JCI The Journal of Clinical Investigation

Effects of Acute Bilateral Ureteral Obstruction on Deep Nephron and Terminal Collecting Duct Function in the Young Rat

John Buerkert, … , Mary Head, Saulo Klahr

J Clin Invest. 1977;59(6):1055-1065. https://doi.org/10.1172/JCI108728.

Research Article

The effects of acute bilateral ureteral obstruction (BUO) of 18-h duration on deep nephron and collecting duct function were studied by micropuncture in 11 weanling rats. After release of BUO glomerular filtration rate was reduced (178±15 vs. 1,343±119 μl/min per g kidney weight in shams), while urine flow was increased averaging 17.5±1.3 vs. 6.8±0.72 μl/min per g kidney weight in controls. There was a marked increase in the absolute and fractional excretion of Na. Single nephron glomerular filtration rate of deep nephrons was reduced in the BUO group, mean 19.4±3.5 vs. 77.0±7.7 nl/min per g kidney weight in shams. Single nephron glomerular filtration rate of superficial nephrons fell to the same extent after relief of BUO. Mean tubular fluid to plasma inulin ratio of fluid from Henle's loop was 2.46±0.20 after relief of BUO vs. 8.23±0.85 in shams. This suggested a reduction in the reabsorption of Na and water before the bend of the loop of Henle, most likely in both the proximal tubule and descending limb. Fluid osmolality was depressed due to a decline in both Na and nonelectrolyte solute content. After release of BUO the percentage of filtered water remaining in the collecting duct (CD) at the base of the papilla was greater than in controls (13.3±2.0 and 1.72±0.01%, respectively) but fell significantly by the tip of the […]

Effects of Acute Bilateral Ureteral Obstruction on Deep Nephron and Terminal Collecting Duct Function in the Young Rat

JOHN BUERKERT, MARY HEAD, and SAULO KLAHR

From the Renal Physiology Laboratory, John Cochran Veterans Administration Hospital and the Renal Division, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT The effects of acute bilateral ureteral obstruction (BUO) of 18-h duration on deep nephron and collecting duct function were studied by micropuncture in 11 weanling rats. After release of BUO glomerular filtration rate was reduced (178±15 vs. 1,343±119 μl/min per g kidney weight in shams), while urine flow was increased averaging 17.5±1.3 vs. 6.8±0.72 μl/min per g kidney weight in controls. There was a marked increase in the absolute and fractional excretion of Na. Single nephron glomerular filtration rate of deep nephrons was reduced in the BUO group, mean 19.4±3.5 vs. 77.0±7.7 nl/min per g kidney weight in shams. Single nephron glomerular filtration rate of superficial nephrons fell to the same extent after relief of BUO. Mean tubular fluid to plasma inulin ratio of fluid from Henle's loop was 2.46±0.20 after relief of BUO vs. 8.23±0.85 in shams. This suggested a reduction in the reabsorption of Na and water before the bend of the loop of Henle, most likely in both the proximal tubule and descending limb. Fluid osmolality was depressed due to a decline in both Na and nonelectrolyte solute content. After release of BUO the percentage of filtered water remaining in the collecting duct (CD) at the base of the papilla was greater than in controls (13.3±2.0 and 1.72±0.01%, respectively) but fell significantly by the tip of the papilla to 7.92±1.12 vs. 1.17±0.02% in controls. These results indicate that water was reabsorbed along the terminal CD after relief of ureteral obstruction. In fact, a greater fraction was reabsorbed in this segment after release of BUO (5.37±1.58%) than after sham operation (0.55±0.15%). Similar changes were seen in Na excretion. Thus alterations in deep nephron function appear to contribute to the natriuresis and diuresis which follow release of BUO while terminal CD function in this model appears intact.

INTRODUCTION

It is well known that a marked natriuresis and diuresis occur after the release of complete bilateral ureteral obstruction (BUO).[1] These alterations occur in the face of a marked decline in glomerular filtration rate (GFR) and therefore must represent a decrease in the reabsorption of fluid by one and most likely multiple anatomical segments of the renal tubule.

Micropuncture studies of cortical renal tubules have demonstrated that after release of acute BUO there is a decrease in the amount of water reabsorbed along proximal (1, 2) and distal tubules (1–3) of superficial nephrons, while after release of unilateral ureteral obstruction quite the opposite changes have been found. Under these conditions the reabsorption of tubular fluid is actually increased along the accessible length of superficial renal tubules (1, 4). Recent reinfusion (5) and cross-circulation studies (6) have demonstrated that a major factor in the pathophysiologic events which occur after relief of BUO is the accumulation of substances in the blood during the period of anuria which suppress the reabsorption of

Portions of this work have been previously presented in abstract form in 1976: *Clin. Res.* **24**: 395A. (Abstr.)

Dr. John Buerkert is a research associate of the Veterans Administration.

Received for publication 25 August 1976 and in revised form 9 February 1977.

[1] *Abbreviations used in this paper*: BUN, blood urea nitrogen; BUO, bilateral ureteral obstruction; CD, collecting duct; CD_{tip}, collecting duct at the tip of the papilla; CD_{prox}, collecting duct at the base of the papilla; DLH, descending limb of the loop of Henle; GFR, glomerular filtration rate; KW, kidney weight; NES, nonelectrolyte solute; SNGFR, single nephron glomerular filtration rate; TF/P In, tubular fluid to plasma inulin; V, tubular flow rate.

sodium and water by the renal tubule after release of obstruction. Taken as a whole these micropuncture and clearance studies suggest that the intrinsic reabsorptive capabilities of cortical renal tubules may not be adversely affected by ureteral obstruction of short duration per se.

The effects of acute ureteral obstruction on deep nephron filtration rate and reabsorptive capacity have not been adequately characterized. It has been suggested (1, 3) that these nephrons are more adversely affected by obstruction and that this might be the explanation for the proportionately greater fall in whole kidney GFR than in the single nephron GFR (SNGFR) of superficial nephrons. On the other hand the studies of Jaenike (7) suggest that SNGFR of these superficial nephrons may not be uniform despite the fact that they have a homogeneous appearance. Further studies in rats by Wilson (8) with a modification of Hanssen's technique and renal tubular microdissection failed to reveal a redistribution of SNGFR after release of BUO. No information is available concerning alterations in the reabsorption of salt and water by juxtamedullary nephrons. It is well known that the concentrating ability of the kidney is markedly diminished after release of obstruction (9–11). While it has been suggested that this may be a result of a direct effect of obstruction on collecting duct function (12), there is little direct evidence to differentiate an intrinsic collecting duct defect from alterations in papillary interstitium which might alter, in a passive way, collecting duct function.

We have studied the effects of acute BUO on deep nephron and terminal collecting duct function in the weanling rat. We found that deep nephron GFR is decreased to the same extent as that of superficial nephrons, and that the reabsorption of sodium and water to the bend of the loop of Henle is suppressed. Further, fractional sodium and water reabsorption along the terminal segment of the collecting duct was significantly greater after release of BUO than in controls, suggesting that the terminal collecting duct is functionally intact.

METHODS

Approximately 18 h before preparation for micropuncture 11 Sprague-Dawley rats weighing 40–85 g were lightly anesthetized with ether. Both ureters were then exposed through a mid-line abdominal incision and ligated with 4-0 silk at a point approximately one-third of the distance from the bladder to the renal pelvis. In another 12 rats the ureters were visualized in a similar fashion but not touched. Until the time of the initial surgical procedure all rats were fed standard rat chow. Thereafter, all rats were deprived of food and water.

On the day of micropuncture the rat was anesthetized with Inactin (Promonta, Hamburg, Germany) intraperitoneally (100–120 mg/kg body weight). A tracheostomy was then performed and the rat was intubated with a piece of polyethylene tubing (PE no. 90). Polyethylene catheters were placed in both jugular veins and the right femoral artery. The left kidney was exposed through a mid-line abdominal incision and carefully dissected free of the surrounding peritoneal attachments. The renal papilla was exposed by temporarily pushing it into the renal pelvis and carefully removing the ureter near its origin (13). The kidney was then placed in a glass cup and bathed in mineral oil which was warmed to 38°C.

Shortly before the left kidney was prepared for micropuncture, ureteral obstruction was relieved by severing the ureter above the ligature. To obtain clearance measurements from the right unmicropunctured kidney, the right ureter of the BUO group was cannulated with polyethylene tubing (PE no. 50), and a catheter was placed in the bladder of sham-operated rats. Immediately after the cannulation both groups of rats were given an inulin prime followed by an infusion of normal saline containing inulin in sufficient amounts to maintain plasma levels between 50–100 mg/100 ml. In sham-operated rats the mean infusion rate was 70.9±5.9 μl/min per 100 g body weight (SE). A slightly higher infusion rate (81.5±2.1 μl/min per 100 g body weight) was necessary in the BUO group due to the greater urinary losses. An equilibration period of approximately 45 min was allowed before micropuncture.

The renal papilla was illuminated with a small fiber optics light guide. A loop was identified and punctured near the bend with an acid-washed micropipette (5–6 μm outside diameter) and a droplet of oil, stained with Sudan Black and approximately 2–3 tubule diameters in length, was injected. A timed tubular fluid sample was then obtained at a rate so as to maintain the oil droplet in a stationary position. After completion of one–three loop collections, samples were obtained from collecting ducts, with pipettes of slightly larger diameter (8–10 μm). Collections were made from sites as close to the base of the papilla as possible (CD_{prox}) and from points closer to the tip of the papilla (CD_{tip}). The distance between the two collecting duct sites was estimated with an eyepiece micrometer. In almost all cases duplicate collections were obtained. Either before or after these collections, the cortical surface of the kidney was illuminated with a fiber optics light guide attached to a quartz rod. Random proximal tubules were then punctured and fluid was obtained in a way similar to that described for loop collections.

Blood samples and blood pressure measurements were either obtained at 30-min intervals or immediately after the collection of tubular fluid. Body temperature was determined with a rectal thermometer and maintained between 36.5 and 38°C. One or two timed urine collections were made in preweighed test tubes and their volumes were determined gravimetically.

The volume of proximal tubule samples and loop collections was determined with a quartz capillary of constant bore previously calibrated with ^{14}C-inulin. The samples were then placed in a plexiglass trough containing mineral oil. The concentration of inulin was determined with the fluorometric method described by Vurek and Pegram (14). Sodium and potassium content was measured with a helium glow photometer (American Instrument Co., Travenol Laboratories Inc., Silver Spring, Md.). The osmolality of tubular fluid was measured by the method of Ramsay and Brown (15). Recoveries for these micromethods (mean±SD) were as follows: Na, 97.6±3.7% (n = 17); K, 101.8±7.4% (n = 17); inulin, 100±4.0% (n = 20); and osmolality, 103±8.1% (n = 28). They were determined from samples handled in an identical fashion to tubular fluid collections by individuals

TABLE I

*Weights, Blood Pressure, and Plasma Parameters in Control and Experimental Rats**

	Body weight	Blood pressure	Hematocrit	Na	K	Osmolality	BUN
	g	*mm Hg*	*ml/100 ml*	*meq/liter*	*meq/liter*	*mosmol/kg*	*mg/100 ml*
Sham n = 12	52.9±4.4	115±4	38.0±0.7	146±1	4.6±0.2	286±3	13.3±2.0
BUO n = 11	44.0±1.1	112±3	37.5±1.0	142±1	7.1±0.3	320±3	125±11
P	NS	NS	NS	NS	<0.001	<0.001	<0.001

* Values are mean±SEM; n, number of rats studied in each group.

who were unaware of the actual content of the substance to be measured.

The concentration of inulin in plasma and urine was determined with an anthrone method (16). Plasma and urinary sodium and potassium concentrations were estimated using standard flame photometry (Instrumentation Laboratory, Inc., Lexington, Mass.). The osmolality was measured with a vapor pressure osmometer (Wescor, Inc., Morgan, Utah). Blood urea nitrogen (BUN) was determined with an enzymatic method (17).

Tubular fluid to plasma inulin ratios (TF/P In) permitted a calculation of fractional water reabsorption to the site of micropuncture. SNGFR was calculated from the TF/P In ratio and the timed tubular flow rates (V), (SNGFR = TF/P In × V). The fraction of filtered water remaining in the tubule was calculated as: (P/TF) In × 100. The percentage of filtered sodium, potassium, or solute remaining was determined with the general formula:

$$\frac{\text{TF/P X}}{\text{TF/P In}} \times 100,$$

where X represents the variable. In addition, collecting duct function was assessed by determining the percentage of the filtered load of solute, sodium, and potassium reabsorbed between the two sites of puncture. This was done by utilizing the formula:

$$(Y)_{CD\ prox} - (Y)_{CDtip},$$

where (Y) represents percentage of the filtered load of the variable remaining in the fluid collected at the tip (CD_{tip}) and from a more proximal site (CD_{prox}) of the collecting duct segment. The concentration of nonelectrolyte solute (NES) in urine and tubular fluid was estimated utilizing the following equation: NES = Osm − (1.84 Na + 2 K), where Osm, Na, and K represent the osmolality and concentrations of sodium and potassium, respectively.

Mean differences in whole kidney and superficial nephron function were tested for significance with the Student's t test for unpaired data when comparing the two groups of animals studied and for paired data when comparing superficial and deep nephron function in the same animal or when comparing proximal to tip measurements along collecting ducts (18).

RESULTS

There were no differences in mean values for body weight, blood pressure, or hematocrit in the two groups of rats studied (Table I). Plasma potassium concentration was significantly higher in the BUO group averaging 7.1±0.3 meq/liter as compared to 4.6±0.2 meq/liter (SE) in controls. As expected mean values for BUN were markedly greater in the experimental group and because of this plasma osmolality was also significantly higher in those rats who had undergone bilateral ureteral ligation.

Infusion rates averaged 81.5±2.1 μl/min per 100 g body weight in BUO as compared to 70.9±5.9 μl/min per 100 g body weight in controls. As stated earlier a greater rate of infusion was necessary to maintain adequate blood pressure and preserve the viability of the BUO preparation. Despite this the mean difference between the total amount infused and the total urine output, that is the total amount that either accumulated in the rat or was lost by other routes, was significantly less in the postobstructed group.

In general, kidneys in the two groups appeared the same. The surface tubules in the BUO rats were widely patent and did not differ significantly from those of controls or from previous published reports in larger rats (1, 7). As in the studies of Jaenike (7), tubule fluid collections from superficial nephrons were frequently difficult because of decreased flow.

There were striking differences in the appearance of the papilla after relief of ureteral obstruction. The degree to which the papilla protruded beyond the renal parenchyma was greatly diminished in the BUO group. The papilla appeared somewhat pale and the rate of blood flow through the vasa recta was uniformly slowed. Tubular fluid collections from loops of Henle were frequently difficult because of reduced flow and the necessity for somewhat prolonged collecting times. In some cases the collection had to be abandoned because of this. The collecting ducts were widely patent and flow, as judged by the difficulty in controlling the movement of an injected droplet of oil, was increased. The length of papillary collecting duct accessible to micropuncture averaged 0.5–0.6 mm whereas 1.0–1.5 mm was available in control studies. Because of this, an attempt was made to assess a comparable length of collecting duct. Hence CD_{prox} in both groups was as close to the base of the papillae as possible while CD_{tip} in the BUO group was close to the tip but in shams the sampling site was 0.4–0.6 mm from the base of the papilla.

TABLE II

Renal Function of the Unmicropunctured Kidney after Sham Operation or Release of BUO of 18 Hours Duration

	V	GFR	V/GFR	$U_{Na}V$	FE_{Na}	U_KV	FE_K	U_{Osm}	$U_{Osm}V$	FE_{Osm}	U_{NES}	$U_{NES}V$	$U\frac{NES}{Osm}$
	μl/min/g KW	*μl/min/g KW*	*%*	*neq/min*	*%*	*neq/min*	*%*	*mosmol/ kg*	*μosmol/ min*	*%*	*mosmol/ kg*	*μosmol/ min*	*%*
Sham													
$n = 12$	6.8±0.72	1,343±119	0.25±0.17	238±75	0.40±0.11	424±38	29.0±4.0	1,515±117	2.96±0.32	2.57±0.26	923±116	1.75±0.21	59.0±3.3
BUO													
$n = 11$	17.5±1.3	178±15	10.7±1.0	767±83	8.99±1.24	241±23	55.6±6.7	426±9	2.68±0.78	14.3±1.5	130±5	0.81±0.06	30.5±0.8
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	<0.001	NS	<0.001	<0.001	<0.001	<0.001

V/GFR, fraction of the filtered load of water excreted; $U_{Na}V$, absolute rate of sodium excretion; FE_{Na}, fraction of filtered load of sodium excreted; U_KV, absolute rate of potassium excretion; FE_K, fraction of filtered potassium excreted; U_{Osm}, urinary osmolality; U_{NES}, concentration of nonelectrolyte solute [U_{Osm} − (1.84 Na + 2 K)] in the urine; $U_{Osm}V$, absolute rate of solute excretion; FE_{Osm}, fraction of filtered solute excreted.

* Data are the mean±SEM; *n*, number of rats studied in each group.

Whole kidney function. The mean values for the clearance studies obtained from the right unmicropunctured kidney after sham operation and release of BUO are presented in Table II.

Urine flow from the postreleased kidney averaged 17.5±1.3 μl/min per g kidney weight (KW), a value significantly higher than that of the sham-operated group (6.80±0.72 μl/min per g KW). GFR of the postobstructed kidney was decreased compared to the GFR of controls (178±15 and 1,343±119 μl/min per g KW, respectively). Accordingly the fractional excretion of water was greater after release of BUO than after sham operation.

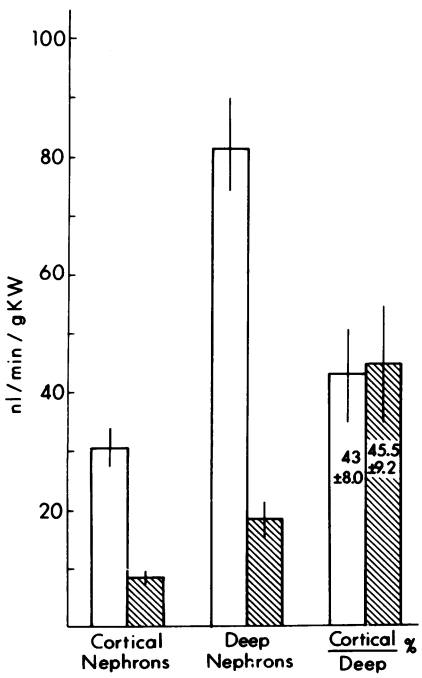

FIGURE 1 Depicts the mean and SEM for the SNGFR of cortical and deep nephrons of sham-operated rats (clear bar) and after relief of BUO (hatched bar). The two bars to the right represent the mean and SEM of the ratio of cortical to deep nephron SNGFR in the two groups expressed as a percentage.

The absolute rate of sodium excretion was increased in the postobstructed group. Nearly 9% of the filtered load of sodium was excreted in this group compared to only 0.40% in controls. Potassium excretion was significantly reduced in the BUO group, although in fractional terms it was greater averaging 55.6±6.7% compared to 29.0±4.0% of filtered load in the sham-operated group.

Urine osmolality was markedly depressed after release of BUO. The absolute rate of solute excretion in the two groups was not significantly different, although fractional excretion of solute was significantly greater in the BUO group. The concentration of nonelectrolyte solute was reduced in the experimental animals both in absolute and in fractional terms. The rate of excretion for NES was significantly reduced averaging only 0.81±0.06 μosmol/min in contrast to 1.75±0.21 μosmol/min in normal rats.

Nephron function. In 9 control and 10 experimental rats timed tubular fluid samples were collected from both surface and deep nephrons. Mean values for SNGFR in these rats are graphically depicted in Fig. 1. In controls the mean of 16 SNGFR determinations was 31.3±2.7 nl/min per g KW and was significantly greater ($P < 0.001$) than the superficial SNGFR after release of BUO which averaged 6.7±1.2 nl/min per g KW for 19 measurements. TF/P In ratios were not different in the two groups (2.01±0.24 in controls vs. 1.94±0.24 after release of obstruction). Deep nephron GFR was also significantly greater ($P < 0.001$) in the sham-operated group (81.1±9.6 nl/min per g KW, $n = 21$) than in the BUO group (18.5±3.9 nl/min per g KW, $n = 20$). However, in both groups deep nephron GFR was significantly greater ($P < 0.01$) than superficial nephron GFR. The decline in SNGFR in the two populations of nephrons after relief of BUO was proportional, as evidenced by the fact that the ratio of

TABLE III

Micropuncture Data Obtained from Deep Nephrons 18 Hours after Release of Bilateral Ureteral Obstruction and in Sham-Operated Rats

BUO	V	$\frac{TF}{P}$ In	SNGFR	Osm	Na	K	NES	$\frac{TF}{P} \cdot \frac{Na}{In}$ × 100	$\frac{TF}{P} \cdot \frac{K}{In}$ × 100
	nl/min/g KW		*nl/min/g KW*	*mosmol/ kg*	*meq/liter*	*meq/liter*	*mosmol/ kg*	%	%
1	2.84	2.86	8.11	—	—	—	—	—	—
2	24.2	1.12	26.9	620	155	15	304.0	88.1	197.8
	13.0	1.75	22.9	440	180	16	76.8	65.9	121.7
3	14.54	3.66	53.3	690	330	19	44.8	60.9	79.80
	6.42	2.42	15.5	440	213	20	8.1	57.2	142.0
4	5.01	2.64	13.5	670	255	18	164.8	59.0	93.40
5	3.30	4.40	14.5	450	235	12	−6.4	35.2	34.10
	16.55	1.58	26.2	430	215	14	7.9	89.3	106.1
6	3.07	2.35	7.36	470	230	20	7.8	65.0	143
	3.98	2.28	9.54	490	172	13	147.5	50.3	98.0
7	6.80	1.26	8.58	460	190	10	91.4	101.0	108
	1.24	1.11	1.38	—	—	—	—	—	—
8	3.32	2.20	7.31	505	225	11	69.0	70.6	57.7
	1.19	2.07	2.46	540	200	14	144.0	67.7	80.0
9	17.59	3.25	57.1	420	190	10	50.4	39.5	41.8
	10.1	2.99	30.2	502	205	8	108.8	46.3	35.7
10	0.89	1.81	1.61	560	262	15	47.9	98.5	76.2
	10.96	3.38	36.9	520	255	15	19.8	48.3	34.7
11	10.18	2.43	24.7	420	210	10	13.6	58.2	57.5
	4.91	2.62	12.9	465	190	11	94.4	48.1	53.9
	7.57	3.48	26.4	380	165	10	57.4	31.4	36.4
Mean	7.98	2.46	19.4	499	214	13.7	76.4	62.1	84.3
SE	±1.40	±0.20	±3.5	±20	±10	±0.9	±17.7	±4.6	±11.2
Sham (n = 11)									
Mean	13.4	8.23	77.0	1,152	378	36.1	412	35.7	107.7
SE	±2.5	±0.85	±7.7	±84	±29	±5.8	±54	±2.9	±17.2
P*	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS

$\frac{TF}{P} \cdot \frac{Na}{In} \times 100$, percentage of filtered sodium remaining; $\frac{TF}{P} \cdot \frac{K}{In} \times 100$, percentage of filtered potassium remaining at the site of micropuncture.

* Level of significance for differences between the BUO and sham-operated group.

mean cortical to deep nephron GFR was not different (43±8.0% for control vs. 45.5±9.2% for the experimental group).

In Table III all the micropuncture data obtained after release of BUO and the mean values of 22 loop collections obtained after sham operation are presented.

The changes seen in the SNGFR of deep nephrons after release of BUO were not different from the data presented graphically in Fig. 1. Tubular fluid flow rate was reduced after release of BUO. More striking, however, was the marked fall in TF/P In ratios which was uniformly seen in all of the rats studied in the BUO group. The mean was 2.46±0.20 and as such represents less than one-third of the average ratio in the sham-operated group (8.23±0.85).

The osmolality of fluid collected near the bend of the loop of Henle was significantly less ($P < 0.001$) after release of obstruction than after sham operation. Although this difference was due in part to a decrease

TABLE IV

Micropuncture Measurements of Collecting Duct Function in the Two Groups of Rats

	$\frac{P}{TF}$ In × 100			$\frac{TF}{P} \cdot \frac{Na}{In}$ × 100			$\frac{TF}{P} \cdot \frac{K}{In}$ × 100			$\frac{TF}{P}$ Osm		$\frac{TF}{P} \cdot \frac{Osm}{In}$ × 100		
BUO	CD_{prox}	CD_{tip}	Difference	CD_{prox}	CD_{tip}	Difference	CD_{prox}	CD_{tip}	Difference	CD_{prox}	CD_{tip}	CD_{prox}	CD_{tip}	Difference
1	22.56	15.6	6.94	—	—	—	—	—	—	2.04	2.02	46.4	31.6	14.8
2	7.09	6.75	0.34	6.28	6.16	0.12	69.1	69.8	−0.76	1.43	1.50	10.2	10.1	0.01
3	6.35	4.51	1.83	7.60	5.38	2.04	84.3	54.7	28.1	1.59	1.76	9.5	7.62	1.89
4	18.9	12.6	6.33	23.0	6.13	16.8	83.8	57.0	26.8	1.82	2.11	20.2	19.3	0.90
5	15.3	9.67	5.63	24.6	12.3	12.3	87.6	80.6	7.02	1.60	1.66	23.7	15.8	7.91
6	9.95	7.56	2.39	10.3	7.13	3.22	103.5	88.2	15.4	1.43	1.48	14.1	11.1	2.96
7	8.45	3.91	4.54	5.53	1.72	3.81	55.0	43.9	11.6	1.47	1.70	11.5	6.32	5.14
8	12.8	7.83	5.00	7.10	2.44	4.66	99.03	83.9	15.2	1.37	1.43	17.6	11.2	6.36
9	12.5	7.84	4.65	14.3	9.42	4.96	61.0	75.5	−14.54	1.65	1.75	20.3	13.6	6.74
10	7.43	5.84	1.59	5.92	4.13	1.79	31.6	36.13	−4.52	1.43	1.59	9.93	8.7	1.18
11	25.1	5.26	19.85	26.6	4.07	22.6	33.5	37.8	−4.3	1.33	1.38	33.3	7.23	26.1
Mean	13.3	7.92‡	5.37	13.2	5.88‡	7.24	70.8	62.8	8.08	1.56	1.67‡	19.7	13.0	6.73
SEM	±2.0	1.12	1.58	±2.8	±1.06‡	±2.86	±8.9	±6.9	±5.03	±0.07	±0.08	±3.6	2.3	2.31
Sham (n = 11)														
Mean	1.72	1.17‡	0.55	1.65	1.19‡	0.47	40.5	30.93§	9.66	3.30	4.17*	1.75	1.69	0.81§
SEM	±0.01	±0.02	±0.15	±0.22	±0.35	±0.13	±5.07	±4.0	±6.30	±0.30	±0.44	±0.06	±0.05	0.47
P	<0.001	<0.001	<0.01	<0.001	<0.001	<0.01	<0.005	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.01

$\frac{P}{TF}$ × 100, percentage of filtered water remaining; $\frac{TF}{P} \cdot \frac{Na}{In}$ × 100, percentage of the filtered sodium remaining; $\frac{TF}{P} \cdot \frac{K}{In}$ × 100, percentage of filtered potassium remaining at the site of micropuncture; $\frac{TF}{P}$ Osm, tubular fluid to plasma osmolality.

* Mean values obtained at CD_{prox} and CD_{tip} are significantly different at $P < 0.001$, ‡ $P < 0.01$, or § not significantly different.

in the sodium content and to a decrease in potassium concentration, the major contributing factor seemed to be a marked decline in NES in the BUO group. As expected the fraction of the filtered sodium remaining was significantly higher in experimental rats (62.1±4.6%) than in the hydropenic controls (35.7 ±2.9%, $P < 0.001$). The percentage of potassium remaining tended to be less in the BUO group than in the shams but this difference did not achieve statistical significance.

Papillary collecting duct function. Individual measurements made at proximal and tip collecting duct sites of the BUO group and the mean values for the sham-operated group are presented in Table IV. The distance between these two sites averaged 0.3 mm (range 0.2–0.5 mm) in the postrelease group and was not significantly different from controls (mean, 0.4 mm; range, 0.2–0.8 mm).

As expected the fraction of the filtered load of water remaining at proximal and tip collecting duct sites (13.3±2.0 and 7.92±1.12%, respectively) was significantly greater than the values found in sham-operated rats in which the mean proximal value was 1.72±0.01% and tip values averaged 1.17±0.02%. In both groups the fraction of the filtered water remaining fell significantly between proximal and tip collecting duct sites. This is graphically depicted in the left hand panel of Fig. 2. The data indicate that water was reabsorbed in both shams and after release of BUO. The fractional amount of water reabsorbed along a comparable length of collecting duct averaged 5.37±1.58% after release of BUO (Table IV, Fig. 3) as compared to only 0.55±0.15% in the control group.

The fraction of the filtered load of sodium remaining at proximal segments of the papillary collecting duct after relief of obstruction was significantly greater than what remained at this site in controls (13.2 ±2.8 vs. 1.65±0.22%, $P < 0.001$). In both groups there was a significant fall ($P < 0.01$) in the fraction of filtered sodium remaining near the papillary tip (central panel, Fig. 2). As in the case for water, sodium was reabsorbed along this segment to a much greater extent in the experimental group (7.24±2.86% in the BUO group vs. 0.47±0.13% in shams, Table IV, Fig. 3).

Although the fraction of the filtered potassium remaining at proximal and tip collecting duct sites was greater in the postrelease group there was no consistent change along the collecting duct segment studied in either group.

In both groups papillary osmolality rose significantly along the segment studied (right panel, Fig. 2). As expected osmolality of fluid collected in the sham-operated group was significantly greater at proximal (TF/P Osm = 3.30±0.30) and tip sites (TF/P Osm = 4.17±0.44) than after relief of BUO (TF/P Osm = 1.56±0.07 and 1.67±0.08, respectively). Fractional solute presented to both proximal and tip sites of the collecting duct was greater in the postobstructed group

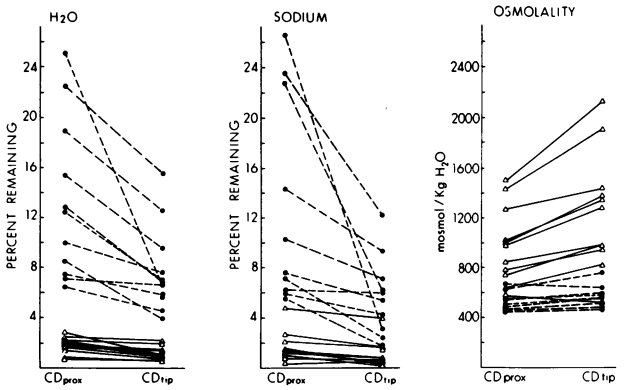

FIGURE 2 Graphically presents the individual results for the fraction of the filtered water (left panel) and sodium (center panel) remaining in sham-operated rats (△ —— △) and after relief of the BUO (● - - - ●) at a site close to the base (CD_{prox}) and near the tip of the papilla (CD_{tip}). The osmolality of fluid at these sites in the two groups of rats is shown in a similar fashion in the right panel.

of rats than in shams but fell significantly from the papillary base to the tip (19.7±3.6% and 13.0±2.3%, $P < 0.01$). Fractional solute remaining did not change along the collecting duct in the sham-operated group.

DISCUSSION

The present study demonstrates that in the weanling rat release of BUO of 18-h duration results in a marked increase in the excretion of sodium and water. Mean urine flow from the unmicropunctured postrelease kidney was almost three times greater than in hydropenic controls studied under nearly identical conditions. The absolute excretion of sodium was increased to the same extent. These events occurred in face of a nearly 10-fold decrease in GFR and as such represent a marked increase in the fractional excretion of sodium and water. The alterations seen in these experiments are comparable to results reported previously in more mature rats studied after unilateral release of bilateral ureteral obstruction of 24 h duration (1). Thus, the weanling rat is an adequate model for studying papillary collecting duct and juxtamedullary nephron function under conditions of postobstructive diuresis.

Single nephron function. After release of ureteral obstruction SNGFR fell significantly in superficial nephrons. As in previous studies (1–3, 7) the extent of the fall (approximately 30% of control values) was not as great as the decline in whole kidney GFR (nearly 10-fold). SNGFR of deep nephrons also fell significantly, but to the same extent as cortical nephrons (approximately 30%). While these studies provide the first direct measurements of SNGFR of juxtamedullary nephrons under conditions of postobstructive diuresis, the results were predictable from the ferrocyanide-microdissection studies of Wilson (8). In his studies, Wilson found that the ratio of superficial to juxtamedullary nephrons with respect to ^{14}C-ferrocyanide content immediately after release of acute ureteral obstruction was similar to controls. The present studies do not provide an explanation for the lesser fall in SNGFR in superficial or deep nephrons as compared to whole kidney GFR. However, the frequent difficulty in tubular fluid collection from both cortical and deep nephrons implies that some nephrons are not functioning and would thus support the hypothesis put forth by Jaenike (7). That is, that the differences seen between whole kidney GFR and SNGFR are due to heterogeneity in the filtering capacity of nephrons in both populations rather than to selective loss of juxtamedullary nephron function.

After release of ureteral obstruction the TF/P In ratios at the end of the descending limb of the loop of Henle (DLH) were markedly depressed when compared to values in control rats. Two possibilities exist to explain this decline. One is that these tubules are no longer impermeable to inulin and that these changes are the consequence of a significant inulin backleak. This possibility cannot be entirely excluded in the present studies, and the evidence for inulin backleak in adult models is inconclusive (1, 2). Alternatively, this fall in TF/P In ratios could reflect a marked decrease in the reabsorption of both sodium and water proximal to the site of micropuncture.

In absolute terms the amount of water reabsorbed

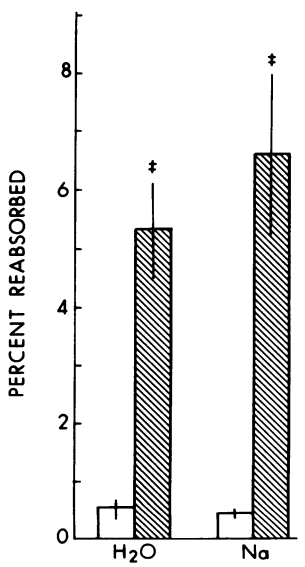

FIGURE 3 The mean and SEM for the fraction of the filtered water (left) and sodium (right) reabsorbed in the terminal collecting duct after sham operation (clear bar) and after release of BUO (hatched bar) are presented in this figure. $P < 0.01$.

TABLE V

*Function of Descending Limb of the Loop of Henle in Controls and after Relief of Complete Ureteral Obstruction**

	TF/P In		Filtered H_2O reabsorbed			Osmolality			Factors affecting osmolality	
	^{B}DL	^{E}DL	$^{T}H_2O$	$^{B}DL - {}^{E}DL$‡	$\frac{^{B}DL - {}^{E}DL}{^{B}DL}$§	^{B}DL	^{E}DL	Δ OSM	H_2O extraction	Solute entry
			nl/min/g KW	%	%		*mosmol/kg*		%	%
Control (n = 21)	3.23±0.30	7.88±0.71	63.6±5.9	21.3±4.5	57.3±2.6	286±2.5	1,118±93	870±80	53.1±4.1	46.9±4.1
BUO (n = 19)	1.69±0.13	2.47±0.20	11.4±2.6	17.5±2.8	27.9±3.5	320±2.6	506±25	178±21	77.5±9.6	19.6±10.5
P	<0.005	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.02	<0.02

* Data are the mean±SE; n, number of tubules; ^{B}DL, beginning of the descending limb; ^{E}DL, end of descending limb; $^{T}H_2O$, absolute amount of water reabsorbed to the site of collection.

‡ Percentage of water reabsorbed between ^{B}DL and ^{E}DL.

§ Percentage of water reabsorbed which was presented to this segment.

in the BUO group averaged 11.4±2.6 nl/min per g KW, significantly less ($P < 0.001$) than the amount reabsorbed in the sham-operated group (63.6±5.9 ng/min per g KW, see Table V). If one assumes that the permeability characteristics of the descending limb are unchanged by ureteral obstruction and that therefore the reflection coefficient for sodium chloride is 0.96, a value based on the in vitro findings in the rabbit (19), then the TF/P In ratio at the beginning of the DLH may be estimated by factoring the TF/P In ratio at the point of micropuncture by the TF/P sodium ratio measured at the same site (20). In controls the mean value was 3.23 ±0.30, indicating that approximately 69.1% of the filtered sodium was isotonically reabsorbed by the end of the proximal tubule of deep nephrons (Table V). This value is similar to what has been previously reported utilizing the same calculations (20, 21). In the BUO group the ratio was significantly less, averaging 1.69±0.13. Although the explanation for this decline cannot be determined from the present studies, it is possible that such changes may not reflect an intrinsic defect in renal tubular function.

One factor which might be playing a role is the accumulation of natriuretic factors in the blood during the interval of complete ureteral obstruction. Recent studies by Wilson and Honrath (6) support this possibility. They found that a natriuresis and a diuresis comparable to that seen after relief of acute BUO could be produced in normal rats by crosscirculation with rats which had been subjected to BUO. Similar conclusions were obtained from the studies of Harris and Yarger (5). They demonstrated that release of unilateral ureteral obstruction in face of reinfusion with urine from the contralateral kidney resulted in a marked increase in the excretion of sodium and water similar in magnitude to that observed after release of BUO. It is therefore quite possible that the decrease in the tubular reabsorption of sodium and water in deep nephrons described in these studies may reflect, at least in part, the result of a generalized response of the kidney to circulating natriuretic factors.

It seems likely that the effects of physical forces in proximal tubular reabsorption of sodium have been altered in the BUO group, since it has been shown that both single nephron and whole kidney filtration fraction are significantly decreased in this experimental model (3, 7). These alterations would tend to further reduce the net reabsorption of sodium and water in the proximal tubule.

The data presented here suggest that the reabsorption of sodium and water in the proximal segments of deep nephrons is more profoundly affected than in proximal tubules of cortical nephrons. One possible cause for this disparity may be increased medullary blood flow. In rats, results of measurements of total renal blood flow after release of BUO are conflicting (3, 7, 22); however most studies report data which are compatible with an absolute increase in medullary blood flow (3, 7, 23). Since SNGFR fell significantly in deep nephrons it can be implied that there was a greater proportional fall in filtration fraction in juxtamedullary than in cortical nephrons. This could account for the even greater reduction in fractional reabsorption of sodium and water in the proximal tubular segments of these nephrons.

The filtered load of water reabsorbed along the DLH is equal to the difference between the percentage remaining at the beginning and at the bend of the loop of Henle. In both controls and the experimental group this was highly variable. In shams approximately 21% was reabsorbed. This was not significantly different from the mean value of 17% obtained after release

of BUO (Table V). However, a significantly greater fraction of the filtered water presented to the DLH was reabsorbed in the sham-operated group. In this group 57% of the filtered water available was reabsorbed while only 28% left the tubular lumen of the DLH after ureteral release. This decline is not unexpected since the papillary osmolality which is the driving force for the movement of water out of the loop is significantly reduced.

If it can be assumed that the osmolality of tubular fluid at the beginning of the DLH approximates that of plasma, then the mean increase in osmolality along this segment can be determined by subtracting the plasma osmolality from the tubular fluid osmolality measured at the bend of the loop of Henle ($\Delta Osm = TF_{Osm} - P_{Osm}$). As might be expected the mean increase in osmolality along the DLH was significantly greater in the control group (870±80 mosmol/kg H_2O) than in the experimental group (178±21 mosmol/kg H_2O, $P < 0.001$). The relative contributions of solute entry and water extraction to the osmolality achieved by the bend of the loop of Henle may be estimated utilizing the following formula (21):

Percentage contribution to increase in osmolality due to:

Water extraction

$$= \frac{\left(\frac{\text{TF/P In at end of DLH}}{\text{TF/P In at beginning of DLH}} \times P_{Osm}\right) - P_{Osm} \times 100}{\Delta \text{ Osm}};$$

Solute entry

$$= 100 - \frac{\left(\frac{\text{TF/P In at end of DLH}}{\text{TF/P In at beginning of DLH}} \times P_{Osm}\right) - P_{Osm} \times 100}{\Delta \text{ Osm}},$$

where it is assumed that the transtubular flux of sodium is 0 along this segment of the nephron and is not altered by bilateral ureteral obstruction.

The mean results of these calculations for individual nephrons are presented in Table V. In hydropenic rats approximately 53% of the increase in osmolality along the DLH was due to water extraction while that due to solute entry was approximately 47%. These data are quite similar to the results obtained in rats with hereditary diabetes insipidus after the administration of antidiuretic hormone (20) and in normal hydropenic rats (21) when the same basic assumptions are utilized. In rats subjected to ureteral obstruction and release, the relative contributions of solute addition and water extraction were more variable than after sham operation. However, as a group solute entry into this segment of the loop was significantly reduced and contributed only 20% to the total change in osmolality. A concomitantly greater fraction resulted from water extraction (77.5%). The explanation for these changes cannot be determined from these studies. One factor which is likely to have a significant effect on the observed decrease in osmolality along this segment of the loop is an increase in medullary blood flow. This increase in medullary blood flow would lower solute concentration in the papillary interstitium as has been reported previously (24) and is inferred in the present studies from the observations that the concentration of NES in loop and collecting duct fluid are considerably less than controls. Another factor may be the higher concentration of urea in tubular fluid at the beginning of the DLH after relief of obstruction. This can be inferred from the fact that BUN values were 10-fold greater after ureteral release than in shams. This would further decrease the chemical gradient for urea from tubular interstitium to tubular lumen and affect solute (urea) entry into the DLH.

Papillary collecting duct function. In all the sham-operated rats the fraction of filtered load of water and sodium remaining fell from the proximal to tip collecting duct sites. The amount reabsorbed between these two sites was approximately 0.5% of the filtered sodium and water or 1.4% of the filtered load per millimeter of collecting duct. As expected, this decline was associated with a concomitant rise in osmolality. In these studies nearly 1.2% of the filtered sodium and water remained at distal collecting duct sites. This is significantly greater than the amount excreted in the final urine of the contralateral kidney or than previously reported collecting duct data obtained under comparable experimental conditions (13). One major reason for this disparity is that the site of collection designated CD_{tip} in the control animals was 0.5–1 mm proximal to the tip of the papilla. This was done in an attempt to assess a comparable length of collecting duct in controls as that available to micropuncture in the BUO group. If one assumes that the rate of reabsorption of sodium and water along the remaining portion of the collecting duct in controls is roughly the same as it was in the proximal segment and that the length of this segment was 0.5 mm, then the amount of water remaining at the tip would be approximately 0.48%. This estimate is similar to the results of studies reported by Jamison (13), although still greater than the percent of filtered water excreted by the contralateral kidney. It is thought that the disparity between the fraction of the filtered load of sodium and water excreted by the contralateral hydropenic kidney and that found at CD_{tip} is the result of removing the ureteral pelvis from the

renal papilla. It has been shown by Schütz and Schnermann (25) that removal of the ureteral pelvis and therefore the urine bathing the papilla impaired the efficiency of this structure to generate high urinary osmolalities and reduce the amount of water excreted in the final urine.

Somewhat surprising was the finding that the function of the terminal collecting duct appears intact in rats undergoing a postobstructive diuresis. In all the rats studied in the experimental group, the fraction of the filtered water and sodium remaining fell from proximal to tip collecting duct sites indicating that water and sodium were reabsorbed along this segment. These changes were associated with a slight but significant increase in tubular fluid osmolality from base to tip.

In fractional terms the amount of water and sodium presented to the terminal collecting duct after release of BUO was significantly greater than hydropenic controls (13 vs. 1.7%). By the tip nearly 8% remained, significantly greater than the amount remaining at a comparable distance from CD_{prox} in the sham-operated group. This value is slightly less than 10.7% excreted by the contralateral kidney. It is likely that this difference is the result of the manipulations involved in the preparation of the renal papilla for micropuncture. A slight decrease in renal blood and concomitant drop in GFR would decrease tubular flow and increase the reabsorption of water along the renal tubule and collecting duct. Such changes might be readily apparent in the BUO group where papillary osmolality is significantly reduced but might not be seen in hydropenic controls where flow through the collecting duct is maximally reduced and the overriding effect is likely to be a slow loss of medullary tonicity secondary to the removal of the ureteral pelvis.

Nevertheless, the data indicate that 5.4% of the filtered load of water was reabsorbed between these two sites, a value 10-fold greater than the amount reabsorbed along a comparable length in the controls. In absolute terms it is likely that these differences are not as striking. If one assumes that the GFR of the micropunctured kidney is the same as that of the contralateral untouched kidney, then the absolute amount of water reabsorbed can be calculated by multiplying contralateral whole kidney GFR by the fraction of the filtered load of water reabsorbed between the two sites of micropuncture. In the sham-operated group the mean value was 3.3 ± 0.9 μl/min per mm collecting duct (CD). In the BUO group 8.6 ± 1.4 μl/min per mm CD was reabsorbed. This difference was significant ($P < 0.01$) but reflected only a threefold increase in the reabsorption of water along this segment.

Similarly the absolute rate of sodium reabsorption is probably not as great as fractional changes would imply. An estimate of absolute reabsorption can be obtained by multiplying the filtered load of sodium by fractional reabsorption between CD_{prox} and CD_{tip}. In controls 566 ± 135 μeq/min per mm CD were reabsorbed. This was significantly ($P < 0.001$) less than the absolute rate of sodium reabsorption in the BUO group where the mean was $1{,}672 \pm 405$ μeq/min per mm CD. These calculations suggest that while absolute reabsorption of sodium is greater after release of BUO than in controls in the terminal segment of the CD, the magnitude of the difference is not as great as fractional differences might indicate.

While the observations made in this study do not rule out the possibility that there is a functional defect in the CD after relief of obstruction, the data indicate that the changes in CD function are at least not contributing to the ensuing diuresis. The magnitude of the fractional changes seen between base and tip CD sites in this study are comparable to what has been reported previously when the terminal CD has been studied under conditions of water diuresis (26) and saline expansion (27) where the flow through this segment is of a similar degree.

These findings appear not to agree with the papillary microcatherization studies reported by Sonnenberg and Wilson (12). These investigators found that unlike hydropenic controls mean TF/P In ratios after release of BUO decreased as a function of the distance along the medullary CD from the cortex. Similar conclusions were reached by McDougal and Wright (2) because of the findings that the percentage of filtered sodium remaining at end distal sites was less than the amount excreted in the urine. In the studies presented here fractional reabsorption of sodium and water to the bend of the loop of Henle was strikingly reduced when compared to hydropenic rats or when compared to the effects of BUO on cortical tubular segments previously reported (1, 2). It is therefore conceivable that the differences seen between the fractional sodium excretion and the amount remaining at distal tubular sites in the studies of McDougal and Wright (2) might be the result of an admixture of fluid from superficial and deep nephrons. It is difficult however to explain the different results obtained by Sonnenberg and Wilson (12) on this basis. It is clear that part of the difficulty may be related to differences in the magnitude of the diuresis and natriuresis obtained in these two studies. It is also possible that medullary segments respond in a different fashion to obstruction than the terminal segments of the CD.

In summary, the data presented here suggest that a number of alterations underly the postobstructive diuresis observed after release of BUO. One factor is a depression of fractional reabsorption of sodium and water to the bend in the loop of Henle. This probably

reflects changes at two levels. It is likely that, as in cortical nephrons proximal tubular reabsorption of sodium and therefore water is depressed. Further movement of water out of the DLH is diminished. This is likely the result of a diminished tonicity of the surrounding medullary interstitium perhaps as a result of increased medullary blood flow. An unusually high concentration of urea in the tubular fluid would further impair the concentrating ability to the extent that urea entry into the DLH is diminished. The tonicity of the medullary interstitium may also be affected by the decrease in SNGFR seen in our studies and the fact that the number of functioning nephrons per total mass of papilla is significantly reduced as implied by other studies. The net effect of these changes would be a marked decline in the concentration of solute in the papilla and therefore a decrease in the ability of this structure to generate high urinary osmolalities.

ACKNOWLEDGMENTS

We thank Ms. Jackie Fitzsimmons and Ms. Edna Grandberry for their assistance in the preparation of this manuscript. We thank Dr. Rex Jamison for critically reading this manuscript.

This work was supported by grants from the Veterans Administration (6930-02) and the U. S. Public Health Service AM 09976, AM 07126).

REFERENCES

1. Buerkert, J., E. Alexander, M. L. Purkerson, and S. Klahr. 1976. On the site of decreased fluid reabsorption after release of ureteral obstruction in the rat. *J. Lab. Clin. Med.* **87:** 397–410.
2. McDougal, W. S., and F. S. Wright. 1972. Defect in proximal and distal sodium transport in post-obstructive diuresis. *Kidney Int.* **2:** 304–317.
3. Yarger, W. E., H. S. Aynedjian, and N. Bank. 1972. A micropuncture study of postobstructive diuresis in the rat. *J. Clin. Invest.* **51:** 625–637.
4. Harris, R. H., and W. E. Yarger. 1974. Renal function after release of unilateral ureteral obstruction in rats. *Am. J. Physiol.* **227:** 806–815.
5. Harris, R. H., and W. E. Yarger. 1975. The pathogenesis of post-obstructive diuresis. The role of circulating natriuretic and diuretic factors including urea. *J. Clin. Invest.* **56:** 880–887.
6. Wilson, D. R., and U. Honrath. 1976. Cross-circulation study of natriuretic factors in postobstructive diuresis. *J. Clin. Invest.* **57:** 380–389.
7. Jaenike, J. R. 1972. The renal functional defect of post-obstructive nephropathy. The effects of bilateral ureteral obstruction in the rat. *J. Clin. Invest.* **51:** 2999–3006.
8. Wilson, D. R. 1975. Nephron functional heterogeneity in the postobstructive kidney. *Kindey Int.* **7:** 19–26.
9. Winberg, J. 1959. Renal function in water-losing syndrome due to lower urinary tract obstruction before and after treatment. *Acta Pediatr.* **48:** 149–163.
10. Roussak, N. J., and S. Oleesky. 1954. Water-losing nephritis. A syndrome simulating diabetes insipidus. *Q. J. Med.* **23:** 147–164. (and plates 16, 17).
11. Earley, L. E. 1956. Extreme polyuria in obstructive uropathy. Report of a case of "water-losing nephritis" in an infant, with a discussion of polyuria. *N. Engl. J. Med.* **255:** 600–605.
12. Sonnenberg, H., and D. R. Wilson. 1976. The role of the medullary collecting ducts in postobstructive diuresis. *J. Clin. Invest.* **57:** 1564–1574.
13. Jamison, R. L. 1970. Micropuncture study of superficial and juxtamedullary nephrons in the rat. *Am. J. Physiol.* **218:** 46–55.
14. Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination of nanogram quantities of inulin. *Anal. Biochem.* **16:** 409–419.
15. Ramsay, J. A., and R. H. J. Brown. 1955. Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. Sci. Instrum.* **32:** 372–375.
16. Führ, J., J. Kaczamarczyk, and C-D. Krüttgen. 1955. Eine einfache colorimetrische Method zur Inulinbestimmung für Nieren-clearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin. Wochenschr.* **33:** 729–730.
17. Fawcett, J. K., and J. E. Scott. 1960. A rapid and precise method for the determination of urea. *J. Clin. Pathol.* **13:** 156–159.
18. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 593 pp.
19. Imai, M., and J. Kokko. 1973. Sodium chloride, urea, and water transport in the thin ascending limb of Henle: Generation of osmotic gradients by passive diffusion of solutes. *J. Clin. Invest.* **52:** 42A. (Abstr.)
20. Jamison, R. L., J. Buerkert, and F. Lacy. 1973. A micropuncture study of Henle's thin loop in Brattleboro rats. *Am. J. Physiol.* **224:** 180–185.
21. Pennell, J. P., F. B. Lacy, and R. L. Jamison. 1974. An *in vivo* study of the concentrating process in the descending limb of Henle's loop. *Kidney Int.* **5:** 337–347.
22. Safirstein, R., and F. W. Wright. 1975. Renal vessel and tubular pressures during and after obstruction of one or both ureters. *Fed. Proc.* **34:** 393. (Abstr.)
23. Feldman, R. A., N. J. Siegel, M. Kashgarian, J. P. Hayslett, and B. Lytton. 1974. Intra renal hemodynamics in post-obstructive diuresis. *Invest. Urol.* **12:** 172–175.
24. Eknoyan, G., W. N. Suki, M. Martinez-Maldonado, and M. A. Anhalt. 1970. Chronic hydronephrosis: observations on the mechanism of the defect in urine concentration. *Proc. Soc. Exp. Biol. Med.* **134:** 634–639.
25. Schütz, W., and J. Schnermann. 1972. Pelvic urine composition as a determinant of intermedullary solute concentration and urine osmolality. *Pfluegers Arch. Eur. J. Physiol.* **334:** 154–166.
26. Jamison, R. L., J. Buerkert, and F. Lacy. 1971. A micropuncture study of collecting tubule function in rats with hereditary diabetes insipidus. *J. Clin. Invest.* **50:** 2444–2452.
27. Stein, J. H., R. W. Osgood, and R. T. Kunau, Jr. 1976. Direct measurement of papillary collecting duct sodium transport in the rat. Evidence for heterogeneity of nephron function during ringer loading. *J. Clin. Invest.* **58:** 767–773.