A Micropuncture Study of Postobstructive Diuresis in the Rat

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ABSTRACT In order to investigate the syndrome of postobstructive diuresis, clearance and micropuncture studies were carried out in rats after relief of 24 hr of bilateral (BUL) or unilateral (UUL) ureteral ligation. In rats with BUL, a striking diuresis and natriuresis occurred when the obstruction to one kidney (the experimental kidney) was relieved. The results were not influenced by administration of vasopressin or *d*-aldosterone. Whole kidney clearances of inulin and *p*-aminohippuric acid (PAH) in the experimental kidney were reduced to 10% and 20% of normal, respectively. Superficial nephron inulin and PAH clearances were also reduced, but only to 40% and 45%, respectively. These findings suggest a heterogeneity of nephron function in which deep nephrons were functioning poorly or not at all. To investigate the site of impaired tubular reabsorption in the surface nephrons, absolute and fractional water reabsorption was measured. Absolute reabsorption was found to be decreased all along the nephron. Fractional reabsorption in proximal tubules was normal, as indicated by an average end-proximal tubular fluid per plasma inulin (TF/P_{In}) of 2.16 vs. 2.30 in controls. TF/P_{In} was markedly decreased in distal tubules (2.91 vs. 8.02) and final urine (5.56 vs. 263). These observations indicate that the major sites of impaired sodium reabsorption leading to the diuresis were beyond the proximal tubule.

Rats with 24 hr of UUL did not demonstrate a comparable natriuresis or diuresis either spontaneously when the obstruction was relieved or after i.v. infusion of urea. A major difference between the BUL and UUL rats was that prerelease intrarenal hydrostatic pressure was markedly elevated (30.1 mm Hg) in the former but was below normal free-flow values (9.2 mm Hg) in the latter. Thus, elevation of intrarenal pressure during the period of obstruction may be causally related to the natriuresis and diuresis which occurs after the obstruction is relieved.

INTRODUCTION

It is well known that complete or partial obstruction of the urinary tract in man can result in a massive diuresis and natriuresis after the obstruction is relieved (1–6). Measurements of glomerular filtration rate (GFR)[1] in such patients have shown that filtration rate is usually moderately reduced (1–5). Thus, the large amount of sodium excreted in the urine cannot be accounted for by an increase in the filtered load of sodium, indicating that a defect in sodium reabsorption by the renal tubules is present. The specific site of the defect in sodium reabsorption, as well as the mechanism of the diuresis and natriuresis, remain unclear. Bricker et al. (3) and

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[1] *Abbreviations used in this paper:* BUL, UUL, bilateral and unilateral ureteral ligation, respectively; C_{In}, C_{PAH}, C_{urea}, whole kidney clearance of inulin, PAH, and urea, respectively; E_{PAH}, PAH extraction; EF_K, EF_{Na}, excreted fraction of filtered potassium and sodium, respectively; FF, whole kidney filtration fraction; GFR, glomerular filtration rate; NSPF, nonsecretory plasma flow; PAH, *p*-aminohippuric acid; RA, RV, renal artery and vein, respectively; RPF, renal plasma flow; SNC_{PAH}, single nephron clearance of PAH; SNFF, single nephron filtration fraction; SNGFR, single nephron glomerular filtration rate; $T^c_{H_2O}$, solute-free water reabsorption; TF/P_{In}, tubular fluid per plasma inulin; U/P_{In}, U/P_{PAH}, urine per plasma inulin and PAH, respectively; U_KV, $U_{Na}V$, rate of potassium and sodium excreted in urine, respectively; V, rate of urine flow.

Witte, Short, and Hollander (6) have concluded, on the basis of clearance data, that the primary site of inhibited sodium reabsorption is in the proximal tubule. On the other hand Roussak and Oleesky (1), Earley (2), Winberg (4), and Berlyne (5) have suggested a distal tubular defect, based upon their observations of abnormalities in urinary acid excretion and concentrating ability.

In the present report, an experimental model of postobstructive diuresis in the rat (7) was studied in order to determine the site in the nephron of the defect in sodium reabsorption and the mechanism(s) of the natriuresis. Micropuncture experiments were carried out in rats after a 24 hr period of bilateral ureteral ligation (BUL). The results suggest that the sites of impaired sodium reabsorption predominantly responsible for the natriuresis are beyond the proximal tubule. GFR and plasma flow of superficial nephrons were moderately reduced, but much less so than whole kidney clearances of inulin and *p*-aminohippuric acid (PAH). This suggests that deeper nephron function was either markedly decreased or entirely absent. Clearance studies in rats with unilateral ureteral ligation (UUL) showed that neither a diuresis nor natriuresis occurred in the presence of a normally functioning contralateral kidney. A striking difference between the UUL kidney and the BUL kidney was that prerelease intrarenal hydrostatic pressure was below normal free-flow values in the former but was markedly elevated in the latter. This high hydrostatic pressure may have been casually related to inhibition of sodium and water reabsorption observed in this experimental model.

METHODS

23 male white rats weighing 220–390 g were anesthetized lightly with i.p. Inactin (Promonta, Hamburg, West Germany) and both ureters were ligated with cotton thread through a small midline abdominal incision (BUL rats). The wound was sutured and the animal returned to a metabolic cage. Food and water were withheld for the next 24 hr. In five other rats weighing 240–380 g the ureter of only the left kidney was ligated (UUL rats) and in a sixth animal, the left ureter was ligated and the right kidney excised. Food was withheld from only two of the five UUL rats. The control group consisted of nine normal rats weighing 245–360 g and three rats in which the ureter of the right kidney was ligated 24 hr before study. The purpose of right ureteral ligation in these three control animals was to evaluate the effects of the i.v. infusion during the micropuncture study on sodium and water excretion and renal hemodynamics in the absence of a functioning contralateral kidney. As analysis of clearance and micropuncture data revealed no significant differences between these three rats and the nine normal animals, the data from all 12 rats have been pooled. Food but not water was withheld from all control animals during the 16 hr preceding the micropuncture experiment. All micropuncture and clearance data described below were obtained from the left kidney (experimental kidney) except in those few instances (see Results) where clearance data were obtained from both kidneys.

Micropuncture and clearance experiments were carried out in 20 of 23 bilaterally obstructed rats, and in the 3 rats with only right ureteral obstruction approximately 24 hr after ureteral ligation. The methods of anesthesia and surgical preparation of all the animals for micropuncture study have been described previously (8). A polyethylene PE 50 catheter was placed in the left renal pelvis cephalad to the ureteral ligature 1 hr before urine collections or tubular fluid collections were begun. The experimental observations were then made over the following 3–4 hr. All animals received Ringer's lactate solution i.v. at a rate of 0.05 ml/min containing carboxyl inulin-^{14}C and, in most, glycyl-2-^{3}H-*p*-aminohippuric acid (PAH) (New England Nuclear Corp., Boston, Mass.). The concentrations of these compounds were estimated to provide a count at least three times that of the background in the smallest samples measured. Vasopressin (9 mU/kg per hr) (Parke, Davis & Co., Detroit, Mich.) and *d*-aldosterone (21 μg/kg per hr) (Ciba Pharmaceutical Co., Summit, N. J.) were added to the infusion in nine control rats and seven BUL rats. In three of the five UUL animals, urine was collected from the bladder as well as the left renal pelvis. In all animals, arteriolized blood was collected from the cut end of the tail at approximately 45-min intervals throughout the experiment. In experiments in which PAH extraction (E_{PAH}) was measured, renal venous blood was slowly withdrawn from the renal vein using a No. 28 gauge needle.

Timed tubular fluid samples were collected from end-proximal convolutions on the surface of the kidney, identified by both their anatomical location (9) and by i.v. injection of a bolus of 10% Lissamine green (9). Timed tubular fluid samples were also collected from distal tubules on the surface of the kidney, identified by their thin glistening epithelium and by i.v. Lissamine green injection. In order to insure complete collections in both proximal and distal tubules, a long column of castor oil stained with Sudan black was injected distally and maintained in a constant position by regulating the rate of tubular fluid collection. Although the site of collection in the distal tubule was not determined precisely by microdissection, the approximate location was judged from the injection of small oil droplets from the collecting pipet. If oil droplets appeared in convolutions distal to the pipet tip, we assumed that the pipet was in an early segment of the distal tubule. If injected droplets disappeared immediately below the surface of the kidney, we assumed that the pipet was in a late distal segment close to a collecting tubule. Using these criteria as a base, we estimated that collections were obtained from both early and late portions of the distal tubule. Thus, the mean values for tubular fluid per plasma inulin (TF/P_{In}) for the distal tubule probably represent mid-distal values. This assumption is supported by the similarity between our values in the normal rats and those reported by others (10) for the middle third of the distal tubule. In addition to tubular fluid collections, Lissamine green transit times were measured from the first appearance of the dye in the surface blood vessels to the end-proximal tubule (proximal transit time) or to the site of puncture in the distal tubule (distal transit time).

In addition to the above experiments, hydrostatic pressure was measured in proximal and distal tubules and in efferent arterioles at the center of vascular stars (9) in three BUL and three UUL rats before relief of ureteral obstruction. Neither urine nor tubular fluid was collected from these rats. Pressures were also measured in six BUL animals 1 hr after

release of left ureteral obstruction. The technique used to measure hydrostatic pressure was that described by Gottschalk and Mylle (11, 12).

After a 1 hr control clearance measurement in two of the UUL animals, an i.v. urea load (300 mg/ml at 0.1 ml/min for 20 min) was administered in order to elevate the plasma urea level without expanding body fluids by more than 1% (13). Two additional 30–45 min urine collections were then obtained.

All tubular fluid, plasma, and urine samples were transferred to a constant bore capillary tube and their volumes measured by a prevously described method (14). The samples were then washed into liquid scintillation vials with five drops of water. The liquid scintillation solution consisted of 88% toluene, 9% Bio-Solv Solubilizer (Beckman Instruments, Inc., Fullerton, Calif.), and 3% Liquifluor (New England Nuclear Corp.). The activities of ^{3}H and ^{14}C in tubular fluid, plasma, and urine were determined by liquid scintillation techniques. Quench correction was calculated by channels ratio using ^{133}Ba as an external standard. Plasma ^{14}C and ^{3}H levels were expressed as a function of time so that the values for the midpoint of tubular fluid and urine collections could be determined.

Urine and plasma sodium and potassium concentrations were determined by flame photometry using lithium as an internal standard. Osmolalities of urine and plasma were determined by freezing point depression. Urea concentrations were determined by the urease method of Chaney and Marbach (15).

Clearances of inulin and PAH for single nephrons (SNGFR and SNC_{PAH}) were determined from:

$$SNGFR = TF/P_{In} \times TF \text{ flow rate}, \quad (1)$$

and

$$SNC_{PAH} = TF/P_{PAH} \times TF \text{ flow rate}. \quad (2)$$

In the case of SNGFR, calculation was based upon individual collections of either proximal or distal tubular fluid samples. All data on SNC_{PAH} were calculated from distal tubular samples only. Whole kidney clearances were calculated from the expressions:

$$GFR = U/P_{In} \times V, \quad (3)$$

and

$$C_{PAH} = U/P_{PAH} \times V, \quad (4)$$

where U/P_{In} and U/P_{PAH} are urine per plasma inulin and PAH, respectively, and V is urine flow rate. No correction was made for plasma water content or PAH binding by proteins in the above calculations. As a result, the inulin clearances in the present study are slightly higher than those previously reported from this laboratory (16). However, in expressing fractional reabsorption of filtered water in the various nephron segments, TF/P_{In} and U/P_{In} were corrected for a plasma water content of 94%. Total renal plasma flow (RPF) was calculated from Wolf's equation (17)

$$RPF = \frac{(U_{PAH} - RV_{PAH}) \times V}{(RA_{PAH} - RV_{PAH})}, \quad (5)$$

where RV and RA are the renal artery and renal vein, respectively. The flow of plasma through kidney tissue not capable of secreting PAH, nonsecretory plasma flow (NSPF), was calculated from

$$NSPF = RPF - C_{PAH}. \quad (6)$$

Filtration fraction for single nephrons (SNFF) was calculated from

$$SNFF = SNGFR/SNC_{PAH}. \quad (7)$$

SNFF was calculated from the simultaneously determined SNGFR and SCN_{PAH} of individual distal tubular samples.

All statistics were calculated according to methods described by Steel and Torrie (18). The statistics are presented as mean ±SE. In the analysis of micropuncture data, "N" refers to the total number of samples. However, in whole kidney clearance data, all values for a given animal were averaged and considered as a single value in order to avoid a bias in favor of the BUL animals where the number of urine collections per experiment was greater than in the control animals. All calculations were carried out by appropriate computer programs on an Olivetti Programma 101.

RESULTS

Excretory data in BUL and control rats. After 24 hr of BUL, a striking diuresis and natriuresis occurred from the left kidney when the obstruction to its ureter was relieved. As shown in Table I, the V from the experimental kidney of the BUL rats was more than three times greater than that from the left kidney of the control rats. V approximated 18% of the GFR (V/GFR) in the BUL rats as compared with less than 1% in the control animals. The difference between the two groups is even more striking when the fact is considered that the experimental animals were deprived of water overnight whereas the control animals were not. The addition of vasopressin to the i.v. infusion in seven of the experimental rats did not significantly alter the magnitude of the diuresis (133.8 ±49.6 vs. 124.2 ±19.8 μl/min per kg). Qualitatively, a similar result was seen with respect to sodium excretion. The experimental rats excreted 2½ times more sodium in their urine ($U_{Na}V$) than did the control rats. The excreted fraction of filtered sodium (EF_{Na}) in the BUL rats was 13%, in comparison with 0.6% in the control rats. The presence of *d*-aldosterone in the infusion of 7 of the 19 BUL rats did not abolish the natriuresis (EF_{Na} = 11.2 ±1.8% vs. EF_{Na} = 17.9 ±3.2%). Although absolute potassium excretion (U_KV) was not significantly higher in the BUL animals, the excreted fraction of filtered potassium (EF_K) was increased to nearly 90%. In about one-third of the individual urine samples from the BUL rats, net potassium secretion ($EF_K > 100\%$) was observed. The presence of aldosterone in the infusion of seven BUL rats was without significant effect on potassium excretion. (EF_K = 90.8 ±15.6% vs. 87.5 ±11.0%). Average urine osmolality in the experimental rats was only 420 mOsm/kg H_2O in comparison with 1290 mOsm/kg H_2O in the control animals. Within the group of BUL rats, vasopressin had no significant effect on urine osmolality (446 ±10 vs. 410 ±13 mOsm/kg H_2O).

Renal hemodynamic data in BUL and control rats. In Table II, the clearance data for inulin (GFR) and

TABLE I

Excretory Data for Experimental Kidney of BUL Postobstructive and Control Animals

	Control rats	BUL rats	P
V, *μl/min per kg*	34.6 ±5.2 (12)*	121.1 ±17.3 (19)‡	<0.001
(V/GFR) × 100, %	0.62 ±0.12 (12)	17.81 ±1.86 (19)	<0.001
$U_{Na}V$, *μEq/min per kg*	4.8 ±1.1 (11)	11.3 ±1.6 (19)	<0.01
$EF_{Na} \times 100$, %	0.6 ±0.1 (11)	13.0 ±1.7 (19)	<0.001
U_KV, *μEq/min per kg*	3.2 ±0.5 (11)	3.7 ±0.5 (18)	NS
$EF_K \times 100$, %	13.0 ±2.2 (11)	89.6 ±11.5 (18)	<0.001
U_{Osm}, *mOsm/kg* H_2O	1290.0 ±268.0 (7)	420.0 ±17.0 (11)	<0.001

V, rate of urine flow; (V/GFR) × 100, per cent of filtered water excreted; $U_{Na}V$, rate of sodium excretion in urine; $EF_{Na} \times 100$, per cent of filtered sodium excreted; U_KV, rate of potassium excretion in urine; $EF_K \times 100$, per cent of filtered potassium excreted; U_{Osm}, urinary osmolality; NS equals $p > 0.05$.

* The numbers in parentheses represent the number of animals.

‡ Part of the urine volume was lost from one rat in a laboratory accident.

PAH (C_{PAH}) obtained from the left kidney of BUL and control animals are shown. As can be seen, GFR in the experimental animals was reduced to about 10% of control and C_{PAH} to about 17% of the control values. Since the E_{PAH} was also decreased in the BUL rats, total RPF fell less than C_{PAH}. The difference between RPF and C_{PAH} has been used to estimate medullary blood flow (19, 20). Since this component of total RPF may not represent flow to the medulla exclusively, particularly in our experimental animals, we have designated it "nonsecretory plasma flow" (NSPF). As seen in Table II, this value was slightly, but not significantly, higher in the experimental animals. When expressed as a per cent of total RPF, however, NSPF constituted a much higher fraction in the BUL animals than in the controls (56.9 vs. 17.4%). As shown in the table, whole kidney filtration fraction (FF) was significantly lower in the experimental animals than in the controls.

Plasma urea concentration was measured in six BUL and six normal animals. The average value in the BUL rats was 38.8 ±1.9 mM compared with 5.5 ±0.10 mM in the normal rats ($P < 0.001$). The ratio of urea clearance to inulin clearance (C_{Urea}/C_{In}) in the six BUL rats was 0.36 ±0.07. This ratio is close to that reported in normal nondiuretic rats (21).

Micropuncture data in BUL and control rats. At the time of micropuncture 1 hr after relief of the left ureteral obstruction, the experimental kidney of the BUL animals appeared larger than normal. The surface tubules were all patent and in most kidneys the distal tubules were 3–4 times wider in diameter than in normal kidneys. Blood flow through the surface capillaries appeared markedly slowed. When Lissamine green was injected i.v., the dye appeared in all surface tubules. The transit time of Lissamine green to the end-proximal tubule was significantly prolonged in the experimental rats (29.9 ±2.0 vs. 10.4 ±0.5 sec; $P < 0.001$). In some BUL animals in which GFR was greatly reduced, it was difficult to determine the transit time to the distal tubule accurately. In those instances in which it could be measured, the transit time to the distal tubule was markedly prolonged (115.1 ±9.6 vs. 44.2 ±1.2 sec; $P < 0.001$). In order to determine fractional reabsorption of water along the nephron, TF/P_{In} in proximal and distal tubules and U/P_{In} were measured. The results are shown in Fig. 1. As can be seen, end-proximal TF/P_{In} in BUL rats (E) did not differ signifi-

TABLE II

Renal Hemodynamics of Experimental Kidney in BUL Postobstructive and Control Rats

	Control rats	BUL rats	P
GFR, *ml/min per kg*	6.64 ±0.51 (12*)	0.76 ±0.11 (19)	<0.001
C_{PAH}, *ml/min per kg*	17.53 ±1.40 (7)	2.91 ±0.58 (14)	<0.001
FF	0.41 ±0.02 (7)	0.28 ±0.01 (14)	<0.001
E_{PAH}	0.80 ±0.01 (7)	0.40 ±0.04 (10)	<0.001
RPF, *ml/min per kg*	21.48 ±1.55 (7)	7.44 ±1.58 (10)	<0.001
NSPF, *ml/min per kg*	3.74 ±0.63 (7)	4.22 ±0.88 (10)	NS

* The numbers in parentheses refer to the number of animals.

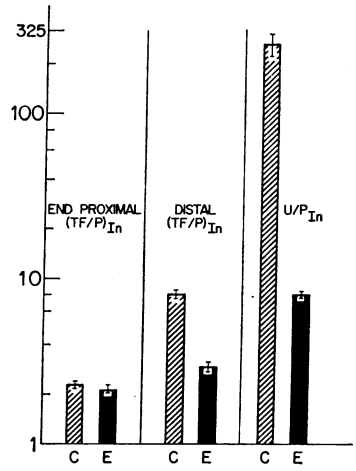

FIGURE 1 TF/P_{In} ratios in control rats (C) and BUL experimental rats (E) undergoing postobstructive diuresis. U/P_{In} ratios are shown at the right. The vertical line inserts represent SE.

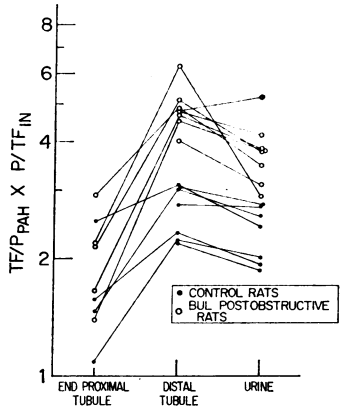

FIGURE 2 TF/P_{PAH} ratios corrected for water reabsorption (P/TF_{In}). Each point represents the average of 2–5 measurements in a single animal and the lines connect the means from individual animals. The data indicate addition of PAH to the tubular lumen between the late proximal convolutions and the distal tubule in both control and BUL rats.

cantly from that in the control animals (C) (2.16 ±0.13 vs. 2.30 ±0.13). On the other hand, distal TF/P_{In} in the experimental animals was significantly lower (2.91 ±0.20 vs. 8.02 ±0.54; $P < 0.001$). U/P_{In} was also markedly lower in the BUL animals than in the controls (5.56 ±0.37 vs. 263 ±42).

SNGFR on the surface of the kidney was calculated from both proximal and distal tubular fluid timed collections (Table III). Within the group of control animals there was no significant difference in SNGFR calculated from proximal and distal tubular fluid samples. In the BUL rats there was considerable variation of SNGFR from on animal to another, but within individual animals there was again no apparent difference of SNGFR calculated from proximal and distal tubular samples. As shown in Table III SNGFR in the BUL rats was reduced to about 40% of that found in the control animals. Although the decrease in SNGFR was highly significant ($P < 0.001$), it was relatively less than the 90% reduction in whole kidney GFR.

In addition to SNGFR, we determined the SNC_{PAH}. In order for this value to be representative of nephron plasma flow, the addition of PAH to the tubular lumen must have been completed at a site proximal to the point of tubular fluid collection. This segment of the nephron was determined by comparing the concentration of tubular fluid PAH, corrected for water reabsorption, in the various nephron segments by multiplying $TF/P_{PAH} \times P/TF_{In}$. These calculations are shown in Fig. 2. In both experimental and control rats tubular fluid PAH was found to rise between the end of the proximal convolution and the distal tubule. Presumably, the additional PAH secretion occurred in the pars recta of the proximal tubule (22, 23). The value failed to rise between distal tubules and the final urine, and actually fell in every case but two. The data confirm the observations of Cortney, Mylle, Lassiter, and Gottschalk (22). These authors, using microdissection techniques, found no significant change in $TF/P_{PAH} \times P/TF_{In}$ between early and late distal tubu-

TABLE III

Single Nephron Glomerular Filtration Rate (SNGFR) and PAH Clearance (SNC_{PAH}) in BUL Postobstructive and Control Rats

	Control rats	BUL rats	P
SNGFR, *nl/min per kg*	130 ±7 (48)*	51 ±4 (64)	<0.001
SNC_{PAH}, *nl/min per kg*	377 ±21 (16)	171 ±18 (19)	<0.001
SNFF‡	0.36 ±0.02 (16)	0.23 ±0.01 (19)	<0.001

* The numbers in parentheses represent the total number of tubular fluid samples. In the case of SNC_{PAH}, the samples were collected from the distal convoluted tubule. In the case of SNGFR, samples were collected from both the proximal and distal convoluted tubules.
‡ SNFF equals single nephron filtration fraction as defined in equation number 7.

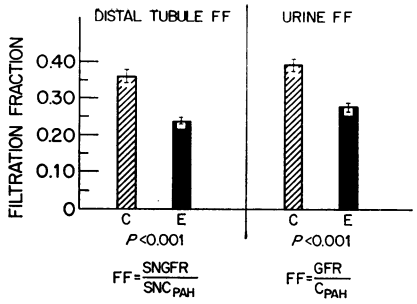

FIGURE 3 Filtration fraction determined from measurement of PAH and inulin in distal tubules and urine. "C" represents the control rats and "E" the BUL experimental rats.

lar samples, which suggests that secretion of PAH is completed before the accessible portion of the distal tubule. SNC_{PAH} was therefore calculated only from distal tubular fluid collections.

The validity of the measured SNC_{PAH} as an estimate of actual single nephron plasma flow depends upon several different theoretical variables. These include the number of secretory sites for PAH per unit area, the total proximal tubular length of individual nephrons, the concentration of PAH in peritubular blood, and the relative admixture of postglomerular blood from different nephrons. However, as shown in Table III, when SNFF was estimated from SNC_{PAH} and SNGFR, the value was in reasonable agreement with the FF (Table II). Furthermore, SNFF determined by this method agrees fairly well with that determined from postglomerular protein concentrations (24). Thus it appears that SNC_{PAH} provides a reasonable estimate of single nephron plasma flow. As shown in Table III, the average value for SNC_{PAH} in normal control rats was 377 nl/min per kg. The value in the BUL rats was reduced to 171 nl/min per kg. This 55% reduction is slightly less than the 60% reduction in SNGFR.

SNFF and the comparable FF for the control and BUL rats are shown in Fig. 3. In the control rats, the mean SNFF of 0.36 was slightly lower than the value of 0.41 for the whole kidney. In the BUL rats, the SNFF was reduced to 0.23 and the FF was reduced to 0.26. The differences between the BUL and control animals for both SNFF and FF are highly significant ($P < 0.001$). Brenner and Troy (24) have calculated SNFF in normal rats from the protein concentration in efferent arteriolar blood. Their mean value of 0.32 is comparable to our value of 0.36 in control rats. The slightly higher value calculated by the present method may be due to protein binding of PAH (22) which, by artificially lowering C_{PAH}, yields a higher calculated filtration fraction. If we correct our average SNC_{PAH} value for 10% protein binding of PAH (22), the calculated value for SNFF in our control rats is 0.32, identical to that obtained by Brenner and Troy (24).

In addition to tubular fluid collections, hydrostatic pressure was measured in proximal and distal tubules and in efferent arterioles approximately 1–3 hr after relief of the obstruction to the left ureter in BUL rats. The pressure in 46 proximal tubules was 14.5 ±0.5 mm Hg, and that in 19 distal tubules was 10.0 ±0.4 mm Hg. Pressure in 19 efferent arterioles was 13.9 ±0.6 mm Hg. The mean pressures measured in the proximal and distal tubules are slightly higher, and that in the efferent arteriole is slightly lower than those found by Gottschalk and Mylle (11, 12) in normal rats, using the same technique.

Studies on unilateral ureteral obstruction. Clearance experiments were carried out in three rats in which only the left ureter (experimental kidney) had been ligated for 24 hr. During the experiment, urine was collected from both the left renal pelvis above the obstruction and the right kidney via the bladder. The results are shown in Table IV where "E" refers to the left experimental kidney and "C" refers to the right control kidney. As can be seen in part A, the rates of V, $U_{Na}V$, and EF_{Na} were lower on the previously obstructed side than on the control side. Thus no diuresis or natriuresis occurred in these animals. The observations are in agreement with the data reported by Wilson (25, 26) for rats in which chronic partial unilateral ureteral occlusion had been produced. In general, although GFR and C_{PAH} were lower on the previously obstructed side, the reductions were less extreme in two out of the three animals than that which was observed in the BUL rats. Filtration fraction was lower on the previously obstructed side in two of the three rats. As seen in the last column, the plasma urea concentration, although higher than normal in two of the three rats, was much lower than that found in the bilaterally obstructed animals. The data following i.v. urea infusion in two of these three animals are shown in part B. Again, FF, GFR, and C_{PAH} on the previously obstructed side were lower than on the contralateral normal side. Although $U_{Na}V$ and EF_{Na} rose on both sides after urea infusion, the average EF_{Na} of 1.2% for the previously obstructed kidney was far lower than the 13.0% observed in the BUL rats (Table I), despite a comparable elevation of plasma urea. The urine volume, although increased by urea infusion, averaged only 2.35% of the filtered water in the previously obstructed kidney and 2.12% in the contralateral normal kidney. Both of these values differ greatly from the 17.8% of filtered water excreted by the BUL animals. Thus, although elevating plasma urea concentration caused a slight increase in fractional water and sodium excretion in the UUL rats, the values fell far short of

TABLE IV

Clearance and Excretory Data from Experimental Kidney (E) and Contralateral Control Kidney (C) in UUL Rats

Rat No.	V*		GFR		C_{PAH}		FF		$U_{Na}V$		EF_{Na}		Plasma urea
	E	C	E	C	E	C	E	C	E	C	E	C	
	μl/min per kg		*ml/min per kg*		*ml/min per kg*				*μEq/min per kg*		%		*mmoles/liter*
Part A: Before urea loading													
1	21.1	26.5	3.80	7.20	14.43	31.47	0.26	0.23	3.24	6.84	0.57	0.65	5.66
2	10.1	34.1	2.30	4.01	10.21	11.62	0.23	0.34	0.71	4.51	0.19	0.75	10.01
3	4.1	25.7	0.50	4.62	1.51	10.61	0.33	0.44	0.40	2.23	0.51	0.93	12.30
Part B: After an i.v. urea load													
1	49.9	170.9	2.86	6.62	8.44	14.79	0.34	0.45	6.40	18.02	1.50	1.87	22.01
	50.1	138.3	3.31	8.10	10.08	17.33	0.33	0.47	6.14	18.26	1.23	1.54	22.52
2	90.1	208.8	2.73	4.44	5.90	7.48	0.43	0.59	5.27	8.04	1.23	1.15	47.63
	97.5	166.5	3.43	4.68	8.29	9.28	0.42	0.50	4.29	3.25	0.79	0.44	56.61
Part C:‡ Data from a left postobstructive kidney in a rat with right nephrectomy													
4‡	131.8		1.26		4.51		0.28		9.62		5.26		
	141.1		1.36		3.93		0.35		12.06		6.11		
	123.8		1.55		4.22		0.37		9.35		4.15		65.6
	130.3		1.80		5.08		0.35		8.99		3.44		

* Abbreviations represent the same quantities as defined in Table I and Table II.

‡ Rat No. 4 underwent left ureteral ligation and right nephrectomy 24 hr before study. The clearances were obtained beginning 1 hr after release of the left ureter. Urea was not administered to this animal.

that seen in the bilaterally obstructed animals with comparable plasma urea levels.

In part C of Table IV are the data obtained from a single animal in which the right kidney was excised at the same time that the left ureter was ligated 24 hr before the experiment. As can be seen, the diuresis and natriuresis that developed from the remaining left kidney were of the same order of magnitude as that which was observed in the BUL rats (Table I). The EF_{Na} was 4–5 times higher than that observed in the UUL rats with urea loading.

Intrarenal hydrostatic pressure before relief of obstruction. Intrarenal hydrostatic pressure was measured before relief of 24 hr of obstruction in three BUL and three UUL rats. The results are shown in Table V. As can be seen, both proximal and distal intratubular pressures in the BUL rats were markedly higher than the postrelease free-flow pressures (see above). The pressure in efferent arterioles in the prerelease period was consistently and significantly lower ($P < 0.001$) than both the proximal and distal intratubular pressures. In sharp contrast, the intrarenal pressures in the kidneys of the UUL animals before release of the obstruction were all reduced below normal free-flow pressures, and were significantly lower than the pressures observed in the BUL rats. As shown in the last column, the plasma urea level was markedly elevated in the BUL rats but much less so in the UUL rats.

TABLE V

Hydrostatic Pressure in Surface Tubules and Efferent Arterioles Before Release of Ureteral Obstruction in BUL and UUL Rats

Group	No. of rats	Proximal tubular pressure	Distal tubular pressure	Efferent arteriolar pressure	Plasma urea
		mm Hg	*mm Hg*	*mm Hg*	*mmole/liter*
BUL	3	30.1 (32)* ±2.3	27.7 (25) ±2.1	19.0 (8) ±2.1	54.7 (3) ±7.0
UUL	3	9.2 (20) ±0.6	6.5 (11) ±1.0	5.5 (11) ±0.6	10.0 (3) ±1.1
P		<0.001	<0.001	<0.01	<0.001

* The numbers in parentheses represent the total number of measurements.

DISCUSSION

This model of postobstructive diuresis in the rat is similar in several respects to the syndrome that has been described in man after relief of urinary tract obstruction. In patients with this syndrome, urine flow is frequently massive (1–4, 6) as is the excretion of sodium and potassium (3, 6). In addition, there is a concentrating defect which is effected little or not at all by the administration of vasopressin (1–6). The experimental model which we have studied here differs to the extent that GFR was reduced to a greater degree than is usually observed in man, and that the obstruction was of considerably shorter duration than is usually present in patients (1–3, 5, 6). In spite of these differences, the features of the natriuresis in our animals seem to resemble closely those which are seen in patients with the postobstructive syndrome. The reason for the lower GFR is not certain but may be related to the fact that water was withheld from our experimental animals for 24 hr whereas patients with the postobstructive syndrome are usually not dehydrated and are often overhydrated. The site of impaired sodium reabsorption is unknown in either man or experimental animals but on the basis of clearance data various investigators have assigned it to either the proximal (3, 6, 27–29) or the distal tubules (1, 4, 5).

In the present investigation in rats, three findings seem to us to be most important. First, ureteral ligation led to a heterogeneity of nephron function in which GFR and the perfusion of surface nephrons were moderately reduced, whereas the function of the deeper nephrons was apparently much more severely impaired. Second, the natriuresis and diuresis resulted from impaired sodium reabsorption in segments of the nephron beyond the proximal tubule. Third, the fact that a diuresis occurred after BUL but not UUL suggests that a high plasma urea concentration during the period of obstruction may play some role in the mechanism of the diuresis. Each of these findings will be discussed below.

The suggestion that nephrons were functioning in a heterogeneous fashion is based upon the observation that whole kidney clearances of inulin and PAH were reduced to a much greater extent than the comparable clearances of these substances by individual surface nephrons. Since all of the tubules that were visible on the kidney surface were widely patent, suggesting that they were all functioning, we assume that the deeper nephrons were particularly affected. We have illustrated this in Fig. 4 by plotting SNGFR against the simultaneously determined GFR of the whole kidney. It has been demonstrated (30–32) that there are approximately 30,000 nephrons in the rat kidney. If all are functioning at the same rate, the product of SNGFR × 30,000 should yield a close approximation of whole kidney GFR and the observed values should fall on or about the solid line shown in Fig. 4. As can be seen however, all observed values for the normal control animals fell above the line. In other words, the observed whole kidney GFR in these nondiuretic rats was greater than the product of superficial SNGFR × 30,000. A number of other laboratories have demonstrated by direct micropuncture of the long loop of Henle at the tip of the papilla that the juxtamedullary nephrons in nondiuretic rats have a filtration rate which is roughly twice as high as the nephrons on the surface of the kidney (33–35). The data for our normal animals shown in Fig. 4 are thus compatible with that finding. In contrast, most of the observed data for the experimental animals fell below the line. In this case, the product of superficial SNGFR × 30,000 yields a value greater than that observed for the whole kidney. This suggests that a population of nephrons beneath the surface of the kidney was functioning at a rate even lower than the superficial nephrons. We estimated the maximum number of nephrons which could be filtering at the rate observed in the superficial nephrons by calculating the regression coefficient of the line passing through the data for the BUL rats and the origin. The slope of this line indicates that at most 14,300 nephrons were filtering at a rate comparable to the observed surface nephrons. Therefore, at least 50% of the nephrons were contributing little or nothing to the final urine. As several anatomical studies (33, 36, 37) have estimated that the juxtamedullary nephrons comprise only 20–30% of the total nephron population in the rat kidney, this calculation suggests that the function of many mid-cortical nephrons as well as juxtamedullary nephrons must have been severely impaired by the obstruction to the urinary tract.

The data on SNC_{PAH} clearance may be compared to C_{PAH} in the same fashion, as shown in Fig. 5. Again, the values for the normal control animals all fall above the line of $SNC_{PAH} \times 30{,}000$. This implies that in normal nondiuretic rats, the PAH clearance (i.e. plasma flow) of the deeper nephrons, like their rate of filtration, is greater than that of the superficial nephrons. In contrast, all values for the BUL experimental animals fall below the line. Although this can be interpreted to mean that plasma flow was severely reduced to the deeper nephrons, an alternative explanation is that the reduction in filtration rate in the deep nephrons precluded their contributing PAH to the final urine, irrespective of the amount of blood flowing through their peritubular capillaries. A similar conclusion can also be drawn from the filtration fraction data. If filtration fraction is calculated as C_{In}/RPF, rather than C_{In}/C_{PAH}, the value for FF becomes 0.336 ±0.015 for the control animals and 0.122 ±0.017 for

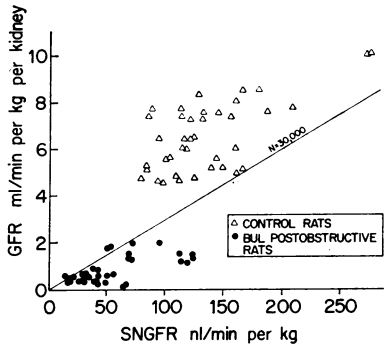

FIGURE 4 Relationship between SNGFR of surface nephrons and simultaneously measured whole kidney GFR. The diagonal line was derived by multiplying SNGFR × 30,000, the approximate total number of nephrons in the rat kidney. The experimental data are from BUL rats undergoing postobstructive diuresis.

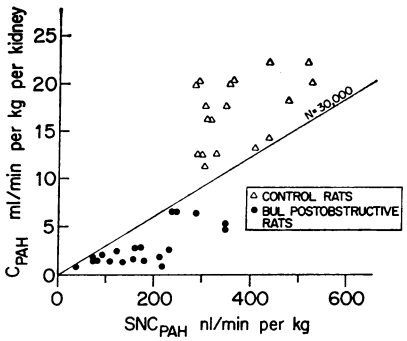

FIGURE 5 Relationship between SNC_{PAH} of surface nephrons and simultaneously measured whole kidney PAH clearance. The diagonal line was derived by multiplying SNC_{PAH} × 30,000. The single nephron data for both the control and BUL experimental rats were obtained from distal tubular fluid collections.

the BUL animals. Although the FF of the control kidneys is similar to that calculated from their individual distal tubules, the FF for the BUL animals is quite markedly lower than their surface SNFF. This strongly suggests that in the BUL rats a significant portion of the renal plasma flow was delivered to areas of the kidney which were not able to form glomerular filtrate. These areas are presumably the medulla and the lower portions of the cortex whose nephrons, although perfused, were filtering very poorly. Why these deeper nephrons were more severely impaired than the superficial nephrons is not clear, but it may relate to the functional and anatomical differences between the juxtamedullary and surface nephrons. For example, the relative deficiency of renin around the afferent arteriole of the juxtamedullary nephrons under normal conditions (38) might make them respond differently to stop-flow conditions than the vasculature of the superficial nephrons. In addition, the efferent vasculature of the juxtamedullary glomeruli differs from that of the superficial nephrons. The postglomerular blood of these deep nephrons may flow through the subcortical capillary plexus or directly to the vasa recta (39). Thurau has suggested that resistance to blood flow through the long vasa recta may provide most of the efferent resistance of these nephrons (40). One of the determinants of resistance to flow is fluid viscosity. In the normal medulla, the hypertonic environment probably causes blood viscosity to rise due to the crenation of red cells (41) and the threefold increase in protein concentration that occurs in the vasa recta (42–46). Therefore, medullary hypertonicity may determine, in part, the efferent resistance of the juxtamedullary glomeruli. There is good evidence that acute ureteral occlusion leads to a washout of medullary solutes (28, 47, 48) and this may in turn decrease the resistance to flow through the vasa recta. If resistance falls in the vasa recta, there could result a sharp decrease in filtration pressure in the juxtamedullary glomeruli. Several authors have demonstrated that medullary blood flow increases during acute ureteral occlusion (49, 50) which would be in accord with this idea. Our data on NSPF, although not strictly a measure of medullary blood flow, are compatible with the view that medullary blood flow may not be decreased after relief of chronic bilateral ureteral obstruction, at least in comparison to total renal blood flow. If this assumption is correct, it could reflect a reduced resistance in the vasa recta and a fall in filtration pressure in the juxtamedullary glomeruli. Whatever the mechanism, it seems likely that nephron heterogeneity is probably not in itself a cause of the natriuresis and diuresis, since Wilson (25) found that rats with unilateral ureteral occlusion exhibit a heterogeneity of nephron function similar to that observed in our BUL animals. In contrast to our BUL rats, spontaneous natriuresis and diuresis did not occur in his animals nor in unilaterally obstructed rats studied by Jaenike (51).

The second point of interest in this model of postobstructive diuresis is that the sites in the nephron responsible for sodium loss were beyond the end of the proximal tubule. Calculations of absolute and fractional water reabsorption for the different nephron segments of the superficial nephrons are shown in Table VI. The data show that absolute reabsorption was decreased along the entire nephron in BUL rats. Fractional reabsorption by the end of the proximal convolutions, however, was not significantly different from that measured in the normal control animals. In the loop of Henle, however, fractional reabsorption was only about one-third of that in the control animals, in spite of the fact that the volume of fluid entering the loop was

TABLE VI

*Calculated Volumes Delivered and Reabsorbed by Various Nephron Segments in Control (C) and BUL Rats**

Segment	Volume entering		Volume reabsorbed		$\left(\frac{\text{Volume reabsorbed}}{\text{Volume entering}}\right) \times 100$	
	C	BUL	C	BUL	C	BUL
	nl/min per kg		*nl/min per kg*		%	
Proximal‡	130.0	51.0	73.2	27.3	56.3	53.5
Loop§	56.8	23.7	40.4	6.1	71.1	25.7
Distal‖	16.4	17.6	15.7	8.3	95.7	47.2
Urine¶	0.7	9.3				

* Calculated from mean SNGFR (Table III) and mean TF/P_{In} in proximal and distal tubules and U/P_{In}.

‡ This segment contains only the initial two-thirds (convoluted portion) of the proximal tubule.

§ This segment contains the pars recta (distal one-third) of the proximal tubule, the medullary and cortical portions of the loop of Henle and the initial portion of the distal tubule (see Methods).

‖ This segment contains the latter portion of the distal tubule (see Methods) and the collecting duct.

¶ This volume represents the portion of the GFR of a single nephron which would, theoretically, reach the urine.

greatly reduced. The volume delivered to the distal tubule was approximately equal in BUL and control rats, whereas fractional reabsorption was reduced to about one-half of normal in the BUL animals. Since approximately 13% of the filtered sodium appeared in the final urine, and since a normal fraction of filtered sodium was reabsorbed in the proximal tubule, we assume that these observations on distal TF/P_{In} reflect impaired sodium reabsorption in the loop of Henle and more distal segments. As the segment which lies between the end of the proximal convoluted tubule and the distal tubule consists of the pars recta and the medullary and cortical sections of the loop of Henle, we cannot state definitely which one of these sites was primarily responsible for the observed defect in this middle portion of the nephron. However, several observations suggest that sodium reabsorption was impaired in the ascending limb rather than in the pars recta. First, there was evidence of continued secretion of PAH by the pars recta (Fig. 2). Although perhaps not directly related to sodium reabsorption, intact PAH transport suggests that the pars recta cells were still metabolically active. Second, it has been shown that delivery of an increased volume of fluid out of the end of the proximal tubule results in a sharp rise in the ratio of urea to inulin clearance toward unity (52, 53). Our observed C_{urea}/C_{In} values, which were actually slightly lower than the values reported for normal nondiuretic rats (21) are compatible with the view that delivery of fluid out of the proximal tubule was not increased. Finally, we observed a defect in the concentrating mechanism which supports the conclusion that transport of sodium in the ascending limb of Henle's loop was impaired. Although it has been shown that a significant reduction in GFR can, in itself, impair the concentrating process (54), such impairment can be completely corrected by elevating plasma urea concentration (54). As the concentrating defect in our experimental animals coexisted with an elevated plasma urea concentration, the reduction in GFR would not seem to be the main cause for impaired urinary concentration. It might be argued that the low U_{max} was due to the increased rate of solute excretion (55). However, in 11 BUL rats, 7 of which received vasopressin, C_{osm} was measured and the rate of solute-free water reabsorption ($T^c_{H_2O}$) was calculated from $T^c_{H_2O} = C_{osm} - V$. For the observed level of C_{osm} (190 ± 27 μl/min per kg) the rate of $T^c_{H_2O}$ (33 ± 7 μl/min per kg) was less than one-fifth of that previously reported in normal rats (56). This marked decrease in $T^c_{H_2O}$ might have been due to several factors, such as medullary solute washout and reduced or absent filtration in the juxtamedullary nephrons, but the finding is also consistent with impaired reabsorption in the medullary segment of the ascending limb of Henle's loop (57, 58).

The underlying mechanism(s) responsible for impairing sodium reabsorption in the BUL rats is unknown, but several possibilities can be considered. If filtration rate was greatly reduced or absent in deep nephrons and medullary blood flow was increased, a washout of medullary interstitial hypertonicity should have occurred. Several authors have shown that the

activity of Na^+-K^+-ATPase in the outer medulla of the kidney is maximal only under hypertonic conditions (59, 60). Washout of medullary solutes surrounding the thick ascending limb in the outer medulla might result in a decline in the activity of this enzyme which is thought to be related to active sodium transport (61). Thus, reduced enzyme activity might have been responsible for impaired sodium reabsorption in this segment of the nephron.

There are at least four additional factors which might have acted to decrease sodium reabsorption in the BUL rats. First, the lower filtration fraction in these animals (Fig. 3) suggests that colloid oncotic pressure may have been lower in the peritubular capillaries of the BUL rats than in the controls. Although arterial plasma protein concentrations were not measured, there was no significant difference in arterial hematocrits between the BUL and control rats (42.8 ±1.3% vs. 44.0 ±2.1%) suggesting that no major change had occurred in intravascular volume in the BUL animals. Decreased colloid oncotic pressure has been shown to reduce net sodium reabsorption, at least in the proximal tubule (62). However, as FF was also reduced in the ligated kidney of UUL animals without an associated natriuresis (Table IV), it is doubtful that a lower colloid oncotic pressure in itself can account for the observed diuresis in the BUL rats. Likewise there was no significant difference in arterial blood pressure between the BUL and control rats (128.1 ±2.5 mm Hg vs. 122.9 ±2.9 mm Hg) and hydrostatic pressures in surface efferent arterioles were slightly lower than those reported in normal rats (11, 12). Thus, the natriuresis observed in the BUL animals cannot be attributed to an elevated renal perfusion pressure or peritubular capillary pressure. It has been suggested that postobstructive diuresis is due to the osmotic effect of urea (3). However, this seems an unlikely explanation in our animals for several reasons. As mentioned above, C_{urea}/C_{In} in the BUL rats was not elevated as it is in normal rats undergoing a urea diuresis (21). Second, the transit time of Lissamine green to the distal tubule was increased, not decreased, as it is after i.v. infusion of urea to normal rats chronically loaded with urea (21). Finally, although the infusion of urea to UUL rats did produce a slight natriuresis and diuresis, the excreted fractions of sodium and water were only about 2% of that filtered as compared with 13% and 18%, respectively, in the BUL rats (Table IV). Thus urea acting as an osmotic diuretic, cannot be the sole cause of postobstructive diuresis. Another possible cause of impaired sodium reabsorption in the BUL rats might be the release of a natriuretic substance such as renin-angiotensin (63, 64) or prostaglandin (65–67) from either the previously obstructed experimental kidney or its obstructed contralateral mate. Both angiotensin and prostaglandin have been shown to inhibit sodium reabsorption in the distal nephron (64, 66). However, it seems unlikely that these hormones can be the entire explanation since the UUL rats did not exhibit a natriuresis from either kidney whereas the single animal in which the right kidney had been excised did undergo a natriuresis from the previously obstructed left kidney (Table IV).

A fourth possibility to explain the impaired sodium reabsorption in the BUL rats is related to the high intrarenal hydrostatic pressure which was present during the period of obstruction (Table V). It is known that when ureteral obstruction is produced during an osmotic diuresis, renal blood flow falls (68). In contrast, acute ureteral obstruction in the nondiuretic animal usually results in no change or a significant rise in renal blood flow (68). Gottschalk and Mylle (11) have shown that acute ureteral ligation leads to increased intratubular hydrostatic pressure during an osmotic diuresis but not under nondiuretic conditions. As shown in Table V, prerelease intratubular pressures in the BUL rats were markedly elevated whereas those in the UUL rats were lower than normal free-flow values. It seems possible that in chronic bilateral ureteral obstruction, the elevated plasma urea concentration acts as an endogenous osmotic agent, leading to both a high intrarenal pressure and a reduction in renal blood flow. A prolonged increase in hydrostatic pressure, in concert with a reduced renal blood flow, might cause damage to the nephrons with consequent impairment of sodium reabsorption after the obstruction is relieved.

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