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Acute human pyelonephritis: leukocytic infiltration of tubules and localization of bacteria

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Summary. The fine structural details of how leukocytes appear in the lumen of tubules and the localization of bacteria in the tubulo-interstitial space were studied by light and electronmicroscopy in renal cortical biopsy specimens from three patients with acute pyelonephritis. The cells of interstitial infiltrates infiltrated and sometimes disrupted the cortical collecting tubules preferentially, while inflammatory infiltration of the proximal and distal convoluted tubules occurred more rarely. Since the emigration of tubular wall-localized individual leukocytes into the lumen was not observed even in long series of thin sections, focal inflammatory disruption of the uriniferous ducts was considered to be the morphological basis of the intratubular accumulation of leukocytes. The structural simplicity of the collecting tubular cells is suggested to be the reason for their preferential involvement in the drainage of the interstitial suppuration, although a role for specific carbohydrate receptors cannot be excluded. The bacteria were usually found within the neutrophilic granulocytes and macrophages of the interstitial infiltrates, and within and among the cells of leukocyte casts. Additionally, pure bacterial colonies were noticed in the lumen of a few collecting tubules. The problem of the adherence of the bacteria to the surface of the tubular cells is discussed.

Key words: Acute pyelonephritis – Tubule infiltration – Cortical collecting duct rupture – Bacterial localization

Introduction

The histological hallmarks of acute bacterial pyelonephritis in humans (patchy suppurative interstitial inflammation, tubular necrosis, leukocyte casts) are well known in practice (see Gloor 1966; Heptinstall 1983). To our knowledge the process by which neutrophils and macrophages appear in the lumen of tubules, leading to the formation of leukocyte casts, or to the localization of bacteria in the tubulo-interstitial space, has not been investigated in human material. For this reason, renal biopsy specimens from three adult patients with acute oliguric renal failure secondary to acute pyelonephritis were studied by light- and electronmicroscopy. This report describes the fine structural details of how interstitially localized inflammatory cells infiltrate the tubules. The findings show that the proximal, distal and collecting tubules are not uniformly involved in the transtubular passage of leukocytes in the kidney cortex. With regard to the localization of bacteria, observations on bacterial adherence to the tubular epithelium seem noteworthy, since practically nothing is known about this topic in human kidney tissue (Harber and Asscher 1985).

Materials and methods

The cases have been described in detail in a previous paper dealing with peritubular capillary damage in acute human bacterial pyelonephritis (Iványi and Thoenes 1987). Biopsy specimens were prepared for light microscopy by fixation in 4% formaldehyde or Dubosq-Brazil solution and subsequently embedded in paraplast. Slides were stained with haematoxylin and eosin, PAS, Pearse-trichrome and Jones-Chromotrop 2R. For electron microscopy, specimens with or without previous formaldehyde fixation were fixed in 3% glutaraldehyde and 1% osmium tetroxide and embedded in Epon. Semithin sections were stained with Azur II-methylene blue (A-M). Uranyl acetate and lead citrate contrasted thin sections were examined with an EM 301 (Philips) electronmicroscope. The localization of intraluminal leukocytes within the different parts of the nephron was determined on two PAS-stained paraplast sections from each case. The sections were taken from various depths of the embedded kidney tissue, with a minimum topographic distance of 55–60 μm between them. The total numbers of proximal tubules (tall, columnar or cuboidal cells with nuclei located

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near the base and a PAS-positive brush border) and of "distal tubules" of the nephron (distal convoluted tubules and cortical collecting tubules: lower, cubical or flattened cells without a brush border) with and without leukocytes in the lumen were recorded. Since some features of interstitial inflammatory infiltrates have already been summarized (Iványi and Thoenes 1987), we will only describe inflammatory changes confined to the space immediately ajacent to the tubules.

Results

Light microscopic findings

The biopsies were taken from the renal cortex. There was a patchy, but massive inflammatory infiltration of the widened interstitium (Figs. 1–4), sometimes forming discrete abscesses. The infiltrates were composed mainly of neutrophilic granulocytes and macrophages, with smaller numbers of plasma cells and lymphocytes, and a few eosinophils. Many neutrophils and macrophages had incorporated bacteria in their cytoplasm. Rarely, small colonies of bacteria were found between the inflammatory cells (Fig. 4). At sites of less severely affected areas of inflammation and even around discrete abscesses, the leukocytes either did not appear in the wall or in the lumen of the proximal tubules, or were present very occasionally (Figs. 1 and 2). In contrast, they infiltrated the distal tubular portions of the nephron preferentially. The lumina of these tubule segments very often contained leukocytes, usually forming cellular casts (Figs. 2–4). The localization of intratubular leukocytes is shown in Table 1.

The cellular casts consisted of aggregated polymorphonuclear leukocytes and macrophages (mostly containing a great number of bacteria) as well as necrotic inflammatory cells, debris and, rarely, a few erythrocytes. Among the cellular debris, several bacteria were seen lying free in the urinary fluid. Most of the "distal tubules" (definition see above) were filled by these casts like a plug, resulting in dilatation of tubules and reduction in the thickness of their walls (Fig. 4). At times, the inflammatory cell-infiltrated distal segments of the nephrons exhibited a circumscribed (approx. 5–12 μm) wall rupture and the interstitial inflammation had burst into the lumen (Fig. 3). No rupture was found in the proximal tubules.

A few "distal tubules" contained masses of bacteria in their lumen only. Accumulation of neutrophils in the adjacent interstitial space and in the tubular wall was not seen. The cells of the proximal tubules appeared normal or displayed apical blebs, fine cytoplasmic vacuolization or hyalin droplets, respectively. At sites of confluent abscesses, the tubules are extensively destroyed.

Table 1. Numbers of tubules with and without leukocytes in their lumina recorded in paraplast sections of the biopsies

	lumen without leukocytes	lumen with leukocytes
proximal tubules	1734	24*
distal portions of nephrons ("distal tubules")	678	574
undefined tubules	112	132

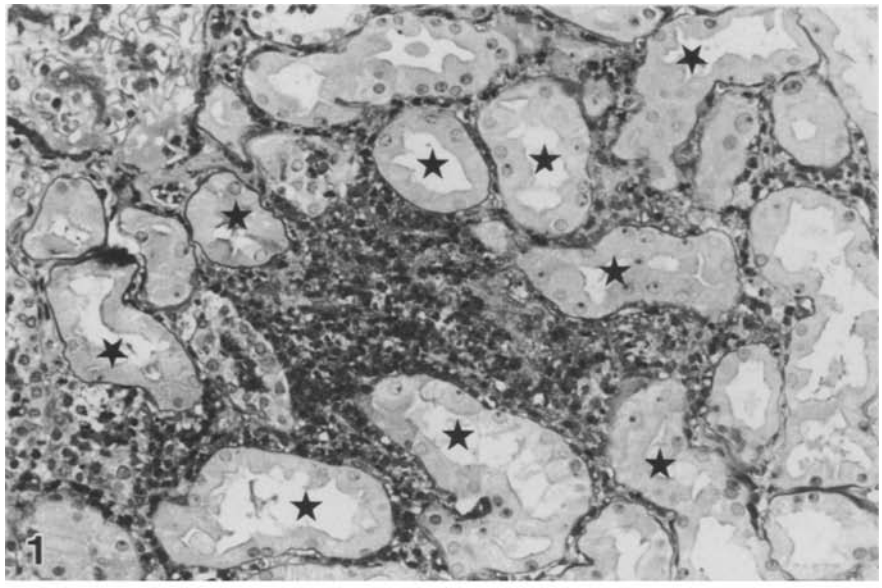
* Maximum 3 leukocytes in lumen

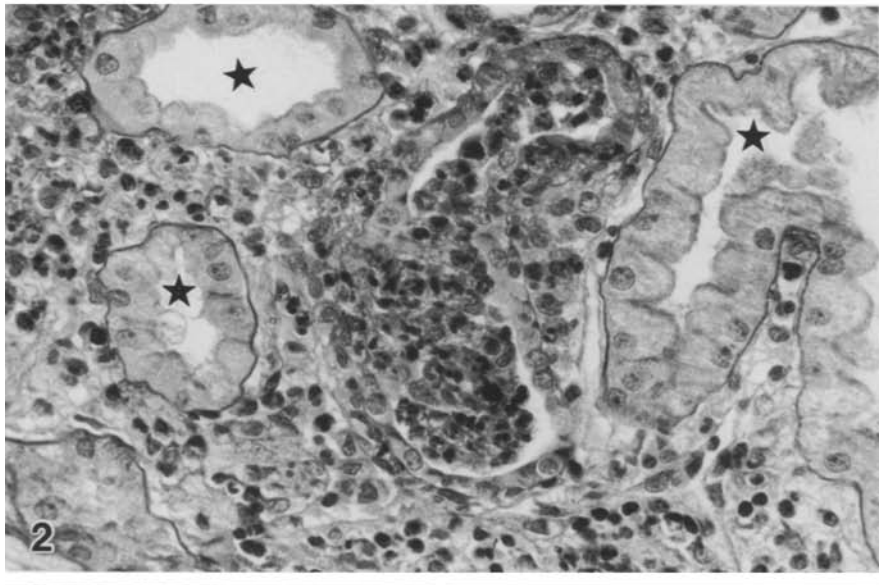
Electronmicroscopic findings

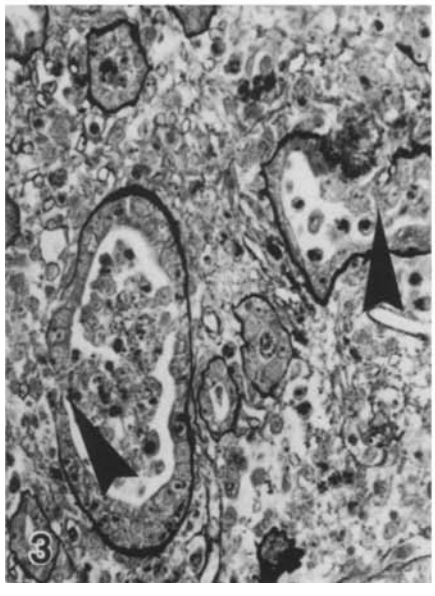
Inflammatory activated neutrophil granulocytes (for details see Iványi and Thoenes 1987), macrophages and a small number of lymphocytes had accumulated in the peritubular interstitium. The inflammatory cells were often found in close contact to the tubular basement membrane (Figs. 8 and 10). Many granulocytes were degranulated and incorporated bacteria into lytic vacuoles. The phagolysosomes of the macrophages were densely filled with cellular debris, dense bodies resembling remnants of erythrocytes, crumpled membranes, and bacteria. The majority of the microorganisms were usually found within granulocytes. Occasionally, bacteria were lying free in the interstitium between the inflammatory cells, forming small colonies (compare Fig. 4 in light microscopy).

The sequential steps of infiltration by individual inflammatory cells and rupture of the tubular wall were observed only in the cortical collecting tubules. In some places individual inflammatory cells had opened the collecting tubular basement membrane by a cytoplasmic process (Fig. 5). In more advanced stages they were found in a position lying between the epithelial cells or still in the interstitium (Fig. 6). The emigrated cells, which lay in the basal region of the tubular wall (Fig. 8), showed close cell-to-cell contacts with the tubular cells and with other emigrated leukocytes. Very often, a narrow layer of the epithelial cytoplasm was situated between the tubular basement membrane and the tubular wall-localized inflammatory cells (Fig. 8). We did not observe an individual leukocyte opening a tight junction by penetration into tubular lumen.

In areas of massive inflammatory infiltration collecting tubules showed circumscribed wall rupture (Fig. 7), including breaks of the regional basement membrane. The sites of rupture were filled by neutrophilic granulocytes and mononuclear







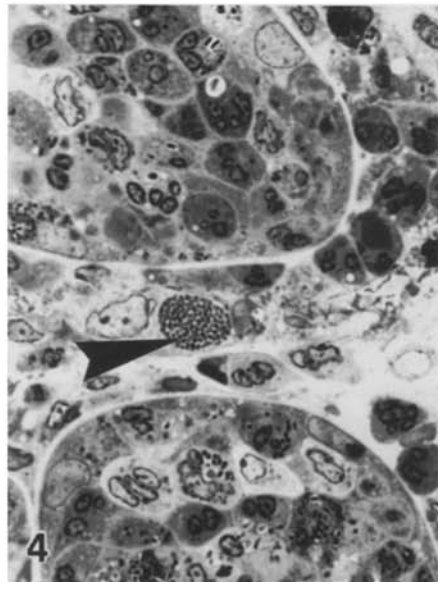


Fig. 1. The interstitial inflammation (*in the centre*) does not involve the surrounding proximal tubules (*stars*). PAS, 700×

Fig. 2. The inflammatory cells do not infiltrate the proximal tubules (*stars*). Instead, they accumulate around and in a "distal" tubule (*in the centre*). Pearse, 1120×

Fig. 3. Through a rupture (*arrowhead*), the interstitial inflammation bursts into the lumen of "distal" tubules. Jones, 640×

Fig. 4. Bacterial colony (*arrowhead*) in the interstitium between two tubules tightly filled by leukocyte casts. A-M, 1400×

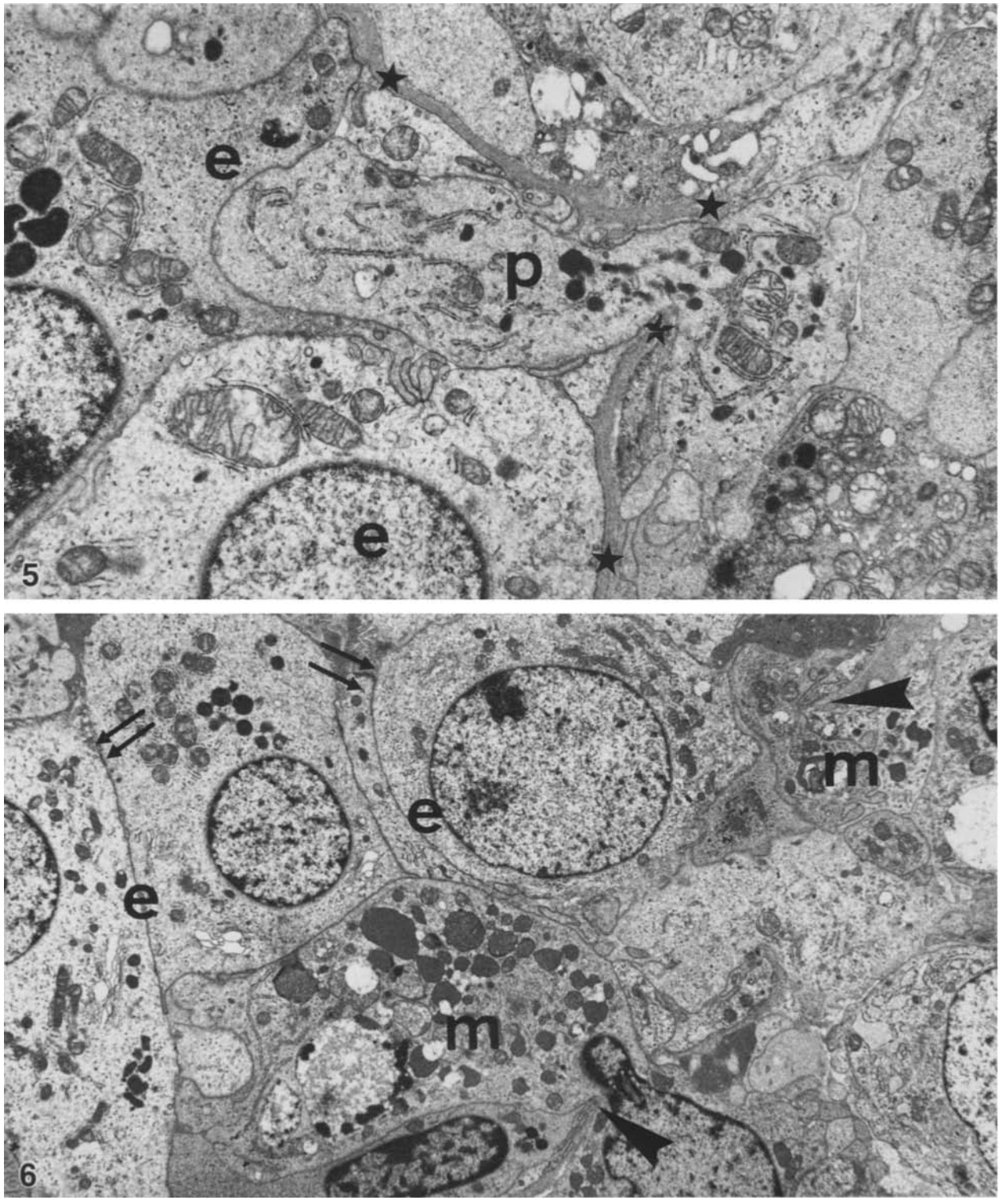


Fig. 5. The basal region of a collecting tubule. Between two epithelial cells (*e*), the basement membrane (*stars*) is opened by an infiltrating pseudopodium (*p*) of an inflammatory cell. 9000 ×

Fig. 6. Macrophages (m) massively emigrate from the interstitum (*right, below*) into the collecting tubular wall (*left, above*) through the widely opened (*arrowheads*) basement membrane. The epithelial cells (*e*) are connected by junctional complexes (*arrows*). 5000 ×

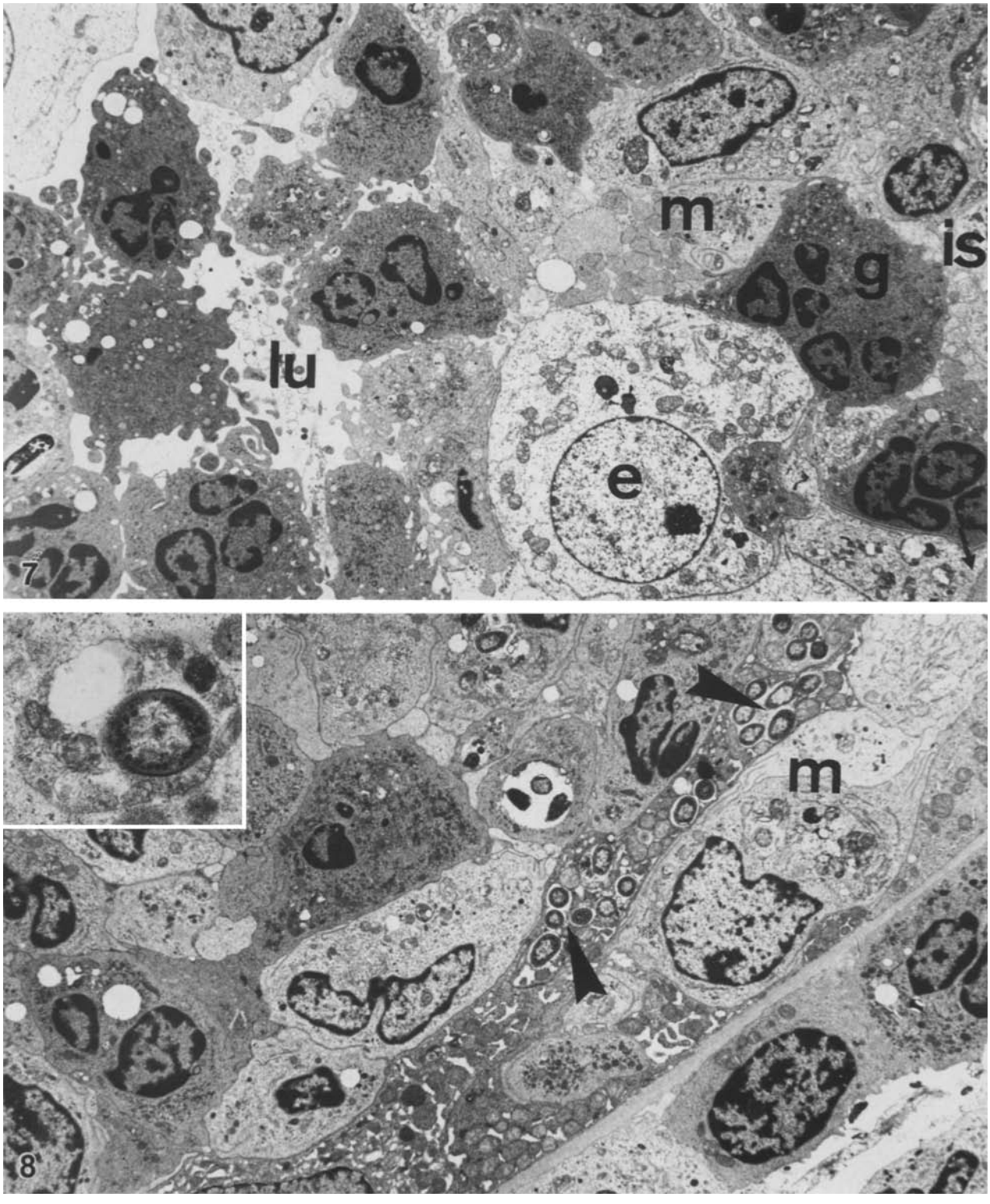
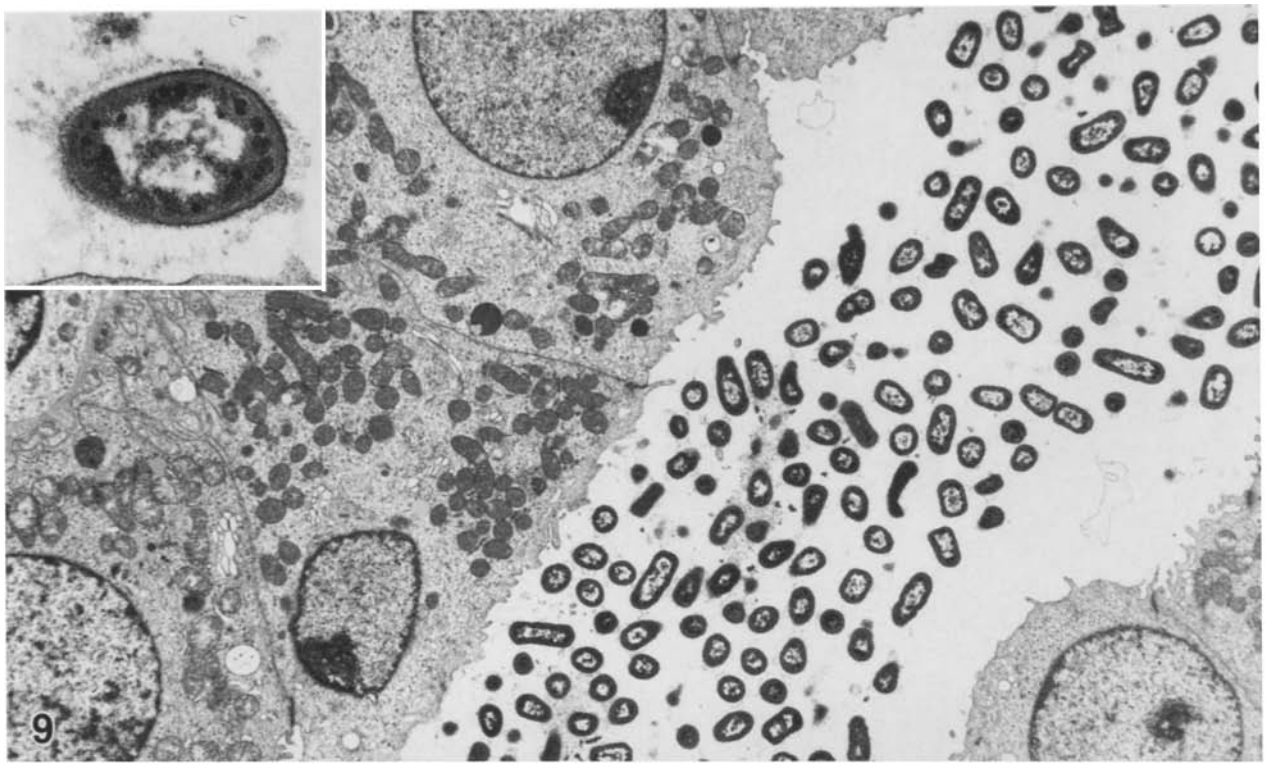


Fig. 7. Rupture of the collecting tubule showing epithelial cell (*e*) hydrops. In the upper part of the micrograph, there is a "bridge" of aggregated neutrophils (*g*) and macrophages (*m*) between the tubular lumen (*lu*) and the interstitium (on the *right side*). Tubular basement membrane (*arrow*). 3800 ×

Fi. 8. A cellular cast fills the lumen of a collecting tubule. Macrophages (*m*) have infiltrated the tubular wall. The epithelial cells contain numerous bacteria (*arrowhead*). 3800 ×. *Inset:* A bacterium in the phagolysosome of a collecting tubular cell. 18200 ×



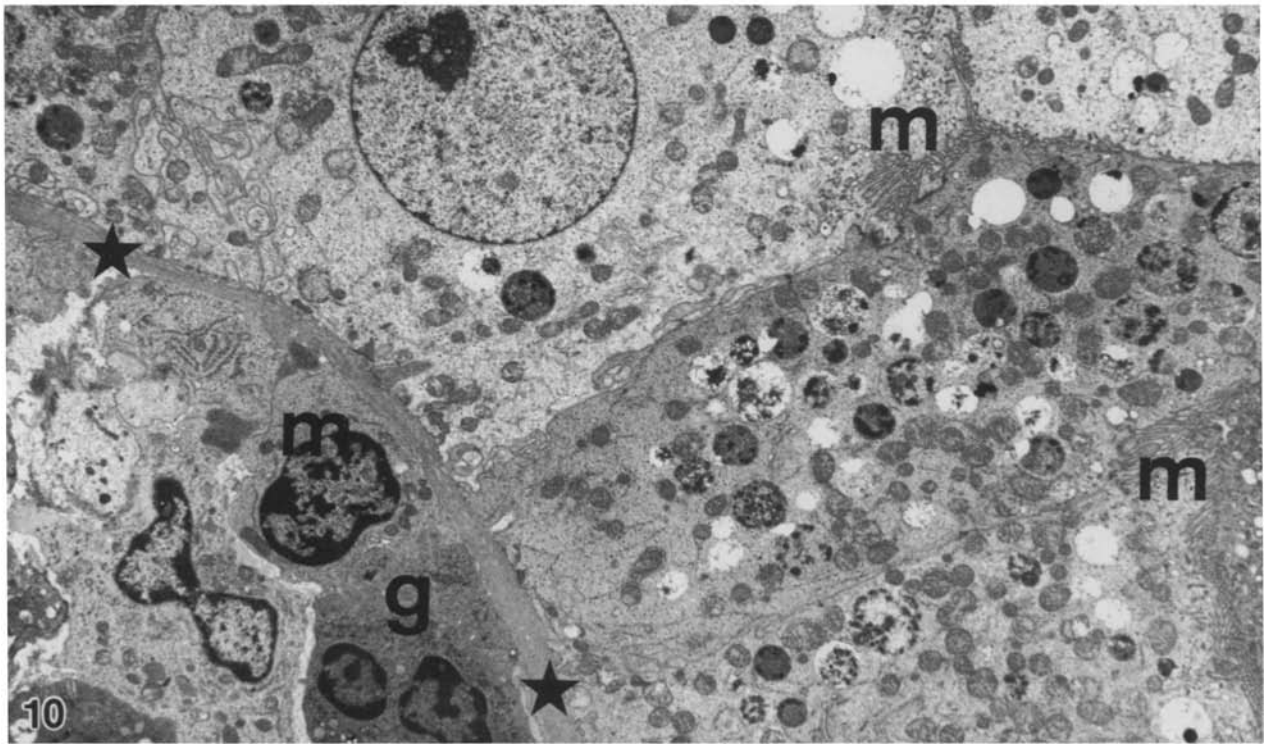


Fig. 9. Bacterial colony in the lumen of a collecting tubule with a low tendency to epithelial adherence. 5000×. *Inset:* Rarely pilous structures of the bacterial wall mediated attachment. 30000×

Fig. 10. A granulocyte (*g*) and a mononuclear cell (*m*) adhere closely to the basement membrane of a proximal tubule containing several vacuoles filled with electron dense material. *m*=microvilli, 3800×

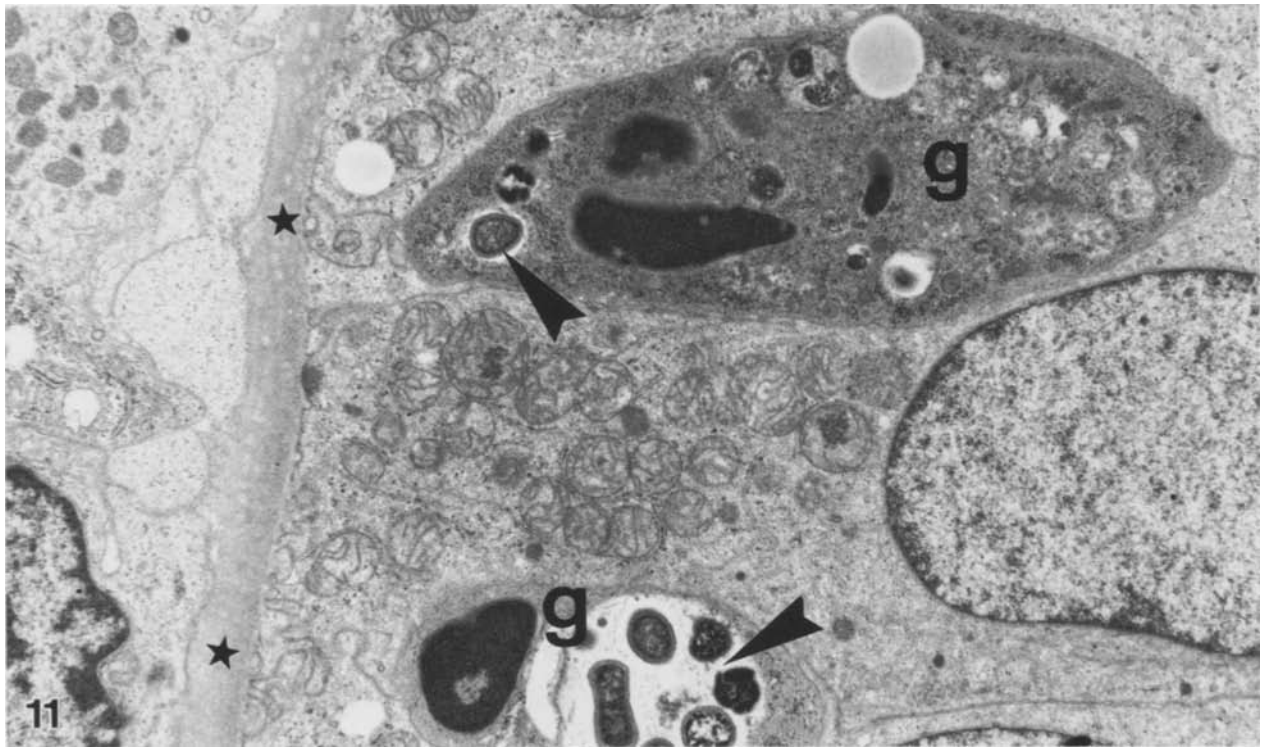


Fig. 11. Neutrophilic granulocytes (*g*) with phagocytosed bacteria (*arrowhead*) at the basal region of the distal convoluted tubule wall. The basallateral interdigitations are simplified; the basement membrane is continuous (*star*). 9000 ×

cells. At the margins of the rupture, the tubular cells exhibited hydropic changes in the cytoplasmic matrix and swelling of the mitochondria indicating severe damage (Fig. 7).

When a collecting tubule was tightly filled by cast of inflammatory cells (Fig. 8), the apical surface of the ductal cells was flattened and the basal infoldings were reduced. Both the principal and the intercalated cells had several bacteria enveloped by a vacuolar membrane in their cytoplasm (Fig. 8). Not infrequently, the microorganisms were clearly localized in phagolysosomes (Fig. 8, inset). In contrast, when the lumina of collecting tubules were only filled with bacteria, the microorganisms were not incorporated into the epithelial cells (Fig. 9), but were loosely distributed in the centre of the tubular lumen, sometimes adhering to each other and only occasionally to the surface of the epithelial cells by the fine pilous structures of the bacterial wall (Fig. 9, inset).

Although leukocytes also had contact with the basement membrane of proximal (Fig. 10) and distal convoluted tubules, inflammatory cells were rarely found in the wall of these segments. If infiltration had occurred, the cells localized in the basal region of the tubular wall (Fig. 11) as described for the collecting tubules. Massive inflammatory cell infiltration of the tubular wall or the migration of the tubular-wall localized individual leukocytes to the lumen were not found.

In most of the tubular segments, the cells exhibited a slight-to-moderate simplication of the cell shape. In particular, the extent of the basal-lateral interdigitations was reduced. The proximal tubular cells showed alterations in focal distribution, varying from one proximal tubule to the next and from one cell to another. Apical blebs, decreases in the height and number of microvilli, focal loss of the brush border, slight swelling of mitochondria and a relatively high number of cytoplasmic vacuoles were common findings. The vacuoles were either electron-lucent or were filled by electron-dense, proteinaceous material (Fig. 10). In any segments of the cortical tubules some cells displayed cellular swelling, while the neighbouring ones often exhibited cytoplasmic and nuclear shrinkage.

Discussion

From in vitro studies (Cramer et al. 1980; Cramer et al. 1986), it appears that leukocytes can traverse

the tubular epithelium from both interstitial and luminal directions. On the basis of our investigations on human material, it is likely that the majority of the intratubularly accumulated leukocytes have emigrated from the interstitium to the lumen.

In all parts of the nephron, but especially in the collecting tubules a common finding was leukocytes in the tubular wall with a continuous underlying basement membrane. However, it was not easy to demonstrate the sequential steps of tubular wall infiltration, indicating that the process was rapid and that after leukocytes had passed through the basement membrane, the epithelial cells immediately restored the discontinuity of the membrane. When leukocytes were found localized in the basal region of the tubular wall, they were usually separated from the basement membrane by a thin epithelial layer. The emigrated cells exhibited a close cell-to-cell contact with the tubular cells. These features appear to be important in the maintenance of the tubular wall integrity during emigration. In vitro, the close contacts prevented leakage of tracers into the tubular wall across penetration sites (Milks et al. 1983). In this study on human pyelonephritis and in that on the inflammatory cell-infiltrated tubules in experimental pyelonephritis of rats (Iványi et al. 1983), the emigration of the individual leukocytes into the tubular lumen through the junctional complexes was not found even in long series of thin sections. Since very rarely 1–3 leukocytes were in fact observed light microscopically within proximal tubules (see Table 1), we suppose that the migration from the epithelial wall into the lumen is quicker than the penetration process through the tubular basement membrane.

Resistance of proximal tubules to inflammatory infiltration, frequent disruptions of collecting tubules, and elective localization of leukocytes within the "distal tubules" are not emphasized in acute pyelonephritis (Zollinger and Mihatsch 1978; Heptinstall 1983; Bohle et al. 1984; Churg et al. 1985). Only Ooi et al. (1975) mention the prevalence of damage in distal tubules in cases of acute lymphocytic interstitial nephritis. In renal allografts undergoing rejection, Cohen et al. (1984) noticed that the "distal tubules" were the sites of inflammatory infiltration and disruption, but the distal convoluted and the collecting tubules were not differentiated in that study. Electronmicroscopically, in our material only the collecting tubules and particularly the cortical ones displayed disruption, whereas the proximal and distal convoluted tubules showed a low tendency to inflammatory infiltration, and no rupture was found in their tubular wall. Reexamination of the electronmicrographs of the inflammatory injury of the cortical tubules in experimental pyelonephritis (Iványi et al. 1983) revealed that all the tubular wall ruptures observed were confined exclusively to the collecting tubules. To explain this finding in acute pyelonephritis, it may be recalled that the basal-lateral interdigitations and the number of desmosomes formed by the neighbouring tubular cells are scanty in the collecting tubules compared with the proximal and distal convoluted tubules (Thoenes and Langer 1969; Kriz and Kaissling 1985; Tisher and Madsen 1986). This structural simplicity of the collecting tubules might be one reason for the preferential inflammatory infiltration and subsequent rupture. Another reason could be that only collecting duct epithelium (unlike epithelium in other parts of the nephron) possess carbohydrate receptors on their surface which interact with certain factors produced by bacteria. Analogous binding reactions are known for other inflammatory mediators such as interleukines (Hession et al. 1987).

The distribution of bacteria in the cortical peritubular capillaries was considered earlier (Iványi and Thoenes 1987). In this study, microorganisms were usually found within neutrophilic granulocytes and macrophages of the interstitial exsudates, as well as within and between the cells of leukocyte casts. Additionally, pure bacterial colonies were noticed in the lumina of a few collecting tubules. Thus it could not be determined from the localization patterns of the microorganisms whether they had originally infected the kidneys *via* the ascending or the haematogenous route.

The endocytosis and phagolysosomal incorporation of bacteria by the tubular epithelium is a well known finding in experimental pyelonephritis (Shimamura 1981; Fussell and Roberts 1984; Iványi et al. 1985). In our material, the microorganisms were often found to be incorporated by the cells of the collecting tubules containing a leukocyte cast. In contrast, they were not encountered in those tubules having only pure bacterial colonies in the lumen. That means that some influence from the inflammatory cells seems to be a prerequisite for incorporation of bacteria into epithelial cells. Indeed, the process of incorporation itself was never observed. However, the intracellularly localized bacteria were very often found within lysosomal vacuoles (Fig. 8 inset) thus indicating endocytosis by the cell from the extracellular space, or intussusception of the bacteria into preexistent lysosomal vacuoles from the cytoplasmic matrix after penetration through the damaged cell membrane.

In pure bacterial colonies in the lumen of collecting tubules (Fig. 9) the bacteria seemed to be situated in the centre of the lumen rather than adhering to the epithelial cells. This observation may be due to the routine fixation procedure by which the carbohydrates of the bacterial capsules are not well preserved. Only in few bacteria were remnants of these substances seen, and in connection with the surface of the tubule epithelia (Fig. 9 inset). Thus it may possible that the bacterial colonies observed in the tubular lumina are attached by the capsular substances (not seen in the electron micrograph) to each other and to the epithelium. The adherence to the latter may be mediated by the Tamm-Horsfall protein or uromucoid (Øskov et al. 1980).

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