the luminal fluid. We were not able to observe the exact, serial endocytotic events of the apical cell membrane because of the very large numbers of bacteria attached to the cell surface. Cytoplasmic vesicles with engulfed organisms (phagosomes) also appeared in the middle and basal portions of the tubular cells (Fig. 4) and occasionally phagosomes were filled with dividing bacteria (Fig. 6). Rarely, fusion with cytoplasmic dense bodies containing AcPase were observed (Fig. 7); the large apical vesicles and phagosomes were free of lead phosphate reaction product. Cytoplasmic bodies showing AcPase activity (phagolysosomes), lytic vacuoles with well-preserved E. coli (Fig. 8), and amorphous granular substance or membrane-bound inclusions were found in moderate number around the nucleus and in the basal part of the cells. Bacterial replication was not encountered in these phagolysosomes. The number of phagosomes considerably exceeded the number of lysosomes and phagolysosomes (Fig. 4). Peroxisomes seemed to be intact and the lateral intercellular spaces had preserved outlines. Where marked bacterial uptake had taken place the endocytic apparatus had expanded and increased in amount. The mitochondria showed condensation of the inner compartments, with an increased density of the matrix and moderate expansion of the intracristal spaces (Fig. 9). Under the SEM, in some dilated proximal tubules around cortical abscesses, microorganisms were seen to be attached longitudinally to the surface of the microvilli, but we were not able to find a fractured surface where clear-cut bacterial uptake could be observed. The lumina of some other tubular segments were filled with bacteria and cellular debris. The epithelium having no distinct apical cellular boundaries also contained microorganisms. The underlying basement membranes were intact and the adjacent interstitium was sometimes free of inflammatory cells (Fig. 10).

Accumulation of E. coli with tubular damage (Fig. 11)

A moderate acute inflammatory infiltrate was situated on the interstitial side of the basement membrane of proximal tubules without infiltration of the tubular wall. A large number of *E. coli* patently filled the lumina of the proximal tubules which were dilated and thin walled. The microvilli of the epithelial cells were partially or totally lost, and the number of small apical vesicles was increased. The large apical vesicles containing bacteria had fused with each other and it was difficult to distinguish which organisms were in the lumen and which were within the apical vesicles. Merging phagosomes with replicating *E. coli* were distributed widely in the swollen, electron-lucent cytoplasm, often separated in large areas the basal cytoplasmic

region from the underlying basement membrane (Fig. 11). At severely injured sites the whole cytoplasm had become vacuolated; the nucleus had become pycnotic, and the mitochondria were disorganized, showing rounded profiles, pale matrix and dense flocculent material within the inner compartment (Fig. 12). The endoplasmic reticulum was dilated and the phagolysosomes, peroxisomes and Golgi profiles seemed to be reduced in number. Peroxisomes were still present even when the cell showed severe (sublethal) injury. They were in close vicinity to the phagosomes, but they did not protrude into them.

Discussion

In the pathogenesis of the parenchymal damage in hematogenous acute pyelonephritis following ureteric obstruction, on the basis of our previous data (Iványi et al. 1983), the polymorphonuclear leukocytic and monocytic infiltration of the peritubular interstitium seems to be the most important feature. The inflammatory process may cause circumscribed necrosis of the tubular wall in all nephron segments. This corresponds to the conclusions in other publications (Shimamura 1981; Bille and Glauser 1982). In our former study we concluded that the connection between the tubular epithelium and its basement membrane must be very strong, because inflammatory cells, after opening the basement membrane from the interstitial side, immediately move in between adjacent epithelial cells, but never between epithelial cells and their underlying basement membrane. We have now seen that the large numbers of phagosomes, sometimes even containing dividing bacteria, break this strong connection and detach the damaged tubular cells from their basement membrane over wide areas, where it remains denuded.

Epithelial ischemia is an important factor in bacterial tubular damage. In complete unilateral ureteric obstruction, the blood flow to the obstructed kidney decreases and by 24 h is about half the normal (Wilson 1980). The inflammatory broadening of the peritubular interstitium and stasis in the capillaries further decrease the blood supply to the tubules at the site of bacterial accumulation. These factors might explain the ultrastructural signs of ischemic injury (marked cytoplasmic swelling, mitochondrial alterations, loss of brush border, electronlucent cytoplasm, vacuolization). The large number of interiorized bacteria, accompanied by ischemic changes of the cell, destroy the normal cell architecture and disturb cellular functions so that irreversible cell damage develops. This lesion involves only proximal tubular segments which have significant endocytic capacity.

It is difficult to analyze the morphological events in bacterial tubular injury, since hydronephrotic changes and the biological properties of *E. coli* may also contribute to the development of parenchymal damage. To produce acute pyelonephritis with ureteric obstruction, highly virulent *E. coli* strains must be used (Brooks et al. 1974). The potential liberation of endotoxin from virulent bacteria may cause severe permeability changes on the cell surface or within the cell. In *E. coli*-infected kidneys of leukocyte-depleted rats, Shimamura (1981) observed ultrastructurally degenerative

changes in tubular cells containing bacteria, without any marked inflammatory infiltration of the peritubular intersitium. He did not detect epithelial necrosis, but the latest phase he investigated was only 40 h after bacterial infection. He also presumed the degenerative changes to be due to bacterial injury of the tubular cell ultrastructure.

One must also consider the altered function of the endocytic apparatus in the evolution of bacterial tubular injury. The increased periapical vacuolization of proximal tubules occurs both in ischemic (Venkatachalam et al. 1978) and in hydronephrotic conditions (Shimamura et al. 1966) but the pathophysiologic explanation for this cellular alteration is still lacking (Møller et al. 1984). Proximal tubular pressure after 24 h of complete unilateral obstruction is normal or moderately decreased in the rat (Wilson 1980). Thus, the effect of the intraluminal pressure probably does not play a role in the promotion of bacterial interiorization.

The experimental conditions were not sufficient to describe more exactly the fine structural details of bacterial endocytosis. However, the work showed evidence that incorporated bacteria, like reabsorbed proteins involve the lysosomal apparatus of the proximal tubular cells. Renal lysosomes are not able to generate hydrogen peroxide, which is the most important mechanism in killing bacteria in granulocytes and macrophages. The discharge of lysosomal contents into phagocytic vacuoles is without effect and proximal tubular epithelium is not able to degradate the great number of incorporated bacteria. Thus it falls victim to its own phagocytotic activity.

References

Bariéty J, Druet Ph, Laliberté F, Belair M-F, Paing M, Sapin C (1980) Ultrastructural evidence by immunohistochemical techniques for a tubular reabsorption of endogenous albumin and certain circulating proteins in normal rat. In: Maunsbach AB, Olsen TS, Christensen EI (eds) Ultrastructure of the kidney. Academic Press, London pp 303–314

Barka T, Anderson PJ (1962) Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J Histochem Cytochem 10:741–753

Beard ME, Novikoff AB (1969) Distribution of peroxisomes (microbodies) in the nephron of the rat. J Cell Biol 42:501–518

Bille J, Glauser MP (1982) Protection against chronic pyelonephritis in rats by suppression of acute suppuration: effect of colchicine and neutropenia. J Infect Dis 146:220–226

Bordeau JE, Carone FA, Ganotte ChE (1972) Serum albumin uptake in isolated perfused renal tubules. J Cell Biol 54:382–398

Brooks SJD, Lyons JM, Braude AI (1974) Immunization against retrograde pyelonephritis. I. Production of an experimental model of severe ascending Escherichia coli pyelonephritis without bacteriemia in rats. Am J Pathol 74:345–358

Christensen EI (1976) Rapid protein uptake and digestion in proximal tubule lysosomes. Kidney Int 10:301–310

Ericsson JLE (1965) Transport and digestion of hemoglobin in the proximal tubule. II. Electron microscopy. Lab Invest 14:16–39

Ericsson JLE, Trump BF (1966) Electron microscopic study of the epithelium of the proximal tubule of the rat kidney. III. Microbodies, multivesicular bodies, and the Golgi apparatus. Lab Invest 15:1610–1633

Graham RC, Karnovsky MJ (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302

Iványi B, Ormos J, Lantos J (1983) Tubulointerstitial inflammation, cast formation, and renal parenchymal damage in experimental pyelonephritis. Am J Pathol 113:300–308

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A

Maunsbach AB (1966) The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. J Ultrastruct Res 15:242–282

Miller F, Palade GE (1964) Lytic activities in renal protein absorption droplets. J Cell Biol 23:519–552

Møller JChr, Skriver E, Olsen S, Maunsbach AB (1984) Ultrastructural analysis of human proximal tubules and cortical interstitium in chronic renal disease (Hydronephrosis). Virchows Arch [Pathol Anat] 402:209–237

Novikoff AB, Novikoff PM, Davis C, Quintana N (1972) Studies on microperoxisomes II. A cytochemical method for light and electron microscopy. J Histochem Cytochem 20:1006–1023

Shimamura T, Kissane JM, Györkey F (1966) Experimental hydronephrosis. Nephron dissection and electron microscopy of the kidney following obstruction of the ureter and in recovery from obstruction. Lab Invest 15:629–640

Shimamura T (1981) Mechanisms of renal tissue destruction in an experimental acute pyelonephritis. Exp Mol Pathol 34:34–42

Straus W (1964) Cytochemical observations on the relationship between lysosomes and phagosomes in kidney and liver by combined staining for acid phosphatase and intravenously injected horseradish peroxidase. J Cell Biol 20:497–507

Tan HK, Heptinstall RH (1969) Experimental pyelonephritis. A light and electron microscopic study of the periodic acid-Schiff positive interstitial cell. Lab Invest 20:62–69

Thoenes W, Langer KH (1969) Die Endocytose-Phase der Eiweißresorption im proximalen Nierentubulus. Virchows Arch [Cell Pathol] B 2:361–379

Tisher CC, Bulger RE, Trump BF (1966) Human renal ultrastructure. I. Proximal tubule of healthy individuals. Lab Invest 15:1357–1394

Trump BF (1961) An electron microscope study of the uptake, transport, and storage of colloidal materials by the cells of the vertebrate nephron. J. Ultrastruct Res 5:291–310

Venkatachalam MA, Bernard DB, Donohoe JF, Levinsky NG (1978) Ischemic damage and repair in the rat proximal tubule: Differences among the S_1, S_2 and S_3 segments. Kidney Int 14:31–49

Wilson DR (1980) Pathophysiology of obstructive nephropathy. Kidney Int 18:281–292

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