Renal Hemodynamics and Medullary Osmolal Gradient in Ischemic Acute Renal Failure in Rabbits

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Summary

The effects of 2 hours of renal artery occlusion were studied in previously uninephrectomized rabbits. Oliguric renal failure by arterial clamping was produced. Renal blood flow was maintained during observation periods, except for an early decrease in outer cortical blood flow without change elsewhere. During the oliguric stage medullary osmolality was markedly diminished, concomitantly with reduced (U/P)osm. C.in, E.PAH and sodium reabsorption were decreased, accompanied by tubular necrosis and intratubular casts. During the early diuretic stage C.in, medullary osmolality and (U/P)osm were still reduced. Intratubular casts disappeared while regenerated tubular cells were focally observed. Two weeks after the occlusion, improved C.in, E.PAH, sodium reabsorption, medullary osmolality and (U/P)osm, and regenerated tubular epithelium were found. In 7 weeks C.in and E.PAH had returned to the preclamping values whereas medullary osmolality remained decreased with the appearance of medullary fibrosis. (U/P)osm was not completely restored. The findings indicate that during the oliguric stage of acute renal failure of rabbits renal blood flow is maintained, except for a decrease in outer cortical flow, and urine concentrating ability is restored more slowly than other measured functions.

Additional Indexing Words:
Renal artery occlusion Renal failure Urine concentrating ability Intrarenal blood flow distribution

With prolonged renal ischemia, tubular damage, uremia, and death in acute renal failure appear to be the usual outcome. However, anesthetized dogs with previous uninephrectomy uniformly survive 2 hours of clamping of the contralateral renal artery,1,2 despite development of oliguric renal failure. Friedman and his coworkers,3 and Roof et al4 demonstrated that renal blood flow, estimated by para-aminohippurate clearance and the Fick...
application, is fairly well restored in 24 hours after release of the clamp, whereas other renal functions return slowly over a period of weeks. However, less information is available concerning effects of prolonged renal ischemia on the intrarenal hemodynamics and medullary osmolal gradient. These problems are of special interest because of the reported intrarenal blood flow redistribution and urinary concentrating defect in acute renal failure in the human.

The present work was performed in the previously uninephrectomized rabbit, to reinvestigate the recovery pattern of renal hemodynamics and urinary concentrating ability in acute renal failure, caused by 2 hours clamping of the renal artery.

**Methods**

Sixty-six New Zealand white rabbits, weighing from 2.7 to 3.6 Kg and allowed free access to a commercial rabbit chow and drinking water, were used in the experiments.

Right nephrectomy was performed after anesthesia with intravenous sodium pentobarbital (25–30 mg/Kg). After a minimum of 10 days of recovery from surgery, the left kidney was aseptically exposed through an experitoneal flank incision, with care taken to avoid damage of the renal nerves, and then the kidney and the clamp were returned to the abdomen. After 2 hours the clamp was released and surgical wounds were sutured. Chloramphenicol (0.5 Gm) was used to prevent bacterial infection. The animals were not restricted in water or food intake during the observation periods. Any animal, in which macroscopic renal infarction produced by the arterial occlusion was noted either immediately following the occlusion or when the rabbits were studied, was discarded.

The uninephrectomized rabbits were divided into 7 groups. In each group data were obtained in the preocclusion state, immediately after release of the clamp, 3 days, 7 days, 2 weeks, 3 weeks, and 7 weeks after release of the occlusion, respectively. Data are presented as mean±1 SD.

**Pressure-flow measurements and clearance studies**

The left kidney area was exposed through a midabdominal incision and the left renal artery cleared of all surrounding tissues. The renal nerves were left intact whenever possible. After stabilization of blood flow and pressure, renal blood flow was measured using a square wave electromagnetic flowmeter (MF-5, Nihon Koden, Co, Japan). A flow transducer of suitable size (1.5 mmø, MF-2T, Nihon Koden, Co) was placed on the artery. Baseline was determined by brief occlusion of the artery distal to the flow probe. Abdominal aortic pressure was measured below the origin of the renal artery with a Statham pressure transducer (P23AA), connected to a catheter inserted into the abdominal aorta through the right femoral artery. All transducers were connected to appropriate amplifiers in a pen-writing recorder (WI-180M, Nihon Koden, Co). The values of the blood flow and pressure were expressed as the means of 1 hour measurement periods.
Intracortical blood flow distribution was estimated by means of a $^{85}$Sr-labeled microsphere technique, described by McNay and Abe. After a catheter was introduced into the left ventricle via the right common carotid artery and blood pressure stabilized, approximately 20 $\mu$C of $^{85}$Sr-labeled microspheres (3M Company, St Paul, Minn, USA) with a diameter of 15±5 $\mu$ were injected through the ventricle catheter. The injecting catheter was flushed with saline. At the end of the experiment the kidney was removed, weighed, measured in 3 dimensions and middle triangular renal tissue block, containing cortex and papilla, was quick-frozen in a dry ice-acetone mixture. Cortex thickness was measured. While still frozen, the renal cortex was separated from the medulla with a razor blade and divided into 4 zones of equal thickness, perpendicular to the longitudinal axis to the papilla: superficial layer (C-1) and deep layer (C-2) of the outer cortex, and outer layer (C-3) and inner layer (C-4) of the deep cortex. Cortical zone C-4 included some outer medulla tissue due to the scalloped margin between the cortex and medulla. The tissue slices were weighed, and their radioactivities were counted in a well scintillation counter. More than 97% of microspheres, which entered the kidney, were trapped in the glomerular capillaries while the remaining were detected in the medulla or renal venous blood. McNay and Abe's studies suggest that axial migration of microspheres does not occur within the interlobular arteries. So the quantity of $^{85}$Sr-spheres per unit tissue weight in each cortical zone is a function of regional glomerular blood flow. The percent of total blood flow perfusing each cortical zone was calculated as follows:

$$\text{Percent flow} = \left( \frac{C_i W_i}{\sum C_i W_i} \right) \times 100\% \quad (i=1\sim4)$$

where $C_i$ is a radioactivity per unit gram tissue in each cortical zone and $W_i$ is total weight of each cortical zone. The weight of each cortical zone was approximated by calculations based on the formula for an ellipsoid. A series of volumes were calculated by sequential reductions in each hemiaxis by an amount equal to one-fourth of the cortex thickness. The microsphere technique can not estimate medullary blood flow, since most microspheres are trapped in the juxtamedullary glomerular capillaries before they enter the medulla.

For determinations of extraction ratio of para-aminobiphenurate ($E_{PAH}$) and inulin clearance ($C_{in}$), suitable plasma concentration of para-aminobiphenurate (2–4 mg/100 ml) and $^{14}$C-inulin (0.1–0.3 mg/100 ml or 0.27–0.081 $\mu$C/100 ml) were maintained by infusing in isotonic saline at a constant rate through the marginal vein of the ear. Para-aminobiphenurate concentration in blood samples drawn from the femoral artery and renal vein was measured by the standard chemical method. In the measurement of $^{14}$C-inulin in arterial blood and urine, 0.1 ml of the sample was added to 10 ml of Bray's solution and its radioactivity determined in a Packard liquid scintillation counter. Clearance values were expressed as the mean of 30 min clearance periods.

Sodium and potassium concentrations were measured by flame photometry. Blood urea nitrogen concentration was determined by the diacetyl-monoxime method. Plasma and urine creatinine levels were measured by a standard chemical method.

Medullary osmolal concentration and urine/plasma osmolality ratio

Determinations of medullary osmolar concentration and urinary to plasma
osmolality ratios (U/P)osm were performed after 40 hours of water deprivation.

Several hours prior to blood and urine collections, the bladder was voided by compression of the abdominal wall. Osmolality of plasma and urine samples, drawn from the femoral artery and bladder, was measured using a Fiske osmometer.

After completion of blood and urine collections, the left kidney was removed. The kidney was sectioned and a middle triangular block, containing the cortex and medulla, was frozen in a mixture of dry ice and acetone. The time interval from kidney removal to freezing was less than 2 min. While still frozen, the kidney block was cut with a razor blade into 6 slices, perpendicular to the longitudinal axis to the papilla: superficial and deep cortex, outer medulla, and outer layer, middle layer, and papilla of the inner medulla. The slice analysis was made with the same technique as previously described. The kidney slices were weighed on a Mettler analytical balance and placed in 20 ml Erlenmeyer flasks with 2 ml of distilled water. The flasks with the slices were weighed and then heated to boiling for 5 min in a water bath. After cooling, distilled water was added to the flasks to the same weight as before boiling. The flasks were closed and kept in a refrigerator of 6°C for 24 hours for diffusion to take place. The supernatant was analyzed for sodium, potassium, and urea. Sodium and potassium concentrations were measured by means of a flame photometer. Urea concentration was measured by means of the Conway microdiffusion method. Concentration in renal tissue water was calculated from wet and dry weights of the slices. The osmolal concentration in kidney tissue water was estimated as the sum of the urea concentration and 2 times the sum of sodium and potassium concentrations, though this estimation might be a slight overestimate. The solute content per unit dry tissue weight of the kidney was also calculated.

Histological studies
For histological examination a piece of the kidney tissue, removed for determination of the tissue osmolality, was used. The kidney block was fixed in 10% neutral buffered formalin solution and then embedded in paraffin. The block was cut at 3 to 4 micra and stained for light microscopic examination using hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Mallory Azan staining.

Results
In the uninephrectomized rabbit, 2 hours of clamping of the contralateral renal artery gave rise to oliguric acute renal failure. Fig. 1 shows a typical experiment. Urine output decreased from the average control value of 332 ml/day to less than 50 ml/day during the first several days after release of the clamp. Blood urea nitrogen and creatinine concentrations were significantly increased to average values of 132 and 10.1 mg/100 ml, respectively, 3 days after the occlusion, when compared to control values of 23.5 and 1.62 mg/100 ml. Plasma sodium concentration decreased from the control value of 147 mEq/L to 131 mEq/L, with an increase in potassium concentration from 4.1 mEq/L to 6.6 mEq/L. This oliguric stage was uniformly followed by a
Fig. 1. Effects of 2 hours of unilateral renal artery clamping on blood urea nitrogen (BUN), plasma creatinine (Cr), sodium (Na) and potassium (K) concentrations, and urine output (UV) in a contralaterally nephrectomized rabbit.

Diuretic stage. The duration of the diuretic stage averaged 2 weeks. With progressively increased urine output the above-described parameters returned to the control values. Also, effects of a sham operation and renal artery clamping for less than 1 hour were examined in 3 uninephrectomized rabbits. There were no significant changes when compared with the control state.

Renal hemodynamics and renal clearances following the renal arterial occlusion

In 44 uninephrectomized rabbits, effects on renal hemodynamics and functions of 2 hours clamping of the contralateral renal artery were examined. In these animals control values of mean systemic arterial pressure, left renal blood flow, E_PAH, Cin and C_{Na}/Cin averaged 95.9 ± 7.4 mmHg, 48.8 ± 14.8 ml/min, 0.87 ± 0.03, 6.09 ± 0.33 ml/min, and 0.97 ± 0.14 %, respectively. The values of these parameters following release of the clamp are illustrated in Fig. 2. Systemic arterial pressure gradually increased to an average value of 115 mmHg 3 days after the occlusion, and then, returned to the preoclusion level with increased urine output. Directly measured renal blood flow showed a transient overshoot immediately after release of the occlusion, and then, returned toward the control level. The average values of renal blood flow remained at the preocclusion level throughout the observation periods, except slightly decreased flow for the first week after release of the occlusion.

Two hours of clamping of the renal artery resulted in a redistribution of intracortical blood flow. In the preoclusion state, percent of total blood

Flow perfusing cortical zones was 33.9±6.9% for C-1, 31.4±2.5% for C-2, 21.1±3.9% for C-3, and 13.6±2.5% for C-4. Percent of total flow perfusing the superficial cortex (C-1) decreased significantly during the first week following the occlusion, whereas percent flow in the deep cortex (C-4) increased (Fig. 2). No significant change in percent flow was found in the middle cortex (C-2, C-3). Regional blood flow, calculated from total renal blood flow and percent flow in each cortical zone, decreased in the superficial cortex (C-1) during the oliguric stage without significant change elsewhere. Seven days after the occlusion, renal blood flow and the flow distribution returned toward those in the preocclusion state.

Following release of the arterial occlusion a reduction of \( E_{\text{PAH}} \) was found
(Fig. 2). Also, Cin was reduced, in contrast to fairly well maintained renal blood flow. These parameters began to increase in the second week and showed good recovery in 7 weeks after the occlusion. Ratios of CNa to Cin, which were markedly increased to $21.5 \pm 11.1\%$ 3 days after release of the clamp, decreased to $5.2 \pm 2.3\%$ after 1 week (Fig. 2).

**Urinary concentrating ability following the renal arterial occlusion**

In 19 uninephrectomized rabbits the effects on urinary concentrating ability and medullary osmolar concentration of 2 hours of the contralateral renal artery occlusion were studied. In the hydropenic state, caused by 40 hours of water deprivation, urine and plasma osmolality determinations and renal tissue analysis for osmolar and solute concentrations were performed.

Control values of the urinary to plasma osmolar ratios $(U/P)_{osm}$ averaged $5.54 \pm 0.75$ in the hydropenic rabbit with the previous uninephrectomy. Two hours of clamping of the renal artery gave rise to a marked decrement of $(U/P)_{osm}$ toward unity (Fig. 3). This decrement of $(U/P)_{osm}$ appeared to be improved fairly well 2 weeks after the occlusion, but did not completely recover even after 7 weeks.

![Fig. 3. Papillary osmolal, sodium, potassium and urea concentrations (top) and $(U/P)_{osm}$ (bottom) before and after renal artery clamping.](image-url)
In the hydropenic state of uninephrectomized rabbits, osmolal, sodium, and urea concentrations, and sodium and urea contents per unit dry tissue weight were found to increase progressively from the outer medulla through the papilla tip (Fig. 4). Papillary osmolal concentration averaged $1,343 \pm 98$ mOsm/Kg of tissue water in a hydropenia. During the first several days following the arterial occlusion, osmolal, sodium and urea concentrations, and solute content, measured 24 hours and 3 days following the occlusion, were decreased by more than 50% (Figs. 3 and 4). The papillary tissue osmolality ($1,370 \pm 71$ mOsm/Kg of tissue water), measured 2 weeks after release of the occlusion, appeared to recover to the level in the preocclusion state. However, papillary osmolal concentrations in 3 and 7 weeks following the occlusion were $1,042 \pm 64$ and $1,047 \pm 6$ mOsm/Kg of tissue water, respectively, lower values than those in 2 weeks (Fig. 3).

**Histological examination**

Histological examination was performed on 19 kidneys. Light microscopic observations 3 days after release of the occlusion revealed prominent tubular necrosis and intratubular cast formation (Fig. 5). Tubular necrosis was more marked in the cortex, especially in the subcapsular area, than in the medulla. Also, glomerular congestion was observed. Interstitial edema was found in some cases. One week after release of the occlusion, intratubular
casts disappeared (Fig. 6). In this early diuretic stage regeneration of tubular cells was focally observed. Fig. 7 shows the finding observed in 2 weeks after the occlusion. The regeneration process of tubular cells advanced markedly. Interstitial edema and glomerular congestion disappeared. Seven weeks after release of clamping, damaged tubular epithelium was completely repaired. Interstitial fibrosis, which appeared 3 weeks after the occlusion, became more marked in the medulla and cortex (Fig. 8). There was no evidence of glomerular injuries.

**DISCUSSION**

Two hours of clamping of the unilateral renal artery gave rise to severely reduced urine output, hyponatremia, hyperpotassemia, elevated blood urea nitrogen and creatinine concentrations, diminished inulin clearance, a urinary...
concentrating defect and reduction of the medullary osmolar gradient in a contralaterally nephrectomized rabbit. The finding is apparently comparable to that in oliguric acute renal failure in man. Teschan and Mason described that experimental procedures that involve severe ischemia produce renal infarction or extensive cortical necrosis rather than the focal lesions of tubulorrhexis in the dog. They further pointed out that with acute renal failure in man, rarely there is cortical necrosis and renal infarction while focal tubular necrosis is the rule. In the present experiments, rabbits in which macroscopic renal infarction was produced by the arterial occlusion were discarded. The prominent histological changes were tubular necrosis and intratubular cast formation. There was no evidence of glomerular injuries, except glomerular congestion seen during the oliguric stage.

In this model of acute renal failure, oliguria resulted in the face of maintained renal blood flow during the first several days after release of the clamp. The following mechanisms may be proposed to account for oliguria: 1) tubular blockage by casts, 2) primary reduction or cessation of glomerular filtration due to hemodynamic changes, and 3) leakage of tubular fluid to the renal interstitium across damaged tubules. In the present experiments casts were prominent in the tubular lumina during the oliguric stage and disappeared in the diuretic stage. The production of intratubular casts might have resulted from markedly diminished tubular fluid flow during occlusion of the renal artery. However, a major participation of the cast in the development of oliguria remains unsettled, because proximal tubular pressure was not measured. Two hours of clamping of the renal artery gave rise to severely diminished inulin clearance in the face of fairly well maintained blood flow. An estimation of glomerular filtration rate by the inulin clearance in the kidney with tubular damage is open to criticism, because of the possibility of the back transfer of inulin across the damaged tubular epithelium. Although the leakage of inulin and tubular fluid was not proved in the present experiment, a participation of passive backdiffusion of tubular fluid cannot be ruled out. Also, this possibility may be supported by the findings that reduced inulin clearance persisted despite restoration of renal blood flow and intracortical blood flow distribution, and disappearance of intratubular casts in the second week following the arterial occlusion. On the other hand, decreased renal arterial perfusion pressure accelerates renin secretion from juxtaglomerular cells. Renin mediated vasoconstriction during renal failure has been suggested by Schnermann et al as a cause of the reduced glomerular filtration rate. Thus, increased renin secretion, caused by renal artery occlusion, may result in preglomerular arteriolar constriction with resultant reductions of inulin clearance and renal blood flow. However, it ap-
pears that severely reduced inulin clearance, seen in this model of acute renal failure, cannot be completely explained by preglomerular vasoconstriction, since renal blood flow was maintained in the face of the severe decrement of inulin clearance.

Friedman et al. demonstrated that renal plasma flow, estimated by para-aminohippurate clearance and the Fick application, is fairly well restored 24 hours after release of the occlusion. In the present experiments directly measured renal blood flow showed a transient overshoot immediately after release of the occlusion, and then, returned toward the control level. Average values of blood flow remained relatively constant throughout the observation periods, though there was a slight decrement of flow during the oliguric stage. Some investigators demonstrated the intrarenal blood flow redistribution in acute renal failure in man, dog, and rat. In the present experiments decrement in blood flow occurred in the outer cortex during the oliguric stage. On the contrary, there was no significant change of blood flow in the deep cortex, suggesting continued medullary blood flow because the main medullary blood vessels originate from the vas efferens in the juxtamedullary glomeruli.

In contrast to the rapid restoration of renal blood flow, \( E_{PAH} \), \( C_{in} \) and \( C_{Na}/C_{in} \) were gradually improved after damage due to renal ischemia. The findings are consistent with those of Friedman et al. Severely reduced \( E_{PAH} \) and \( C_{in} \), and markedly increased \( C_{Na}/C_{in} \) during the oliguric stage may be partly explained by the tubular damage, because of the possibilities of impaired transport of para-aminohippurate, and the back transfer of inulin across the damaged tubular epithelium. The restoration of these parameters appeared to be in parallel fashion with repair of the damaged tubules.

One of the renal functions not evaluated by Friedman et al. is urinary concentrating ability. Papillary tissue fluid osmolality in a hydropenia was low in the uninephrectomized rabbit as compared to the value of 1,604 ± 126.2 mOsm/Kg of tissue water in the non-nephrectomized normal rabbit, supporting the finding of Boylan and Asshauer. Two hours of clamping of the renal artery resulted in a severe urinary concentrating defect. The concentrating ability did not completely recover even after 7 weeks. Medullary osmolal, sodium and urea concentrations, and solute content were tremendously reduced following the renal arterial occlusion. These decrements in medullary tissue fluid osmolality and solute content might have resulted from more severe curtailment of glomerular filtration and tubular solute reabsorption than medullary blood flow. Ruiz-Giñazú demonstrated that reduced medullary solute concentration in the damaged kidney of dogs, caused by 1 hour occlusion of the renal artery and intravenous injection of acid hematin, is completely restored 21 days after the damage. In the present
experiments the medullary osmolal gradient was apparently restored to the preclamping level 2 weeks after release of the occlusion. However, medullary osmolal concentration measured in 3 and 7 weeks was significantly lower than that in 2 weeks, indicating reappearance of urinary concentrating impairment. In experiments in mice, Fox has described progressive deterioration in renal function, accompanied by production of interstitial fibrosis, after recovery from ischemic acute renal failure. Thus, interstitial fibrosis, which appeared 3 weeks after the occlusion, might have impaired the tubular reabsorption with resultant reduced medullary osmolal gradient.

Contrary to our findings, Briggs et al. reported that after recovery, glomerular filtration rate is reduced more often than urinary concentrating ability, estimated by measurement of urine specific gravity after water deprivation, in acute renal failure in man. The difference between their and our data may be partly due to the difference in the causes of acute renal failure.

CONCLUSION

The pattern of recovery of renal functions was observed in ischemic acute renal failure in rabbits and the following conclusions were obtained: 1) oliguria continues even when renal blood flow is maintained, except for a decrease in outer cortical blood flow, and 2) the urinary concentrating ability and medullary osmolal gradient are restored more slowly than renal blood flow, Cin and EP AH.

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