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Tubular dilatation in the repair process of ischaemic tubular necrosis

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Abstract To elucidate the mechanisms of renal tubular dilatation in acute tubular necrosis (ATN), morphological findings after 60 min ischaemia were studied in rats. The characteristics of the tubular basement membrane (BM) were also examined. A morphometric analysis of cell proliferation, epithelial cellularity and the circumference of damaged tubules was performed. The ischaemic injury resulted in widespread necrosis of renal tubules at day 1, and the BM of damaged tubules appeared thin. The intensity of the immunohistochemical staining for BM components decreased. The epithelial cell proliferation was particularly active in the early phase. Dilatation of the damaged tubules began at day 2, and the degree of dilatation increased up to day 6. Regenerative epithelial hyperplasia occurred and abnormalities of tubular BM were seen. Epithelial hyperplasia and dilatation of damaged tubules was most prominent at day 6 and the tubular BM was thickened by newly produced BM components. Tubular obstruction was not seen and tubules returned to normal size by day 28. Epithelial hyperplasia and abnormalities of tubular BM disappeared progressively. Regenerative tubular epithelial hyperplasia and abnormalities of tubular BM may play an important role in pathogenesis of tubular dilatation in ATN, and tubular dilatation is not due to tubular obstruction.

Key words Acute tubular necrosis · Tubular dilatation
Basement membrane · Epithelial hyperplasia
Repair process

Introduction

It is well known that tubular dilatation is observed during acute tubular necrosis (ATN) [30, 34] but this tubular dilatation has not been investigated. According to some reports, dilatation has been ascribed to tubular obstruction [12, 27, 29]. The pathogenesis of dilatation of tubules has been studied in polycystic kidney disease [2, 16, 35] and in many experimental renal cystic models where the following three factors for tubular dilatation have been noted: tubular epithelial cell hyperplasia, abnormalities of the tubular basement membrane (BM) and accelerated fluid accumulation within cystic cavities [2, 16, 35]. Tubular obstruction by hyperplastic epithelial cells protruding into the tubular lumen is also considered to be one of the causes of tubular dilatation [4, 11, 13]. However, it was recently reported that tubular obstruction is not a condition necessary for tubular dilatation [3, 6, 33].

In this study, morphological and physiological findings were analysed in order to clarify the mechanism of tubular dilatation in ATN, using a well-established rat model of ATN induced by 60 min of ischaemia.

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Materials and methods

Male Wistar rats (Saitama Experimental Animal Supply, Saitama, Japan) weighing 180–200 g were used during the study. They were anaesthetized intraperitoneally with sodium pentobarbital (30 mg/kg) at surgical operation or sacrifice.

Under anaesthesia both renal arteries were completely occluded using Sugita aneurysm clips (Mizuho Ikakogyo, Tokyo, Japan) for 60 min. The clamps were removed after 60 min and the incision was closed. Control 1 group was not subjected to the surgical operation, and control 2 groups were subjected to a sham operation without clamping of the renal arteries.

The experimental and control 2 animals were killed at 1, 2, 3, 6, 8, 14 and 28 days after ischaemia.

For determination of DNA synthesis, the animals of each group were injected intraperitoneally with 4 mg per 100 g body weight bromodeoxyuridine (BrdU; Sigma, St. Louis, Mo., USA), at 1 h before sacrifice.

Each experimental and control group consisted of three rats.

In renal function studies the urine of all animals was obtained as 12 h overnight collections from metabolism cages before sacrifice. After the volume was measured, the urine was centrifuged for 5 min at 1500 rpm. Urine osmolarity determinations were performed on the supernatant from centrifuged urine by the auto-analyser (Osmotic pressure auto and STAT OM-6030, Kyoto Daiichi Kagaku, Kyoto, Japan). Casts in urinary sediments were

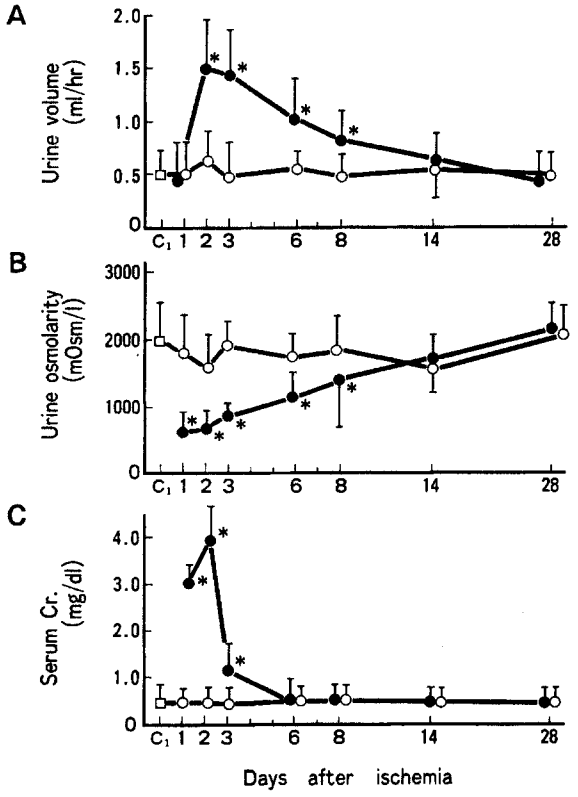


Fig. 1A–C Renal function parameters in experimental (●), control 1 (□), and control 2 (○) animals during experimental period. (*Cr.* creatinine) Values are expressed as mean±standard deviation (SD). * $P<0.05$ when compared to control

Fig. 2A, B One day after ischaemic injury. **A** Extensive necrosis and occasional nonnecrotic cells with bromodeoxyuridine (BrdU)-positive nuclei (*single arrow*) in damaged tubules (immunohistochemistry for BrdU, ×600). **B** The basement membrane (BM) of damaged tubule is thin and wrinkled (*double arrow*) compared to the BM of undamaged tubules (periodic acid-methenamine silver stain, electron microscopy (EM), ×5600; *bar*=2 μm)

examined by light microscopy. Serum creatinine levels were determined from heart blood obtained at autopsy of each animals of each group, using the modified Folin-Wu method (SRL, Tokyo, Japan).

After removal of both kidneys, tissue blocks for light microscopic study were fixed in 20% neutral formalin and embedded in paraffin. Haematoxylin and eosin, periodic acid-Schiff (PAS), and periodic acid-methenamine silver (PAM) stains were used for light microscopy.

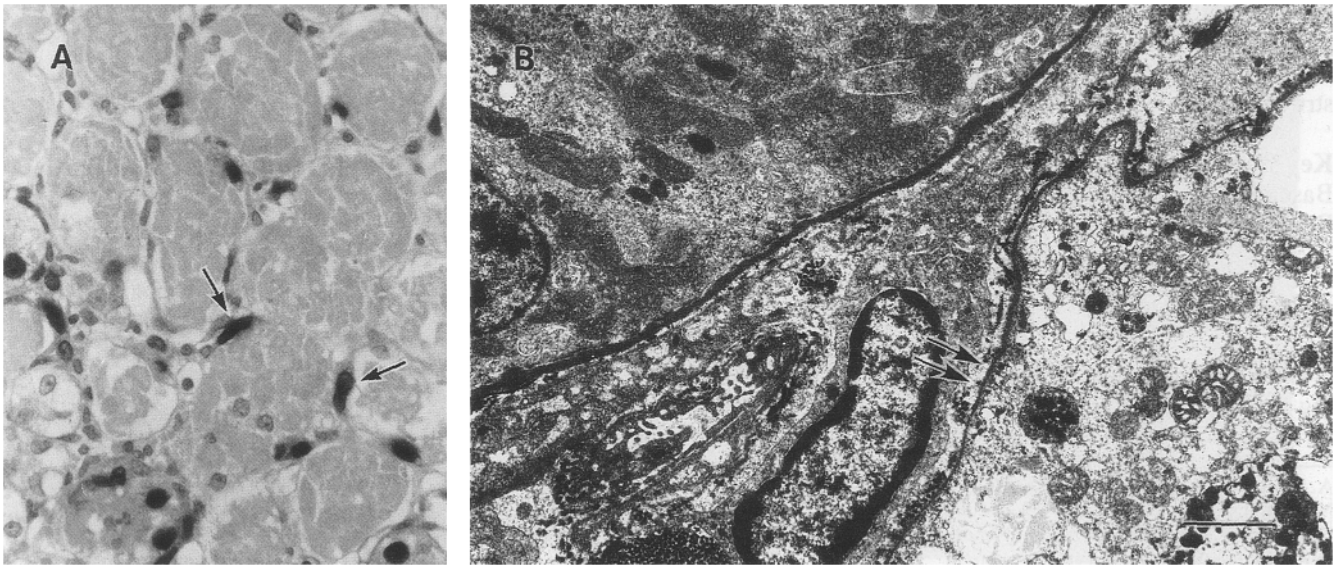
In immunohistochemical studies for the detection of laminin freeze-dried tissue blocks were prepared. Small samples of tissues were frozen in dry ice-acetone and dried at −60° C in a vacuum chamber (TIS-U-DRY, Freeze-dryers Model TFD-3, FTS System) for 5 days. Paraffin was melted gradually by raising the chamber temperature up to 60° C and the paraffin-embedded freeze-dried tissue blocks were cut into sections 2.5 μm thick and deparaffinized. The sections were treated with 0.3% hydrogen peroxide in methanol for 30 min and incubated for 1 h with rabbit anti-mouse laminin antibody (E-Y Laboratories, San Mateo, Calif., USA) at a dilution of 1:200 in phosphate-buffered saline (PBS). They were then incubated with biotinylated anti-rabbit IgG antibody (Dakopatts, Denmark) at a dilution of 1:200 in PBS for 1 h. After incubating in avidin-biotin-peroxidase complex solution (Vector Laboratories, Burlingame, Calif., USA) for 1 h, the reaction products were visualized using hydrogen peroxide containing 3,3′-diaminobenzidine in 0.05 M TRIS buffer, and were finally counterstained with Meyer's haematoxylin.

Immunohistochemistry for detection of BrdU was performed as previously described [32] using the indirect method with monoclonal antibody against BrdU containing nuclease for DNA (Amersham International, Amersham, UK).

For electron microscopic study, small blocks were fixed in 2.5% glutaraldehyde solution in phosphate buffer (pH 7.4) and postfixed with 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, then examined with a Hitachi H7100 electron microscope.

Ultrathin sections from the blocks embedded in epoxy resin were also stained with PAM stain using the modified Jones' method [19], then examined with a Hitachi H7100 electron microscope.

In the electron microscopic study for confirmation of heparan sulfate proteoglycan in tubular BM, the small blocks were reacted with ruthenium red (RR) using the modified Luft's method [22]. Ultrathin sections which reacted with RR were processed for electron microscopy according to routine procedures. Ultrathin sections subjected to RR reaction were stained with only uranyl acetate and then examined with a Hitachi H7100 electron microscope.



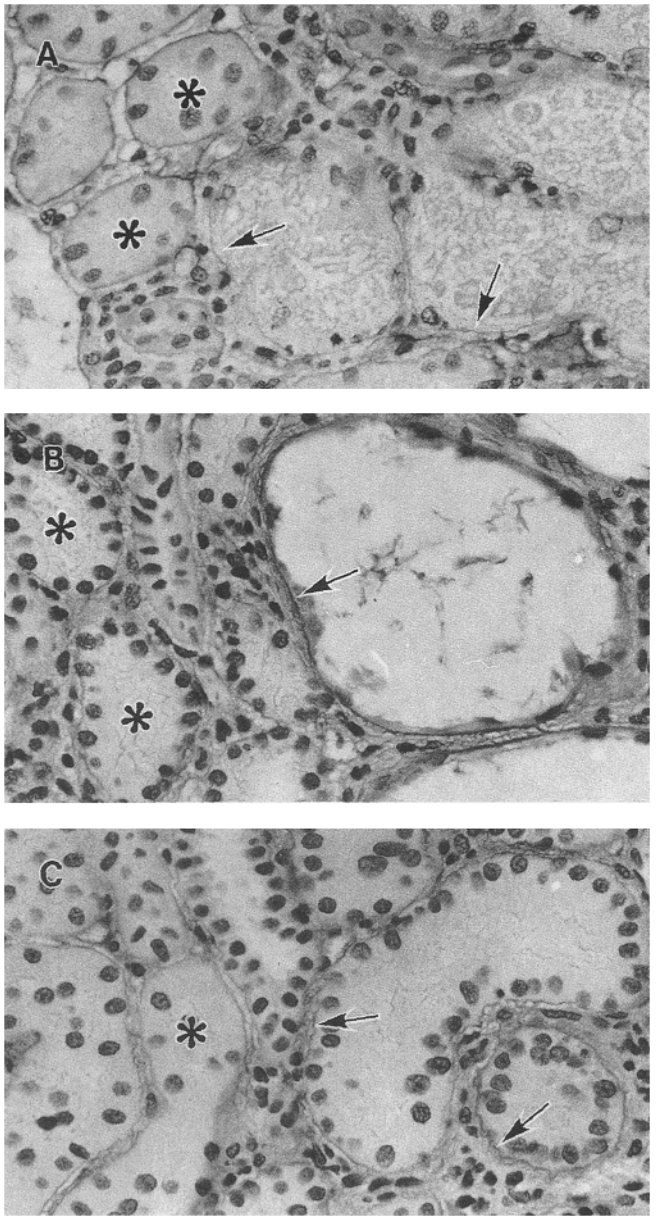


Fig. 3A–C Immunohistochemistry for laminin in renal tubules. **A** One day after ischaemic injury. The intensity of laminin appears to decrease in the damaged tubules (*arrow*) compared to that in the undamaged tubules (*asterisk*). **B** Six days after ischaemic injury. Tubular BM of dilated tubules stained very intensely for laminin (*arrow*) as compared to undamaged tubules (*asterisk*). **C** Fourteen days after ischaemic injury. Immunostaining for laminin slowly recovered to its original appearance (*arrow*). **A**, **B**, **C** ×700

The numbers of BrdU-positive nuclei in tubular epithelium of the outer strip of the outer medulla were counted by light microscopy at ×400 magnification (0.0698 mm^2) in the specimens from control and ischaemic kidneys. More than 60 high-power microscopic fields were examined in each animal. The circumference of tubule cross-sections was measured by using a Luzex IIIU computed image processor analyser (Nireco, Tokyo, Japan) in PAS-stained sections from the outer strip of the outer medulla of control and treated kidneys. The numbers of epithelial nuclei in the same tubules were also counted. Cross sections of more than 200 tubules were examined for each animal in each experimental stage.

These results are expressed as the mean±standard deviation (SD), and statistical analysis was performed by Student's *t*-test.

To elucidate the morphological findings of dilated tubules, 800 PAS-stained serial sections of the kidney on day 6, when tubular dilatation was at its peak, were prepared. The three-dimensional reconstruction of the luminal intersurface of dilated tubules from serial sections was made using an application software TRI (Ratoc System Engineering, Tokyo, Japan) in a personal computer system. The three-dimensional images of dilated tubules were the examined, focussing on the correlation between tubular dilatation and tubular obstruction.

Results

There was no oliguric phase in the experimental animals after ischaemic injury, and the diuretic phase began on day 2 (Fig. 1A). The diuretic phase continued for a few days, after which urine volume gradually decreased as the decreased urinary osmotic pressure recovered (Fig. 1B). The serum creatinine level increased on day 1 after ischaemic injury, and showed a maximum value on day 2 (Fig. 1C). The serum creatinine level then decreased rapidly during the diuretic phase, and no significant difference was observed between the experimental and the control groups on day 6.

One day after ischaemia, 60 min ischaemia and reperfusin resulted in widespread necrosis of the renal tubules, especially in the straight portion of the proximal tubules; however, no tubular dilatation was observed (Fig. 2A). Necrotic cells and their debris were observed in the tubular lumen. DNA-producing BrdU-positive cells appeared among the residual epithelial cells in the damaged zone. Casts were also observed almost throughout the tubular lumen. By electron microscopy, the BM of damaged tubules was thin and wrinkled compared to the BM of undamaged tubules (Fig. 2B). The intensity of immunohistochemical staining of laminin on the BM of damaged tubules decreased compared with that on the tubular BM of the control group (Fig. 3A). Reduced intensity of immunostaining for type IV collagen was also observed in damaged tubules (figure not shown here).

Two days after the ischaemic injury slight tubular dilatation was observed in the damaged straight portion of the proximal tubules (Fig. 4A) wherein the distribution of regenerating epithelial cells became prominent. A large number of BrdU-positive cells were observed among epithelial cells of the damaged tubules. By electron microscopy, flattened regenerated epithelial cells with reduced intracellular organelles, brush borders and basolateral invaginations covered the thin tubular BM (Fig. 4B).

At three days dilatation of tubules progressed in the damaged straight portion of the proximal tubules (Fig. 5A). Regenerated epithelial hyperplasia was seen with numerous BrdU-positive cells in the damaged tubules. The amount of debris as well as the number of casts in the tubular lumen decreased rapidly, and the number of casts in urine increased prominently. By elec-

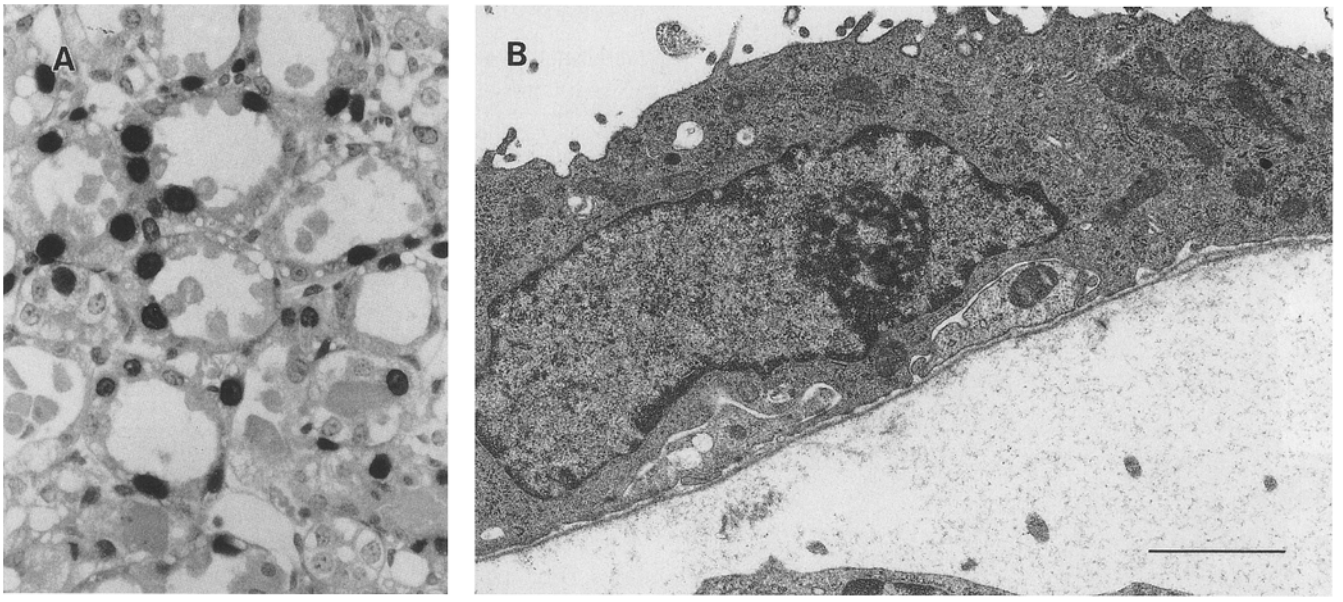


Fig. 4A, B Two days after ischaemic injury. **A** Slight dilatation of tubules and many regenerating epithelial cells with numerous BrdU-positive nuclei in damaged tubules (immunohistochemistry for BrdU, ×600). **B** The flattened regenerating epithelial cell covers the thin tubular BM (EM, ×9300; *bar*=2 μm)

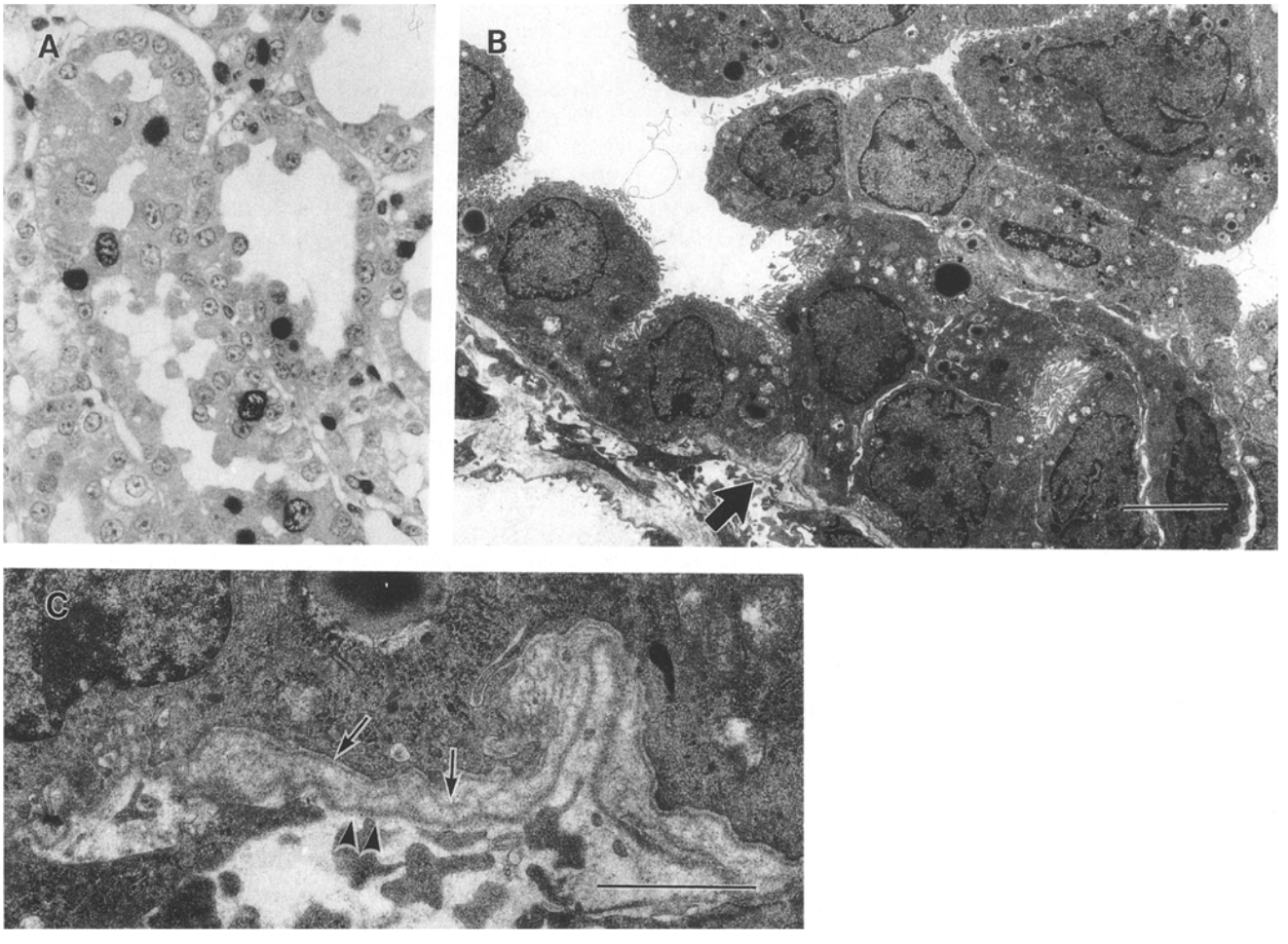


Fig. 5A–C Three days after ischaemic injury. **A** Mild dilatation and stratified epithelial cells in damaged tubules with many BrdU-positive epithelial cells (immunohistochemistry for BrdU, ×600). **B** Stratified regenerating epithelial cells in damaged tubule (EM, ×2600; *bar*=5 μm). **C** The portion is indicated by a *thick arrow* in **B**. New BM-like material (*arrow*) is identified between the existing thin BM (*arrowheads*) and regenerating epithelial cells (*II*10300; *bar*=2 μm)

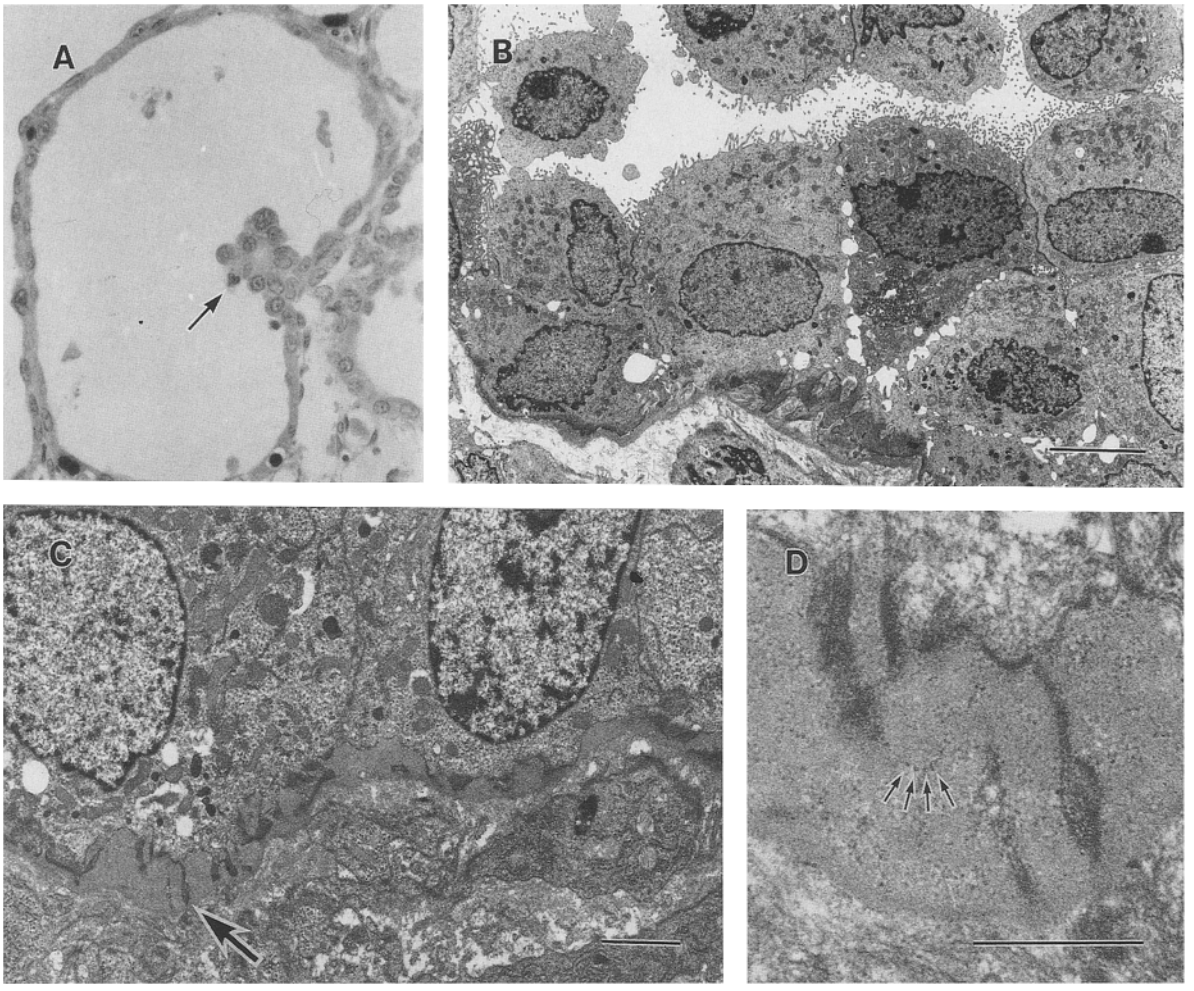


Fig. 6A–D Six days after ischaemic injury. **A** Prominent cystic dilatation of damaged tubules. The papillary epithelial cluster (*arrow*), as well as circumferential epithelial cells covers the dilated tubules. Only a few BrdU-positive cells are noted (immunohistochemistry for BrdU, ×600). **B** Hyperplastic regenerating epithelial cells in damaged tubule (EM, ×2400; *bar*=5 μm). **C** Marked thickening of tubular BM of dilated tubules (RR stain in EM, 5300; bar=2 μm). **D** The portion is indicated by an *arrow* in **C**. The amount of RR-positive material (*arrow*) appears to increase, and the distribution is irregular in the thickened BM (×22500; *bar*=1 μm)

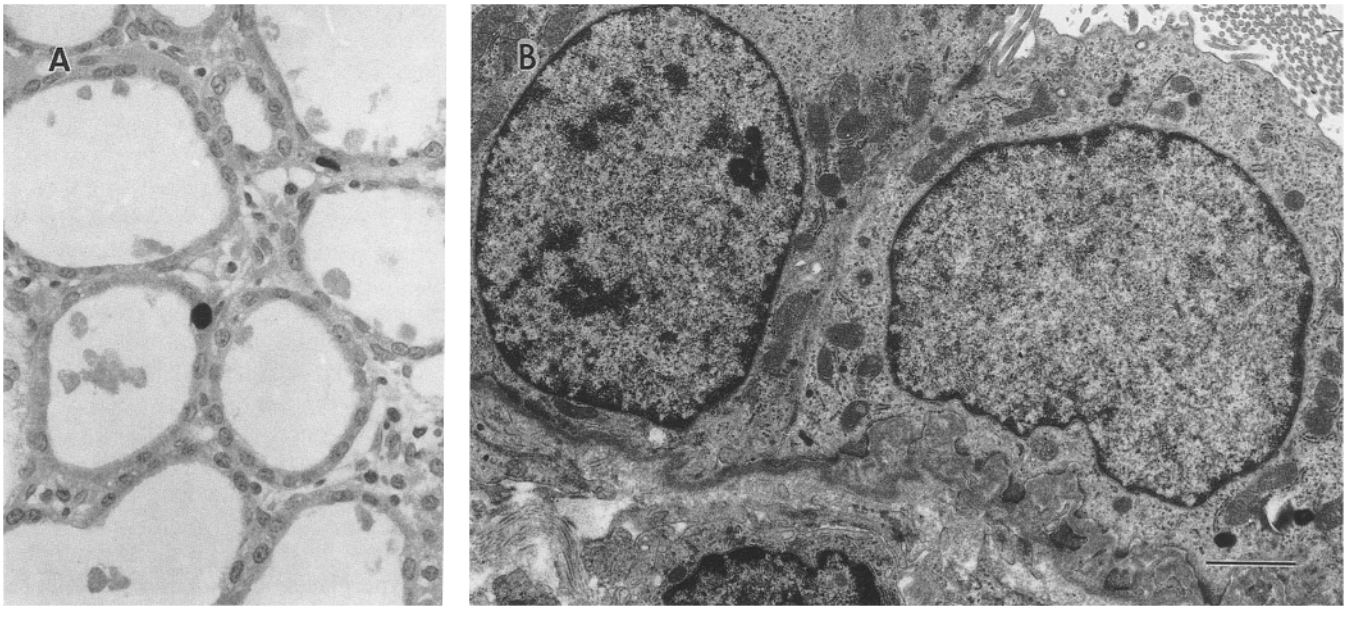


Fig. 7A, B Eight days after ischaemic injury. **A** The degree of dilatation of damaged tubules decrease. BrdU-positive epithelial cells are scarcely observed (immunohistochemistry for BrdU, ×600). **B** The tubular BM shows a tendency to recover to its original structure. However, thickened BM can still be observed. Slightly differentiated regenerating epithelial cells cover the BM (EM, ×6700; *bar*=2 μm)

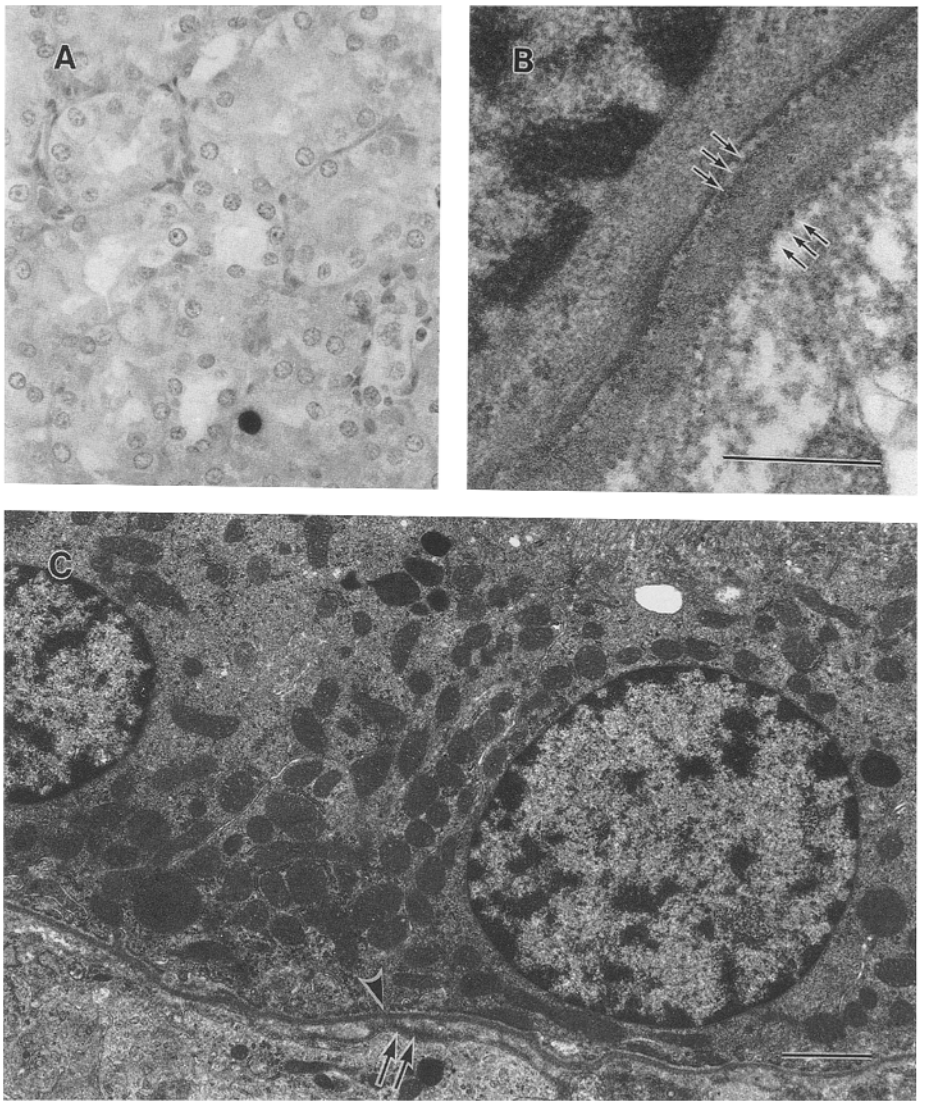


Fig. 8A–C Fourteen days after ischaemic injury. **A** The damaged tubules normalize to the original structure with mild epithelial hyperplasia (immunohistochemistry for BrdU, ×600). **B** The RR-positive material (*arrow*) distributes regularly on both surfaces of lamina densa of BM (RR stain in EM, ×43000; *bar*=0.5 μm). **C** The almost completely differentiated epithelial cells cover the BM with almost normal structure. The previously existing original BM (*arrow*), which appears thin and folded, is also seen beneath the newly formed BM (*arrowhead*) (×5800; *bar*=2 μm)

tron microscopy, the number of regenerated epithelial cells increased, and epithelial cells piled up in the lumen (Fig. 5B). Formation of a new BM-like material was observed between the existing thin BM and regenerated tubular epithelial cells (Fig. 5C).

Six days after ischaemia a large number of damaged tubules in the outer strip of the outer medulla showed mild cystic dilatation. The hyperplastic regenerated epithelial cells in damaged tubules were observed with occasional papillary epithelial clusters extending into the tubular lumen (Fig. 6A, B). The number of BrdU-positive tubular epithelial cells rapidly decreased. Casts in the tubular lumen were scarcely observed. Interstitial oedema around the damaged tubules was reduced. By electron microscopy, the initiation of regenerated epithelial cell differentiation was observed (Fig. 6B). The BM of dilated tubules thickened irregularly (Fig. 6C). The amount of RR-positive material apparently increased (Fig. 6D). The distribution of the RR-positive material was irregular in the thickened BM, however, distribution was regular on both inner and outer surfaces of the lamina densa in the normal BM. The degree of staining of laminin of the thickened tubular BM was increased when compared with the tubular BM of the control kidneys (Fig. 3B).

At eight days dilatation of the damaged tubules decreased rapidly (Fig. 7A). Although the slight tubular epithelial cell hyperplasia continued, the papillary clusters of hyperplastic epithelial cells protruding into the tubular lumen were not seen. BrdU-positive tubular epithelial cells were scarcely found, as in control materials. By electron microscopy, the regenerated epithelia were observed as gradually differentiating cells which eventually develop into brush borders, intracellular organelles and basolateral invaginations (Fig. 7B). The tubular BM which thickened on day 6 showed a tendency of recovery to the normal structure.

Two-to-four weeks after ischaemia tubular epithelial cells were slightly hyperplastic but the dilated tubules had almost returned to their original form (Fig. 8A).

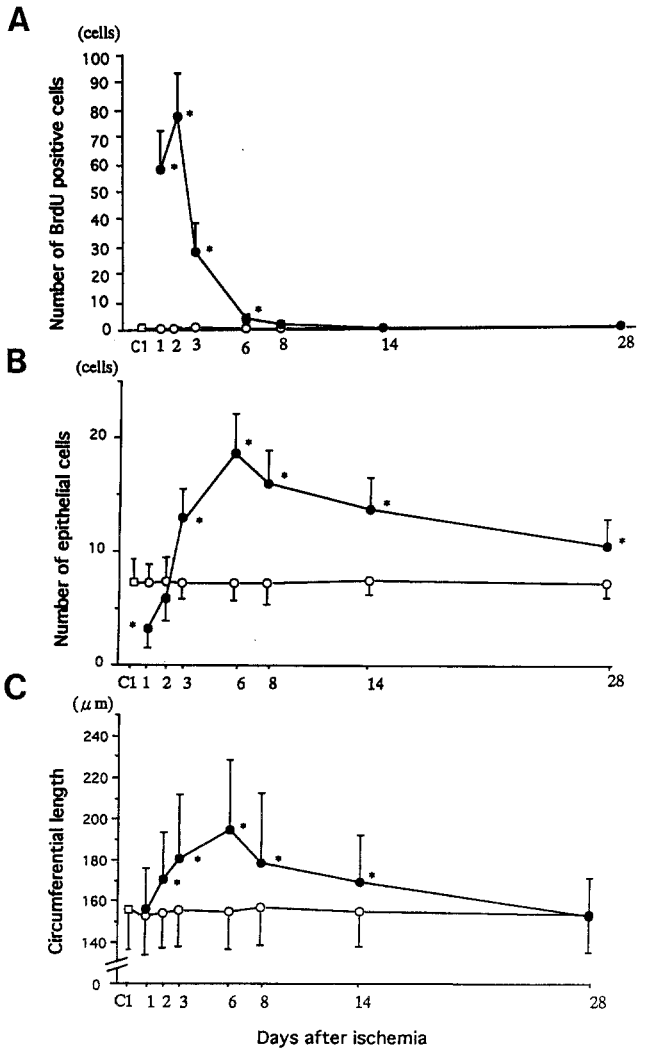


Fig. 9A–C Morphometry of cell proliferation, epithelial cellularity and the circumference of renal tubules. **A** The number of BrdU-labelled tubular epithelial nuclei per ×400 magnification field in experimental (●), control 1 (□), and control 2 (○) kidneys. **B** The number of epithelial nuclei in the cross sections of tubule in experimental (●), control 1 (□), and control 2 (○) kidneys. **C** The cross sections of the circumference of the tubule in experimental (●), control 1 (□), and control 2 (○) kidneys. Values are expressed as mean±SD. * $P<0.05$ when compared to control

Slight interstitial fibrosis was observed around the damaged tubules. By electron microscopy, an almost complete differentiation of regenerated epithelial cells was observed (Fig. 8C). The tubular BM showed improvement toward its original condition, and the thickened BM returned to its normal structure. The formation of a new BM was also observed other than the previously existing BM. In RR stain, distribution of the RR-positive material was regular on both surfaces of the lamina densa in a manner similar to that of the normal BM (Fig. 8B). The intensity of staining of laminin of the tubular BM gradually decreased and almost reached the same level as that of the tubular BM of the control group (Fig. 3C).

Figure 9A shows the number of BrdU-positive nuclei of tubular epithelial cells per high-power microscopic field of control and ischaemic kidneys. A marked increase in the number of BrdU-positive cells was observed at day 1 and maximum values were observed on day 2 which rapidly decreased and were normal by day 8. Figures 9B and C show the number of epithelial nuclei and the circumference of tubular cross-sections in control or ischaemic kidneys. In experimental groups, the number of lining epithelial cells in tubules decreased significantly compared with that of controls at day 1. Thereafter, regenerating cells increased dramatically with tubular dilatation. The number of nuclei per tubular cross section increased significantly compared with that of controls at day 3, and reached the highest value at day 6. During the progress of tubular cell hyperplasia, tubular dilatation developed further. In the later stages, tubules returned to normal size by day 28. During this recovery the number of hyperplastic epithelial cells decreased.

A three-dimensional analysis of ten dilated tubules was conducted using the serial sections obtained on day 6, when tubular dilatation was at its peak. Analysis was successful for three of ten dilated tubules. No obstruction was observed at the initial portion of dilatation (Fig. 10), which confirmed that no relationship exists between tubular dilatation and tubular obstruction.

Discussion

Tubular dilatation is observed during ATN [30, 34], and it has been reported that the cause of dilatation is tubular obstruction [12, 27, 29]. However, our study shows that tubular dilatation in the recovery phase of ATN does not result from tubular obstruction. Hyperplasia of regenerative tubular epithelial cells and abnormalities of the tubular BM greatly affect tubular dilatation in ATN, and dilated tubules recover as remission of these phenomena occurs. Tubular cell hyperplasia and abnormalities of tubular BM are known to be significant in the pathogenesis of polycystic renal disease. We consider that tubular dilatation in both renal cystic disease and ATN has a similar mechanism. However tubular dilatation in ATN is observed during recovery, a very important finding for the diagnosis and prognosis of ATN.

A burst of proliferation and regenerative epithelial hyperplasia is observed after the beginning of tubular dilatation. This development of tubular epithelial cell hyperplasia progressed until day 6, while tubular dilatation increased. Epithelial hyperplasia and dilatation of tubules was most prominent at day 6: tubular dilatation returned to normal as epithelial hyperplasia disappeared. Tubular regeneration and tubular epithelial cell hyper-plasia are thus important in the progress of tubular dilatation in ATN. In experimental models of renal cystic disease, formation of cysts is observed following an increase in the number of tubular epithelial cells. Abnormality in cell proliferation affects cyst formation greatly [14] and enlargement of the cysts is affected by tubular epithelial cell hyperplasia [2, 16, 31, 35]. Furthermore, recent evidence suggests that an increase in the number

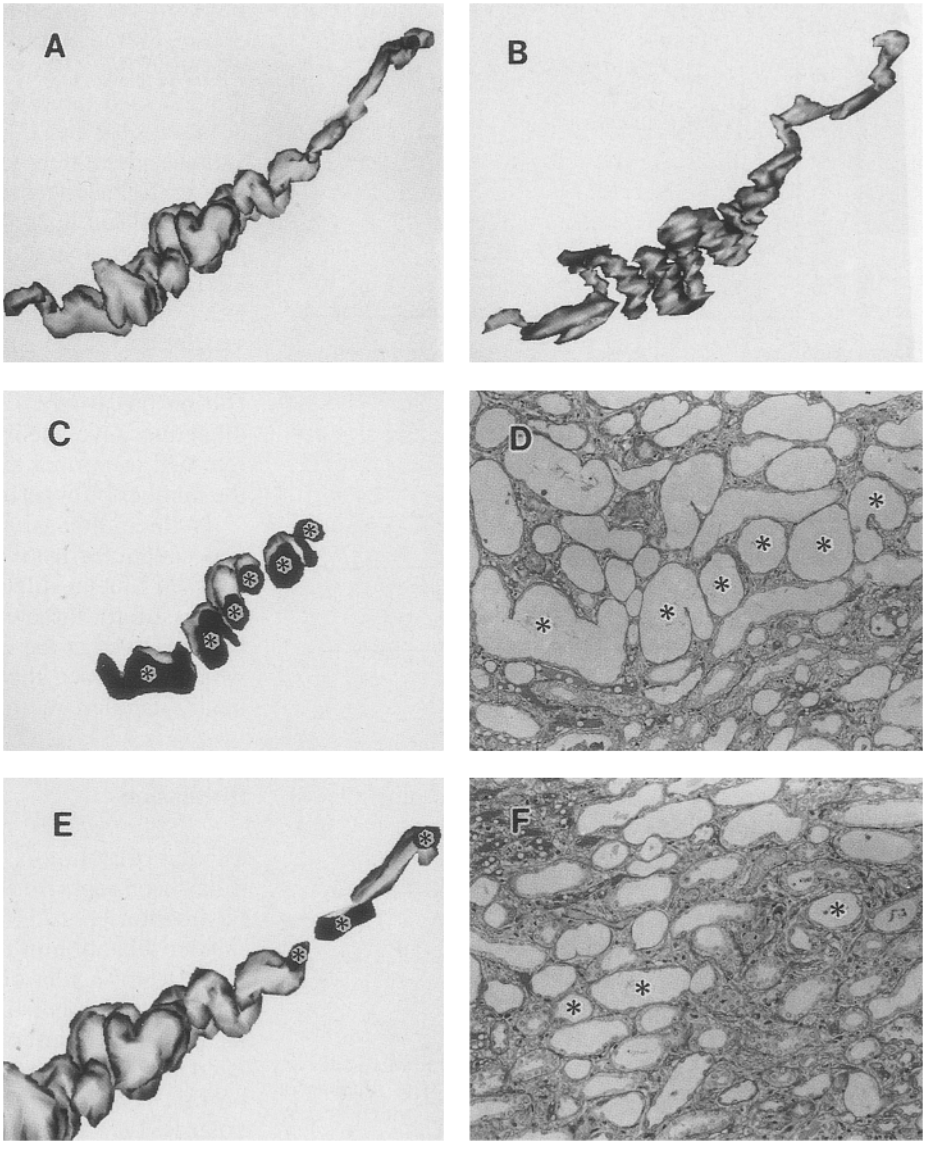


Fig. 10A–F Three-dimensional images of the dilated tubules. **A** A computer-aided reconstructed image of dilated tubule, which was stacked serially from 1 to 160 sections. **B** The same image in **A** at a rotated angle. **C** A three-dimensional reconstructed image of the same dilated tubule in (**A**), which was stacked serially from 1 to 64 sections. **D** The microscopic picture of section 64. The *asterisks* in both (**C**) and (**D**) indicate the same tubules. Many dilated tubular cross sections from one dilated tubule are seen (×60). **E** A three-dimensional reconstructed image of the same dilated tubule in (**A**), which was stacked serially from 1 to 152 sections. **F** The microscopic picture of section 152. The *asterisks* in both (**E**) and (**F**) indicate the same tubules. The diameter of these cross sections of the tubule (*asterisk*) is within normal range, and no obstruction is observed at the initial portion of dilatation (×100)

of cells (cell proliferation) may be a central process in the formation of renal cysts [17, 18, 26]. During the recovery process of tubular dilatation, a decrease in the number of hyperplastic epithelial cells was observed in our model: the decrease in the number of hyperplastic cells is due to the desquamation into the tubular lumen as well as to apoptosis [32].

Tubular dilatation in ATN has been thought to be due to the tubular obstruction caused by hyperplastic epithelial cells, cellular debris or casts [12, 27, 29]. It is also known that one of the mechanisms of cyst formation in polycystic kidney disease is tubular obstruction by papillary clusters of hyperplastic epithelial cells [4, 11, 13]. Papillary clusters of hyperplastic epithelial cells protruding into the lumen, were observed in our model on day 6. Tubular obstruction is thought to be one of the mechanisms affecting the deterioration of renal function in ATN [25] but our results indicated that renal function recovered by day 6 when casts in the tubular lumen had hardly decreased at all. Furthermore, no relationship was observed between tubular dilatation and tubular obstruction by our three-dimensional analysis. Carone et al. [6] reported that even when intratubular hydrostatic pressure is within the normal range, tubular dilatation occurs. Avner et al. [3] indicated that tubular dilatation occurs upon administration of cis-dichlorodiamineplatinum into a metanephric organ culture where neither tubular obstruction nor intratubular hydrostatic pressure elevation takes place, indicating that the cysts can develop without obstruction. Moreover, Tanner et al. [33] showed that tubules tend to be atropic following complete tubular obstruction. Some authors have found that the tubular obstruction is not present in association with cystic changes in experimental and human polycystic kidney disease [23, 24] and we agree; papillary clusters of hyperplastic epithelial cells desquamate rapidly into the tubular lumen [32] and have no effect on tubular obstruction.

The thinning of the BM and reduced intensity of immunohistochemical staining of BM components after tubular necrosis were observed at the damaged tubules indicating abnormalities of BM after epithelial cell damage. Tubular dilatation then began and progressed. In human and the experimental polycystic kidney disease, a thickened and multilayered BM is observed in cystic tubules [8, 21, 28] with changes in the intensity of immunohistochemical staining of BM components [7, 9]. The changes of protein composition are also observed in the BM of cystic tubules [5]. The mRNA expression of components of the BM altered during the development of cystic kidney [10], and the altered BM protein biosynthesis have both been reported in primary polycystic kidney cell culture [15]. It has been reported that the viscoelastic properties of the BM of cystic tubules are not altered [18]. However, it was considered that abnormalities in the BM strongly affect cyst formation.

On day 6 in our model, when tubular dilatation was at its peak, the staining of BM components increased transiently, and a thickened tubular BM was observed. In the formation of the BM, synthesis of BM components is enhanced transiently during development [20]. In the process of tubular BM formation during renal development, newly generated BM-like materials accumulate outside the BM, leading to the thickening [1]. In our results, the thickening of the tubular BM and enhancement of the degree of staining for BM components were found transiently during the normalization of the altered BM.

Dilated tubules after ischaemic injury recovered with normalization of the BM due to the regeneration and differentiation of epithelial cells. It has been reported that normalization of the altered BM is important for recovery of the cysts in the reversible renal cystic models [21, 28]. Our results also indicated that the normalization of the altered BM affects the recovery of dilated tubules in ATN.

The present study demonstrated that tubular dilatation occurs transiently after ischaemic renal damage in the straight portion of proximal tubules, where the epithelial cell damage is at its peak, and that the formation and development of tubular dilatation are due to regenerated epithelial hyperplasia and abnormalities of the BM following epithelial cell damage. Tubular obstruction is not important. Dilated tubules in ATN recover with normalization of the BM due to regeneration and differentiation of epithelial cells, and with decrease in the number of hyperplastic epithelial cells.

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References

1. Abrahamson DR, Leardkamolkarn V (1991) Development of kidney tubular basement membranes. Kidney Int 39:382–393
2. Avner ED (1988) Renal cystic disease. Insights from recent experimental investigations. Nephron 48:89–93
3. Avner ED, Sweeney WE, Ellis D (1983) Cyst formation in metanephric organ culture induced by cis-dichlorodiamine-platinum (II). Experientia 39:74–76
4. Bernstein J, Evan AP, Gardner KD (1987) Epithelial hyperplasia in human polycystic kidney disease. Its role in pathogenesis and risk of neoplasia. Am J Pathol 129:92–101
5. Butkowski RJ, Carone FA, Grantham JJ, Hudson BG (1985) Tubular basement membrane changes in 2-amino-4,5-diphenylthiazole-induced polycystic disease. Kidney Int 28:744–751
6. Carone FA, Rowland RG, Perlman SG, Ganote CE (1974) The pathogenesis of drug-induced renal cystic disease. Kidney Int 5:411–421
7. Carone FA, Makino H, Kanwar YS (1988) Basement membrane antigens in renal polycystic disease. Am J Pathol 130:466–471
8. Carone FA, Hollenberg PF, Nakamura S, Punyarit P, Glogowski W, Flouret G (1989) Tubular basement membrane change occurs pari passu with the development of cyst formation. Kidney Int 35:1034–1040
9. Cowley BD, Gudapaty S, Kraybill AL, Barash BD, Harding MA, Calvet JP, Gattone VH II (1993) Autosomal-dominant polycystic kidney disease in the rat. Kidney Int 43:522–534
10. Ebihara I, Killen PD, Laurie GW, Huang T, Yamada Y, Martin GR, Brown KS (1988) Altered mRNA expression of basement membrane components in a murine model of polycystic kidney disease. Lab Invest 58:262–269
11. Evan AP, Gardner KD (1979) Nephron obstruction in nordihydroguaiaretic acid-induced renal cystic disease. Kidney Int 15:7–19
12. Finn WF, Chevalier RL (1979) Recovery from postischemic acute renal failure in the rat. Kidney Int 16:113–123
13. Gardner KD, Evan AP (1983) Renal cystic disease induced by diphenylthiazole. Kidney Int 24:43–52
14. Gattone VH II, Calvet JP, Cowley BD, Evan AP, Shaver TS, Helmstadter K, Grantham JJ (1988) Autosomal recessive polycystic kidney disease in a murine model. A gross and microscopic description. Lab Invest 59:231–238
15. Granot Y, Putten VV, Przekwas J, Gabow PA, Schrier RW (1990) Intra- and extracellular proteins in human normal and polycystic kidney epithelial cells. Kidney Int 37:1301–1309
16. Grantham JJ (1990) Physiology and cell biology update. Polycystic kidney disease: neoplasia in disguise. Am J Kidney Dis 15:110–116
17. Grantham JJ, Geiser JL, Evan AP (1987) Cyst formation and growth in autosomal dominant polycystic kidney disease. Kidney Int 31:1145–1152
18. Grantham JJ, Donoso VS, Evan AP, Carone FA, Gardner KD (1987) Viscoelastic properties of tubule basement membranes in experimental renal cystic disease. Kidney Int 187–197
19. Jones DB (1957) Nephrotic glumerulonephritis. Am J Pathol 33:313–329
20. Kadoya Y, Yamashina S (1989) Intracellular accumulation of basement membrane components during morphogenesis or rat submandibular gland. J Histochem Cytochem 37:1387–1392
21. Kanwar YS, Carone FA (1984) Reversible changes of tubular cell and basement membrane in drug-induced renal cystic disease. Kidney Int 26:35–43
22. Luft JH (1971) Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat Rec 171:347–368
23. Mandell J, Koch WK, Nidess R, Preminger GM, McFarland E (1983) Congenital polycystic kidney disease. Genetically transmitted infantile polycystic kidney disease in C57BL/6J mice. Am J Pathol 113:112–114
24. Milutinovic J, Agodoa LY (1983) Potential causes and pathogenesis in autosomal dominant polycystic kidney disease. Nephron 33:139–144
25. Myers BD, Moran SM (1986) Hemodynamically mediated acute renal failure. N Engl J Med 314:97–105
26. Neufeld TK, Douglass D, Grant M, Ye M, Silva F, Nadasdy T, Grantham JJ (1992) In vitro formation and expansion of cysts derived from human renal cortex epithelial cells .Kidney Int 41:1222–1236

27. Norlén BJ, Engberg A, Källskog Ö, Wolgast M (1978) Nephron function of the transplanted rat kidney. Kidney Int 14:10–20
28. Ojeda JL, Ros MA, Icardo JM, Garcia-Porrero JA (1990) Basement membrane alterations during development and regression of tubular cysts. Kidney Int 37:1270–1280
29. Oliver J (1953) Correlations of structure and function and mechanisms of recovery in acute tubular necrosis. Am J Med 15:535–557
30. Olsen S (1989) Acute tubular necrosis and toxic renal injury. In: Tisher CC, Brenner BM (eds) Renal pathology with clinical and functional correlations. J.B. Lippincott, Philadelphia, pp 657–699
31. Schaffner DL, Barrios R, Massey C, Banez EI, Ou CN, Rajagopalan S, Aguilar-Cordova E, Lebovitz RM, Overbeek PA, Lieberman MW (1993) Targeting of the *ras*T24 oncogene to the proximal convoluted tubules in transgenic mice results in hyperplasia and polycystic kidneys. Am J Pathol 142:1051–1060
32. Shimizu A, Yamanaka N (1993) Apoptosis and cell desquamation in repair process of ischaemic tubular necrosis. Virchows Arch [B] 64:171–180
33. Tanner GA, Evan AP, Summerlin PB, Knopp LC (1989) Glomerular and tubular morphology after single nephron obstruction. Kidney Int 36:1050–1060
34. Venkatachalam VA (1981) Pathology of acute renal failure. In: Brenner BM, Stein JH (eds) Acute renal failure. Churchill Livingstone, New York, pp 79–107
35. Wilson PD, Sherwood AC (1991) Tubulocystic epithelium. Kidney Int 39:450–463