Effects of Renal Lymphatic Occlusion and Venous Constriction on Renal Function

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The effects of renal lymphatic occlusion or increased lymph flow due to renal vein constriction on renal function were investigated in rats. In each experiment, the renal lymphatics or vein of the left kidney were occluded or constricted and the right kidney served as a control. Occlusion of renal lymphatics caused renal enlargement, no change in glomerular filtration rate, a marked increase in urine flow and solute excretion without any change in urine osmolality, and enhanced urinary loss of urea, potassium, sodium and ammonium. Urea concentrations in medullary and papillary tissues were significantly elevated. Renal vein constriction caused renal enlargement and a marked drop in glomerular filtration rate, urine volume, urine osmolality and solute excretion. Tissue concentrations of urea and potassium were decreased in the medulla and papilla and total tissue solute was significantly decreased in the papilla. The data indicate that in the rat, renal lymphatic occlusion traps urea in the medulla and induces a urea diuresis resulting in a large flow of normally concentrated urine. On the other hand, increased lymph flow secondary to renal vein constriction decreases medullary urea and potassium concentrations and papillary osmolality. These changes and the reduced glomerular filtration rate result in a small flow of dilute urine. Thus both renal lymphatic occlusion and enhanced lymph flow have a significant effect on renal function. (Am J Pathol 78:285–296, 1975)

Relatively little work has been done on the physiologic role of renal lymphatics on renal function. Renal lymph flow generally equals urine flow and renal lymph returns considerable quantities of protein and other substances to the blood.[1-6] Increased renal lymph flow prevents or diminishes cell injury secondary to increased ureteral or venous pressure.[4] It has been postulated that the lymphatics play a role in the concentrating process by removing protein from the medullary interstitium, which creates a high oncotic pressure within medullary vessels and permits effective removal of interstitial water.[1] It has been shown that several conditions that increase renal lymph flow cause

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depletion of the osmotic gradient in the medulla and loss of concentrating ability.[2,7-10]

In this paper the effects on renal function of lymphatic occlusion or increased lymph flow secondary to renal venous constriction were studied in rats. Both lymphatic occlusion and enhanced lymph flow have a significant effect on: a) renal excretion of water and certain solutes, b) electrolyte concentrations in renal tissues and c) the renal concentrating mechanism.

Materials and Methods

Male, 300- to 400-g Sprague-Dawley rats (Holtzman Co, Madison, Wisc) which had free access to food and water were anesthetized with sodium pentobarbital, 40 mg/kg intraperitoneally, placed on a thermostatically controlled heated table, and their body temperatures were maintained between 37 and 38 C. An endotracheal tube was inserted, the right external jugular vein was cannulated, and saline was infused at a constant rate of 0.85 cc/hr. The kidneys were exposed by a midline abdominal incision, and the abdominal viscera were covered with saline-soaked gauze. The right and left ureters were catheterized, and urine was collected under oil bilaterally. In each experiment, the renal lymphatics or vein of the left kidney were occluded or constricted and the right kidney served as a control. To determine glomerular filtration rate, the animals were given a priming dose of 50 μCi of inulin-methoxy-^{3}H (New England Nuclear Corp) in 0.4 ml of saline and a sustaining dose of 50 μCi hourly. Blood samples were taken every 30 minutes from the right femoral artery, radioactivity in plasma and urine was determined with a Packard Liquid Scintillation Spectrometer, and inulin clearance values were corrected for plasma water (94%).

Lymphatic Occlusion

In this strain of rats it was found that three to five lymphatics issue from the left renal hilum and join into a single trunk which enters a lymph node located near the origin of the left renal artery. In 14 animals examined microscopically (12.5 ×), this common lymphatic channel was ligated with a fine cotton thread and the fibroadipose tissue at the upper and lower poles of the left kidney was ligated to occlude capsular lymphatics. Within a few minutes, hilar lymphatics became markedly distended, the kidney enlarged (about 1.5 times normal), and urine flow increased on the ligated side. After these changes were well established, urine was collected bilaterally for 2 hours.

Renal Vein Constriction

In 12 animals examined microscopically, the left renal vein was separated from hilar lymphatics and fibroadipose tissue. A suture was placed around the renal vein (distal to the spermatic vein), a polyethylene tube (P.E.10, outer diameter .024 inches) was placed adjacent to the vein, the suture was tightened and tied, and the polyethylene tube was removed. Within a few minutes the renal vein and the hilar lymphatics became markedly distended, urine flow diminished, and the kidney enlarged (about 1.7 times normal). After these changes were well established, urine was collected for 2 hours.

Urine and Tissue Analyses

Following urine collection, the kidneys were removed after clamping the artery, vein and ureter and were quick-frozen at −80 C. The kidneys were dissected with a cold knife in a cryostat at −16 C, and portions of cortex and outer medulla and the entire papilla were accurately weighed and homogenized in 1 ml of ion-free water in a glass tissue grinder. The tubes were capped with parafilm, placed in boiling water for 5 minutes, centrifuged at 3000 rev/min for 20 minutes, filtered and recentrifuged. The supernatant was used for electrolyte determinations. Sodium and potassium levels were determined by internal flame photometry. Urea was determined colorimetrically by the monoxime method [11] and ammonia by the method of Seligson and Seligson.[12]

Results

Morphologic Changes

Lymphatic Occlusion

Occlusion of the renal lymphatics produced, within a few minutes, a marked dilatation of the hilar lymphatics and an increase in renal size to about 1.5 times normal. Microscopically, there was no detectable interstitial edema in the cortex or medulla. Small bloodless endothelium-lined structures, which probably represent distended lymphatics, were seen in the corticomedullary region in association with arcuate arteries and veins (Figure 1). These structures were not detected in control kidneys, which supports the belief that they are dilated lymphatics.

Renal Vein Constriction

Constriction of the renal vein produced, within a few minutes, a marked dilatation of the hilar lymphatics (five to ten times normal diameter) and an increase in renal size to about 1.7 times normal. Attempts to quantitate lymph flow by cannulating the hilar lymphatics with fine polyethylene tubes (outer diameter 40 to 50 mμ) employing microsurgical technics were unsuccessful. Cutting the hilar lymphatics, however, did result in a greatly increased continuous flow of lymph into the surgical field after renal vein constriction as compared to normal kidneys. Microscopically, interstitial edema was not detectable in the cortex or medulla, and tubular degenerative changes or necrosis were not seen. Small bloodless endothelium-lined structures were seen in association with arcuate arteries and veins in kidneys with venous constriction but not in normal kidneys, which supports the belief that they represent distended lymphatics.

Composition of Urine and Renal Tissues

Lymphatic Occlusion

Occlusion of the renal lymphatics caused a threefold increase both in urine volume and solute excretion (Table 1). There was no significant

Table 1—Effect of Lymphatic Occlusion on Composition of Urine

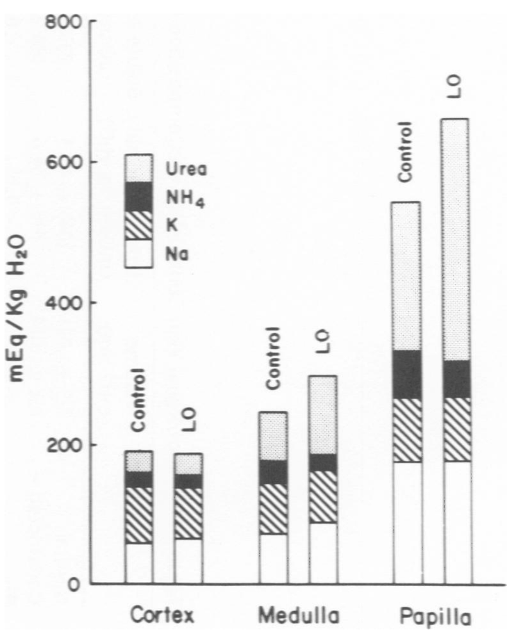
	Volume (ml/2 hrs)	Urine osmolality (mOsm/kg H_2O)	Solute excretion (μOsm/2 hrs)	K (μEq/2 hrs)	Na (μEq/2 hrs)	Urea (μmole/2 hrs)	NH_4 (μEq/2 hrs)
Normal	.182 ± .023	1097 ± 52.9	.200 ± .014	17.8 ± 1.48	11.7 ± 0.74	23.7 ± 2.35	16.3 ± 1.62
Occluded	.546 ± .036	1212 ± 55.2	.662 ± .056	103.0 ± 4.32	44.9 ± 2.16	162 ± 10.4	33.9 ± 2.78
P*	<0.01	NS	<0.01	<0.01	<0.02	<0.01	<0.01

* Derived from paired comparison analysis of 14 animals.
¶ Figures represent means ±SE.

change in urine osmolality, and the levels of urinary excretion of urea, potassium, sodium and ammonium were significantly increased (Table 1). The glomerular filtration rate was unaltered by lymphatic occlusion: mean values ± SE were 2.16 ± 0.13 and 2.10 ± 0.10 ml/min/kg of body weight for normal and lymphatic occluded kidneys, respectively. Tissue analyses revealed no significant change in the electrolyte content of the renal cortex following lymphatic occlusion (Text-figure 1). There was, however, a significant increase in urea concentration in the outer medulla ($P < 0.01$) and in the papilla ($P < 0.05$), so that total solute content exceeded control values in both the medulla and papilla (Text-figure 1).

Renal Vein Constriction

Venous constriction caused a marked decrease in urine volume, osmolality and solute excretion (Table 2). The excretion of potassium, urea and ammonium fell significantly; however, sodium excretion remained unchanged (Table 2). Venous constriction caused a marked, significant drop in glomerular filtration rate: mean values ± SE were 2.32 ± 0.17 and 0.41 ± 0.03 ml/min/kg of body weight for normal and venous constricted kidneys, respectively ($P < 0.01$). Tissue analyses revealed a significant increase in sodium concentration in the renal cortex and outer medulla ($P < 0.02$) but not in the papilla (Text-



TEXT-FIG 1—Tissue concentrations of sodium, potassium, ammonium and urea in cortex, outer medulla and papilla of control and lymphatic occluded (*LO*) kidneys of rats.

Table 2—Effect of Renal Vein Constriction on Composition of Urine

	Volume (ml/2 hrs)	Urine osmolality (mOsm/kg H_2O)	Solute excretion (μOsm/2 hrs)	K (μEq/2 hrs)	Na (μEq/2 hrs)	Urea (μmole/2 hrs)	NH_4 (μEq/2 hrs)
Normal	.223 ± .021	1046 ± 41.3	.233 ± .019	19.3 ± 2.03	9.1 ± 0.57	28.7 ± 2.16	7.35 ± 0.69
Constricted	.112 ± .014	595 ± 24.6	.066 ± .008	4.16 ± .027	9.81 ± 0.64	6.62 ± .044	1.65 ± .011
P*	<0.01	<0.01	<0.01	<0.05	NS	<0.05	<0.01

* Derived from paired comparison analysis of 12 animals

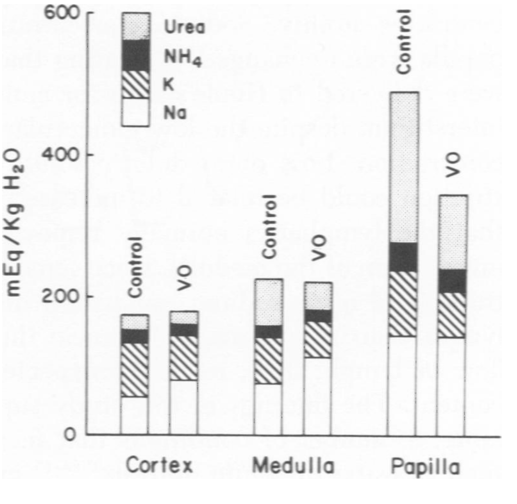
¶ Figures represent means ±SE.

figure 2). Potassium concentration was significantly decreased in all zones of the kidney ($P < 0.05$), and urea concentration was significantly decreased in the outer medulla and papilla ($P < 0.01$). The low potassium and urea concentrations in the papilla resulted in a significant decrease in the level of total tissue solute in the papilla compared to the control value ($P < 0.01$) (Text-figure 2).

Discussion

It is generally accepted that the lymphatic system has an important role in tissues and organs as a drainage and transport system to return substances to the blood and help maintain homeostasis. The kidneys have a well-developed system of lymphatics in the cortex, but there is conflicting data on the presence of lymphatics in the medulla.[4,13–18] Although renal lymph flow is of significant magnitude and generally equals urine flow, few studies have been done on the role of lymphatics in renal function. The present study reveals that occlusion of the renal lymphatics or enhanced renal lymph flow secondary to venous constriction in the rat have a significant effect on renal function.

Lymphatic occlusion produced a marked increase in urine flow and excretion of solutes; however, urine osmolality remained unchanged, so that the increase in solute excretion was proportional to the increase in urine flow. This water and solute diuresis was not due to an altered glomerular filtration rate, since this remained unchanged. Lymphatic occlusion had a prominent effect on the renal handling of urea. Urea clearance increased markedly, and urea concentration in the medulla,



TEXT-FIG 2—Tissue concentrations of sodium, potassium, ammonium and urea in cortex, outer medulla and papilla of control and venous occluded (*VO*) kidneys of rats.

especially the papilla, was greatly increased. It has been shown that a significant quantity of urea normally recycles in the kidney from collecting tubules to the medullary interstitium, the loops of Henle, the distal tubles and back to the collecting tubules.[19,20] An increased concentration of urea in the medulla secondary to lymphatic occlusion could result in more urea diffusing into Henle's loops. A proportional amount of water would diffuse into or less fluid would diffuse out of Henle's loops, so that flow in the loops and the distal tubules would be enhanced. Since distal and collecting tubules are not highly permeable to urea, rapid flow in these segments could greatly enhance urea clearance due to the brief contact time for diffusion. A urea diuresis in the distal nephron could account for inreased excretion of potassium, sodium and ammonium. Increased flow along the collecting tubules in the presence of a normal medullary osmotic gradient would result in a large flow of normally concentrated urine.

Lymphatic occlusion may enhance urea concentration in the medulla by the following mechanisms: a) decreased urea reabsorption by the proximal tubule with increased delivery of urea to the distal nephron and diffusion into the medulla, b) decreased effective medullary blood flow with decreased loss of urea via the medullary vessels and c) decreased loss of urea via lymph flow, assuming the presence of medullary lymphatics in the rat. Data from this study do not permit evaluation of these proposed mechanisms.

Venous constriction caused a marked dilatation of the lymphatics, and there was qualitative evidence of a great increase in lymph flow. Associated with this was a significant drop in total tissue solutes largely due to decreased urea, in the renal papilla and an impairment of concentrating ability. Sodium concentration in the renal medulla and papilla were unchanged, indicating that sufficient quantities of sodium were delivered to Henle's loop for active transport into the medullary interstitium despite the low glomerular filtration rate following venous constriction. Loss of medullary solute content following venous constriction could be related to increased lymph flow. There is evidence that the lymphatics normally remove solute from the hyperosmotic interstitium of the medulla, since some studies indicate that the concentrations of urea, sodium, potassium and chloride are higher in renal lymph than in plasma or thoracic duct lymph.[1,5,7,21,22] An increased flow of lymph, then, might be expected to diminish medullary solute content. The findings in this study support this concept and, furthermore, a number of conditions that increase lymph flow in the kidney such as water or solute diuresis [2,23–25] increased renal vein pressure,[2,7,8,] [25,26] ureteral occlusion [9,10,23,24,27] or stop-flow procedures [9] result in depletion of medullary hyperosmolality and impairment of concentrating abilty.

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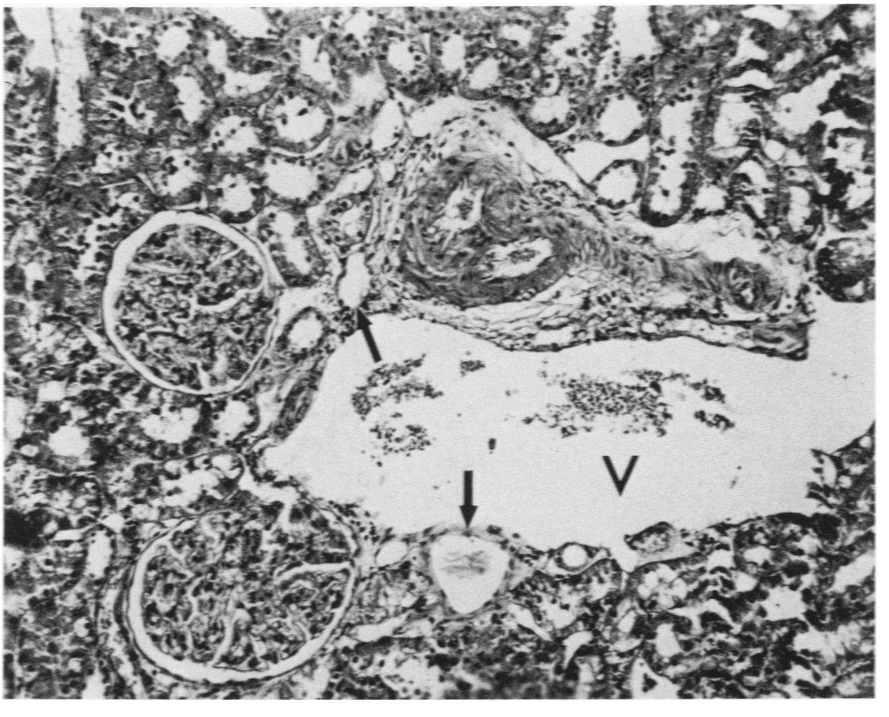

Fig 1—Small bloodless endothelium-lined channels (*arrows*) which are in close proximity to arcuate arteries and vein (*V*) and interpreted as distended lymphatics in a rat kidney with occluded lymphatics (× 130).

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