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Urine-reinfusion natriuresis: Evidence for potent natriuretic factors in rat urine

ROBERT H. HARRIS and WILLIAM E. YARGER

Veterans Administration Hospital and the Division of Nephrology, Department of Medicine, Duke University Medical Center, Durham, North Carolina

Urine-reinfusion natriuresis: Evidence for potent natriuretic factors in rat urine. In awake rats the entire urine output was continuously reinfused i.v. Urine-reinfusion (UR) consistently led to the appearance, within one to two hours, of massive, sustained natriuresis and diuresis, suggesting the existence of potent natriuretic factors in the urine. At the time of maximal natriuresis, mean sodium excretion rate and urine flow rate were 25 and 15 times their respective values in control rats. This "urine-reinfusion natriuresis" could be demonstrated despite treatment with desoxycorticosterone acetate, blockage of prostaglandin synthesis by indomethacin or meclofenamate, reduction of plasma urea by pretreatment with a protein-free diet, or heating the urine to 100°C. The natriuresis was not prevented by the absence of vasopressin (in Brattleboro rats) and was augmented by vasopressin infusion. In the Brattleboro rats, a marked increase in (C_{H_2O} + C_{Na})/GFR with only a slight rise in C_{H_2O}/GFR during UR suggests inhibition of both proximal and distal tubular reabsorption. Renal blood flow and plasma flow increased markedly during UR with a lesser rise in GFR, consistent with post-glomerular vasodilatation. Thus, the phenomenon of urine-reinfusion natriuresis suggests the presence in rat urine of potent, heat-stable natriuretic factors, whose action is largely independent of changes in mineralocorticoids, prostaglandins, urea, or vasopressin. Renal vasodilatation, with decreased sodium reabsorption at both proximal and distal nephron sites, appears to play an important role in the natriuresis.

Natriurèse au cours de la réinjection d'urine: Preuve de l'existence de facteurs natriurétique et diurétique puissants dans l'urine de rat. La totalité du débit urinaire a été réinjectée en continu chez des rats éveillés. La réinfusion d'urine (UR) détermine l'apparition, dans un délai d'une à deux heures, d'une natriurèse et d'une diurèse massive ce qui suggère la présence, dans l'urine, de facteurs natriurétiques puissants. Au moment de la natriurèse maximale les débits de sodium et d'urine sont égaux à 25 et 15 fois leurs valeurs respectives chez les animaux contrôles. Cette natriurèse par réinfusion d'urine peut être obtenue malgré le traitment par l'acétate de désoxycorticostérone, le blocage de la synthèse des prostaglandines par l'indométhacine, la diminution de l'urée plasmatique au moyen d'un pré-traitement consistant en une alimentation sans protéines ou le chauffage de l'urine à 100° C. La natriurèse n'est pas empêchée par l'absence de vasopressine (rat Brattleboro), elle est augmentée par l'administration de vasopressine. Chez le rat Brattleboro une augmentation importante de (C_{H_2O} + C_{Na})/GFR avec une augmentation non significative de C_{H_2O}/GFR au cours de l'UR suggère l'inhibition de la réabsorption tubulaire proximale et distale. Les débits sanguin et plasmatique rénaux augmentent de façon importante au cours de l'UR cependant que le débit de filtration glomérulaire augmente moins, ce qui est compatible avec une vasodilatation post glomérulaire. Ainsi le phénomène de la natriurèse consécutive à la réinfusion d'urine suggère la présence, dans l'urine, d'un facteur natriurétique puissant, thermostable donc l'action est pour une grande part indépendante des modifications des minéralocorticoïdes, des prostaglandines, de l'urée et de la vasopressine. La vasodilatation rénale, qui diminue la réabsorption de sodium à la fois dans les régions proximale et distale du néphron, semble jouer un rôle important dans la natriurèse.

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In recent years, evidence from a number of laboratories has supported the existence of natriuretic factors in plasma or serum [1–3], urine [1, 4–6], and renal tissue [7]. Such factors have been described under conditions of chronic [1, 4, 6] and acute [3, 7] volume expansion, and in chronic uremia [2, 5]. Despite intense investigational efforts, the anatomical source, chemical nature, mechanism of action, and stimulus for the appearance or "release" of these factors have remained unsettled.

Recently, we reported evidence for a major role of circulating natriuretic factors in the pathogenesis of post-obstructive diuresis in rats [8]. In the course of these experiments, we observed that continuous i.v. reinfusion of the entire urine output in conscious rats led to the appearance of massive natriuresis and diuresis [8, 9]. This phenomenon, which we have termed "urine-reinfusion natriuresis" [10], has suggested the presence of potent natriuretic factors in the urine.

The objective of the present study has been to further characterize this remarkably intense natriuresis, and to examine its mechanisms. Our findings support the concept of heat-stable natriuretic factors in urine, and suggest that their action is largely unrelated to changes in mineralocorticoid, vasopressin, or prostaglandin activity. Although urea may play a role in the natriuresis, it appears that other natriuretic factors are probably more important. Our data suggest that both proximal and distal nephron sites may be involved in the natriuresis, and that renal vasodila-

tation may be a major natriuretic mechanism during urine-reinfusion.

Methods

Experiments were performed in a total of 64 rats weighing 175 to 345 g. All rats were Sprague-Dawley males, except for three Long-Evans rats of the Brattleboro strain [11]. The diet was commercial rat chow (Ralston Purina Co., St. Louis, MO) and tap water *ad lib*, except where otherwise noted. All animals, studied while awake and partially restrained, were prepared as follows. On the morning of study, during brief ether anesthesia, polyethylene PE-50 catheters were implanted in a femoral artery and vein, and a PE-90 catheter was implanted in the urinary bladder through a small midline suprapubic incision. In six of the Sprague-Dawley rats, a catheter was implanted in the left renal vein to permit sampling of renal venous blood. In these animals, the left renal vein was exposed via a midline abdominal incision and punctured near the inferior vena cava with a 27-gauge needle. A PE-10 catheter was inserted through the needle hole and held in place with a drop of adhesive (Eastman 910 Adhesive, Eastman Chemical Products Inc., Kingsport, TN). All catheters were brought externally, and the incisions were sutured. After surgery each rat was placed in a restraining cage, described previously [8], and food and drinking water were subsequently withheld. The rats were allowed to recover from anesthesia for approximately 90 min before studies were performed. Renal function was examined by clearance techniques, using carboxyl-^{14}C-inulin and glycyl-^{3}H-para-aminohippuric acid (PAH). Arterial blood was sampled, and arterial blood pressure was measured frequently throughout the experiment via the arterial catheter. In the six rats with renal venous catheters, left renal venous blood was sampled simultaneously with arterial blood. ^{14}C-inulin and ^{3}H-PAH concentration in urine and plasma were measured by liquid scintillation techniques as previously described [12]. Plasma and urine sodium and potassium concentrations were determined by flame photometry, and plasma and urine osmolality were measured with a vapor pressure osmometer (Model 5100, Wescor, Inc., Logan, UT). Plasma urea concentration was determined by the phenate-hypochlorite method described by Fawcett and Scott [13]. A total of approximately 50μl of plasma or urine sufficed for all of these determinations.

The following experimental protocols were carried out:

Urine-reinfusion (UR) in previously normal rats. UR was performed in 34 previously normal rats, using the servo-activated pump apparatus which we have previously described [8]. Urine samples, approximately 0.1 ml, were collected periodically, and simultaneous blood samples were obtained. The rats received i.v. 5% dextrose in water throughout the study at the rate of 5% of body wt per 24 hr in order to replace insensible water loss [12]. Since excreted inulin and PAH were reinfused, relatively stable plasma concentrations of ^{14}C-inulin and ^{3}H-PAH resulted from a single priming dose of these compounds.

In the six rats with renal vein catheters, standard clearance studies [12], consisting of three 20-min collection periods, were performed prior to beginning UR. Left renal venous blood was sampled simultaneously with arterial blood.

Various protocols were followed in the remaining 28 UR rats without renal vein catheters. In five rats, standard clearance studies (three 20-min periods) were performed prior to beginning UR. In four of these five rats, clearance studies were performed after UR had been abruptly discontinued. In nine rats, UR was carried out for varying periods of time, and then vasopressin (ADH) (Pitressin®, Parke-Davis and Co., Detroit, MI) (10 and/or 50 mU/kg of body wt per hr) was infused i.v. while UR was continued. In eight rats during UR, either indomethacin or meclofenamate was given i.v. in order to inhibit prostaglandin synthesis: indomethacin, dissolved in a phosphate buffer (pH, 8; 2 mg/ml), was given in a dose of 8 mg/kg of body wt, while meclofenamate, dissolved in 0.45% saline (5 mg/ml) was given as either one or two doses of 20 mg/kg each. In three rats during UR, urine was circulated through a 30-foot coil of silicone rubber tubing (0.8 mm ID, 1.3 mm OD, initially filled with 0.9% saline) immersed in a water bath at 100°C. The calculated transit time through the heating coil was 5 to 6 min. The heated urine was cooled to room temperature before reinfusion.

In addition to the above, the following rats were subjected to UR:

Rats on a protein-free diet (UR-PF): Nine rats were fed protein-free rat chow (ICN Nutritional Biochemicals, Cleveland, OH), supplemented with 20% sucrose and vitamins, for three to five days prior to study in order to decrease endogenous urea production. Five of the UR-PF rats were given desoxycorticosterone acetate (DOCA) in oil (1.0 mg, i.m.) the evening before and the morning of surgery.

Chronically sodium-loaded rats (UR-Hi-Na): Four rats were given 1% NaCl to drink *ad lib* for 7 to 13 days prior to study.

Chronically sodium-depleted rats (UR-Lo-Na): Five rats were fed sodium-free chow (ICN Nutritional

Biochemicals, Cleveland, OH) and deionized drinking water for 11 to 17 days prior to study.

Rats with diabetes insipidus (UR-DI): Three rats with congenital diabetes insipidus (Brattleboro strain) were given a gastric water lavage, 3% of body wt, immediately prior to study. Standard clearance studies were performed prior to beginning UR.

Sham-manipulated control rats (CONT): Standard clearance studies were performed in nine rats which did not undergo UR. At least three clearance periods were done initially without ADH, and subsequently with ADH i.v. at 10 and at 50 mU/kg of body wt per hr. Data from the periods without ADH administration were compared with the control (pre-UR) data of five of the UR rats without renal vein catheters, and no significant differences were found. Therefore, the results of these 14 rats were combined for comparisons with the other experimental models.

Calculations: In the six rats with left renal vein catheters, total renal plasma flow (RPF) was calculated by the Fick principle, using Wolf's equation [14]:

$$RPF = (U_{PAH} - RV_{PAH}) \times \dot{V}/(RA_{PAH} - RV_{PAH})$$

where U_{PAH}, RA_{PAH}, and RV_{PAH} are concentrations of PAH in urine, arterial blood, and renal venous blood, respectively, and $\dot{V}$ is urine flow rate. The extraction ratio of PAH (E_{PAH}) was calculated as:

$$E_{PAH} = (RA_{PAH} - RV_{PAH})/RA_{PAH}$$

In these calculations, we assumed that the PAH concentrations of left and right renal venous blood were equal. Renal blood flow (RBF) was calculated as RPF/(1-Hct). Renal vascular resistance was calculated as the ratio of mean arterial blood pressure to RBF. Statistics were calculated according to methods described by Steel and Torrie [15]. Analysis of variance, with Duncan's multiple range test as modified by Kramer, or Student's *t* test, were used as appropriate to compare group means. A probability value (*P*) of less than 0.05 was regarded as significant. Values are presented as the means ± 1 SEM.

Results

The effects of urine-reinfusion (UR): A total of 28 previously normal rats without renal vein catheters were studied during UR. Five of these rats were initially studied in the control state, then subjected to UR. The clearance data and arterial blood pressures (BP) of these five animals are depicted in Figure 1. A marked increase in urine flow rate ($\dot{V}$) and sodium excretion rate ($U_{Na}V$) occurred in all five rats within 50 to 70 min after UR was begun. In one rat (denoted by open triangles in Fig. 1), a 12-fold increase in $\dot{V}$ and a 19-fold increase in $U_{Na}V$ were noted after 60 min of UR. In this early phase of "urine-reinfusion natriuresis," glomerular filtration rates (GFR) were relatively stable in four rats, but decreased temporarily in one animal. As UR was continued, $\dot{V}$ tended to plateau temporarily at 180 to 200 min in four rats (denoted by closed circles in Fig. 1) and then continued to rise progressively. In the remaining animal, there was a sustained elevation in $\dot{V}$ as compared with the other rats.

GFR remained relatively stable initially, but grad-

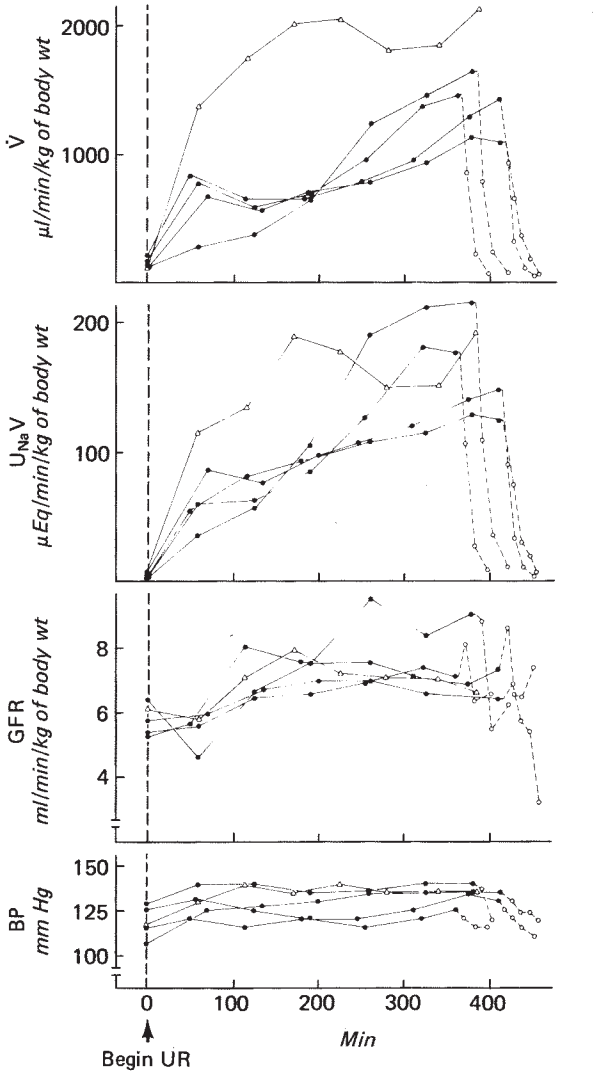

Fig. 1. *Time course of changes in urine flow rate ($\dot{V}$), sodium excretion rate ($U_{Na}V$), glomerular filtration rate (GFR), and mean arterial blood pressure (BP) during urine-reinfusion (UR) in five previously normal rats.* Rates are expressed per kidney. Values at time zero (*vertical broken line*) were observed during control clearance periods prior to UR. The data of one rat, whose course was distinctive by the early onset of massive, sustained diuresis and natriuresis, are indicated by *open triangles*. UR was abruptly discontinued in the four other rats, as indicated by the *open circles* and *broken lines*.

ually increased to levels that were significantly greater than those noted in the control period ($P < 0.05$). BP increased slightly in the early phase of UR in each rat, and remained relatively stable thereafter. BP recorded at the time of maximal $U_{Na}V$ for each rat averaged 12% greater than values observed during the control period.

Most rats exhibited a fairly obvious maximal plateau in urinary sodium and water excretion at some point during UR, even though the magnitude of diuresis and natriuresis observed and the time required to achieve this maximal response were quite variable. Therefore, in order to compare the various experimental groups, the data from the clearance period in which maximal $U_{Na}V$ occurred were analyzed as shown in Table 1. These results refer to UR without the administration of ADH, indomethacin, or meclofenamate, or other experimental maneuvers. These data are compared with the data from 14 control (CONT) rats, also without ADH being given. Maximal $U_{Na}V$ occurred after 339 ± 20 min of UR and was 25 times that of CONT rats. The largest $U_{Na}V$ noted, 317 μEq/min/kg of body wt per kidney, would be equivalent to approximately 30 times the rat's total body sodium content per 24 hr. The fractional excretion of the filtered sodium load (FE_{Na}) exceeded 20% in seven of the UR rats. The animal with the highest FE_{Na}, 30.2%, also had the largest $U_{Na}V$. Mean FE_{Na} in UR animals was increased to 16.5 ± 1.2% compared with 0.93 ± 0.9% for the CONT rats. Urine flow rate at the time of maximal $U_{Na}V$ was 15 times that of the CONT value, and in eight of the UR rats equalled or exceeded 2000μl/min/kg per kidney. The highest value noted, 3730μl/min/kg per kidney, would be the equivalent of 10 times the animal's body wt per 24 hr (total of both kidneys). The fractional excretion of glomerular filtrate ($\dot{V}$/GFR) was 22.4 ± 1.9% in the UR rats (vs. 2.0 ± 0.2% for CONT rats, $P < 0.01$), and attained values greater than 37% in four animals. The rat with the highest $\dot{V}$ and $U_{Na}V$ had a $\dot{V}$/GFR of 47.5%.

Associated with this massive natriuresis and diuresis, mean GFR was increased by approximately 35% over the CONT rats. This increase in GFR was not accompanied by a significant increase in PAH clearance rate (C_{PAH}). However, mean BP in UR animals was significantly elevated to 132 ± 2 mm Hg compared with 121 ± 2 mm Hg for CONT rats.

In addition to the marked natriuresis and diuresis which occurred with UR, there was also a striking kaliuresis. Potassium excretion rate (U_KV) was increased by over six-fold compared with CONT rats. C_K/GFR was increased from 27.2 ± 3.0% in CONT rats to 93.4 ± 3.4% in UR rats, and exceeded 100% in eight UR animals, indicating net tubular secretion of potassium.

Table 2 gives measurements on arterial blood at the time of maximal $U_{Na}V$. As might be expected, the mean plasma urea concentration (P_u) in the UR rats was significantly elevated compared with the CONT mean. Additionally, plasma potassium concentration was significantly increased in the UR animals. There were no significant changes in the plasma sodium concentration, osmolality, or hematocrit of UR rats.

Renal hemodynamics before and during UR. Six previously normal rats with renal venous catheters were studied before and during UR. Control (before UR) values for $\dot{V}$ and $U_{Na}V$ in these rats were significantly less ($P < 0.001$ for both) than those of the 14 CONT rats (Table 1). These differences may have been an

Table 1. Clearance values (per kidney) and blood pressure at the time of maximal urinary sodium excretion[a,b]

Group[c], no. of rats	Duration of urine-reinfusion *min*	$\dot{V}$ *μl/min per kg of body wt*	GFR *ml/min per kg of body wt*	C_{PAH} *ml/min per kg of body wt*	$U_{Na}V$ *μEq/min per kg of body wt*	FE_{Na} %	U_KV *μEq/min per kg of wt*	BP *mm Hg*
Control, 14		110 ± 11	5.47 ± 0.21	12.8 ± 0.9	6.7 ± 0.7	0.93 ± 0.09	5.8 ± 0.6	121 ± 2
UR, 28	339 ± 20	1633 ± 124 ($P < 0.01$)	7.48 ± 0.24 ($P < 0.01$)	14.9 ± 0.9 *NS*	164 ± 12 ($P < 0.01$)	16.5 ± 1.2 ($P < 0.01$)	37.9 ± 2.0 ($P < 0.01$)	132 ± 2 ($P < 0.01$)
UR-PF, 9	341 ± 25	1720 ± 98 ($P < 0.01$)	7.02 ± 0.19 ($P < 0.05$)	19.7 ± 1.5 ($P < 0.01$)	126 ± 10 ($P < 0.01$)	13.7 ± 1.2 ($P < 0.01$)	10.4 ± 1.9 *NS*	124 ± 3 *NS*
UR-HI-Na, 4	366 ± 44	1990 ± 430 ($P < 0.01$)	6.82 ± 0.47 ($P < 0.05$)	10.9 ± 1.4 *NS*	196 ± 53 ($P < 0.01$)	20.6 ± 4.3 ($P < 0.01$)	32.6 ± 3.2 ($P < 0.01$)	131 ± 5 *NS*
UR-Lo-Na, 5	477 ± 23	1040 ± 210 ($P < 0.01$)	6.50 ± 0.45 *NS*	13.9 ± 1.5 *NS*	58 ± 15 ($P < 0.05$)	7.0 ± 1.8 ($P < 0.05$)	31.3 ± 3.1 ($P < 0.01$)	120 ± 2 *NS*
UR-DI, 3	346 ± 70	1380 ± 60 ($P < 0.01$)	5.24 ± 0.17 *NS*	12.2 ± 3.3 *NS*	117 ± 17 ($P < 0.01$)	15.0 ± 1.7 ($P < 0.01$)	15.5 ± 4.9 ($P < 0.05$)	95 ± 1 ($P < 0.01$)

[a] Values are means ± SEM; P values refer to comparisons with Control group means; NS = not significant.
[b] $\dot{V}$ = urine flow rate; GFR = glomerular filtration rate; C_{PAH} = para-aminohippurate clearance rate; $U_{Na}V$ = urinary sodium excretion rate; FE_{Na} = fractional excretion of sodium; U_KV = urinary potassium excretion rate; BP = mean arterial blood pressure.
[c] UR = urine-reinfusion in previously normal rats; UR-PF = rats on a protein-free diet; UR-Hi-Na = chronically sodium-loaded rats; UR-Lo-Na = chronically sodium-depleted rats; UR-DI = rats with diabetes insipidus.

Table 2. Summary of measurements on arterial blood at the time of maximal urinary sodium excretion[a]

Group, Number of rats	[Na] *mEq/liter*	[K] *mEq/liter*	Osmolality mOsm/kg of H_2O	Hematocrit %	[Urea] *mM*
Control, 14	139 ± 1	4.2 ± 0.1	300 ± 3	41 ± 0.4	4.5 ± 0.4
UR, 28	136 ± 1 *NS*	5.7 ± 0.2 ($P < 0.01$)	306 ± 2 *NS*	40 ± 0.4 *NS*	21.8 ± 1.9 ($P < 0.01$)
UR-PF, 9	132 ± 3 ($P < 0.05$)	4.3 ± 0.2 *NS*	292 ± 6 ($P < 0.05$)	45 ± 2.0 ($P < 0.01$)	6.2 ± 0.9 *NS*
UR-Hi-Na, 4	135 ± 1 *NS*	5.3 ± 0.4 ($P < 0.05$)	309 ± 5 *NS*	39 ± 2.0 *NS*	18.9 ± 1.5 ($P < 0.01$)
UR-Lo-Na, 5	136 ± 4 *NS*	6.0 ± 0.3 ($P < 0.01$)	311 ± 4 *NS*	44 ± 0.5 ($P < 0.05$)	18.4 ± 1.1 ($P < 0.01$)
UR-DI, 3	147 ± 1 ($P < 0.05$)	4.3 ± 0.1 *NS*	323 ± 5 ($P < 0.01$)	37 ± 2.0 ($P < 0.05$)	22.9 ± 3.4 ($P < 0.01$)

[a] Values are means ± SEM. *P* values refer to comparisons with Control group means. *NS* = not significant. See Table 1 for explanation of abbreviations.

effect of the somewhat more extensive surgery required for the renal vein catheter implantation. Because of these differences, the data of these six rats have been analyzed separately from the other groups, and the results are shown in Table 3. The UR values represent observations after 379 ± 31 min of UR, the time of maximal $U_{Na}V$. All six rats displayed intense natriuretic and diuretic responses to UR, and the values for $\dot{V}$ and $U_{Na}V$ during UR are quite similar to those of the other 28 UR rats (Table 1). GFR increased significantly during UR, with a slight and non-significant rise in C_{PAH}. A striking degree of renal vasodilatation was noted during UR, the renal vascular resistance decreasing by 28%. Total renal plasma flow (RPF) and renal blood flow (RBF) increased markedly, associated with a substantial decrease in the renal extraction ratio of PAH (E_{PAH}). The increase in RPF was proportionately much greater than that of GFR, with a resultant 28% decrease in whole-kidney filtration fraction (FF). BP in this group increased by 8% ($P > 0.2$).

The effect of decreasing urea production. As noted above, UR rats exhibited a significant elevation of P_u at the time of maximal $U_{Na}V$. In order to examine the role of urea, nine rats (UR-PF) were fed a protein-free diet for five to nine days prior to UR. Mean P_u in the UR-PF rats at the time of maximal natriuresis (Table 2) was not significantly different from that of CONT rats, but was significantly lower than the P_u of UR rats ($P < 0.01$).

Five of the UR-PF rats were treated with desoxycorticosterone acetate (DOCA) shortly before UR, in order to insure a high level of mineralocorticoid activity during the experiment. The results of these five animals at the time of maximal $U_{Na}V$ did not differ significantly from those of the four UR-PF rats that did not receive DOCA, with regard to $\dot{V}$, GFR, $U_{Na}V$, FE_{Na}, or duration of UR prior to maximal $U_{Na}V$ ($P > 0.2$ in each case). Because of the absence of a significant effect of DOCA, the data of all nine UR-PF rats were combined (Table 1).

Although P_u in the UR-PF rats did not differ from P_u in CONT rats, a marked natriuresis occurred. Maximal $U_{Na}V$—noted after 341 ± 25 min (a time not significantly different from the UR rats)—was 19 times the CONT level, and approximately 75% of that of UR animals ($P < 0.05$). The values of FE_{Na} for UR-PF and UR rats did not differ significantly. Mean $\dot{V}$ in the UR-PF rats was nearly 16 times the CONT mean, and slightly greater than the UR value. GFR in the UR-PF rats was significantly increased, to 128% of the CONT value, similar to the results observed in UR animals.

Despite the comparable natriuresis, diuresis, and increased GFR in the UR and UR-PF rats, these two models differed from each other in several respects. In contrast with the relatively unchanged C_{PAH} in the UR rats, C_{PAH} in the UR-PF animals increased to 154% of the CONT mean ($P < 0.01$). Potassium excretion in UR-PF rats was not significantly increased over CONT values. The absence of a significant kaliuresis despite the natriuresis, and the absence of a rise in plasma potassium concentration (Table 2) may have been consequent to decreased dietary potassium intake during treatment with protein-free chow. Plasma sodium concentration in the UR-PF rats was decreased significantly compared with the CONT rats, and plasma osmolality was significantly less than that of either CONT or UR animals. In addition, the hematocrit of UR-PF rats was significantly greater than those of either CONT or UR rats, suggesting that the UR-PF animals may have been relatively sodium depleted.[1] Finally, BP in

[1] The modified protein-free diet contained 42μEq/g of sodium and 134μEq/g of potassium. These quantities are 23% and 56%, respectively, of the amounts in commercial rat chow. Although we did not measure dietary sodium and potassium intake in these rats, it is likely that intakes were considerably below those of rats on commercial chow.

Table 3. Renal hemodynamic data (per kidney) in six rats before and during urine-reinfusion (UR) at the time of maximal urinary sodium excretion[a,b]

	Before UR	During UR	*P*
$\dot{V}$, *μl/min/kg of body wt*	35 ± 9	1576 ± 253	< 0.01
$U_{Na}V$, *μEq/min/kg of body wt*	2.0 ± 0.5	169 ± 20	< 0.01
GFR, *ml/min/kg of body wt*	6.54 ± 0.25	7.07 ± 0.35	< 0.05
C_{PAH}, *ml/min/kg of body wt*	17.4 ± 0.6	19.1 ± 1.4	*NS*
E_{PAH}, %	84.4 ± 1.8	54.4 ± 2.6	< 0.01
RPF, *ml/min/kg of body wt*	20.8 ± 0.7	34.0 ± 2.7	< 0.01
RBF, *ml/min/kg of body wt*	34.3 ± 1.5	53.2 ± 5.1	< 0.01
BP, mm Hg	110 ± 5	119 ± 5	*NS*
RVR, *mm Hg·ml^{-1}·min^{-1}·kg^{-1} of body wt*	3.34 ± 0.28	2.39 ± 0.31	< 0.01
FF (GFR/RPF), %	31.9 ± 1.2	21.8 ± 2.8	< 0.01

[a] Values are means ± 1 SEM. *NS* = not significant.
[b] E_{PAH} = extraction ratio of PAH; RPF = total renal plasma flow; RBF = total renal blood flow; RVR = renal vascular resistance; FF = filtration fraction. Other abbreviations are explained in Table 1.

the UR-PF rats was not significantly different from that of CONT animals.

The effect of chronic sodium-loading. Four rats (UR-Hi-Na) drank 1% NaCl for 7 to 13 days prior to UR, in order to produce a state of chronic sodium-loading. This group exhibited the highest mean values for $U_{Na}V$ and FE_{Na} of any of the models of this study (Table 1), although the degree of natriuresis varied widely among the four animals. Comparison of the UR-Hi-Na and the UR rats did not reveal any significant differences with regard to clearance results or BP (Table 1) or composition of arterial blood (Table 2).

The effect of chronic sodium-depletion. In order to examine the effect of sodium-depletion on the response to urine-reinfusion, five rats (UR-Lo-Na) were pretreated with a sodium-free diet for 11 to 17 days. Despite sodium-depletion, these rats displayed a natriuresis and diuresis ($U_{Na}V$ and $\dot{V}$, both approximately nine times CONT values), as indicated in Table 1. However, the natriuresis of the UR-Lo-Na animals was markedly blunted and delayed in appearance in comparison with the UR rats. Maximal $U_{Na}V$ was not observed until urine-reinfusion had been carried out for 477 ± 23 min in the UR-Lo-Na rats, approximately 30% longer than in the UR animals. Mean $U_{Na}V$ for the UR-Lo-Na rats was only about one-third of that observed in the UR rats, FE_{Na} was comparably reduced ($P < 0.01$ for both). Also in contrast with the UR rats, GFR and BP were not significantly different from CONT values. Hematocrit in the UR-Lo-Na rats was significantly greater than either the CONT or the UR-Hi-Na values, suggesting extracellular fluid (ECF) contraction in these animals.

The effects of ADH on the phenomenon of urine-reinfusion natriuresis were examined in two ways. First, Brattleboro rats (UR-DI) with a complete absence of ADH were subjected to urine-reinfusion. As shown in Table 1, these animals displayed a marked natriuresis ($U_{Na}V$ approximately 17 times CONT) and diuresis. Table 4 shows a comparison of clearance results in the UR-DI rats before and during UR. $\dot{V}$ was increased nearly six-fold and $U_{Na}V$ approximately 26-fold ($P < 0.01$ for both). GFR rose 23%, without a significant change in C_{PAH}, comparable to the results in Sprague-Dawley rats. However, in contrast with all the other models of this study, BP fell significantly. The values of $(C_{H_2O} + C_{Na})/GFR$, an approximation of distal sodium delivery [16], increased six-fold ($P < 0.01$). However, the increase in solute-free water excretion (C_{H_2O}/GFR) did not increase significantly.

To further evaluate the importance of ADH, Sprague-Dawley rats undergoing urine-reinfusion natriuresis were given an i.v. infusion of ADH at two different dose levels. The results of ADH administra-

Table 4. Clearance values (per kidney) and blood pressure in three Brattleboro rats before and during urine-reinfusion (UR) at the time of maximal urinary sodium excretion[a,b]

	Before UR	During UR	*P*
$\dot{V}$, *μl/min/kg of body wt*	245 ± 60	1380 ± 60	< 0.01
GFR, *ml/min/kg of body wt*	4.27 ± 0.29	5.24 ± 0.17	< 0.05
C_{PAH}, *ml/min/kg of body wt*	13.8 ± 1.0	12.2 ± 3.3	*NS*
$U_{Na}V$, *μEq/min/kg of body wt*	4.5 ± 1.0	117 ± 17	< 0.01
FE_{Na}, %	0.71 ± 0.14	15.0 ± 1.7	< 0.01
$(C_{H_2O} + C_{Na})/GFR$, %	3.6 ± 0.1	21.7 ± 0.8	< 0.01
C_{H_2O}/GFR, %	2.9 ± 0.9	6.8 ± 1.1	*NS*
BP, *mm Hg*	111 ± 3	95 ± 1	< 0.05

[a] Values are means ± SEM. *NS* = not significant.
[b] $(C_{H_2O} + C_{Na})/GFR$ = fractional distal sodium delivery; C_{H_2O}/GFR = fractional excretion of solute-free water. Other abbreviations are as shown in Table 1.

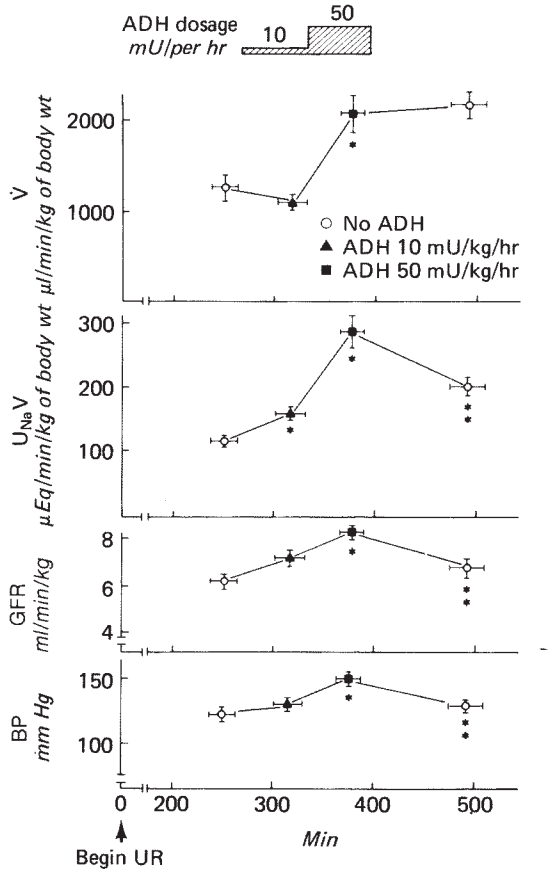

Fig. 2. *The effects of infusing exogenous vasopressin (ADH) at either 10 or 50 mU/kg of body wt per hr in rats undergoing urine-reinfusion.* (*) indicates a significant difference ($P < 0.05$) compared with the initial observations without ADH. (**) indicates a significant difference compared with observations during ADH infusion, 50 mU/kg per hr.

tion are illustrated in Figure 2. At a dose of 10 mU/kg of body wt per hr, ADH infusion led to a 14% fall in $\dot{V}$ ($P > 0.3$), and a rise in the urine-to-plasma osmolality ratio [$(U/P)_{osm}$] from 1.00 ± 0.09 to 1.39 ± 0.17 ($P < 0.01$). Despite this slight blunting of the diuresis, $U_{Na}V$ increased by 39% ($P < 0.01$). This increased natriuresis was associated with modest and nonsignificant rises in both GFR and BP. Increasing the ADH dose to 50 mU/kg per hr was followed by a profound elevation of both $\dot{V}$ and $U_{Na}V$ to 160% and 235%, respectively, of the levels observed without ADH administration. This massive natriuresis and diuresis, the highest levels observed in this study, were associated with a 35% rise in GFR and a 17% increase in BP, both significant changes. Following ADH administration at 50 mU/kg per hr, ADH was discontinued. Compared with values observed in 50 mU/kg per hr, $U_{Na}V$, GFR, and BP all fell significantly after withdrawal of ADH, although $U_{Na}V$ remained elevated above the initial level noted prior to ADH administration. Interestingly, $\dot{V}$ remained virtually unchanged upon discontinuation of ADH, while $(U/P)_{osm}$ decreased to 0.95 ± 0.11.

Clearance studies were also performed in nine sham-manipulated CONT rats without ADH, and with ADH at 10 and 50 mU/kg per hr, the same doses of ADH administered to UR rats. As shown in Table 5, $\dot{V}$ decreased with each dose of ADH to levels significantly below the mean value without ADH administration. In addition, GFR increased significantly above the CONT value at each dose level. For both $\dot{V}$ and GFR, the differences between the levels at 10 and 50 mU/kg per hr were not significant. $U_{Na}V$ and BP increased at both doses of ADH, but these changes were not significant.

The effect of inhibiting prostaglandin synthesis. In order to assess the possibility of a role for the prostaglandins in the phenomenon of urine-reinfusion natriuresis, eight rats were given either of two inhibitors of prostaglandin synthesis, indomethacin or meclofenamate, after varying durations of UR. As illustrated in Figure 3, the eight animals exhibited quite variable changes in $U_{Na}V$ following administration of the drugs. All but two of the animals showed a tendency toward an increase in $U_{Na}V$ following administration of the drugs; however, these changes were not significant.

The effect of heating urine. In three rats, urine-reinfusion was carried out for varying lengths of time, after which the urine was heated to 100°C for 5 to 6 min before reinfusion into the rat. As shown in Figure 4, this manipulation had essentially no effect on $U_{Na}V$. This result indicates that the urinary factors responsible for urine-reinfusion natriuresis are stable in the presence of this degree of heating.

The effects of discontinuing UR. Four rats underwent UR for 360 to 410 min, at which time UR was abruptly discontinued. The ensuing large losses of urinary electrolytes and fluid during this post-UR

Table 5. Renal function (per kidney) and blood pressure of nine Control rats at varying doses of vasopressin (ADH)[a]

ADH *mU/kg of body wt per hr*	$\dot{V}$ *µl/min per kg of body wt*	GFR *ml/min per kg of body wt*	$U_{Na}V$ *µEq/min per kg of body wt*	BP *mm Hg*
None	92.0 ± 11.0	5.31 ± 0.30	7.74 ± 0.78	123 ± 2
10	45.4 ± 7.2 ($P < 0.01$)	6.47 ± 0.22 ($P < 0.01$)	10.72 ± 1.57 *NS*	127 ± 2 *NS*
50	32.5 ± 1.9 ($P < 0.01$)	6.34 ± 0.23 ($P < 0.01$)	8.85 ± 0.56 *NS*	129 ± 3 *NS*

[a] Values are means ± 1 SEM. *P* values refer to comparisons with means during no ADH infusion. *NS* = not significant. Other abbreviations are as shown in Table 1.

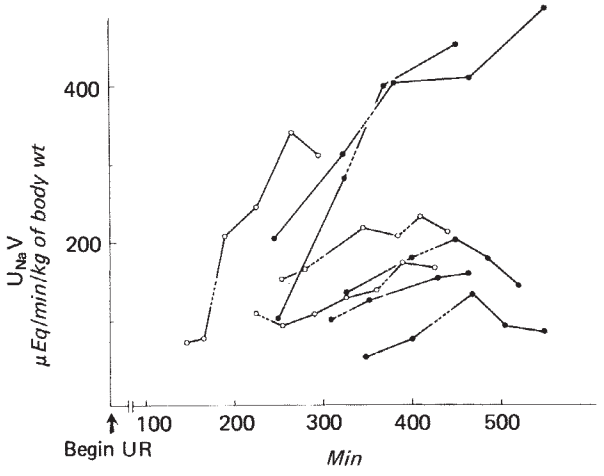

Fig. 3. *The effects on sodium excretion rate ($U_{Na}V$, per kidney) of administering indomethacin in 5 rats (closed circles) or meclofenamate in 3 rats (open circles) during urine-reinfusion. Broken lines* indicate the time of injecting drug.

period were not replaced. The results are shown in Figure 1 by the broken lines and open circles. In each of the four animals, both $\dot{V}$ and $U_{Na}V$ dropped rapidly to initial control levels or below. Associated with the cessation of natriuresis and diuresis, BP decreased to levels comparable to those of the control periods. GFR appeared to fluctuate markedly during this period; however, due to the rapidly changing values of plasma inulin concentration and urinary inulin excretion rate observed, it is uncertain whether the calculated inulin clearance rates accurately reflected GFR. Fluid loss in this post-UR period averaged 1.96 ml/100 g of body wt, a volume equal to approximately 10% of ECF volume.

Discussion

In the present study, as in our earlier experiments [8], the i.v. reinfusion of the entire urine output in rats produced massive and sustained natriuresis and diuresis. Since water and electrolyte balance remained unchanged during UR, it is clear that urine-reinfusion natriuresis is not due to salt or water overload. Rather, this phenomenon appears to be a consequence of one or more potent natriuretic factors in urine. The rapid appearance of the natriuresis in previously normal rats suggests that these factors are constituents of normal urine. This represents a point of difference from findings of other investigators, who have detected natriuretic activity in urine in response to sodium-loading [1, 3, 4, 6, 7] or the need for increased sodium excretion in the uremic state [2, 5].

In an effort to delineate the mechanisms of urine-reinfusion natriuresis, we directed attention in the present study to the possible roles of various factors that are recognized influences upon renal salt or water excretion. These factors include renal hemodynamics, urea, the mineralocorticoids, ADH, the prostaglandins, and dietary sodium intake.

The present data indicate that urine-reinfusion natriuresis is associated with marked changes in renal hemodynamics. In the six rats with renal vein catheters, in which total renal plasma flow (RPF) and renal blood flow (RBF) were determined, a striking degree of renal vasodilatation was observed. RPF increased by 63% during UR, while GFR increased by only 8%, with a resultant marked fall in filtration fraction (Table 3). These results suggest that the primary site of the vasodilatation was the efferent (post-glomerular) vasculature. The mechanism of this efferent vasodilatation is not identified by the present data, but would appear to be mediated by one or more urinary factors. Whether these factors are themselves vasodilators, or whether they induce the appearance of other substances that are vasodilators, remains to be defined. Since, as Brenner et al have shown, glomerular plasma flow is a major determinant of GFR [17], the marked increase of RPF may well account for the increase in GFR during urine-reinfusion natriuresis.

The decrease in the renal extraction of PAH (E_{PAH}) observed during UR in the six rats with renal vein catheters is not clearly explainable by our present data. A fall in E_{PAH} during renal vasodilatation has been well-recognized previously [18–20], and has been attributed both to intrarenal distribution of blood flow [18] and to increased velocity of cortical plasma flow, with reduction in time for transmembrane PAH diffusion to occur [20]. Additionally, the depression of E_{PAH} in the present experiments could be a function of azotemia. It has been

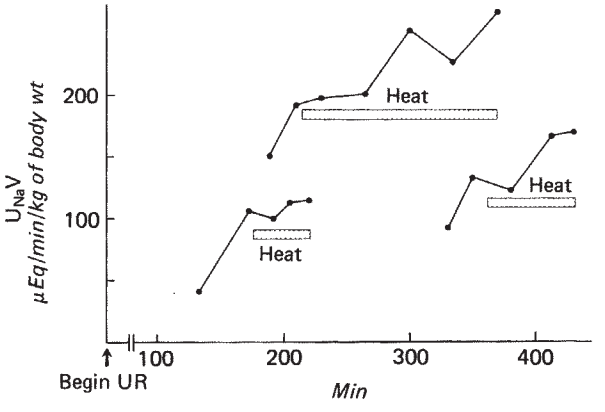

Fig. 4. *The effect on sodium excretion rate ($U_{Na}V$, per kidney) of heating urine to 100°C for 5 to 6 min prior to reinfusion in three rats.*

shown that PAH transport is inhibited by factors, especially organic anions, present in serum in the azotemic state [21–23]. In this regard, it is interesting that C_{PAH} in the present study increased significantly during UR in only one group, the UR-PF rats. The protein-free diet which these rats were fed might be expected to have suppressed the accumulation of organic anions as well as urea. Thus, E_{PAH} in the other groups (on normal protein intake) may have been depressed by the circulatory accumulation of organic anions, as well, possibly, as by hemodynamic factors related to renal vasodilatation.

Previous studies of circulating or urinary natriuretic factors have demonstrated [2, 5–7, 24] or implied [1, 3, 4] an inhibition of tubular sodium reabsorption, rather than increased filtration, as the mechanism or natriuresis. However, the present data indicate that GFR was significantly increased at the time of maximal $U_{Na}V$ in the UR, UR-PF and UR-Hi-Na rats compared with CONT (Table 1), and in the UR-DI rats compared with control values in the same animals. In order to examine the role of hyperfiltration versus decreased tubular reabsorption in urine-reinfusion natriuresis, individual $U_{Na}V$ values for UR and CONT rats are plotted as a function of filtered sodium load in Figure 5. There was a considerable degree of overlap in the filtered load values of UR and CONT rats. In this range (less than 1000 μEq/min/kg of body wt) where filtered loads are comparable, it is clear that the natriuresis in the UR rats is due to decreased absolute sodium reabsorption. In the range of filtered load above 1000μEq/min/kg, it is not evident whether the sodium reabsorptive capacity of the kidney in the UR rats has been further inhibited, or whether a maximal transport rate for sodium has been exceeded, or both. Regardless, these data indicate that a decrease in tubular reabsorption and an increase in filtration may both contribute to the natriuresis during UR. In addition, the present data suggest at least one explanation for a decrease in tubular reabsorption. Both *in vivo* [25, 26] and *in vitro* [27, 28] studies have demonstrated a direct relationship between peritubular oncotic pressure and tubular reabsorption rate. Since, in the present study, filtration fraction (FF) decreased from 32% to 22% (Table 3), it would be expected that post-glomerular plasma protein concentration and peritubular oncotic pressure would be decreased during urine-reinfusion natriuresis.[2] Such a decrease in peritubular oncotic pressure may have contributed to an inhibition of tubular reabsorption during UR, although other mechanisms of decreased tubular reabsorption are possible as well.

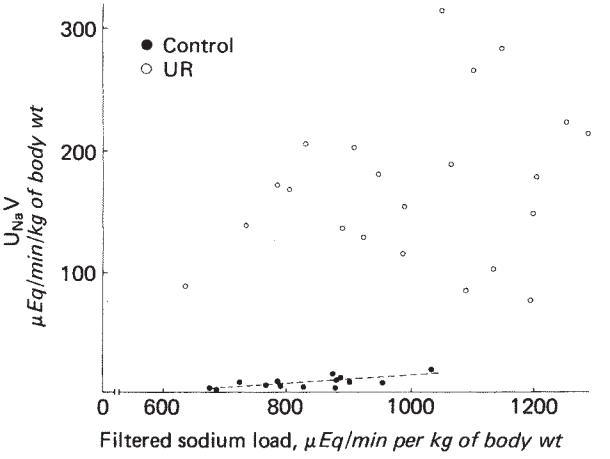

Fig. 5. *The relationship between sodium excretion rate ($U_{Na}V$) and filtered sodium load, for control rats and previously normal rats during urine-reinfusion (UR).* There is a significant correlation for control rats ($r = 0.694$, $P < 0.01$), but not for UR rats ($r = 0.183$, $P > 0.4$).

Elevation of arterial BP may, in itself, inhibit tubular reabsorption [29, 30] and lead to a natriuresis [30, 31]. Even though increased BP was observed in the UR rats (Table 1), our data do not suggest that BP is a primary mediator of urine-reinfusion natriuresis. In Figure 6, $U_{Na}V$ values of both UR and CONT rats are plotted as a function of BP. For neither group is the correlation between $U_{Na}V$ and BP significant. In addition, it can be seen that, although considerable overlap in BP values exists between UR and CONT animals, $U_{Na}V$ values are widely separated. For the ten UR rats with BP in the same range as observed for most CONT rats (112 to 130 mm Hg), the mean $U_{Na}V$ was 175 ± 19μEq/min/kg of body wt, approximately 26 times the CONT value ($P < 0.001$). Moreover, BP was not significantly increased in any of the other urine-reinfusion models (UR-PF, UR-Hi-Na, UR-Lo-Na), and even fell significantly in the UR-DI rats. Changes in BP, therefore, appear qualitatively unimportant in the mechanism of urine-reinfusion natriuresis.

Urea, which is regarded as an osmotic diuretic and natriuretic [32], accumulates in the circulation of rats undergoing UR (Table 2). Previously, we reported that the i.v. infusion of urea into rats resulted in a considerably less intense natriuresis and diuresis than observed with UR, despite comparable elevations of P_u [8]. In the present study, the role of urea in urine-

[2] A decrease in FF from 32% to 22% would be associated with a 13% fall in post-glomerular protein concentration, if one assumes constant arterial protein concentration. Although we did not measure plasma protein concentration, the lack of significant change in arterial hematocrit between CONT and UR rats (Table 2) suggests that protein concentration remained relatively constant.

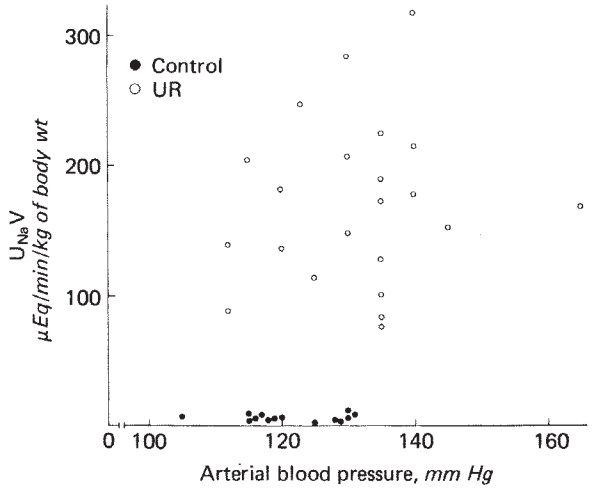

Fig. 6. *The relationship between sodium excretion rate ($U_{Na}V$, per kidney) and mean arterial blood pressure for control rats and previously normal rats during urine-reinfusion (UR).* There is no significant correlation for either group: for control rats, $r = 0.13$, $P > 0.5$; for UR, $r = 0.06$, $P > 0.8$).

reinfusion natriuresis was further examined in the protein-deprived UR-PF rats. The occurrence of a marked natriuresis and diuresis in these rats, despite a very modest rise in P_u and a depression of plasma osmolality (Table 2), makes it unlikely that either urea or other osmotically active solutes are the primary mediators of the natriuresis. However, this result does not exclude a role for urea as a natriuretic factor. Figure 7 illustrates $U_{Na}V$ plotted as a function of P_u for CONT, UR, and UR-PF rats. The majority of P_u values for the UR-PF animals overlap with the CONT values, although $U_{Na}V$ values for the UR-PF rats are consistently much greater. No significant correlation exists between P_u and $U_{Na}V$ for either UR-PF or CONT rats. However, in the UR rats, a positive correlation does occur ($r = 0.628$, $P < 0.001$). It is possible that, in the UR model, the plasma accumulation of urea, while contributing little natriuretic activity itself, may serve as a "marker" for the accumulation of other unidentified natriuretic factors. Alternatively, urea might exert a natriuretic action that is additive to that of other natriuretic factors during UR. The occurrence of natriuresis and diuresis following urea infusion [8] makes the latter hypothesis appear more likely.

The possibility that mineralocorticoid secretion might decline during UR, thereby contributing to an increase in sodium excretion, was evaluated by treating UR-PF rats with pharmacologic doses of the mineralocorticoid DOCA. The failure of DOCA administration to significantly alter $\dot{V}$ or $U_{Na}V$ in comparison with UR-PF rats without DOCA administration appears to exclude changes in mineralocorticoid activity as a mediating factor in urine-reinfusion natriuresis.

ADH, because of its multiple actions, might conceivably play a complex role in urine-reinfusion natriuresis. Although long known for its antidiuretic properties [33], ADH has more recently been recognized as a natriuretic and diuretic agent in pharmacologic doses [34, 35]. In the present study, UR was performed both in the complete absence of ADH (in the UR-DI rats) and during i.v. infusion of exogenous ADH. The appearance of a marked natriuresis in the UR-DI rats (Table 1) indicates that ADH is not necessary for the phenomenon of urine-reinfusion natriuresis. On the other hand, the infusion of ADH in the UR rats produced a remarkable enhancement of the natriuresis (Fig. 2), a phenomenon not observed in the CONT rats (Table 5). Since ADH is excreted in the urine [36], it is likely that recirculation of excreted ADH resulted in much higher blood levels of the hormone in UR rats than in CONT rats receiving the same dose. The further elevation of $U_{Na}V$ during ADH infusion in the UR rats appears to be due to the additional natriuretic action of ADH at high dose levels [35]. In the present experiments, as in previous reports, the mechanism whereby ADH exerts a natriuretic effect is unclear. The exaggerated diuresis at the higher dose rate was probably related to greatly increased delivery of tubular fluid to the medullary collecting ducts, exceeding their ability to abstract solute-free water despite maximal collecting duct water permeability.

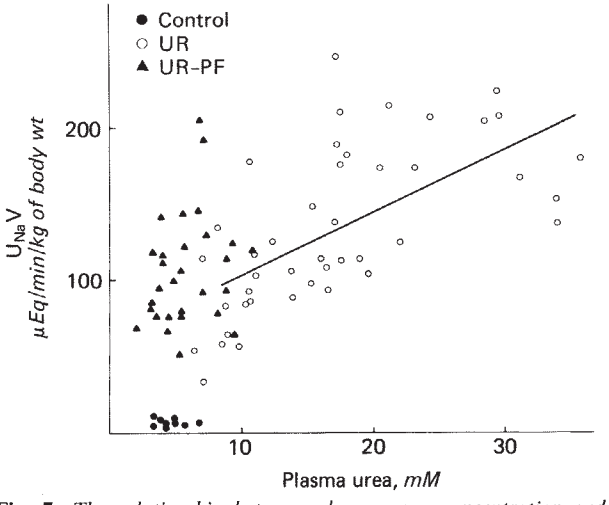

Fig. 7. *The relationship between plasma urea concentration and sodium excretion rate ($U_{Na}V$), per kidney) in sham-manipulated control rats, previously normal rats during urine-reinfusion (UR), and protein-deprived rats during urine-reinfusion (UR-PF).* The *repression line* is given for the UR rats.

Such a phenomenon has been well-described in rats undergoing marked osmotic diuresis [37]. These results indicate that, while endogenous ADH is unlikely to contribute to urine-reinfusion natriuresis, pharmacologic levels of ADH are capable of profoundly intensifying the phenomenon.

The prostaglandin compounds have been shown to have natriuretic and renal-vasodilator properties [38, 39]. The possibility of the prostaglandins playing a mediating role in urine-reinfusion natriuresis was evaluated by administering indomethacin or meclofenamate to UR rats. Renal prostaglandin synthesis has been shown to be markedly inhibited by indomethacin in dogs [40, 41] and rats [42], and by meclofenamate in dogs [40] and rabbits [43] at doses less than those used in the present study. The failure of either indomethacin or meclofenamate to significantly alter the natriuresis in the present experiments (Fig. 3) suggests that the prostaglandins are not responsible for the marked natriuretic response to UR.

The possible relationship between the urine-reinfusion natriuresis demonstrated in the present study and the mechanisms responsible for regulating salt and water homeostasis is of obvious interest. In the UR-Hi-Na rats, prior sodium-loading produced a modest and nonsignificant enhancement of urine-reinfusion natriuresis. In contrast, the natriuresis was markedly attenuated by chronic sodium-deprivation (UR-Lo-Na rats). Such a result appears to parallel the observation of Schmidt, Bourgoignie, and Bricker [44] that a humoral natriuretic factor demonstrable in uremic dogs on normal sodium intake is not detectable when sodium intake is restricted. Taken together, these data suggest that sodium-deprivation in rats might inhibit the production of the urinary factors that mediate urine-reinfusion natriuresis, a concept consistent with a role for these factors in the regulation of sodium balance. However, it appears equally plausible that ECF volume contraction, secondary to the sodium-deprivation, may non-specifically blunt the responsiveness of the kidney to the natriuretic stimuli of urine-reinfusion. Such an antinatriuretic effect of ECF contraction may well explain the rapid cessation of natriuresis that follows the interruption of UR (Fig. 1). Pending further information, it does not seem justified to draw any firm conclusion regarding the role of the urinary factors responsible for urine-reinfusion natriuresis in the regulation of sodium balance.

Except for the inhibition of proximal tubular reabsorption by the uremic serum fraction reported by Weber, Bourgoignie, and Bricker [24], little data exist regarding the site of action of previously reported natriuretic factors. In the present study, the UR-DI rats exhibited a marked rise in fractional distal sodium delivery, as reflected by $(C_{H_2O} + C_{Na})/GFR$, during natriuresis. This finding, as well as the marked elevations of FE_{Na} and $\dot{V}/GFR$ in a number of UR rats, is compatible with inhibition of proximal fractional reabsorption. Additionally, it is possible that the distal nephron is involved in urine-reinfusion natriuresis. Although C_{H_2O}/GFR rose during natriuresis in each of the UR-DI rats, the increase is not significant. In each animal, the increase in C_{H_2O}/GFR is proportionately much less than the increase in $(C_{H_2O} + C_{Na})GFR$.[3] The relative depression of free-water formation is consistent with an additional inhibition of sodium chloride transport in the diluting segment of the nephron.

In summary, the present data support the following conclusions: *a*) potent, heat-stable natriuretic factors appear to be components of normal rat urine; *b*) the natriuretic activity of these factors is probably mediated, to an important degree, by post-glomerular renal vasodilatation; *c*) the mechanism of natriuresis is essentially independent of changes in ADH, mineralocorticoids, or prostaglandin; *d*) although urea may exert a natriuretic action during urine-reinfusion, it appears likely that other natriuretic factors are of much greater importance; and *e*) both proximal and distal nephron sites may contribute to the natriuresis. The anatomic source, chemical composition, and number of the urinary natriuretic factors involved in urine-reinfusion natriuresis are not established by our present data. Additionally, it remains to be determined whether the urinary factors, when reinfused, act directly upon the kidney, or whether their actions are indirect.

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Reprint requests to Dr. R. H. Harris, Box 3014, Duke University Medical Center, Durham, N.C. 27710, U.S.A.

[3] In both Brattleboro and Sprague-Dawley rats, C_{H_2O}/GFR and $(C_{H_2O} + C_{Na})/GFR$ have been shown to increase proportionately during hypotonic saline loading, as $(C_{H_2O} + C_{Na})/GFR$ is increased into ranges comparable to values noted in our UR-DI rats [45].

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