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Further Evidence of Tubular Blockage After Acute Ischemic Renal Failure in *Tupaia belangeri* and Rats*

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Summary. 1. Acute renal failure in man can best be simulated experimentally by using either temporary occlusion of the renal artery or perfusion of noradrenaline into the renal artery.

2. The kidney of *Tupaia belangeri*, a primitive primate, which may allegedly die from psychogenically induced renal failure, is surprisingly more resistant to temporary occlusion of the renal artery than that of the rat.

3. The results of physiological studies (measurements of intratubular pressures, proximal passage-times, inulin clearance) and the intravital microscopic appearances of the renal surface of the *Tupaia* kidney varied little from those of the rat. As intravital microscopic studies showed, however, only rarely are the distal tubuli visible on the surface of *Tupaia* kidneys, and stop-flow pressures measured in them proved remarkably high.

4. In *Tupaia* as in rats, anuria developing 48 hours after temporary ischemia is brought about by intratubular proteinaceous casts blocking the flow of urinary filtrate, most likely at Henle's loop.

Both the diameter of the proximal tubular lumen and the intratubular pressures increase after temporary ischemia but the severity of the diuresis and inulin clearance decrease. To demonstrate that the proteinaceous casts block the tubular lumina, we have shown that when two micropipettes are inserted into the same nephron and that nephron is perfused, the intratubular pressure rises approaching systolic pressures. Renal failure becomes more severe the longer the duration of temporary ischemia (from 1 to 3 h). As renal damage progresses and intratubular pressures fail to rise, tubular permeability increases (leakage).

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5. As with occlusion of the renal artery, infusion of the artery with noradrenaline leads to massive blockage of the nephrons by proteinaceous material in 48 h in the rat. Renal function (clearance of inulin, tubular passage times etc.) decreases as the infusion is prolonged (from 45 to 120 min), whereas the diameter of the tubular lumen and intratubular pressures increase.

As studies with the double insertion of micropipettes and microperfusion have shown, tubular blockage by casts also occurs after perfusion of noradrenaline. Where the tubular damage is greatest, the tubules leak.

6. Histologically, the proteinaceous material blocking the urinary flow is primarily found near the loops of Henle. Depending on the degree of damage, we often observe increasing necrosis of tubules; that represents the morphological equivalent of changes in tubular permeability (leakage).

Key words: Kidney – *Tupaia belangeri* – Acute renal failure – Intravital microscopy – Intratubular pressure.

Introduction

The pathophysiology of acute renal failure is controversial. In analyzing it two distinct questions must be answered:

1. How does acute renal failure develop?
2. After acute renal failure sets in, why does the anuria persist?

Both questions have become all the more perplexing because various groups of investigators have been able to devise different experimental models that simulate the clinical picture of acute renal failure. In humans the signs and symptons of acute renal failure are usually constant, especially those associated with acute oliguria or anuria, but that sympton in turn can be caused by various mechanisms. By listing all these mechanisms under the unspecified name "acute renal failure", the perplexity surrounding the condition has only increased. In man acute anuria may develop after poisoning or circulatory collapse and knowledge of that led to the use of numerous models of poisoning in animal experiments for inducing acute renal failure, including mercuric-chloride poisoning (Bank et al., 1967; Steinhausen et al., 1969a; Flamenbaum et al., 1971), glycerol (Ustimowitsch, 1876; Hsu et al., 1976; Churchill et al., 1977; Flanigan and Oken, 1965), uranyl nitrate (Blantz, 1975), and folic acid (Brade et al., 1969; Schmidt and Dubach, 1976; Schubert, 1976). Although it is possible to produce acute renal failure with these poisons, they do not represent good models when one considers that the anuria seen clinically usually follows circulatory failure (Krian et al., 1976; Minuth et al., 1976).

We should like to concentrate, therefore, on the clinically more relevant acute renal failure due to shock. That cannot be reproduced exactly in animal experiments although bloodletting (especially in rats) does lead to shock and anuria, the anuria does not last longer than the shock. Renal function recovers rapidly despite necrosis of some tubules (Dobyan et al., 1977, as well as unpublished data from personal studies). If one interrupts the blood flow to the kidney for definite periods of time, as for example with a clamp on the renal

artery, then acute renal failure may be simulated with variations in the duration of the oligo-anuria, but the first phase of acute renal failure cannot be reproduced. Nevertheless, to us this model most closely resembles the renal failure seen clinically. It was first employed about one hundred years ago and in the last few years has become increasingly popular (Litten, 1881; Koletsky and Gustafson, 1947; Eisenbach and Steinhausen, 1973; Daugharty and Brenner, 1975; Arendshorst et al., 1975; Tanner and Sophasan, 1976). The first phase of shock-induced acute renal failure in humans is probably brought about by a maximal constriction of the renal arterial vessels via sympathetic nerve stimulation. In animal experiments, however, it has been possible to produce such vascular spasm by renal nerve stimulation for only short periods of time (Steinhausen et al., 1969b). Although the infusion of noradrenaline in the renal artery represents an imperfect model of what actually happens clinically, it none the less seems to us suitable for simulating the pathogenesis of oligo-anuria following shock (Knapp et al., 1972; Cox et al., 1974).

A newer experimental model for inducing an acute renal failure, but only in *Tupaia belangeri*, is by psychological stress (von Holst, 1972). Acute renal failure induced by psychological disturbance alone is unknown in clinical medicine, but this experimental model with *Tapaia* must be considered with our other models since studies in this primitive primate might help explain why it has not been possible to produce an acute renal failure by shock in other animals resembling that caused by shock in man. Studies with *Tupaia* might also help reveal similarities between our experimental models and the course of acute renal failure seen clinically. For these reasons we have also studied normal renal function in *Tupaia* and renal failure due to ischemia, with particular regard to the second question we asked initially: "After acute renal failure sets in, why does the anuria persist?" Four mechanisms exist that either alone or combined with other factors may cause an oligo-anuric response by a nephron:

a) glomerular filtration pressure remains too low because of persistent vasoconstriction (Flamenbaum, 1973; Mason et al., 1978; Kashagarian et al., 1976);

b) glomerular permeability decreases so much that filtration ceases (Cox et al., 1974; Stein and Sorkin, 1976);

c) ischemia so injures the tubular epithelial cells that the urinary filtrate diffuses back across them (Eisenbach and Steinhausen, 1973; Donohoe et al., 1978);

d) proteinaceous casts formed in the tubules block urinary flow and thus glomerular filtration (Tanner et al., 1973; Arendshorst et al., 1975; Tanner and Steinhausen, 1976; Donohoe et al., 1978).

Against a) is the high renal blood flow that can be measured in rats after ischemia, although anuria persists (Steinhausen et al., 1973; Eisenbach et al., 1974; Arendshorst et al., 1975; Churchill et al., 1977). Against b), the concept of suspended filtration, is the fact it is possible to detect filtrate by appropriate tubular aspiration (Tanner and Sophasan, 1976). c) This mechanism of tubular cell-injury is usually considered to be of only minor importance. In our studies we have observed that leakage occurred chiefly when severe tubular necroses were associated with blockage by casts.

Here we present new data supporting mechanism d). We were able to demon-

strate blockage of tubular urinary flow in *Tupaia* after temporarily occluding the renal arteries and in rats after perfusing their renal arteries with noradrenalin for these studies we employed microperfusion and intratubular pressure measurement concurrently in the same nephron (Tanner and Steinhausen, 1976).

Methods

We carried out our studies on 10 male *Tupaia belangeri* from the stock bred at the Battelle Institute and on rats of a Wistar strain. The *Tupaia* weighed between 170 g to 274 g (average weight 208 g). They were from 0.9 to 3.6 years old (average age 2.7 years). The rats weighed about 250 g, plus or minus 43 g). Unless otherwise specified, the experimental techniques we used were those we have developed in our laboratory and described on many occasions (Tanner and Steinhausen, 1976; Steinhausen et al., 1973; Eisenbach et al., 1974). The animals were allowed free access to drinking water until the onset of the experiments, and animal feed (for the rats standard pellets Altromin®, for Tupaias pelled standard diet (Schwaier, 1973)) was withdrawn twelve hours before these began. Sarfan® (12 mg/ml solution) (Glaxo, Chemicals, England) proved to be an ideal narcotic agent for the *Tupaia*. We injected it (36 mg/kg body weight) into a femoral vein while a coworker, protected with leather gloves, held the animal in a supine position. To occlude a renal artery, we exposed the left kidney through a flank incision and compressed its artery for various periods of time with a clamp sheathed by a silicon tubing. Prompt blanching of the renal surface indicated that the clamp was properly situated. We then closed the laparotomy incision and returned the animals to their customary quarters. All animals survived the procedure without evidence of ill-effects. After 48 h we again narcotized the animals by injecting 36 mg/kg Sarfan® in the femoral vein. We then inserted a three-channeled catheter in the left jugular vein and infused 20 mg/kg Sarfan® per hour, enabling us to prolong narcosis for up to 10 h. We used the second catheter for continuous infusion of inulin in a concentration of 1% at a rate of 0.375 ml/h, and determined concentrations with the method of Fuehr, 1955. Through the third catheter we injected purified lissamine green V (Steinhausen and Tanner, 1976), at 5% concentration and in 0.05 ml amounts, for measuring tubular passage times. An additional catheter in the left femoral artery enabled us to withdraw blood for determining plasma concentrations of inulin. We continuously registered systolic and diastolic blood pressures in the left carotid artery with a statham-element and polygraph. The mean arterial pressures were 95 mm Hg. If the blood pressures began to fall, we reduced the rate of infusion of Sarfan®. The airways were kept clear with a tracheal catheter.

The left kidney was exposed by laparotomy, and as usual for micropuncture techniques, held firmly within a kidney-shaped spoon. With a plastic catheter in the left ureter we collected urine for clearance studies. For observing the renal surface and for simultaneous micropunctures we used an 11 power Ultropak objective (Leitz) with appropriate submersion cap. To record the tubular flow of dyes and all micropunctures we photographed with color film (Kodak Ektachrome usually) or with black and white film. Some of the studies were photographed with a motion-picture camera (Steinhausen et al., 1978). The microperfusion studies were carried out with the aid of a microperfusion pump (Hampel, Frankfurt, Max-Planck-Institut für Biophysik, Sonnenberg and Deetjen, 1964). The diameters of the tip-ends of the micropipettes measured between 8–10 μ. For microperfusion we used an equilibrium solution: 68 mg KH_2PO_4, 75 mg NaH_2PO_4, 156 mg Na_2HPO_4, 475 mg NaCl, 1472 mg Mannitol and 100 mg Lissamine green in 100 ml aqua dist. (Tanner and Steinhausen, 1976). We measured intratubular pressure continously with a Wiederhielm servonulling device, with a second micropipette.

To enable us to carry out microphotography and micropuncture studies with two micromanipulators simultaneously we had in addition to the operating platform, to construct a second table, that allowed precise displacement in all directions, which we could move in all horizontal directions under the epi-illuminated microscope. This table also had to support the operating platform with its electronically controlled heating system as well as the two micromanipulators. (The construction of the table and platform was carried out in the institute's workshops.)

For histological fixation at the end of the experiments we perfused the vascular system for 5 min with 5% formalin buffered at pH 7.5 with KH_2/Na_2 HPO_4, whereby the perfusion pressure was always kept below systolic pressure. Tissue samples were prepared for paraffin imbedding,

sections cut at 6 μ and stained with Haematoxilin-Eosin. The operative technique for the rats was identical, except that Inactin® was used as narcotic agent at a dose of 100 mg/kg.

Forty eight hours before onset of the experiments we anesthetized the rats with sodium pentobarbital (Nembutal® at 60 mg/kg), exposed the left kidney through a left laparotomy, restrained it in a kidney spoon and inserted a glass cannula of 30 μ diameter into the renal artery. With a Harvard infusion pump adapted for fine adjustment, we infused noradrenaline (Arterenol®) colored with lissamine green through that cannula at a concentration of 0.6 mM/l, at the rate of 0.57 to 2.64 microliters/min/kg ($0.3–1.6 \cdot 10^{-6}$ mM/min, kg). We determined the dosage by effect, observing changes produced in the renal surface with the stereomicroscope. Pallor of the renal surface, collapse of the proximal tubules, and the non-appearance of lissamine green after injection served as evidences of a sharp decrease in renal blood-flow, resulting at times in stasis of blood in peritubular capillaries. The laparotomy wound in the rats was closed with sutures on the skin brought together by clamps. After this initial operation the rats recovered rapidly.

Results

A. Tupaia belangeri – Temporary Clamping of the Renal Arteries

1. Controls. The intravital microscopic appearance of the *Tupaia* kidney closely resembles that of the rat. As its renal capsule is transparent, it was not necessary to remove it to carry out our studies. Figure 1 shows the normal *Tupaia* kidney at various magnifications during passage of lissamine green through early proximal convoluted tubules. The early and late proximal convoluted tubules are more widely separated than in the rat and so a distinct pattern is even more evident. The 'welling point' is the point of branching of the vas efferens and usually is surrounded by late proximal convoluted tubules (Steinhausen et al., 1970).

The proximal passage time of lissamine green (from the staining of peritubular capillaries to the staining of late proximal convoluted tubules) is 8.04 ± 1.58 s for controls (M ± S.D. for four animals and twenty-three passages of dye). The mean diameter of the proximal tubular lumen in controls is 21.9 ± 4.6 μ (M ± S.D., $N=4$, $n=306$). In *Tupaia* distal tubules only reach the renal surface occasionally. A looptime or a distal passage time cannot therefore be determined. The proximal intratubular pressure under control conditions is 13 mm Hg. The clearance of inulin (in one kidney) is 5.7 ± 2.2 ml/min/kg (M ± S.D., $N=3$, $n=20$). The U/P-inulin here is 198.

In control animals a 5–30 min long blockage of the ureter led to a rise in intratubular pressure to more than 70 mm Hg (cf. Fig. 2). The diameter of the proximal tubular lumen increased at the same time and an osmotic diuresis seemed to augment that increase only slightly. When single nephrons are blocked by oil (Castor oil stained with Sudan Black) and the pressure of the stopped-flow measured with a second capillary (Gertz et al., 1966), high stop-flow pressures were recorded in one *Tupaia* that corresponded with pressures arising from ureteral blockage (Fig. 3). Simultaneously the nephron dilated greatly. In a second *Tupaia* the stop-flow pressures were lower, yet in that animal after a respiratory arrest of short duration the inulin clearance had already diminished. Later, after additional infusions of mannitol (Fig. 2) the animal showed strikingly high ureteral pressures.

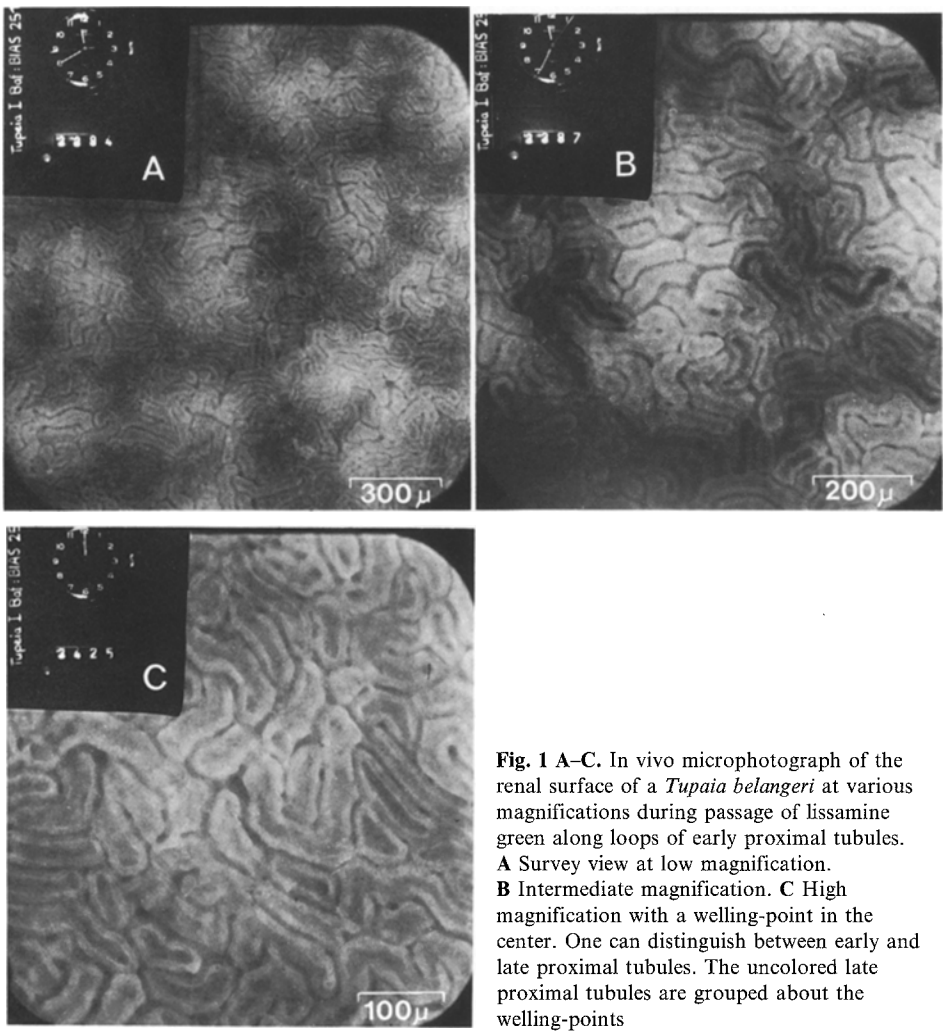


Fig. 1 A–C. In vivo microphotograph of the renal surface of a *Tupaia belangeri* at various magnifications during passage of lissamine green along loops of early proximal tubules. **A** Survey view at low magnification. **B** Intermediate magnification. **C** High magnification with a welling-point in the center. One can distinguish between early and late proximal tubules. The uncolored late proximal tubules are grouped about the welling-points

2. Changes After Temporary Clamping of the Renal Artery. Forty-eight hours after temporary clamping of the renal artery numerous nephrons at the renal surface are dilated. The passage time of lissamine green here is greatly prolonged (4 to 8 times). At times the dye remains in late proximal convoluted tubules (see Fig. 4). Variable numbers of non-dilated nephrons may be seen nearby and by measuring the tubular and luminal diameters of these proximal convoluted tubules, we distinguished dilated from nondilated tubules. Figure 5 presents a graphic summary of these measurements. The lumina of dilated tubules had diameters almost double those of controls, a highly significant change. In calculating the area of the epithelial cross section from the inner and outer tubular radii (see inset of Fig. 5), we found that the epithelial surface of dilated tubules had increased, but not to a degree comparable with the increase in lumen. The

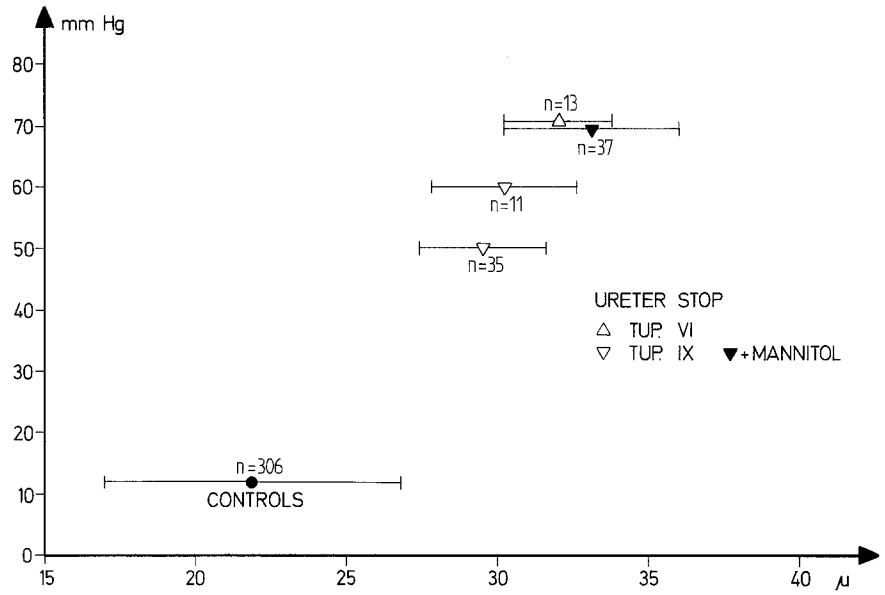


Fig. 2. The diameters of the lumen of proximal tubules (abscissa) are plotted against the ureteral pressures (ordinate); measured simultaneously within thirty minutes of occluding the ureter. Control values are intratubular pressures and lumen diameters measured in the same animal before ureteral occlusion. Mannitol as a 10% solution was infused during 10 min at a rate of 1 ml/min

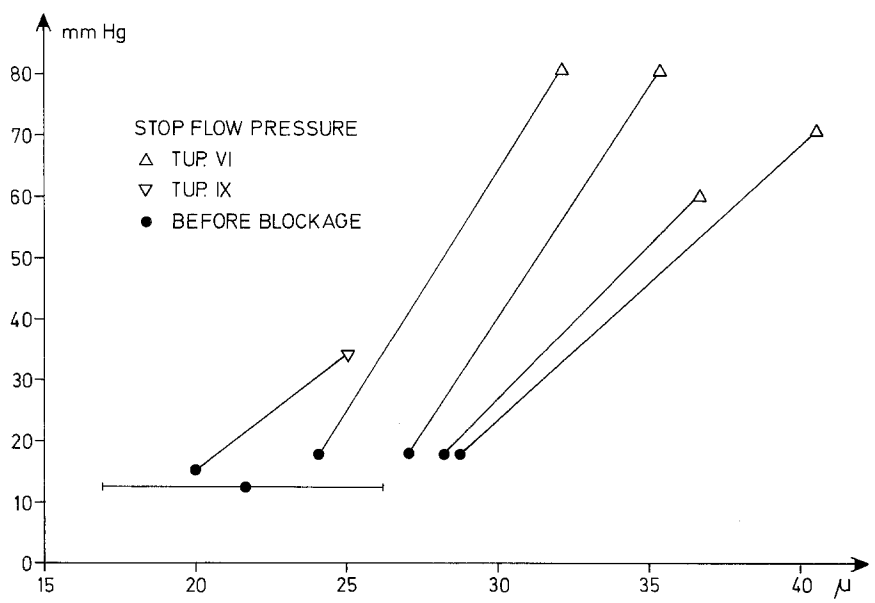


Fig. 3. Dilatation of proximal tubules during local stop-flow studies in single nephrons. Diameter of proximal lumen plotted against the intratubular pressures measured simultaneously

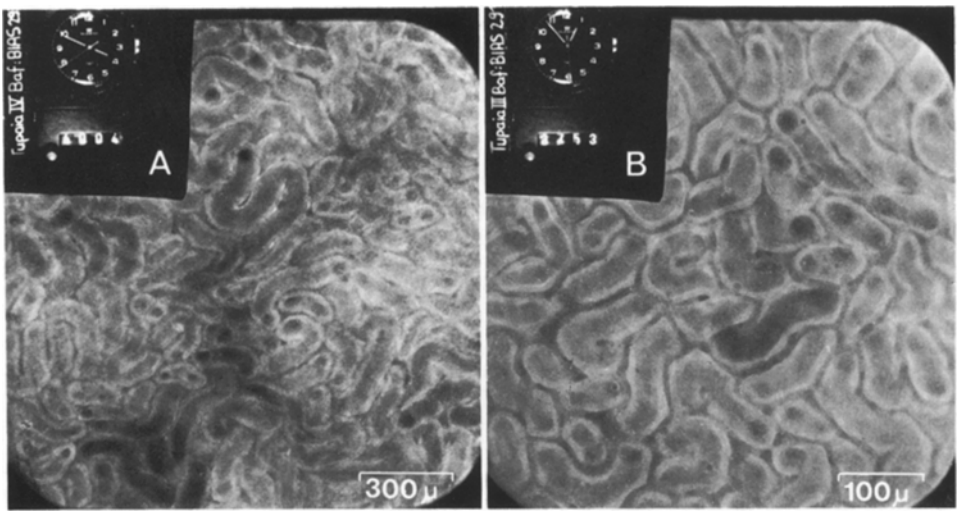


Fig. 4. In vivo microphotographs of the renal surface of *Tupaia* 48 h after occluding left renal artery for two hours (A) and for one hour (B). In the survey magnification at the left, dilated tubules filled with lissamine green abut onto nondilated tubules. At the right at higher magnification some proximal tubules are intensely dilated

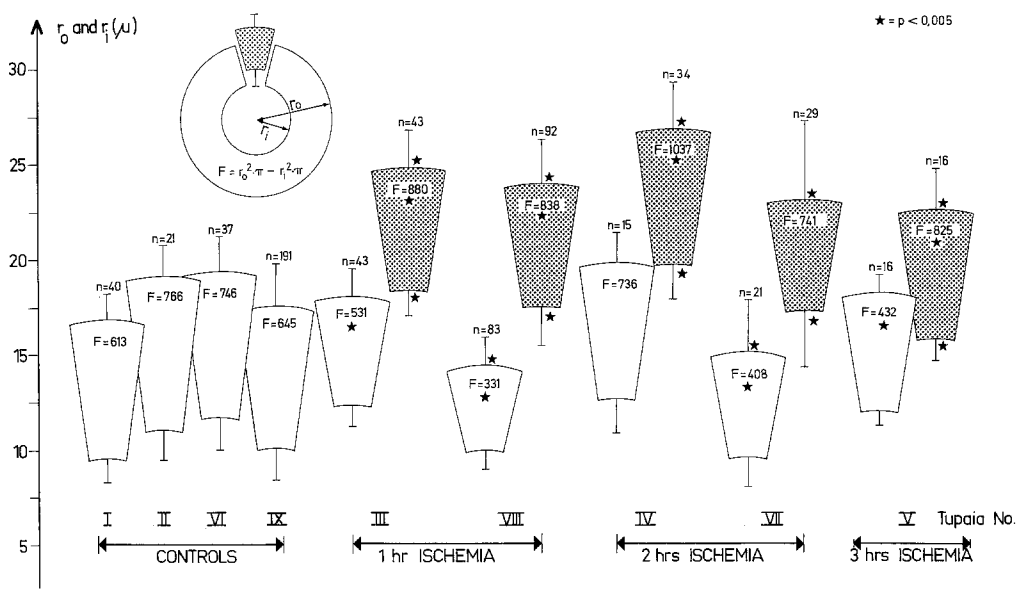


Fig. 5. Schematic representation of the sizes of proximal tubules measured from in vivo microphotographs of *Tupaia* under control conditions as well as after temporary ischemia. The number of the animal is given in Roman numerals. Segments of the tubules are reproduced, as the inset shows. r_i radius of the lumen; r_o half of the entire diameter of the proximal tubule; F area of the cross-sectioned epithelium (M ± S.D., n = number of tubules)

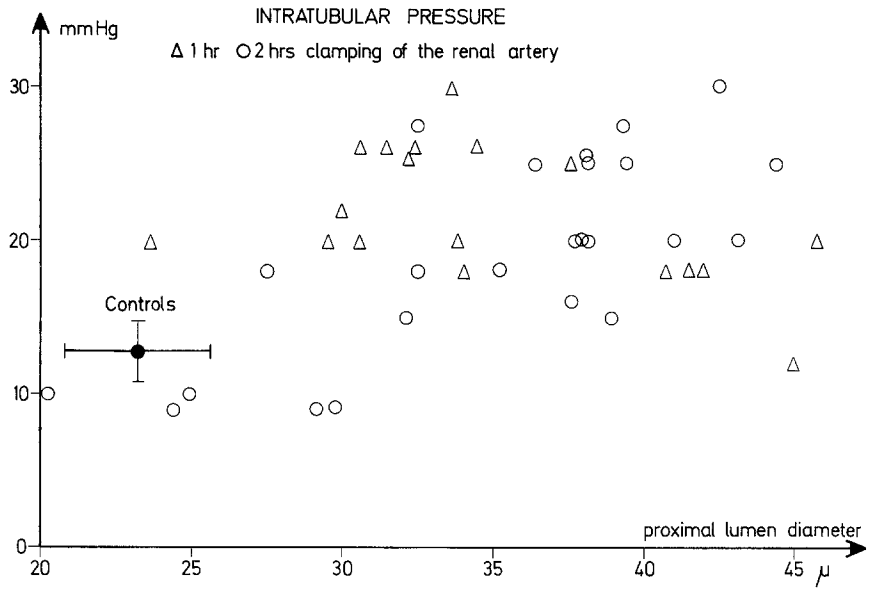


Fig. 6. Intratubular rises in pressure in the proximal tubules of *Tupaia belangeri* after temporary ischemia for one or two hours, plotted against the diameters of the proximal tubules lumina measured simultaneously from microphotographs. Both sets of values are increased over those of controls

increase in cross-sectional area of the epithelial cells might be due to swelling but might also represent a filling-out of the tubule, whereby it approximates to an ideal tube, thus simulating an increase in epithelial surface. The same might hold true for nephrons whose epithelial cross-section seemed to decrease.

The increase in lumen after 1 and 2 h of ischemia is associated with an increase in intratubular pressure. Figure 6 lists the intratubular pressures and the accompanying diameters of the proximal tubules. After 3 h of ischemia (Fig. 7) convoluted tubules observed with the epimicroscope are found to be completely blocked with proteinaceous material.

The longer the ischemia lasts, the more the clearance values for inulin and the concentrating ability of the kidney diminish (Fig. 8).

To be able to prove that tubular blockage after temporary ischemia is a cause of acute renal failure, we measured the intratubular pressure in nephrons as was done previously in rats (Tanner and Steinhausen, 1976). We simultaneously infused an equilibrium solution into the same tubule with the aid of a second micropipette. When the lumen of the nephron is clear, the equilibrium solution flows easily through the loops of Henle without much increase in tubular pressure at even maximal rates of 50 nl/min.

Figure 9 summarizes our results of perfusion studies. Along the abscissa are the perfusion-flow-rates we used, expressed in nl/min, along the ordinate the maximal pressures produced are given. Each animal was assigned its own abscissa. The maximal pressures obtained in the same nephron by comparable perfusions-flow-rates are joined to form curves. As the maximal pressures were

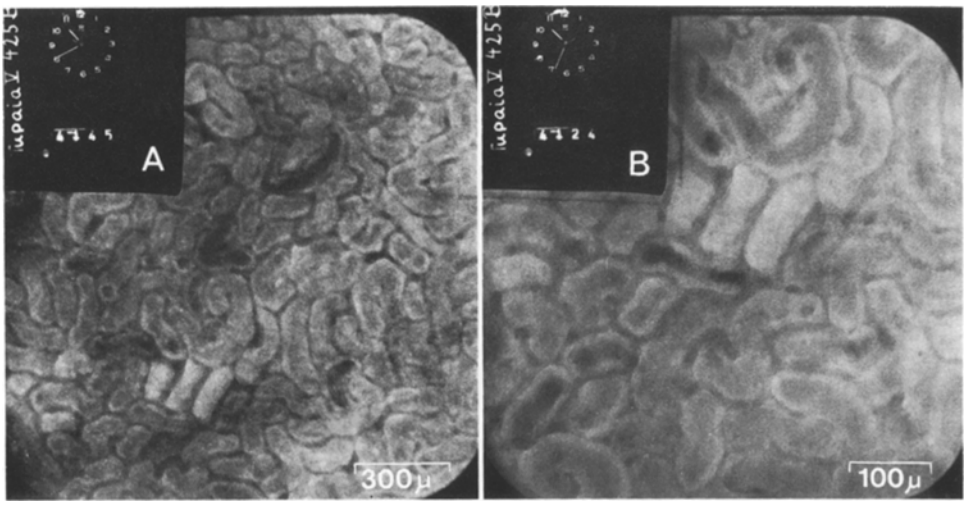


Fig. 7. After temporary ischemia of the *Tupaia belangeri* kidney for 3 h, one can recognize single homogeneous whitened loops of tubules, which fail to stain with lissamine green. These are most likely tubules obstructed with proteinaceous material. At the left, lower magnification; at the right, higher magnification

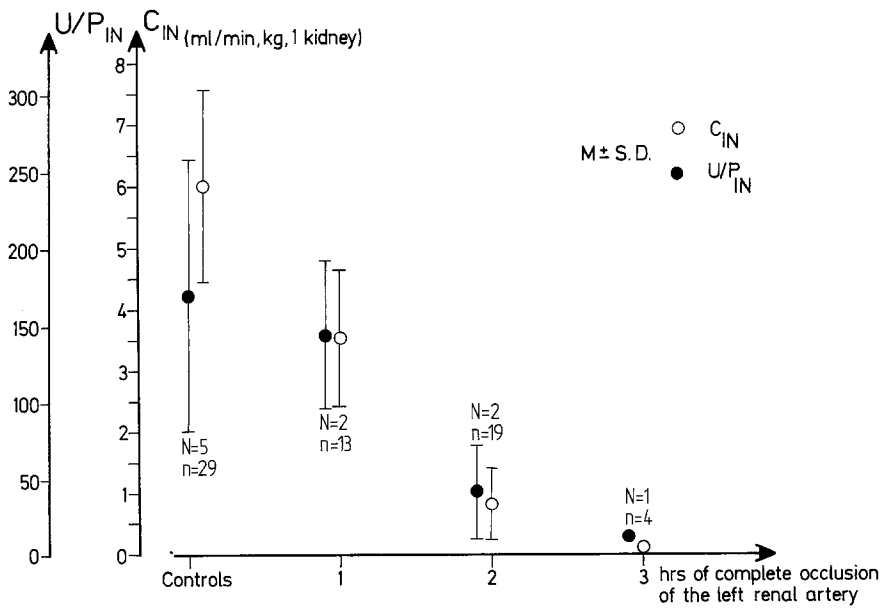


Fig. 8. Glomerular filtration rates (inulin clearance) and urinary concentration (U/P-inulin) of *Tupaia* under control conditions and after temporary ischemia. *N* number of experimental animals, *n* number of urine collections lasting from 15–30 min from which average values were calculated

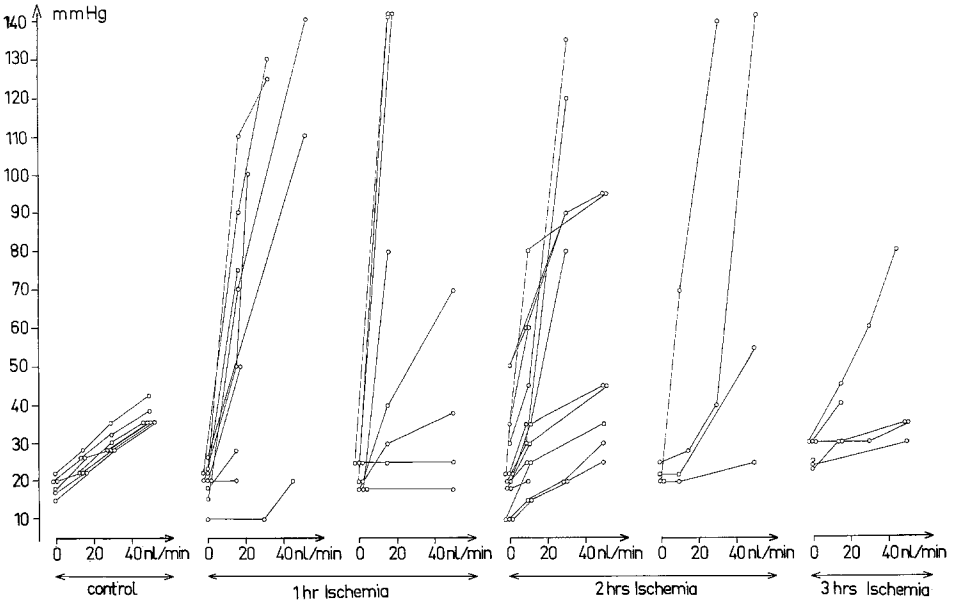


Fig. 9. Microperfusion studies in *Tupaia* with measurements of intratubular pressure in the same nephron under control conditions and after temporary ischemia. The perfusion flow-rates are given along the abscissa; along the ordinate are the corresponding intratubular pressures. Each animal has its own abscissa. The individual curves connect the measurements made in the same nephron. The values give the maximal pressures recorded at the corresponding flow-rates. The duration of perfusion for the flow-rates used (in nl/min) varied from 2 to 5 min

reached the tubular wall often ruptured and dye was seen to escape. After three hours of ischemia the tubular epithelium is so friable that this finding alone can explain why there is no increase in intratubular pressure. Occasionally we succeeded in washing out a nephron and during the perfusion we saw single proteinaceous casts suddenly swim by.

With these perfusions we were often able to produce high intratubular pressures for longer periods of time (5 to 10 min), but could not wash the tubules free, and the pressures never fell. When we suddenly withdrew the micropipette from the tubule, proteinaceous material, now stained green by the perfusion solution, regurgitated up the nephron as the intratubular pressure decreased.

As the pressure increases during microperfusion the dilated tubules become further distended. Figure 10 shows the relationship between pressure and tubular diameter for single nephrons; the same nephrons are connected by lines.

The numbers by the symbols give the perfusion-flow-rates used to produce the increases in pressure. The regurgitation of intratubular material mentioned above that occurs after sudden release of intratubular pressure, most probably results from retraction of the dilated tubule which, despite ischemia, still retains its elasticity.

Histologically the following pathological changes were seen: After a *1 h occlusion of the renal artery,* the cortex of the *Tupaia* kidney showed many

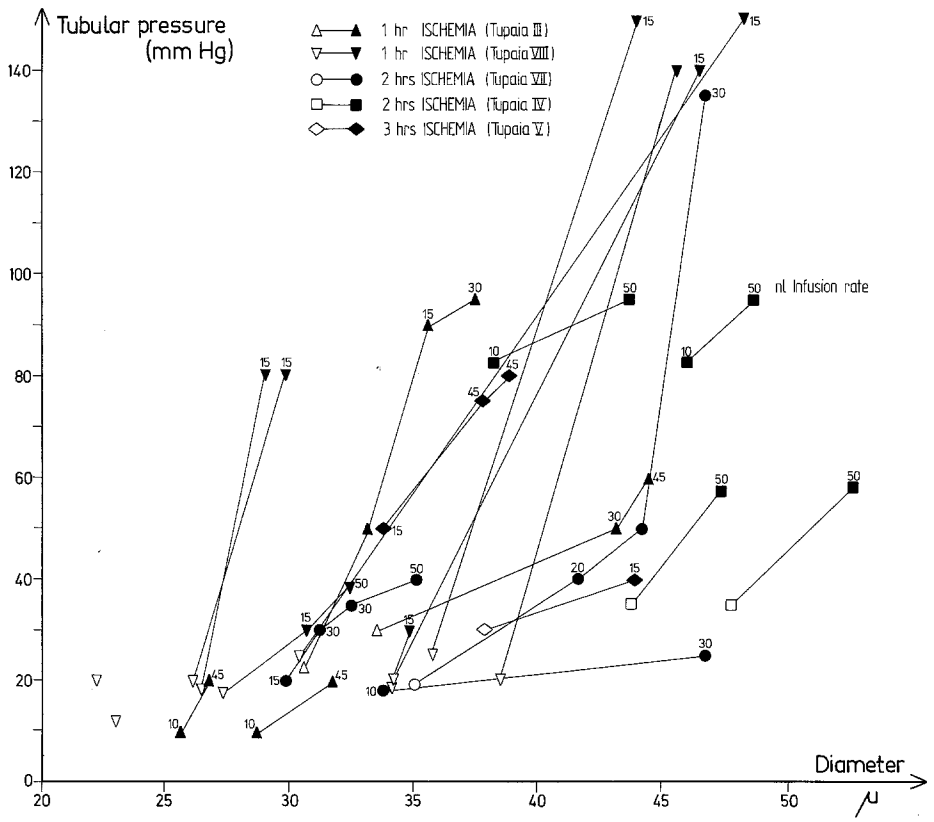


Fig. 10. Diameters of the lumen of proximal tubules of *Tupaia belangeri* measured from in vivo microphotographs taken during intratubular perfusion after temporary ischemia. Diameters correlate with the intratubular pressures used. The values for the same nephron are connected. The open signs give the values prior to perfusion. The small numbers over the closed signs give the values for the perfusion flow-rates used

tubules dilated in both proximal and distal convoluted portions (Fig. 11B). Their lumina were distended two to three times normal, contrasting sharply with those of the adjacent normal nephrons whose small lumina gave the true measure of epithelial shrinkage after death and fixation (Tanner and Steinhausen, 1976; Steinhausen et al., 1963). The epithelial cells lining the dilated tubules were correspondingly flattened, less eosinophilic and granular than the cells of neighboring normal tubules. Occasionally pale-staining, proteinaceous material lay in the dilated lumina of descending segments and in collecting tubules of the medulla. In general, most large blood vessels were empty (washed free of blood by formalin perfusion) but occasional peritubular capillaries and glomeruli still contained blood cells. The interstitium exhibited no abnormalities.

After a *2 h occlusion of the renal artery* (Fig. 11C) virtually all tubules were dilated and at least half contained proteinaceous material, either like the loose, flocculated and amorphous material seen in Bowman's space but more

often homogeneous, darkly-stained hyalin casts that filled the dilated lumina. Excluding the outer cortex, all parts of the kidney, especially the region of the inner cortex and outer medulla showed various and extensive necrobiotic changes, nuclear pyknosis, lysis and cellular necrosis in tubular cells. Adjacent cells were often in mitosis. Many of the descending tubules in the medulla and collecting tubules of the papilla contained proteinaceous deposits. These, in the region of Henle's loop, greatly distended these thin segments. Although most large blood vessels were empty, occasional arterioles, glomeruli and peritubular capillaries were congested with blood containing increased numbers of neutrophilic granulocytes. Patchy regions of the cortical interstitium were enlarged and oedematous, staining a pale blue and separating the tubular segments. The oedema of the medulla was even more extensive, especially in the papilla.

After a *3 h occlusion of the renal artery* nearly all parts, especially at the cortico-medullary junction, revealed extensive coagulative necrosis. Although the tubular epithelium generally remained only as a finely granular ring of proteinaceous material about an ill-defined lumen distended with protein-rich fluid, the contours offered by the residual basement membranes showed clearly that tubular dilation had preceeded cell necrosis. The interstitium was patchily oedematous, but in the medulla and upper papilla was distended with blood-engorged capillaries or extravasations of blood containing increased numbers of polymorphonuclear leukocytes. The glomeruli and blood vessels disclosed no abnormalities.

B. Noradrenaline – Perfusion in the Renal Arteries of Rats

As Figure 12 reveals, 48 h after the intra-arterial perfusion of noradrenaline the inulin clearance is decreased. The degree of injury induced by perfusion depends on its duration. A perfusion of two hours results in almost complete anuria, and the kidney looses its ability to concentrate (cf. U/P-inulin). In contrast to that of the *Tupaia,* the surface of the rat kidney after perfusion of noradrenaline is more uniformly damaged; that uniform appearance, however, may be broken by single proteinaceous casts, which occasionally shine through the tubular walls. The greater the degree of damage, the greater are the passage times for lissamine green. After perfusing with noradrenaline for 45 min, the passage-time for lissamine green for the proximal convoluted tubule (the time it takes lissamine green to reach the late proximal tubules minus the time it needs to reach peritubular capillaries after its i.v. injection) varied from 13 to 16 s (control values 10.7 ± 0.51 s cf. Steinhausen and Tanner, 1976). After 60 min, the passage time varied from 28 to 36 s. After 75 min the passage time was more than 40 s; after 120 min it was not measurable. In the same experiments some of the early proximal tubules near glomeruli are rapidly stained with lissamine green after severe damage (Fig. 13), and the tubules are, in general, uniformly dilated (see Fig. 14). After perfusion of noradrenaline the pressure in the proximal tubules was noticeably increased (see Fig. 15). In these rats we also investigated the flow of urinary filtrate along the same nephron by simultaneously perfusing the nephron and monitoring its intra-

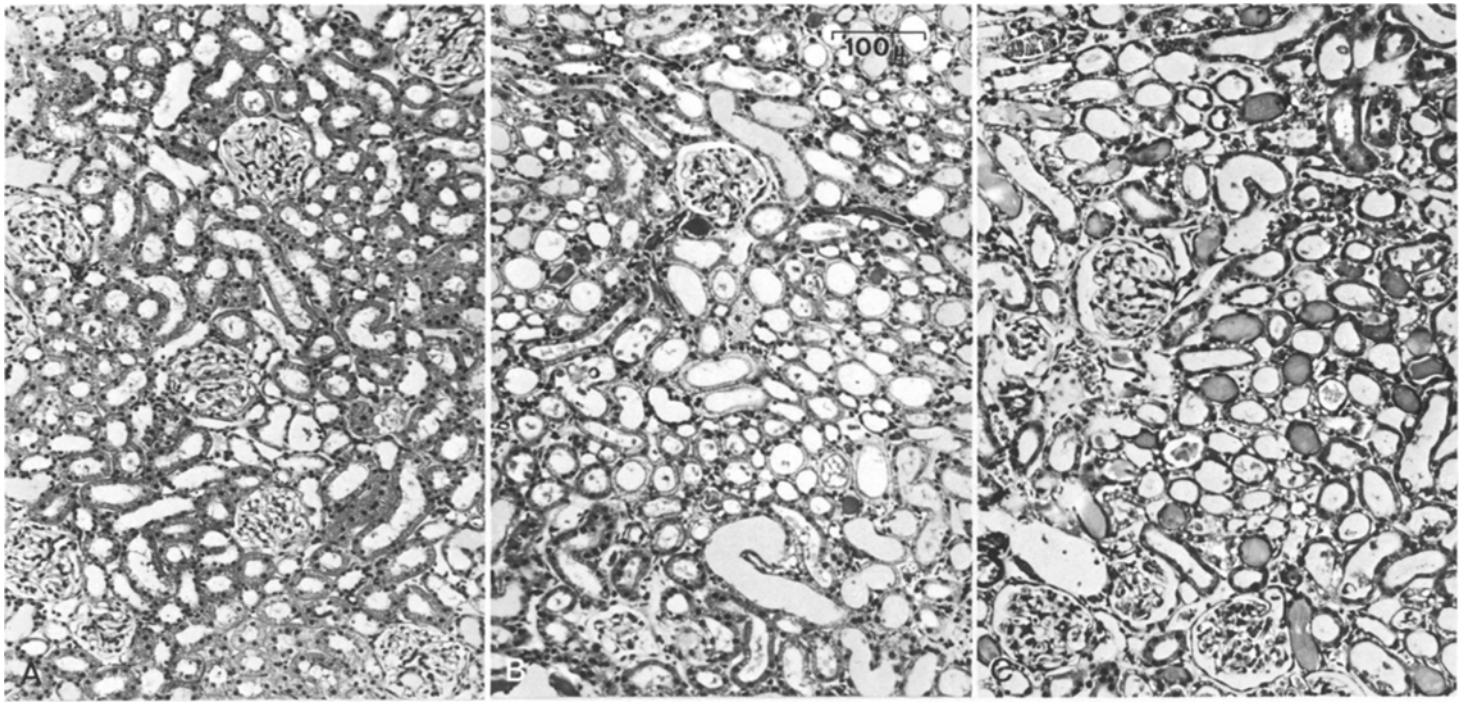
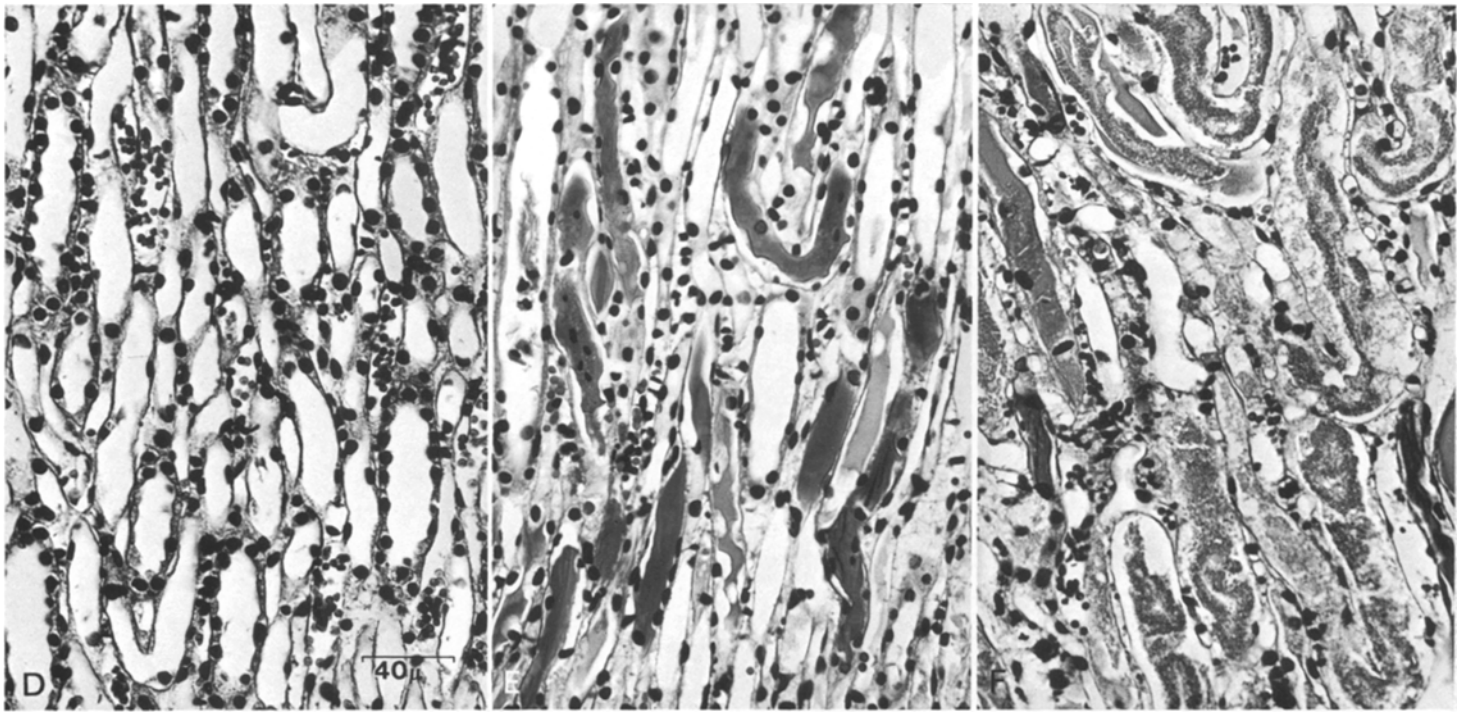


Fig. 11. A Renal cortex of normal *Tupaia* kidney after fixation by formalin-perfusion. Hematoxylin-eosin stain. **B** *Tupaia* renal cortex 48 h after a 1 h temporary ischemia. The tubuli are dilated in general, a few contain proteinaceous casts. **C** *Tupaia* renal cortex 48 h after a 2 h temporary ischemia. The number of tubuli filled with proteinaceous casts is greatly increased when compared with Fig. 11 B.



D Outer medulla of a normal *Tupaia* kidney showing normal loops of Henle. Hematoxylin-eosin stain. **E** Outer medulla of *Tupaia* kidney 48 h after a 2 h temporary ischemia. Descending tubules and Henle's loops blocked by proteinaceous casts. **F** Same part of *Tupaia* kidney as in Fig. 11 D and E, 48 h after a 3 h temporary ischemia. The changes are characterized by stasis of blood in peritubular capillaries, extensive necrosis of tubular epithelium, and abundant proteinaceous material filling tubular lumina

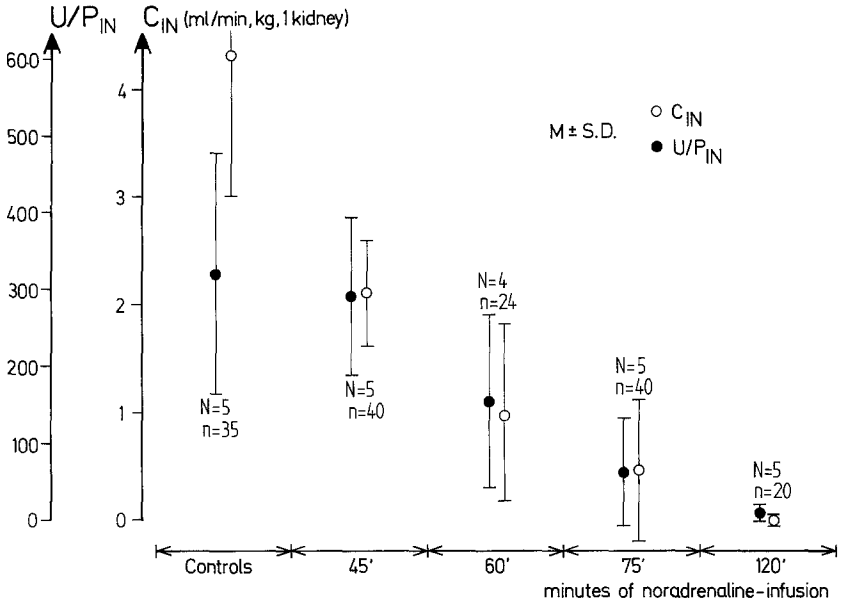


Fig. 12. Glomerular filtration rates (inulin clearance) and concentrating capacities (U/P-inulin) of rat kidneys under normal control conditions, and 48 h after perfusion of noradrenaline into the renal artery for 45 to 120 min. *N* number of experimental animals, *n* number of clearance periods lasting 15 to 30 min

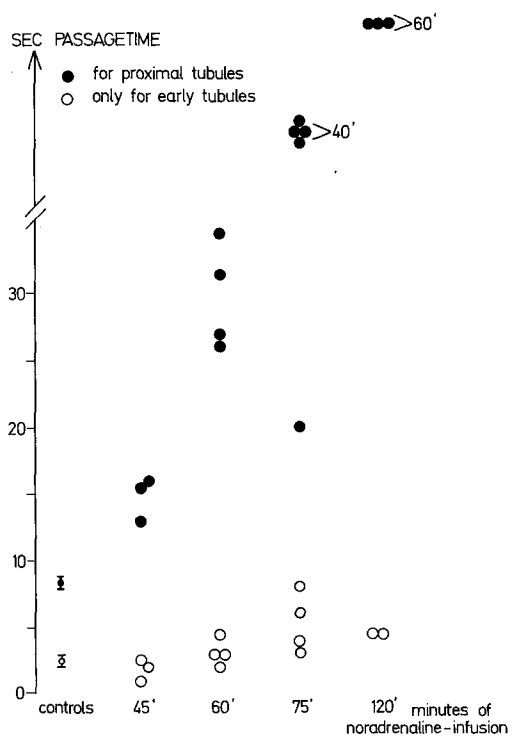


Fig. 13. Passage times in proximal tubules under normal conditions and 48 h after temporary ischemia induced by noradrenaline perfusions in the renal artery. (*Dark circles*). The open circles give the times needed for staining of individual, paraglomerular, early proximal tubules after intravenous injection of lissamine green

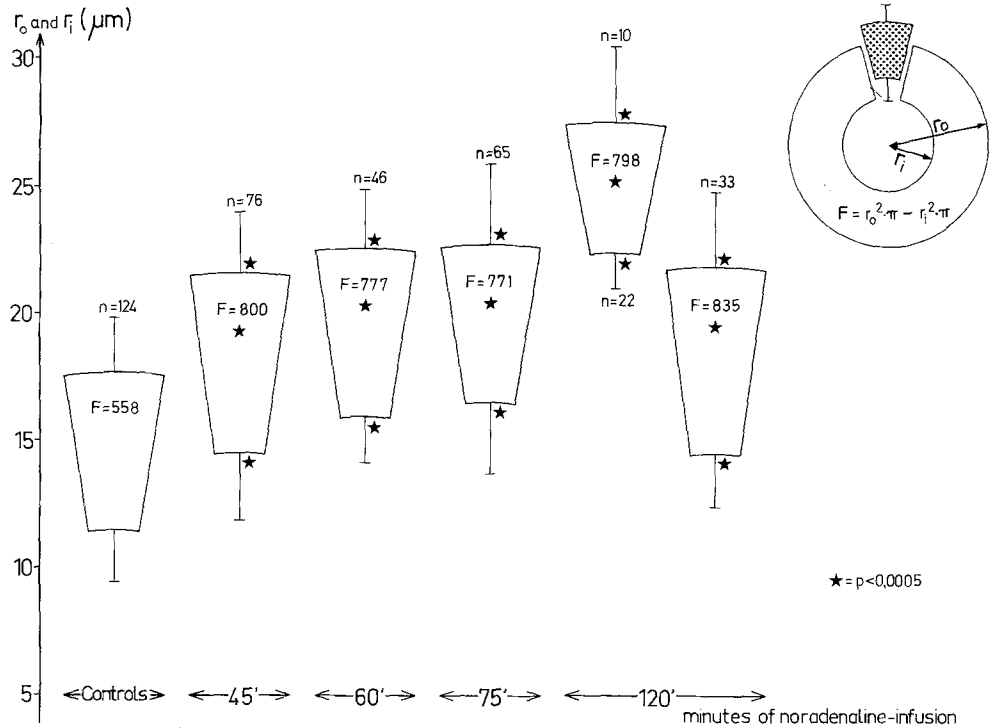


Fig. 14. Schematic representation of the sizes of proximal tubules of rats under control conditions and 48 h after infusion of noradrenaline into the renal artery. The symbols used are the same as in Fig. 5

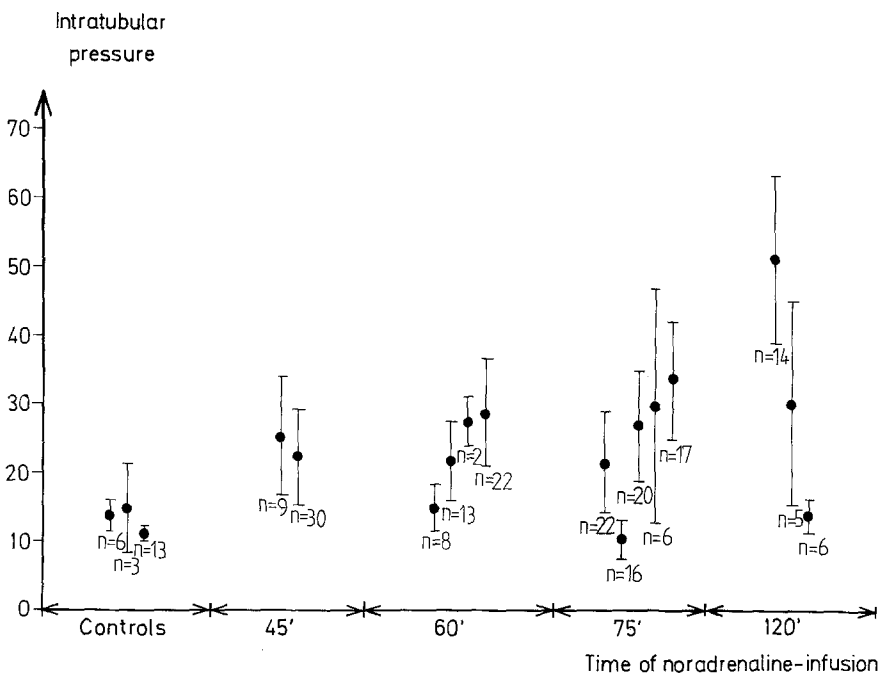


Fig. 15. Intratubular pressures in rat kidneys under normal control conditions and 48 h after infusing noradrenaline into renal artery. Mean value and standard deviation were calculated for each animal. *n* number of tubular punctures made in each animal

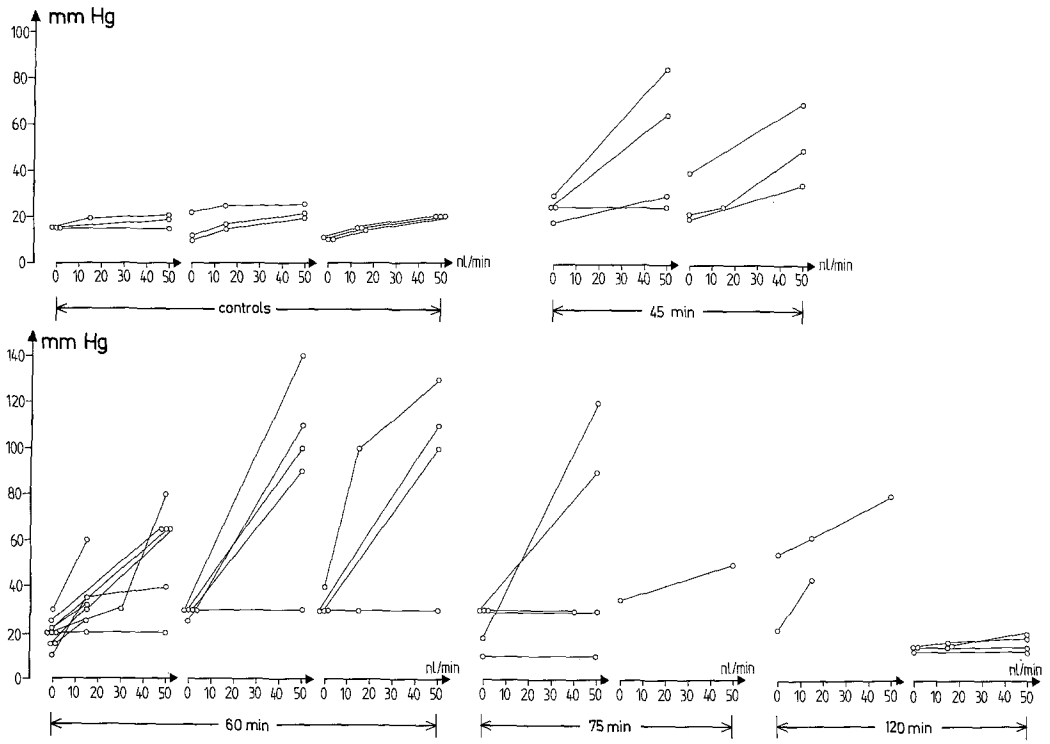


Fig. 16. Microperfusion studies in rats using various flow-rates of perfusate (in nl/min), plotted against the intratubular pressures measured in the same nephron, 48 h after infusing noradrenaline into the renal artery. This graph is equivalent to Fig. 9

luminal pressures. Figure 16 presents the results. Under control conditions there was no increase in intratubular pressure and we could easily follow the downstream flow of the dye along the distal convolution. In most instances when the intratubular pressure increased during microperfusion, the nephron could not be washed out. We found that after severe damage of the tubular system following temporary ischemia, if the pressure failed to rise during perfusion then the tubular wall was abnormally permeable, that is to say, it leaked. In that case colored perfusion-fluid was observed outside the nephron and in the lumen proximal to the site of puncture by the micropipette.

Discussion

The results present here leave no doubt that in these models proteinaceous material in the tubular lumen can block the flow of urinary filtrate. To wash such material out of the tubules intratubular pressures are needed that exceed the systolic blood pressure – pressures that never normally occur in the nephron. Our studies do not allow us to answer the question why psychic stress in *Tupaia belangeri* can cause acute renal failure (von Holst, 1972). In contrast

to what we expected, the *Tupaia* kidney is considerably less sensitive to complete ischemia, as produced by clamping the renal artery, than that of the rat, which becomes completely anuric after only 1 h of ischemia (Eisenbach and Steinhausen, 1973; Eisenbach et al., 1974). As our in vivo and histological observations of tubular dimensions showed, dilatation of the lumen of proximal segments is a characteristic of acute renal failure, a finding in full agreement with Bohle et al. (1976).

The question that remains unanswered is: how is acute renal failure in man brought about by circulatory collapse? From our studies with noradrenaline we are inclined to suggest that the treatment of shock with catecholamines may contribute to renal damage and failure. Of course, when severe hypotension develops after trauma, blood loss, etc. and fails to respond to blood-volume expanders, then the clinicians only recourse is, ultima ratio, to administer vasopressive agents (Merril, 1965; Lindseth et al., 1975). Since stimulation of the sympathetic nervous system reduces the blood-flow through the kidney, therapy with catecholamines may well cause further constriction of renal vessels, further reducing renal blood flow, thereby critically injuring the kidneys. Although the systemic blood pressure may rise and the patient survive, acute renal failure will occur, the sequence of events differing little from that of our experimental models. We have tried to simulate these mechanisms by infusing noradrenaline intravenously into rats. In occasional experiments we indeed were able to produce renal damage that resembled that seen after infusion of noradrenaline into the renal artery. That model, however, proved to be unsatisfactory as a standard method, since too many of the animals died from pulmonary oedema and other complications.

We need to ask why the friable proteinaceous material filling the injured tubules is easily excreted since myoglobin, when precipitated in the collecting tubules, can readily be washed out with infusions of mannitol (as we were able to show in ciné-microphotographic studies (Steinhausen, 1965)). Apparently the bottle-neck is at the loop of Henle and not in the system of collecting ducts. Ischemia damages the proximal convoluted tubules primarily, so that cellular debris is discharged from the relatively long portion of the nephron, from the proximal convolutions to the loop of Henle. In addition, we assume that the cast-material must be more structured than is flocculated myoglobin. Fragments of the brush border, and membranes of the mitochondria and the rich endoplasmic reticulum would supply such structure (Thoenes, 1964; Warning and Thoenes, 1972; Donohoe et al., 1978). It seems also that after temporary ischemia the glomerular filtration membrane becomes more permeable to larger molecules. We found, for example, that when FITC labelled dextran with a molecular weight of 130,000 is injected after ischemia but 24–38 h before the animal is studied, it was found to be admixed with intratubular cast-material, whereas under normal conditions dextran of this molecular size is unable to pass through the glomerular filtration membrane (Steinhausen et al., 1977). These results imply that the friable proteinaceous material forming intratubular casts may also consist of flocculated plasma proteins. The results of our experiments allow us only to estimate roughly how much blockage by tubular casts contributes to oliguria. When the intratubular pressure in our double-puncture studies fails to increase it may mean the nephron is either not blocked, or that it leaks. The leakage may occur either proximal to the puncture site in the nephron or around the puncture-hole for the micropipette. The latter source of error can be excluded in large part by our method of direct observation the Ultropak-Epimicroscope. We estimate that about half of the nephrons of the *Tupaia* are blocked by casts after 1–2 h of ischemia and a follow-up period of 48 h. For rats that would correspond to a perfusion time of noradrenaline of 60 min with a similar follow-up period. With shorter periods of ischemia the proportion of functioning nephrons increases, whereas prolonged ischemia promotes cellular necrosis and hence leakage.

Our results fail to support the idea that glomerular filtration changes in the sense that the membrane becomes impermeable for the normal filtrate, as some investigations based on glomerular changes have suggested. Both lissamine green and FITC-dextrans of low molecular weight can be readily identified in the proximal convoluted tubule after injecting them intravenously; that means they passed through the glomerular filtration membrane.

Finally, we need to explain why the pressures in the proximal tubules in front of the blocked loop of Henle are not greater than those we and others

(Tanner and Steinhausen, 1976; Arendshorst et al., 1975) have found after temporary ischemia? We favor the idea that the lower pressures result from dilatation of the vas efferens; as that dilates the pressure in the glomerular capillaries falls. The longer the hypoxia persists, the more the muscle cells of the vas efferens should lose their ability to contract. It has always been easier for us to imagine that hypoxia is more likely to depress the contractability of a contractile system than to stimulate additional energy needed for increased contraction, especially after injury. Balint and Szöcs (1976) assumed from their high values for renal blood flow associated with falling glomerular filtrate that the postglomerular resistance diminishes. To prove that assumption we carried out the following experiment: In female Wistar-Furth rats, in which glomeruli can be visualized at the renal surface, we measured the stop-flow pressures according to Gertz et al. (1966). With a third micromanipulator we perfused sodium nitroprusside, one of the most potent vasodilators (Kreye et al., 1975), directly into the appropriate glomerulus. The backpressure immediately fell and filtration ceased. Consequently, the vas efferens must regulate and resist the flow of blood and after ischemic injury, its resistance decreases explaining the drastic decline in filtration. It is these aspects of acute renal failure that we are pursuing in our present studies.

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References

Arendshorst, W.J., Finn, W.F., Gottschalk, C.W.: Pathogenesis of acute renal failure following temporary renal ischemia in the rat. Circ. Res. **37**, 558–568 (1975)

Balint, P., Szöcs, E.: Intrarenal hemodynamics following temporary occlusion of the renal artery in the dog. Kidney Int. **10**, S128–S136 (1976)

Bank, N., Mutz, B.F., Aynedjian, H.S.: The role of "Leakage" of tubular fluid in anuria due to mercury poisoning. J. clin. Invest. **46**, 695–704 (1967)

Blantz, R.C.: The mechanism of acute renal failure after uranyl nitrate. J. clin. Invest. **55**, 621–635 (1975)

Bohle, A., Jahnecke, J., Meyer, D., Schubert, G.E.: Morphology of acute renal failure: Comparative data from biopsy and autopsy. Kidney Int. **10**, 9–16 (1976)

Brade, W., Herken, H., Merker, H.J.: Schädigung und Regeneration renaler Tubuluszellen nach Folsäuregabe. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. **262**, 228–250 (1969)

Churchill, S., Zarlengo, M.D., Carvalho, J.S., Gottlieb, M.N., Oken, D.E.: Normal renocortical blood flow in experimental acute renal failure. Kidney Int. **11**, 246–255 (1977)

Cox, J.W., Baehler, R.W., Sharma, H., D'Dorisio, Osgood, R.W., Stein, J.H., Ferris, T.F.: Studies on the mechanism of oliguria in a model of unilateral acute renal failure. J. clin. Invest. **53**, 1546–1558 (1974)

Daugharty, T.M., Brenner, B.M.: Reversible hemodynamic defect in glomerular filtration rate after ischemic injury. Am. J. Physiol. **228**, 1436–1439 (1975)

Dobyan, D.C., Nagle, R.B., Bulger, R.E.: Hypovolemic models of acute tubular necrosis in the rat kidney. Virchows Arch. B Cell Path. **25**, 271–280 (1977)

Donohoe, J.F., Venkatachalam, M.A., Bernard, D.B., Levinsky, N.G.: Tubular leakage and obstruction after renal ischemia: Structural-functional correlations. Kidney Int. **13**, 208–222 (1978)

Eisenbach, G.M., Kitzlinger, B., Steinhausen, M.: Renal blood flow after temporary ischemia of rat kidneys. (Renal venous outflow and clearance techniques). Pflügers Arch. **347**, 223–234 (1974)

Eisenbach, G.M., Steinhausen, M.: Micropuncture studies after temporary ischemia of rat kidneys. Pflügers Arch. **343**, 11–25 (1973)

Flamenbaum, W.: Pathophysiology of acute renal failure. Arch. Intern. Med. **131**, 911–928 (1973)

Flamenbaum, W., McDonald, F.D., DiBona, G.F., Oken, D.E.: Micropuncture study of renal tubular factors in low dose mercury poisoning. Nephron **8**, 221–234 (1971)

Flanigan, W.J., Oken, D.E.: Renal micropuncture study of the development of anuria in the rat with mercury-induced acute renal failure. J. clin. Invest. **44**, 449–457 (1965)

Fuehr, J., Kaczmarczyk, J., Krüttgen, C.D.: Eine einfache Methode zur Inulinbestimmung für Nieren-Clearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. Klin. Wschr. **33**, 729–730 (1955)

Gertz, K.H., Mangos, J.A., Braun, G., Pagel, H.D.: Pressure in the glomerular capillaries of the rat kidney and its relation to arterial blood pressure. Pflügers Arch. **288**, 369–374 (1966)

Holst, D. von: Renal failure as the cause of death in *Tupaia belangeri* exposed to persistent social stress. J. comp. Physiol. **78**, 236–273 (1972)

Hsu, Ch.H., Kurtz, Th.W., Goldstein, J.R., Keinath, R.D., Weller, J.M.: Intrarenal hemodynamics in acute myohemoglobinuric renal failure. Nephron *17*, 65–72 (1976)

Kashgarian, M., Siegel, N.J., Ries, A.L., DiMeola, H.J., Hayslett, J.P.: Hemodynamic aspects in development and recovery phases of experimental postischemic acute renal failure. Kidney Int. **10**, S160–S168 (1976)

Knapp, R., Hollenberg, N.K., Busch, G.J., Abrams, H.L.: Prolonged unilateral acute renal failure induced by intraarterial norepinephrine infusion in the dog. Invest. Radiol. **7**, 164–173 (1972)

Koletsky, S., Gustafson, G.E.: The effects of temporary cessation of renal blood flow in rats. J. clin. Invest. **26**, 1072–1078 (1947)

Kreye, V.A.W., Baron, G.D., Lüth, J.B., Schmidt-Gayk, H.: Mode of action of sodium nitroprusside on vascular smooth muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. **288**, 381–402 (1975)

Krian, A., Grabensee, B., Rümpf, P., Zumfelde, L., Lennartz, H.: Das akute postoperative Nierenversagen. Langenbecks Arch. Chir. **342** (Kongreßbericht 1976), p. 49

Lindseth, R.E., Hamburger, R.J., Szwed, J.J., Kleit, S.A.: Acute renal failure following trauma. J. Bone Joint Surg. **57-A**, 830–835 (1975)

Litten, M.: Über pathologische Verkalkungen und Kalkmetastasen in den Nieren. Virchows Arch. **83**, 509–547 (1881)

Mason, J., Takabatake, T., Olbricht, Ch., Thurau, K.: The early phase of experimental acute renal failure. III. Tubuloglomerular feedback. Pflügers Arch. **373**, 69–76 (1978)

Merril, J.P.: The treatment of renal failure. Therapeutic principles in the managment of acute and chronic uremia. New York and London: Grune and Stratton 1965

Minuth, A.N., Terrell, J.B., Jr., Suki, W.N.: Acute renal failure: a study of the course and prognosis of 104 patients and of the role of furosemide. Am. J. Med. Sci. **271**, 317–324 (1976)

Schmidt, U., Dubach, U.C.: Acute renal failure in the folate-treated rat: Early metabolic changes in various structures of the nephron. Kidney Int. **10**, S39–S45 (1976)

Schubert, G.E.: Folic acid-induced acute renal failure in the rat: Morphological studies. Kidney Int. **10**, 46–50 (1976)

Schwaier, A.: Breeding Tupaias (*Tupaia belangeri*) in captivity. Z. Versuchstierk. **15**, 255–271 (1973)

Sonnenberg, H., Deetjen, P., Hampel, W.: Methode zur Durchströmung einzelner Nephronabschnitte. Pflügers Arch. ges. Physiol. **278**, 669–674 (1964)

Stein, J.H., Sorkin, M.J.: Pathophysiology of a vasomotor and nephrotoxic model of acute renal failure in the dog. Kidney Int. **10**, S86–S93 (1976)

Steinhausen, M.: Mikrozirkulation von Lissamingrün an der Warmblüterniere. Film D 886. Göttingen: Inst. Wiss. Film 1965

Steinhausen, M., Eisenbach, G.-M., Böttcher, W.: High-frequency microcinematographic measurements on peritubular blood flow under control conditions and after temporary ischemia of rat kidneys. Pflügers Arch. **339**, 273–288 (1973)

Steinhausen, M., Eisenbach, G.-M., Galaske, R.: A countercurrent system of the surface of the renal cortex of rats. Pflügers Arch. **318**, 244–258 (1970)

Steinhausen, M., Eisenbach, G.-M., Helmstädter, V.: Concentration of lissamine green in proximal tubules of antidiuretic and mercury poisoned rats and the permeability of these tubules. Pflügers Arch. **311**, 1–15 (1969a)

Steinhausen, M., Tanner, G.A.: Microcirculation and tubular urine flow in the mammalian kidney cortex (in vivo microscopy). Sitzungsberichte der Heidelberger Akademie der Wissenschaften, Mathematisch-naturwissenschaftl. Klasse, 3. Abhdl. Berlin-Heidelberg-New York: Springer 1976

Steinhausen, M., Thederan, H., Nolinski, D., Dallenbach, F.D., Schwaier, A., Höfling, K.-H.: Postischämisches akutes Nierenversagen an *Tupaia belangeri* und *Rattus norvegicus*. Göttingen: Scientific film by Inst. f. d. Wiss. Film (in prep.)

Steinhausen, M., Wayland, H., Fox, J.R.: Renal test dyes V. Quantitative analysis of tubular passage of FITC-dextrans in kidney of rats. Pflügers Arch. **369**, 273–279 (1977)

Steinhausen, M., Weidinger, H., Ross, H.J., Eisenbach, G.M.: Incident-light microscopy of the renal surface during stimulation of the sympathetic renal nerves. In: Progress in nephrology, (G. Peters and F. Roch-Ramel, eds.) Berlin-Heidelberg-New York: Springer 1969b

Tanner, G.A., Sloan, K.L., Sophasan, S.: Effects of renal artery occlusion on kidney function in the rat. Kidney Int. **4**, 377–389 (1973)

Tanner, G.A., Sophasan, S.: Kidney pressures after temporary renal artery occlusion in the rat. Am. J. Physiol. **230**, 1173–1181 (1976)

Tanner, G.A., Steinhausen, M.: Tubular obstruction in ischemia-induced acute renal failure. Kidney Int. **10**, S67–S73 (1976)

Thoenes, W.: Mikromorphologie des Nephron nach temporärer Ischämie. In: Zwanglose Abhandlungen aus dem Gebiet der normalen und pathologischen Anatomie (Bargmann, W., Doerr, W., eds.), Heft 15. Stuttgart: Georg Thieme 1964

Ustimowitsch, C.: Über die angebliche zuckerzersetzende Eigenschaft des Glycerins. Pflügers Arch. ges. Physiol. **13**, 453–460 (1976)

Warning, A., Thoenes, W.: Morphologische Untersuchungen zur Okklusion und Wiedereröffnung proximaler Nierentubuli bei kurzfristiger Ischämie. Virchows Arch. Abt. B Zellpath. **11**, 310–325 (1972)

Wiederhielm, C.A., Woodbury, J.W., Kirk, S., Rushmer, R.F.: Pulsatile pressures in the microcirculation of frogs mesentery. Am. J. Physiol. **207**, 173–176 (1964)

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