Kidney International, Vol. 25 (1984), pp. 33–41

Glomerular filtration and tubular reabsorption during anuria in postischemic acute renal failure

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Glomerular filtration and tubular reabsorption during anuria in postischemic acute renal failure. Complete occlusion of the left renal artery for 60 min in the rat produced anuric acute renal failure after 1 day. Using fluorescence microscopy, a television system combined with double slit densitometry, and micropuncture techniques, tubular pressure and tubular flow rates were determined in different segments of superficial nephrons. Intratubular pressures in proximal convolutions of the postischemic kidney were largely heterogeneous due to abnormally increased flow resistance in proximal tubules which were filled with loose obstructive material. Proximal tubular pressure in the control kidney was independent of the site of its measurement and had a mean value of 14.1 mm Hg. In the postischemic kidney pressure decreased gradually along the proximal tubule, its value in the early and late segments being 16.3 and 9.7 mm Hg, respectively. Low pressure in late proximal convolutions excludes a significant flow impediment due to obstruction in more distal segments. The mean nephron filtration rate (SNGFR) obtained by extrapolation of tubular flow data was 62% of the control value, whereas tubular reabsorption was estimated to be 50% above normal. Reduced SNGFR and increased outflux caused a total reabsorption of tubular fluid within 60% of proximal convoluted tubule length. The partial reduction of SNGFR can be explained by increased pressure in early proximal convolutions and reduced glomerular plasma flow known for these kidneys, without postulating a change in glomerular permeability. Tubular obstruction and increased passive outflux in proximal tubules due to cellular damage appear to be crucial mechanisms responsible for the loss of renal function in this model of acute renal failure.

Filtration glomérulaire et réabsorption tubulaire pendant l'anurie due à une insuffisance rénale aiguë postischémique. Une occlusion complète de l'artère rénale gauche pendant 60 min chez le rat a entraîné une insuffisance rénale aiguë anurique après 1 jour. En utilisant la microscopie par fluorescence, un système de télévision combiné avec une densitométrie à double faisceau, et des techniques de microponction, la pression tubulaire et les débits tubulaires ont été déterminés dans différents segments de néphrons superficiels. Les pressions intratubulaires dans les convolutions proximales du rein postischémique étaient largement hétérogènes, en raison d'une augmentation anormale de la résistance au flux dans les tubules proximaux qui étaient remplis avec un matériau lâche obstructif. La pression tubulaire proximale dans le rein contrôle était indépendante du lieu de sa mesure et avait une valeur moyenne de 14,1 mm Hg. Dans le rein postischémique, la pression diminuait graduellement le long du tubule proximal, ses valeurs dans les segments proximal et terminal étant 16,3 et 9,7 mm Hg, respectivement. La faible pression dans les convolutions proximales tardives exclut un ralentissement significatif du flux par obstruction dans les segments plus distaux. La filtration glomérulaire moyenne par néphron (SNGFR), obtenue par extrapolation des valeurs de flux tubulaire était de 62% de la valeur contrôle, tandis que la réabsorption tubulaire était estimée à 50% au-dessus de la normale. La réduction de SNGFR et l'augmentation du flux sortant entraînaient une réabsorption totale du liquide tubulaire dès 60% de la longueur du tubule contourné proximal. Cette réduction partielle de SNGFR peut être expliquée par l'augmentation de pression dans les convolutions proximales précoces et la réduction du flux plasmatique glomérulaire connue dans ces reins, sans postuler de modification de la perméabilité glomérulaire. L'obstruction tubulaire et l'augmentation passive du flux sortant dans les tubules proximaux par l'altération cellulaire semblent être des mécanismes cruciaux responsables de la perte de la fonction rénale dans ce modèle d'insuffisance rénale aiguë.

Recent studies on the pathogenesis of acute renal failure (ARF) emphasize a multifactorial nature of the syndrome [1, 2]. In ARF, GFR may decrease due to reduced glomerular plasma flow [3, 4], decreased glomerular capillary pressure [5–7], reduced glomerular permeability [8–10], and increased tubular pressure as a result of tubular obstruction [11–14]. Furthermore, glomerular filtration may fail to contribute to renal excretory function due to enhanced transepithelial passive backdiffusion [9, 11, 15–19] leading to total reabsorption of the filtrate. Importance of these factors, which have received experimental support, seem to vary to a certain extent with the choice of the experimental model and the time interval after the initial injury. Relative importance of tubular disorders in ARF is stressed by the finding that in different experimental models single nephron (SN) GFR measured by standard micropuncture technique was much higher than expected from renal inulin clearance [9, 11, 12, 15, 19, 20]. However, it remains unclear to what extent different nephrons in a single damaged kidney have a heterogeneous or homogeneous combination of disorders. Heterogeneous tubular pressure observed in different models of ARF [5, 7, 11–14, 16, 21, 22] supports functional heterogeneity at the single nephron level.

In this study, unilateral anuric ARF was induced by temporary renal ischemia 1 day prior to the experiments. The aim of our experiments was to obtain a quantitative survey of superficial nephron parameters such as SNGFR, filtration pressure, and tubular reabsorption rate. A new method [23, 24] using fluorescence microscopy and microphotometry of video recording, allowed us to measure tubular flow rates in the different segments of a nephron without influencing the hydrostatic pressure inside the tubules.

Received for publication March 8, 1982
and in revised form May 3, 1983

Methods

General. Experiments were performed on Wistar rats with a body weight of 180 to 230 g. The control group consisted of 14 normal rats. In ten rats belonging to the experimental group ARF was produced by clamping the left renal artery for 1 hr. They were anesthetized with sodium pentobarbital (Nembutal), 50 mg/kg body wt i.p., the left renal hilus was exposed by a small subcostal incision, and the renal artery was isolated and occluded for 1 hr by a small bulldog clamp with polyethylene tubing covering the jaws. Thereafter, the incision was closed and the animals were returned to their cages for 24 hr.

The animals were fasted the evening prior to the experiments but had free access to water. They were anesthetized with sodium thiobutalbarbital (Inactin), 100 mg/kg body wt i.p. A tracheotomy was performed. Cannulae were placed into the right femoral artery for continuous recording of BP, into the abdominal aorta via the left femoral artery for rapid dye injections and for collecting blood samples, and into the right femoral vein for continuous infusion and dye injections. A solution containing 0.9% sodium chloride, 1% inulin, and 0.1% PAH was infused at a rate of 2.25 ml/hr throughout the experiment. The arterial pressure was continuously recorded with a pressure transducer (Statham P23Db, Oxnard, California). The left kidney was exposed through a flank incision and placed in a micropuncture cup. The left ureter was cannulated. The kidney surface was decapsulated, covered with a dark stained cellophane film with a 1- to 2-mm hole in it. At this opening the kidney surface was either covered with a small round glass plate or rinsed with a warm (37°C) plasma isotonic and isooncotic polypeptide solution (Haemaccel). For measuring renal clearance of inulin and PAH in control kidneys two or three urine samples were collected for 30-min periods. Arterial blood samples (0.3 ml) were collected usually at the mid-point of the first and the end of the last urine collection period. Inulin, PAH, plasma proteins, and hematocrit were measured as previously described [23]. Unless otherwise stated, the data are presented as mean ± SD (N = number of measurements) and statistical comparisons performed by Student's t test.

Microscopy. Observations were made by means of a television monitor using a high sensitivity television camera (Siemens K5B) mounted on a 45° epiillumination microscope (Leitz) and connected with a video tape recorder (Grundig BK 401), a television-mixture system (Sony), and an external camera focused on an oscilloscope. In addition to the microscopic picture, the monitor frame contained a digital clock and analogue pressure measurements (BP and tubular pressure). The light with a wave length of 540 nm for normal observation and of 490 nm for fluorescence excitation was obtained from a high pressure xenon lamp (Leitz XBO 150) with intereference filters and was focused on the kidney surface by an objective (Leitz, UM 32). The emitted fluorescent light was filtered at 530 nm and observed with an immersion objective (Leitz, UO 11 W).

Dye transit time. To visualize tubular flow a 10 μl bolus of a 4% solution of fluorescein isothiocarbamyl labelled dextran with a molecular weight of 3000 daltons (FITC-3K) was rapidly injected into the abdominal aorta. Unlike normally used renal test dyes such as lissamine green [25], FITC-3K is not bound to proteins and the tubular wall. This property of FITC-3K allows a more precise location of the intratubular dye bolus since surrounding renal structures are practically invisible in the fluorescent image. The filtrable dye appeared within 1 sec in peritubular capillaries and subsequently in proximal convolutions. Arrival time (AT) of the dye at a convolution was defined as the time-lag between the dye maxima at the superficial star vessels and at the given convolution.

Identification of nephrons. To identify proximal convolutions belonging to the same nephron and to determine AT in the first superficial convolutions of different nephrons, passage of FITC-3K was recorded from a microscopic field covering a 0.56 mm^2 surface area. The convoluted tubules in the observed image were drawn on a transparent sheet placed on the television monitor. AT in different convolutions was noted. The convolution with the shortest AT was injected with 4% FITC-3K solution using a micro-pipette (1 to 2 μm OD). The pipette was stained at the outer tip with a platinum containing porcelain glaze [26] to make it visible and was connected air-free by a long narrow teflon tube to a glass syringe. The convolutions in which the microinjected dye appeared were identified as belonging to the same nephron. This procedure was repeated on remaining convolutions to identify further nephrons (Fig. 1).

Pressure measurements. Hydrostatic pressures in convoluted proximal tubules and in peritubular capillaries were measured with a servo nulling device [27] [Instrumentation for Physiology and Medicine (IPM), San Diego, California], using a pipette with a stained tip (1 to 2 μm OD) filled with 2 M sodium chloride solution. Stop-flow pressure was measured in the early proximal convolution after filling the nephron retrogradely with Sudan red-stained castor oil. The convolution chosen for this measurement was that with the shortest AT (always less than 2 sec) within the observed frame.

Tubular flow measurements. The mean flow velocity in a convoluted tubule was determined through video recording by measuring the velocity of the intensity maximum of an intraluminal dye bolus (compare with [24]). A dye bolus was produced either by injecting the dye directly into a proximal convolution or by an intra-arterial injection. A rapid microinjection was possible with a pressure wave produced by tapping the syringe connected to the micropipette with a thin metal rod. Application time and bolus volume were estimated from single video frame analyses to be about 20 msec and 0.01 nl, respectively. The dye intensity was measured with a double slit densitometer (IPM) connected to a coordinate display system for locating the position of each slit and a two-channel chart recorder. The time-lag between maxima was determined after placing densitometer slits at the furtherest ends of a plane tubular section beginning 50 μm distal to the injection site. The tubular length between the two ends was computed from the coordinates at different points along the tubule. The luminal diameter of the tubule was measured with a shearing monitor (IPM) which had been calibrated for the given magnification. An intra-arterial dye injection produced a sufficiently slender intratubular intensity peak to allow a precise velocity measurement. Intratubular dye application which produced the same results as intra-arterial injections had the advantage that the measurements in early proximal convolutions were not disturbed by a residual fluorescence in peritubular capillaries and that a gradual smearing of the dye bolus could be avoided by injecting the dye at a more distal tubular site. To examine the equivalence of the mean tubular flow velocity and that of dye maxima, the former was measured with an oil droplet separating the tubular fluid col-

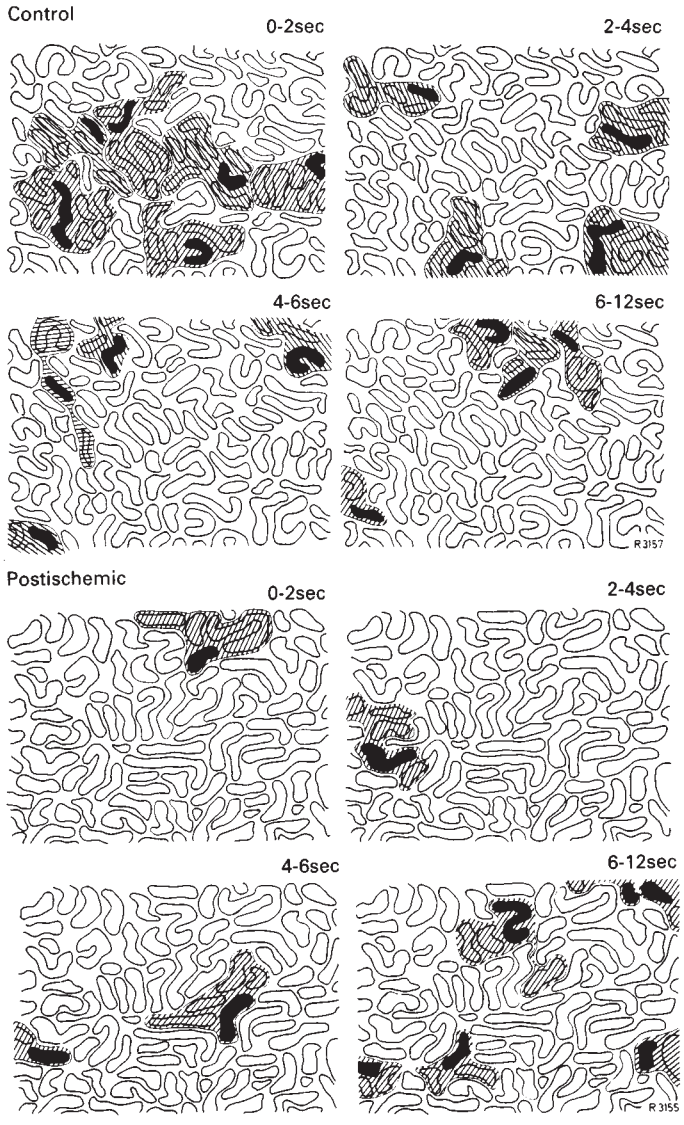

Fig. 1. *Nephrons within a renal surface area of 0.56 mm² from a control and a postischemic kidney.* The first proximal convolutions *(dark)* had AT within the time interval given at right top of each inset. Following convolutions are *shaded* (see **Methods**).

umns. Hydrostatic pressure was measured continuously in an early proximal convolution, and a small Sudan red-stained droplet of low viscosity (40 cSt) watch oil was injected with a micropipette into this convolution. In a number of cases the pressure rise during the droplet flow along the entire proximal tubule was less than 0.5 mm Hg. In these cases droplet velocity was not significantly different from that of the dye maximum indicating the validity of the dye flow method for mean tubular flow measurements.

Two additional series of experiments were made for specific investigations: *(1)* In four rats AT in different proximal convolutions were determined before as well as 24 hr after temporary renal ischemia. In nonfasted Nembutal-anesthetized rats, the left kidney was exposed for microscopy and the left femoral vein cannulated for dye injections. A superficial convoluted tubule, serving as a reference point, was filled with a mixture of epoxy resin and hardener stained with Sudan-red [23]. Prior to renal artery occlusion and wound closure, AT were determined in convolutions within the renal surface adjacent to the reference point. On the next day, using the usual preparation, the same area was identified with help of the reference point, and AT in the corresponding convolutions were determined. *(2)* Three rats were examined on day 3 after temporary renal ischemia. In these rats the investigations consisted of recording tubular dye passage and measurement of intratubular pressures under free-flow and stop-flow conditions.

Results

The mean values and SD for arterial BP, hematocrit, and plasma protein concentrations were 107 ± 9 mm Hg, 43.6 ± 3.9%, and 5.0 ± 1.0 g/dl in 14 control rats and 113 ± 7 mm Hg, 42.0 ± 2% and 5.3 ± 1.1 g/dl in 10 experimental rats, respectively, showing no significant difference of the data in the two groups. In the control rats values for urine flow, inulin clearance, and PAH clearance for the left kidney were 2.2 ± 0.9 μl/100 g body wt/min, 310 ± 70 μl/100 g body wt/min, and 750 ± 150 μl/100 g body wt/min, respectively. A minute urine flow from the experimental kidney which partially filled the ureter cannula was estimated to be about 2 μl/hr/kidney. Very small values for inulin and PAH clearance have been reported previously [12, 28, 29] under these circumstances but were not determined in this study.

Microscopic appearance of peritubular circulation seemed to be normal in the experimental kidney. The size of the tubular lumen was variable. Both slightly dilated and collapsed convoluted tubules were seen on the kidney surface. A number of them were filled with foamy material indicative of tubular obstruction. In control rats passage of intra-arterially applied dye bolus was seen in all of the observed superficial convolutions. The mean proximal transit time, that is, dye arrival time in the majority of the last proximal convolutions, was 9.9 ± 1.0 sec (N = 16). In the postischemic kidneys the bolus passage could be observed in a number of convolutions after the dye injection, but both flow velocity and fluorescence intensity of the intratubular bolus decreased gradually to zero with time. The loss of intratubular dye, labeled dextran with a molecular weight of 3,000 daltons, indicates an abnormally high tubular permeability in postischemic kidneys. Analysis of bolus passage in 12 microscopic fields showed that within the first 10 sec clear cut dye maxima were found in 26 ± 9% of superficial convolutions. In the following 10 sec a distinctly slower flow of fainter dye bolus, still allowing an unequivocal AT determination, was observed in an additional 12 ± 5% of the convolutions. In 16 ± 7% of convolutions a perceivable entry of fluorescent dye was observed later, but a reliable AT measure was generally not possible.

Lack of flow in nearly half of the superficial convolutions in the postischemic kidneys may either be due to an increased outflux of ultrafiltrate along the proximal tubules or due to extremely low GFR in many nephrons. To investigate this question the proximal convolutions having short AT were microinjected with a dye pulse, allowing flow in subsequent convolutions of the same nephron to be visualized. The velocity of bolus flow as well as dye intensity were found to decrease remarkably in subsequent convolutions. Dye injection in the last identified convolution revealed that intratubular flow at this

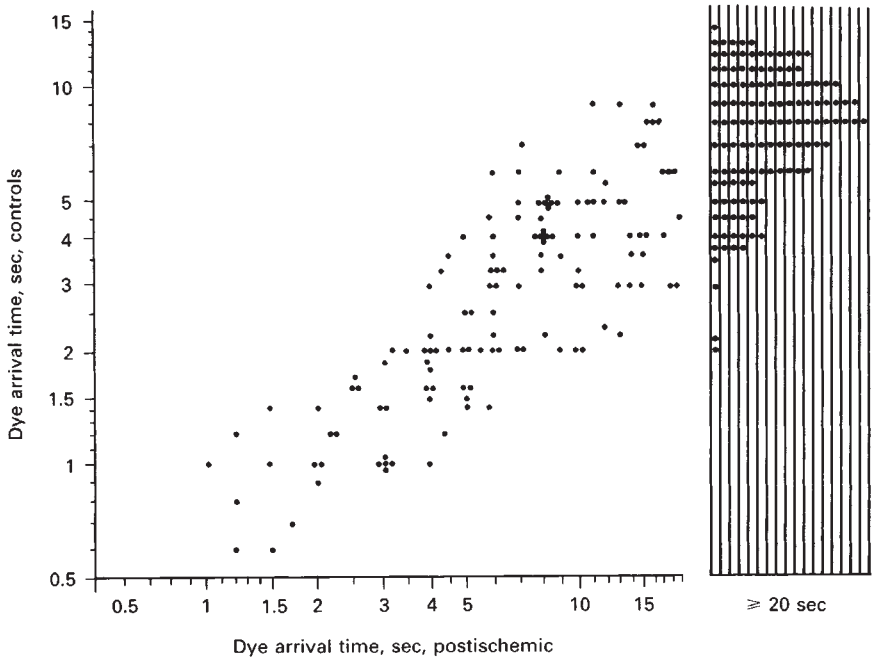

Fig. 2. *Logarithmic plot of dye arrival times in identical proximal convolutions before and after temporary ischemia.* The postischemic values of 20 sec and above are grouped together to indicate convolutions having practically no flow.

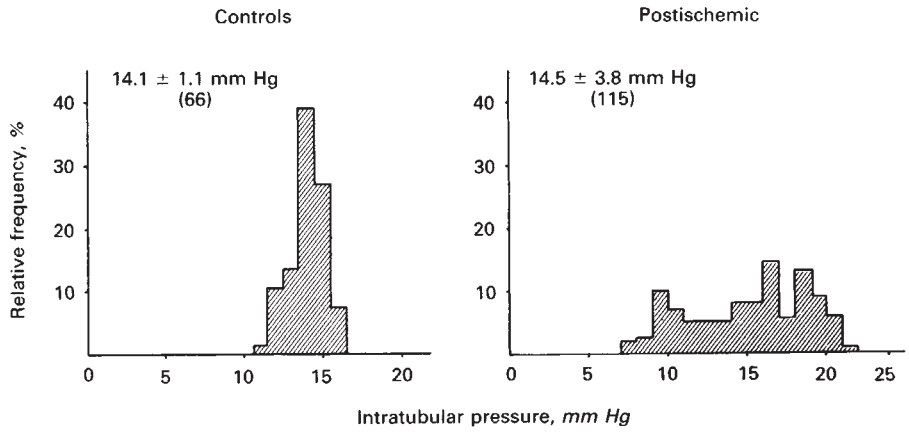

Fig. 3. *Relative frequency distribution of pressure measurements in proximal tubules of control and postischemic kidneys.* Means ± SD (number of measurements) are given as insets.

site was extremely slow. Moreover, further late proximal convolutions having no natural flow could be identified by an excessive application of dye solution in the earlier segment. These findings demonstrate that in the nephrons with substantial GFR, the tubular fluid is completely reabsorbed within early portions of the proximal convoluted tubule.

In the following investigations superficial nephrons in control and postischemic kidneys within the microscopic field of 0.56 mm^2 were identified with the aid of sequential intratubular dye injection (see **Methods**). Figure 1 shows examples of such determinations in a control and an experimental kidney in which all convolutions with a distinctly visible flow of systemically applied dye were considered. The first superficial convolutions are represented dark and subsequent ones are shaded. For sake of clarity, each field with newly identified nephrons has been drawn at four different intervals of AT in their first superficial convolutions. Superficial convolutions belonging to 18 ± 2 (N = 7) different nephrons were identified in the control kidney, whereas in the postischemic kidneys only 8 to 10 (N = 11) nephrons could be recognized easily with this procedure. Identification of further nephrons became increasingly difficult since a precise determination of AT in their first convolutions, required for this procedure, was difficult to determine due to sluggish movement of a faint dye bolus in them.

A second series of experiments was done to decide if in the damaged kidney all superficial nephrons had a finite SNGFR and the convolutions in which systemically applied dye did not appear belonged to distal portions of proximal tubules. In these experiments AT in each proximal convolution within a defined field was determined once before and then after temporary ischemia. Figure 2 gives a logarithmic plot of pre- versus postischemic AT in these convolutions. There is an apparent positive correlation between the pre- and postischemic AT; all early proximal convolutions having a sufficiently short preischemic AT also had relatively short postischemic values. The convolutions in which the dye maxima did not appear within 20 sec, during the postischemic phase, are grouped together since they had no or practically no intratubular flow; an exact AT

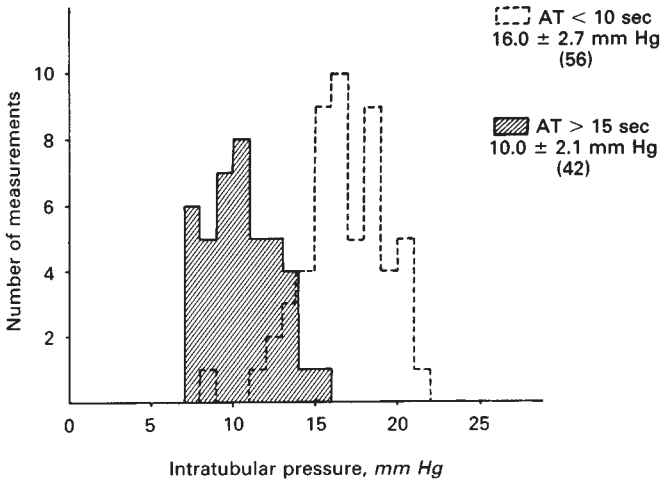

Fig. 4. *Frequency distribution of pressure measurements in proximal convolutions having AT < 10 sec and AT > 15 sec.* Means ± SD (number of measurements) are given as insets.

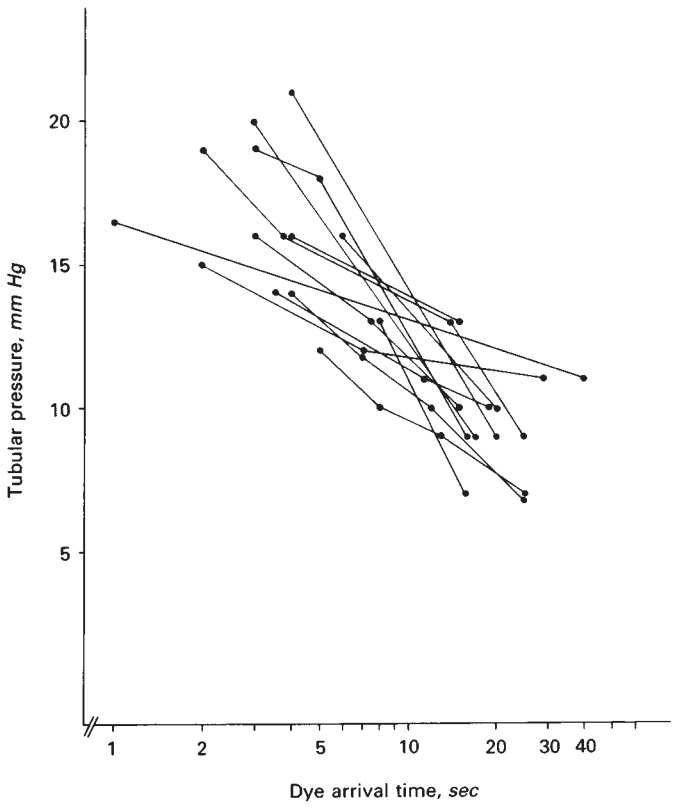

Fig. 5. *Semilogarithmic plot of pressure versus dye arrival time in proximal convolutions of postischemic kidneys.* Values from convolutions of the same nephron are joined together.

measurement was mostly not possible. Furthermore, Figure 2 shows that the number of such nephron segments without tubular fluid flow increases with increasing proximal tubular length, indicated by longer preischemic AT. These findings can be explained by postulating a sizeable SNGFR in all superficial nephrons and total reabsorption of the filtrate within the proximal tubule. In these experiments the proximal transit time during the preischemic phase was longer (13.0 ± 0.8, $N = 4$ rats) than in the normal controls (9.9 sec), probably due to altered experimental protocol (see **Methods**).

Figure 3 represents histograms and mean values of intratubular pressure measured in randomly chosen proximal convolutions of control and experimental kidneys. In comparison to control values, a wide distribution of intratubular pressure was found for postischemic kidneys, also described by other investigators [5, 12, 16]. Prior to a second series of pressure measurements in the experimental kidney, a video recording of the renal surface was carried out during the tubular passage of the dye bolus, and the AT for different convolutions was determined. The pressure measurements were performed in two groups of convolutions; the first group had an AT of less than 10 sec and the second one an AT of more than 15 sec. Figure 4 shows the intratubular pressure distribution in the two groups. The mean value from the first group (16.0 ± 2.7 mm Hg) was significantly higher than in the second one (10.0 ± 2.1 mm Hg, $P < 0.001$). These data suggest a pressure gradient along the proximal tubule in the postischemic kidney. To clarify this issue, the proximal convolutions in the experimental kidney having an AT of less than 10 sec were microinjected with FITC-3K solution, and subsequent convolutions were identified. The pressure in different convolutions of the same nephron was measured in random order, without removing the microinjection pipette. In Figure 5 intratubular pressure is plotted against AT, and the values from the convolutions belonging to the same nephron are joined. The results show a gradual reduction in intratubular pressure along the tubular length. Furthermore, the mean pressure in the first and the last convolutions of the 13 nephrons was 16.3 ± 2.6 and 9.7 ± 1.8 mm Hg; the paired difference was 6.5 ± 2.9 mm Hg ($P < 0.001$). These values are practically identical with those from the two groups of convolutions chosen according to AT only (Fig. 4). In control kidneys no significant difference was found between the pressure in early and late proximal convolutions of the same nephrons. The pressure difference in nine paired measurements was 0.1 ± 0.9 mm Hg.

Further pressure measurements were done to estimate driving forces for glomerular filtration. Table 1 represents the mean values of hydrostatic pressure in the early proximal convolutions (AT < 2 sec) under stop-flow and free-flow conditions, peritubular capillary pressure, and tubular radius in experimental and control kidneys. The mean effective filtration pressure at the afferent end of the glomerulus, given by SFP-P_T, in the postischemic kidney was 17.2 mm Hg or 75% of the corresponding control value (22.7 mm Hg). The reduction was both due to a reduced SFP (−2.1 mm Hg) and increased tubular pressure (3.4 mm Hg). The peritubular capillary pressure in the experimental kidney was significantly lower (−2.6 mm Hg) than the corresponding control value.

Fluorescence microscopic technique allows a reliable measurement of undisturbed intratubular flow rate (TFR) for the first time, which can be used for estimating the mean SNGFR in the postischemic kidney. In Figure 6 TFR in proximal convolutions of control and postischemic kidneys are plotted on a logarithmic scale against AT. For flow measurements in the control kidney all proximal convolutions were considered, whereas in the experimental kidney convolutions having an AT of more than 13 sec were excluded. For each group the mean tubular radius of the convolutions in which TFR was measured

Table 1. Pressure and tubular radius in control and postischemic kidneys[a]

	Controls	Postischemic
SFP,[b] *mm Hg*	36.5 ± 2.4 (16)	34.4 ± 3.2[d] (15)
P_T,[b] *mm Hg*	13.8 ± 0.8 (16)	17.2 ± 2.7[e] (15)
P_{cap}, *mm Hg*	9.8 ± 1.3 (6)	7.2 ± 1.1[e] (10)
r_T,[c] *μm*	12.0 ± 0.8 (58)	14.2 ± 1.3[e] (67)

Abbreviations are: SFP, stop flow pressure; P_T, tubular pressure; P_{cap}, peritubular capillary pressure; r_T, tubular radius; ( ), number of measurements.

[a] The values shown are mean ± SD.

[b] SFP and P_T were determined in the same early proximal convolutions.

[c] Flow data from these convolutions are given in Figure 6.

[d] $P < 0.05$ } significantly different from control.
[e] $P < 0.001$ }

is given in Table 1. Assuming the fluid reabsorption rate per unit of tubular length and the tubular diameter to be constant, it can be shown [30] that

$$\ln \text{TFR} = \ln \text{SNGFR} - \frac{C}{\pi r^2} \text{AT} \qquad (1)$$

where C and r represent reabsorption rate per length and tubular radius, respectively. Accordingly, TFR and AT would yield a semilogarithmic correlation. Although the validity of the above assumptions cannot be readily proven for the experimental kidney, their data were also used to calculate a semilogarithmic regression to allow a comparison with control data as shown in Figure 6. To minimize bias due to possible nonconstancy of C and r in postischemic kidneys, the convolutions with long AT were rejected. The SNGFR obtained by extrapolation of TFR data from the postischemic kidney was 62% of the corresponding control value (10.9 ± 0.9 nl/min vs. 17.6 ± 1.0 nl/min in control, $P < 0.001$). Furthermore, TFR in early proximal convolutions having AT ≤ 2 sec was 15.7 ± 2.7 nl/min ($N = 17$) in control and 10.2 ± 3.1 nl/min ($N = 20$, $P < 0.001$) or 65% of control in the postischemic kidney. The latter values represent mean SNGFR except for small changes due to fluid reabsorption in more proximal segments but are independent of above assumptions. The slope of the regression line ($-C/\pi r^2$) given in Figure 6 was 0.105 ± 0.009 sec^{-1} for the control and 0.116 ± 0.015 sec^{-1} for the experimental group. Tubular reabsorption rates (C) derived from these values and tubular radii given in Table 1 were 2.9 nl/mm/min in the control and 4.4 nl/mm/min in the experimental group. The linear flow velocity at the beginning of the proximal tubule in control kidneys, 650 ± 120 μm/sec, was 2.25 times higher than the corresponding value of 290 ± 80 μm/sec ($P < 0.001$) obtained for postischemic kidneys.

Change in flow velocity at the beginning of the proximal tubule due to renal ischemia could also be estimated from the AT data of the second series of experiments shown in Figure 2. In the second series, unlike the first (Fig. 6), there was no possible exclusion of nephrons with very low SNGFRs, because all of the nephrons were studied both pre- and postischemia. The AT ratio ($R = AT_{post}/AT_{pre}$), reciprocal to the corresponding velocity ratio, was determined for early proximal convolutions with small preischemic AT. The following R values were obtained by selecting different AT_{pre} intervals: $AT_{pre} \leq 3$ sec, R = 2.79 ± 1.25 ($N = 72 + 3$ exceptions with $AT_{post} \geq 20$ sec); $AT_{pre} \leq 2$ sec, R = 2.48 ± 0.94 ($N = 53 + 1$ exception); and $AT_{pre} \leq 1$ sec, R = 2.40 ± 0.76 ($N = 15$). Extrapolation of these values to $AT_{pre} = 0$ should produce R of a little less than 2.4, which is comparable to the inverse velocity ratio of 2.25 found in the first series. The agreement of these results supports the previously stated contention that there is a sizeable SNGFR in all superficial nephrons.

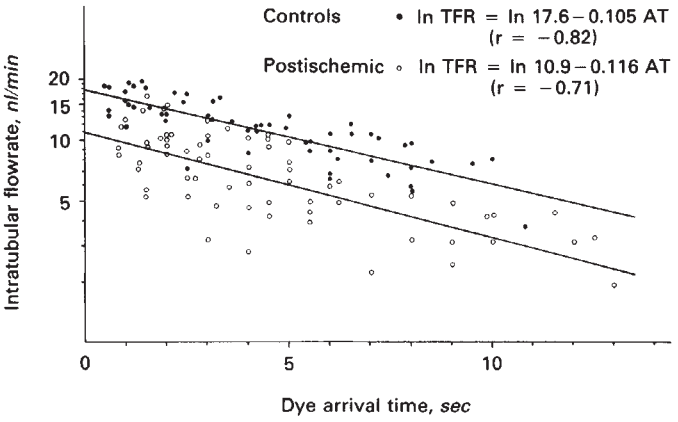

Fig. 6. *Semilogarithmic plot of intratubular flow rates (TFR) in superficial proximal convolutions in control and postischemic kidneys against the dye arrival time (AT).* Regression *lines* and correlation coefficients *(r)* were calculated by using absolute values of TFR.

The limited observations available on kidneys 3 days after temporary ischemia differed in several respects from the observations made 1 day after ischemia. Thus, there was a number of markedly distended convolutions. Dye passage through early proximal convolutions was distinctly slower, and dye persisted within some convolutions for as long as 10 min after injection. Intratubular pressures in convolutions having AT < 10 sec, 23.7 ± 5.3 mm Hg ($N = 60$), was significantly higher than the corresponding value on day 1 ($P < 0.001$). Pressure along the proximal convoluted tubule decreased more irregularly and abruptly than on day 1. However, mean stop-flow pressure in early convolutions (AT < 2 sec) was not significantly different, 35.6 ± 2.3 mm Hg ($N = 5$), than that on day 1.

Discussion

This study indicates that the large variation in tubular pressure observed in different experimental models of ARF is not necessarily due to a heterogeneity of glomerular pressure. In the postischemic kidney we found an inverse correlation between pressure and AT in the superficial proximal convoluted tubules by measuring pressure at two different AT intervals (Fig. 4). This may either be explained by assuming *(1)* that hydrostatic pressure decreases from early to late proximal convolutions, or *(2)* that different populations of nephrons have characteristically high or low intratubular pressure and flow rates due to variable glomerular pressure. The first assumption is supported by a consistent observation that pressure decreased progressively along single proximal tubules. In addition, intratubular pressure was nearly equal at two different AT intervals, irrespective of whether they were derived from paired measurements in single nephrons or not. Similarity of stop-flow pressure variations in the early proximal convolutions (AT < 2 sec) in the two groups (Table 1) provides evidence against a

markedly increased heterogeneity of glomerular capillary pressure in the postischemic kidney.

A gradual pressure drop seen along the proximal tubule in the postischemic kidney indicates an abnormal increase in tubular resistance over an extended length. The foamy material seen in the tubular lumen could represent a loose extended matrix increasing the flow resistance. Tubular obstruction in the region of Henle's loops and collecting ducts has also been repeatedly demonstrated for such kidneys [12, 14, 17]. However, the present data suggest that this obstruction is of little importance for TFR. The near equivalence of pressure in late proximal convolutions and the surrounding capillaries does not suggest appreciable flow impediment due to obstruction in more distal segments. The experiments performed 3 days after temporary ischemia suggest that the nature of the tubular obstruction may have altered. Thus, at this time pressure changes along the tubules were abrupt, indicating the presence of discrete areas of obstruction offering considerable resistance to the driving forces rather than the more diffuse obstruction found earlier.

A substantial rate of glomerular filtration in experimental models of ARF has been confirmed repeatedly for superficial nephrons by observing intravenously applied lissamine green in proximal convolutions [5, 15, 29], for deeper nephrons by using ferrocyanide injection technique [31], and in humans by observing nephrograms after injecting contrast media, which are predominantly excreted by filtration [32]. Values for SNGFR have been reported by other investigators using a standard micropuncture technique for collecting tubular fluid in which intratubular pressure was not controlled. Within a few hours after unilateral temporary ischemia SNGFR was about 70% of control [11, 20] and was normal 1 day after bilateral ischemic damage [12]. In our experiments evidence for sizeable SNGFR in all superficial convolutions was obtained by measuring AT in the same convolutions before and after temporary ischemia. As shown in Figure 2 all early proximal convolution having a sufficiently small preischemic AT also had a finite postischemic AT value. The mean SNGFR in the postischemic kidneys was calculated to be 62% of the control value by assuming a semilogarithmic correlation between TFR and AT. Also, TFR in early proximal convolutions with an AT $\leq$ 2 sec was 65% of the corresponding control value.

The effective filtration pressure at the afferent end of the glomerulus ($SFP - P_T$) in the experimental group was 75% of the control value (Table 1). Using the same experimental model, we found in previous studies renal cortical, and hence glomerular, blood flow in the experimental group to be 75% of the control value [29, 33]. From the above data, it can be easily seen that the upper limit of SNGFR ratio in the experimental to control group tends to 75% for identical but small values for glomerular permeability coefficients in both groups. The lower limit of 62% for this ratio can be calculated by assuming permeability to be high enough to allow filtration equilibrium [34] in both groups, and by using the Landis-Pappenheimer equation [35] for changes in colloid osmotic pressure due to filtration. The lower limit coincides with the experimental value. In regard to the controversial issue, as to whether glomerular permeability is reduced [8, 10] or not in postischemic kidneys [3, 4], our data indicate the permeability alteration to be of little importance, if at all, for the loss of renal function in the rat.

In contrast to SNGFR, tubular reabsorption in the experimental kidney was increased so that tubular fluid was reabsorbed completely within the proximal tubule. At a given AT, fractional tubular flow and length can be calculated from present data (compare [30] for detail). In the control group for AT = 10 sec, measured at the end of proximal convolutions, TFR and tubular length were 35% of SNGFR and 4.0 mm, respectively. In the experimental kidney the corresponding values at AT = 20 sec were 9% of SNGFR and 2.2 mm, and TFR decreased to zero at 2.5 mm with increasing AT. This would explain the nonappearance of systemically applied dye bolus in nearly half of the superficial convolutions in the postischemic kidney despite a significant filtration in its nephrons.

The results shown in Figure 2 lead qualitatively to the same conclusion, that the proportion of convolutions without tubular fluid flow in postischemic kidneys rises with increasing proximal tubular length as indicated by longer preischemic AT. Theoretically, in these kidneys with a proximal transit time of 13 sec (at 4 mm tubular length), an AT = 7 sec corresponds to a proximal tubular length of 2.5 mm, at which filtrate should have been reabsorbed completely after ischemic damage. Indeed, Figure 2 shows that 90% of the convolutions having a preischemic AT $\geq$ 7 sec had no detectable flow, and thus indirectly supports the validity of the tubular reabsorption rate obtained by semilogarithmic correlation of TFR and AT data from postischemic kidneys.

The presence of tubular obstruction in a nephron would naturally impose an equilibrium between the filtered and reabsorbed fluid. The elevated reabsorption in proximal convolutions of the postischemic kidney must be due to increased tubular permeability since frank damage of tubular epithelium known for these kidneys hardly indicates an increased active tubular transport. However, evidence for active sodium transport in ARF persisting at a substantial rate can be derived indirectly from metabolic studies [29, 36–41]. Its value has been estimated from oxygen consumption data in a previous investigation using the same model to be 40% of the control value [29]. Micropuncture studies also demonstrate a high reabsorption capacity in postischemic kidneys, based on the ability of proximal tubules to concentrate inulin [11, 12]. Since the proximal tubule by and large reabsorbs the filtered fluid under normal conditions, a marginal rise in the tubular permeability in the damaged kidney may cause a total efflux of tubular fluid. The increased transtubular pressure difference, which has been shown to increase permeability of small, usually nonpermeant molecules in normal kidneys [42], as well as cellular damage, may contribute to promote passive tubular outflux. In our experiments abnormal permeability for large molecules in the damaged kidney was evident from the rapidly decreasing intratubular concentration of fluorescent labelled dextran (3,000 daltons) on day 1 and to a lesser extent on day 3. A variable increase in tubular permeability has also been reported for different models of ARF by tracing excretion of inulin or other solutes with varying molecular radii [11, 15–18, 28, 43, 44] suggesting that complete reabsorption occurs even in other forms of ARF.

In conclusion, the present model represents a severe form of ARF in which tubular obstruction is obvious for all nephrons. Heterogeneity of tubular pressure in the postischemic kidney

can be explained by a relatively large SNGFR and increased flow resistance in proximal tubules. The reduction of SNGFR due to the alteration of glomerular hemodynamics plays a subordinate role for the loss of renal function. Increased permeability of the proximal tubule one day after temporary ischemia causes a complete reabsorption of the filtrate. Hence, at this time the driving force which may have helped to wash out tubular obstruction dissipates within the first small portion of the tubular system. Evaluation of SNGFR and reabsorption rates in the proximal tubules in other models of ARF may reveal the importance of total reabsorption for loss of renal function.

Acknowledgments

This study was supported by the German Research Foundation within the SFB 90 'Cardiovasculäres System.' Parts of this work were presented at the 54th Meeting of the German Physiological Society, Innsbruck, March 1981. The authors thank Mr. R. Dussel for his excellent technical assistance, Mrs. E. Mack-Kühn for chemical analysis, and Mrs. L. Kroon for secretarial assistance.

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