Cross-Circulation Study of Natriuretic Factors in Postobstructive Diuresis

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ABSTRACT To study the role of circulating natriuretic factors in the postobstructive diuresis that occurs after relief of bilateral, but not unilateral, ureteral ligation, cross-circulation was carried out between normal recipient rats and donor rats having either 24-h bilateral (BUL) or unilateral (UUL) ureteral ligation. With BUL donors, there was a rapid marked increase in sodium and water excretion in the recipient rats, sustained for 80-140 min, with a peak approximately 10 times control values. With UUL donors, no significant natriuretic response occurred. Changes in glomerular filtration rate, renal plasma flow, blood pressure, hematocrit, or circulating levels of aldosterone or Pitressin did not explain the diuresis-natriuresis produced by cross-circulation with BUL donors. Differences in the intrinsic renal damage produced by bilateral as compared to unilateral ureteral obstruction did not appear to account for this response, since UUL donors given an acute urea load and urine reinfusion caused a similar diuresis-natriuresis. Moreover, normal donor rats given a urea load also caused a diuresis-natriuresis nearly equal to that produced by BUL rats, and the relationship between increased urea excretion and sodium excretion or urine flow in the recipients was not different in the two groups. Total urine reinfusion for 3 h in donor rats produced a significant, although less marked, diuresis-natriuresis in recipient animals, with only a slight elevation of the blood urea nitrogen level, much less increase in urea excretion rate, and no significant relationship between urea excretion and sodium excretion or urine flow.

The results indicate that potent natriuretic factors, which act by decreasing the tubular reabsorption of sodium and water, are present in the blood of rats with bilateral, but not unilateral, ureteral ligation. High blood and urine urea levels appear to be the factors responsible for the marked natriuresis-diuresis occurring in normal rats during cross-circulation with BUL donors, although suggestive evidence of other natriuretic factors in urine reinfused intravenously was also obtained. The data suggest that urea osmotic diuresis is an important mechanism for determining the striking difference between the postobstructive diuresis observed after relief of bilateral as compared to unilateral ureteral ligation.

INTRODUCTION

The role of circulating natriuretic factors in postobstructive diuresis has not been established. Clinical experience indicates that massive natriuresis and diuresis occur only after the relief of total, or nearly complete, urinary tract obstruction. In the rat, a marked postobstructive diuresis occurs after the relief of 24-h bilateral ureteral ligation (BUL),[1] and the resulting intrinsic renal defect in salt and water reabsorption has been the subject of several studies (1-4). In contrast to the results after relief of BUL, the relief of unilateral ureteral ligation (UUL) is not associated with natriuresis and diuresis, the postobstructive kidney excreting much less salt and water than the contralateral normal kidney (1, 5, 6).

It is not known whether the striking difference in postobstructive diuresis between these two experimental models of BUL and UUL is primarily due to retention in the circulation of natriuretic factors normally excreted in the urine or to differences in renal hemodynamics and in the intrinsic renal defect that have been described (3, 5, 6). Secondly, if circulating natriuretic factors can be demonstrated in BUL and not in UUL rats, the means

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[1] *Abbreviations used in this paper:* BUL, bilateral ureteral ligation; BUN, blood urea nitrogen; PAH, sodium para-aminohippurate; UUL, unilateral ureteral ligation.

by which such factors increase salt and water excretion in a normal kidney would be of interest. Finally, the relative importance of urea or of other natriuretic factors in the phenomenon of postobstructive diuresis should be determined. In the present study the technique of isovolemic cross-circulation in the rat, described by Pearce, Sonnenberg, and their colleagues (7, 8), was used to examine these questions.

METHODS

Cross-circulation experiments were carried out in pairs of male Wistar rats weighing 300–400 g. In all groups, the recipient rats were normal animals fasted overnight in which standard clearance experiments were carried out immediately before cross-circulation. The preparation of the donor animals varied (Table I). In group A ($n=9$), cross-circulation was carried out between normal recipient rats and donor rats with BUL performed 24 h previously. Ureteral ligation was done under light Nembutal anesthesia (sodium pentobarbital, Abbott Laboratories, North Chicago, Ill.) through a small lower abdominal incision and the animals were given no food or water during the 24 h before the experiment. Group B ($n=6$) consisted of normal rats cross-circulated with animals subjected to UUL 24 h previously; these donor animals were allowed water but no food for the 24 h between ureteral ligation and the experiment. Group C ($n=6$) consisted of normal rats given aldosterone and Pitressin (Vasopressin, Parke, Davis & Co., Detroit, Mich.) for 90 min before and during cross-circulation with BUL donor rats. D-Aldosterone (G. D. Searle & Co., Chicago, Ill.) and aqueous Pitressin were given in doses of 8.5 μg/h and 0.67 nm/h, respectively. Groups D to H were designed to determine the role of structural damage due to BUL, of urea osmotic diuresis, and of other possible urinary natriuretic factors in postobstructive diuresis. Group D ($n=6$) consisted of pairs of rats in which the donor rat had UUL 24 h previously, as in group B, and then received an acute urea load combined with urine reinfusion from the contralateral normal kidney for 1 h before and during cross-circulation with the normal recipient rats. Urea was given in a priming dose of 35 mg in 0.5 ml saline, and in a sustaining dose of 140 mg/100 g/h, stopped at the time of initiation of cross-circulation. During the urea loading and cross-circulation, the urine of the contralateral normal kidney was reinfused intravenously by inserting a small catheter from the bladder into the femoral vein. The patency of the catheter was checked periodically by the injection of lissamine green through a side arm of the catheter. Group E ($n=6$) consisted of pairs of rats in which the normal recipient animal was infused with urea during the clearance periods before the initiation of cross-circulation, in a dose similar to that used in group B, in order to initiate a urea diuresis. Urea-loaded recipient rats were then cross-circulated with rats subjected to BUL 24 h previously, as in group A. Group F ($n=5$) consisted of pairs of animals in which the normal donor rat was given an acute urea load before cross-circulation with a normal recipient. Urea was given in a priming dose of 200 mg in 0.75 ml saline and in a sustaining dose of 200 mg/100 g/h for 1 h before and for the first 40 min of cross-circulation, to mimic the blood urea nitrogen (BUN) levels observed in group A. Group F ($n=5$) consisted of pairs of rats in which the donor was subjected to UUL 24 h previously and urine reinfusion for 1 h before and during cross-circulation, as described for group D. Group H ($n=4$) consisted of pairs of rats in which the donor was a normal animal subjected to urine reinfusion for 3 h before and during cross-circulation.

For cross-circulation all animals were anesthetized with 80 mg/kg Inactin (Promonta, Hamburg, W. Germany) and had tracheotomies; blood pressure was monitored with a femoral artery cannula connected to a mercury manometer, and rectal temperature was maintained from 36.5 to 38°C by a heat lamp. Cannulas were inserted in one femoral vein for the administration of intravenous solutions and in one jugular vein and carotid artery for the cross-circulation connections. Before cross-circulation, clearance experiments were carried out in the normal recipient rats of each pair with a bladder catheter and standard techniques. After recovery from the surgery and achievement of a stable urine flow rate, [^{14}C]inulin and [^{3}H]PAH (sodium para-aminohippurate, used only in groups A and B) were given in a priming dose of 3 μCi and 5 μCi, respectively, with the sustaining dose being 4 μCi and 15 μCi/ml administered in isotonic (0.9%) saline at a rate of 2 ml/h. After a 30–45-min equilibration period, three 20–30-min urine collections were obtained and blood samples were taken from the femoral artery at the beginning and end of each period.

Cross-circulation was carried out by the method of Pearce et al. (7) after completion of the control periods in the

TABLE I
Identification of Groups of Animals

Group	Experiments	Donor	Recipient
A	9	BUL (24 h)	Normal
B	6	UUL (24 h)	Normal
C	6	BUL (24 h)	Normal + Pitressin and aldosterone
D	6	UUL (24 h) + acute urea load +urine reinfusion (1 h)	Normal
E	6	BUL (24 h)	Normal + acute urea load (2 h)
F	6	Normals + acute urea load (1 h)	Normal
G	5	UUL (24 h) +urine reinfusion (1 h)	Normal
H	4	Normals + urine reinfusion (2 h)	Normal

normal recipient rats. Each rat of the pair occupied one pan of a simple balance and all connections to the accessory apparatus were by fine flexible tubing that caused minimal damping of the balance movement. Urine excreted by each rat was collected in tubes fixed to the balance pan so that during cross-circulation any weight changes represented a net gain or loss of blood. Heparin was given in a dose of 1,200 U/kg intravenously to each rat and, after centering of the balance, the cross-circulation clamps were removed and constant adjustment of the blood flow was carried out as required by slight compression of the appropriate connection, to maintain the balance needle in the central position. The balance was sufficiently sensitive to register two scale divisions for a weight difference of 0.2 g. The intravenous infusion rates were equal in the two rats except when the donors had no urine output (groups A, E, F, G, and H), in which case the infusion rate was 0.2 ml/h and the remainder of the infusion entered a small beaker on the balance to keep the added weight constant. After the start of cross-circulation, seven 20-min urine collections were made in the recipient animals with arterial blood samples obtained at the end of each period.

Urine flow rate was determined by weighing. Plasma and urine sodium were measured with a flame photometer and plasma and urine osmolality with a nanoliter osmometer. Urea was measured by the urease method on 10-μl samples of plasma (9). ^{14}C activity and ^{3}H activity in plasma and urine were determined by adding 2-μl samples to a toluene-based scintillation fluid and counting in a dual-channel liquid scintillation counter. At the end of the experiment in groups A and B, the kidneys were removed, chilled, and sliced, and the cortex and outer medulla were separated as previously described (4), and a heavy microsomal fraction was isolated according to the method of Post and Sen (10) by heparin treatment. The Na-K-dependent ATPase activity of this fraction was assayed with the assistance of Drs. A. Sen and W. Knox (4). Standard methods of statistical analysis for paired or unpaired data were used.

TABLE II

Changes in Renal Function of Normal Rats after Cross-Circulation with BUL or UUL Rats

		Cross-circulation time						
	Control	20 min	40 min	60 min	80 min	100 min	120 min	140 min
A. Normals with BUL Rats (n = 9)								
C_{IN},§ *ml/min/kg body wt*	6.0±1.1	—	+1.4±1.8	+0.7±1.2	+0.3±1.0	−1.7±1.2	−2.5±1.4	−1.7±1.6
C_{PAH}, *ml/min/kg body wt*	16.6±3.2	—	+2.3±5.6	+1.3±2.7	−0.6±2.9	−2.1±3.1	−5.0±3.0	−1.9±4.2
Urine flow, *µl/min/kg body wt*	29.8±4.7	+152±30*	+252±50*	+161±21*	+120±22*	+86±15*	+52±10*	+41±11*
Sodium excretion, *µeq/min/kg body wt*	3.3±0.9	+11.5±4.2‡	+23.3±7.9‡	+11.6±2.2*	+8.8±2.4*	+5.1±1.5*	+2.0±0.7‡	+0.6±0.9
FENa (×100) %	0.37±0.11	—	+2.34±0.92	+1.26±0.47‡	+0.76±0.34	+0.65±0.23‡	0.40±0.13‡	+0.21±0.09
U_{osm}, *mosmol/kg*	890±145	−272±151	−297±151	−182±160	−91±172	−66±145	−40±122	+37±119
T_{cH_2O}, *µl/min/kg body wt*	60±16	+144±26*	+231±42*	+235±53*	+160±29*	+121±27*	+77±30	+91±33
BUN, *mg/100 ml*	121±11¶	—	—	—	—	—	—	+66±4‖
Hct, %	48.5±0.8	−2.8±0.8‡	—	−2.8±1.1	—	−5.5±0.7*	—	−6.9±1.3*
Blood pressure, *mm Hg*	122±4	−2±4	—	−5±5	—	−12±4	—	−13±4‡
B Normals with UUL Rats (n = 6)								
C_{IN},§ *ml/min/kg body wt*	7.1±1.0	—	+1.4±0.6	+0.1±0.9	+0.5±0.8	+0.8±0.6	−0.2±0.6	−0.3±1.0
C_{PAH}, *ml/min/kg body wt*	16.0±2.5	—	+2.5±2.0	+1.1±2.3	+4.1±3.1	+4.5±2.2	+1.2±2.0	+1.8±3.3
Urine flow, *µl/min/kg body wt*	29.4±9.5	−12.7±5.1	−11.2±7.4	+1.4±2.6	+10.0±3.8‡	+6.6±1.9‡	−0.4±4.7	+2.0±9.6
Sodium excretion, *µeq/min/kg body wt*	2.6±1.0	−0.8±0.7	−1.0±0.6	−0.3±0.7	+1.9±1.8	+3.7±2.7	+2.7±2.0	+2.4±1.2
FENa, (×100) %	0.26±0.08	—	−0.06±0.06	−0.04±0.08	+0.10±0.12	+0.23±0.17	+0.18±0.12	+0.24±0.12
U_{osm}, *mosmol/kg*	846±120	+88±127	+140±181	−9±111	+42±65	+187±70‡	+291±152	+313±146
T_{cH_2O}, *µl/min/kg body wt*	51.6±14.2	−10.6±10.5	−2.7±10.4	+10.0±15.5	+30.2±18.5	+46.7±23.1	+48.4±32.3	+47.1±18.0‡
BUN, *mg/100 ml*	22±1¶							31±2‖
Hct, %	50.4±0.6	−6.9±1.0*	—	−6.1±0.9*	—	−4.6±0.8*	—	−5.3±0.9‡
Blood pressure, *mm Hg*	116±5	−0.3±4	—	−0.3±4	—	−2±5	—	−2±6
C. Normals given Aldosterone and Pitressin with BUL Rats (n = 6)								
C_{IN}, *ml/min/kg body wt*	9.0±1.0	—	−0.6±0.8	−0.1±1.0	−0.5±1.2	−0.8±1.4	−2.7±0.7‡	2.1±0.8‡
Urine flow, *µl/min/kg body wt*	14.6±1.3	+117±27*	+185±40*	+172±32*	+143±28*	+105±18*	+74±14*	+63±10*
Sodium excretion, *µeq/min/kg body wt*	0.63±0.24	+5.8±2.1*	+13.9±4.6*	+14.7±4.0*	+11.3±3.1*	+8.0±2.3*	+4.9±1.7*	+3.7±1.4
FENa (×100), %	0.05±0.02	—	+1.0±0.32*	+1.15±0.24*	+0.83±0.18*	+0.63±0.14*	+0.50±0.10*	0.35±0.07*
U_{osm}, *mosmol/kg*	1,522±244	−767±249‡	−771±262‡	−680±291	−577±256	−559±245	−448±203	−383±164
T_{cH_2O}, *µl/min/kg body wt*	53±10	+122±22*	+210±37*	+236±31*	+251±36*	+197±30*	+171±35*	+164±37*
BUN, *mg/100 ml*	123±8¶	—	—	—	—	—	—	70±9‖
Hct, %	44.5±0.4	+2.8±1.9	—	+1.1±1.2	—	−2.0±2.4	—	+1.8±1.5
Blood pressure, *mm Hg*	115±2	−1±4	—	−3±6	—	−7±7	—	−11±7

* $P < 0.01$ or less compared to control, values expressed as mean ±1 SEM.

‡ $P < 0.05$ compared to control.

§ C_{IN}, inulin clearance (glomerular filtration rate); C_{PAH}, PAH clearance (renal plasma flow); FENA(×100), fractional excretion of sodium; T_{cH_2O}, free water reabsorption.

‖ Recipient rat at end of experiment.

¶ Donor rat at start of cross-circulation.

RESULTS

Comparison of the effects of cross-circulation of normal rats with BUL or UUL rats. Normal rats developed a marked diuresis and natriuresis, approximately 10 times greater than control values, within 20 min after the institution of cross-circulation with 24-h BUL animals, (group A), as seen in Table II and Fig. 1. These results contrast with the lack of diuresis and natriuresis in normal rats undergoing cross-circulation with UUL donors (group B). Glomerular filtration rate ([^{14}C]-inulin clearance) did not change significantly in the normal rats undergoing natriuresis and diuresis during cross-circulation with BUL donors. Renal plasma flow ([^{3}H]PAH clearance) was similarly unaltered. The natriuresis and diuresis were associated with a slight decrease in urine osmolality and a marked increase in free water reabsorption. One of the major differences between the two groups was the high BUN level in the BUL donors of 121±11 mg/100 ml (±1 SEM), which resulted in an increase in BUN level in the normal recipients from 22±2 mg/100 ml at the beginning of cross-circulation to 66±4 mg/100 ml at the end of the 140-min cross-circulation. Changes in blood pressure and hematocrit in the two groups of recipient animals were similar and of small magnitude.

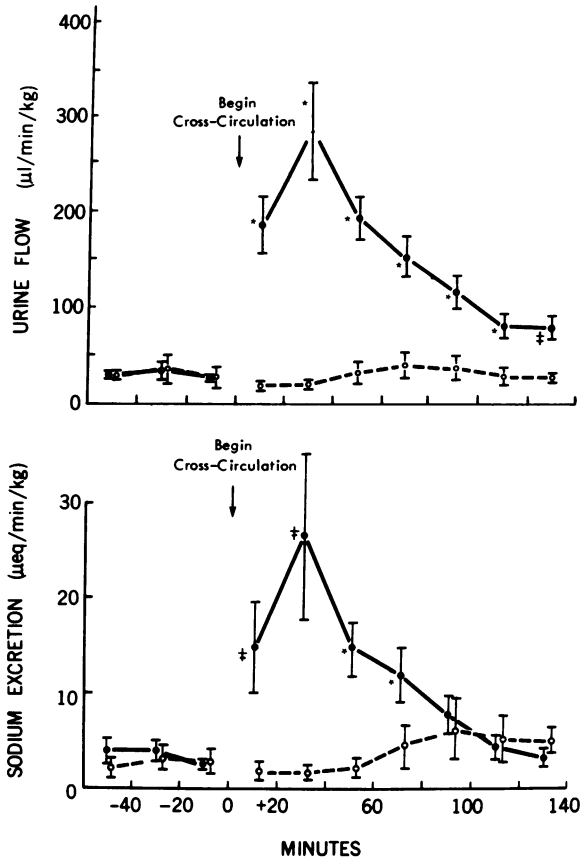

FIGURE 1 Changes in urine flow (upper panel) and sodium excretion (lower panel) in normal rats undergoing cross-circulation with donor rats having bilateral (●, group A) or unilateral (○, group B) ureteral ligation of 24 h duration. Standard error of mean value is shown, and significance of the difference from the mean control value.
* $P < 0.01$ or less.
‡ $P < 0.05$.

TABLE III

Comparison of Changes in Sodium and Water Excretion after Cross-Circulation

	20 min	40 min	60 min	80 min	100 min	120 min	140 min
B:A V§	↓*	↓*	↓*	↓*	↓*	↓*	↓‡
$U_{Na}V$	↓‡	↓‡	↓*	↓‡	NS	NS	NS
C:A V	NS	↓‡	NS	NS	NS	NS	NS
$U_{Na}V$	NS	NS	NS	NS	NS	NS	NS
D:A V	NS	NS	NS	NS	↑‡	↑‡	NS
$U_{Na}V$	NS	NS	NS	NS	↑*	↑*	↑‡
F:A V	↓*	↓‡	NS	NS	↑*	↑*	↑*
$U_{Na}V$	NS	NS	NS	NS	NS	NS	NS
F:D V	↓‡	NS	NS	NS	NS	NS	NS
$U_{Na}V$	NS	NS	NS	NS	NS	NS	NS
G:A V	↓*	↓*	↓‡	NS	NS	NS	NS
$U_{Na}V$	↓‡	↓‡	NS	NS	NS	↑*	↑*
G:B V	NS	NS	↑*	↑*	↑*	↑*	NS
$U_{Na}V$	NS	NS	↑*	↑‡	NS	NS	NS
H:A V	↓‡	↓‡	↓*	NS	NS	NS	NS
$U_{Na}V$	NS	NS	NS	NS	NS	NS	NS
H:F V	NS	NS	NS	NS	NS	NS	NS
$U_{Na}V$	NS	NS	NS	NS	NS	NS	NS

* $P < 0.01$ or less, ↓ means B less than A, ↑ means B greater than A, and similarly for other groups.
‡ $P < 0.05$.
§ V, urine flow; $U_{Na}V$, sodium excretion rate, comparing values from Tables II and IV.

The role of changes in aldosterone or antidiuretic hormone levels. When normal recipient rats were given large doses of aldosterone and Pitressin before and during cross-circulation with BUL donor rats, there was a slight but insignificant decrease in the natriuresis and diuresis which occurred (group C, Tables II and III, C:A). The difference was significant only for the diuresis in the period 40 min after cross-circulation started.

The role of changes in renal Na-K-ATPase activity. Previous studies have shown that there was a decrease in renal Na-K-ATPase activity, especially in the outer medulla, 24 h after relief of BUL (4). For this reason renal Na-K-ATPase activity was assayed in the heavy microsomal fraction of kidneys removed from normal recipient rats after 140 min of cross-circulation with BUL or UUL donor rats (groups A and B). There

TABLE IV

Effect of Cross-Circulation with BUL or UUL Donors on Renal Na^+-K^+ ATPase Activity in Normal Rats (Heavy Microsomal Fraction)

	UUL donors (n = 5)	BUL donors (n = 6)	P value
	μM P_i/mg protein/h		
Cortex	26.1±5.3	28.9±8.8	NS
Medulla (outer)	66.0±9.4	59.3±7.8	NS

was no detectable difference in enzyme activity at this time in the cortex or outer medulla of kidneys from recipients cross-circulated with BUL or UUL donor rats (Table IV), despite the marked diuresis and natriuresis observed in group A.

The role of failure of urine excretion. This group of experiments (group D) was carried out to determine whether failure of urine excretion or the intrinsic renal damage associated with bilateral ureteral obstruction was responsible for the diuresis-natriuresis produced by BUL, but not UUL, donor rats. To attempt to mimic BUL, rats with UUL were given urea acutely and the urine from the contralateral kidney was reinfused during the experiment to prevent volume contraction from urea diuresis (group D). These animals produced a natriuresis in normal recipient rats which, although less in the first two periods, was not significantly different from that resulting from cross-circulation with BUL donors (Tables III, D: A, and V and Fig. 2). The results suggest that failure of urine excretion was responsible for the transferred natriuresis with BUL donors, rather than any difference in the intrinsic renal damage of BUL as compared to UUL.

Role of urea. Failure of urine excretion results in the accumulation of urea and possibly other natriuretic factors in the circulation. Two groups of rats were studied to attempt to clarify the role of urea. When urea was administered to the normal recipient rats before cross-circulation with BUL donors (group E), the BUN level was increased to 131±14 mg/100 ml and marked diuresis and natriuresis was induced (Table V). During cross-circulation with BUL donors, natriuresis and diuresis gradually declined, rather than increased. Thus urea diuresis in the normal recipients obscured any effect of cross-circulation with BUL donors.

Urea was also administered acutely to normal donor rats before the initiation of cross-circulation (group F) and the BUN level was increased to 105±10 mg/100 ml at the beginning and 72±5 mg/100 ml at the end of cross-circulation. These values were similar to those observed in group A (BUL donors). Marked diuresis and natriuresis occurred in the recipient rats, similar to that seen in group A. The onset of marked diuresis was more rapid in group A, as was the natriuresis, but the latter differences were not statistically significant (Fig. 2, Tables III, F: A, and V).

The role of urea diuresis was further examined by comparing the urea excretion rate with the sodium excretion rate and urine flow for the same clearance periods during cross-circulation in groups A and F. The urea excretion rates were higher in group A, although the difference was not significant (Fig. 2, Table VI), and likewise the diuresis and natriuresis were greater

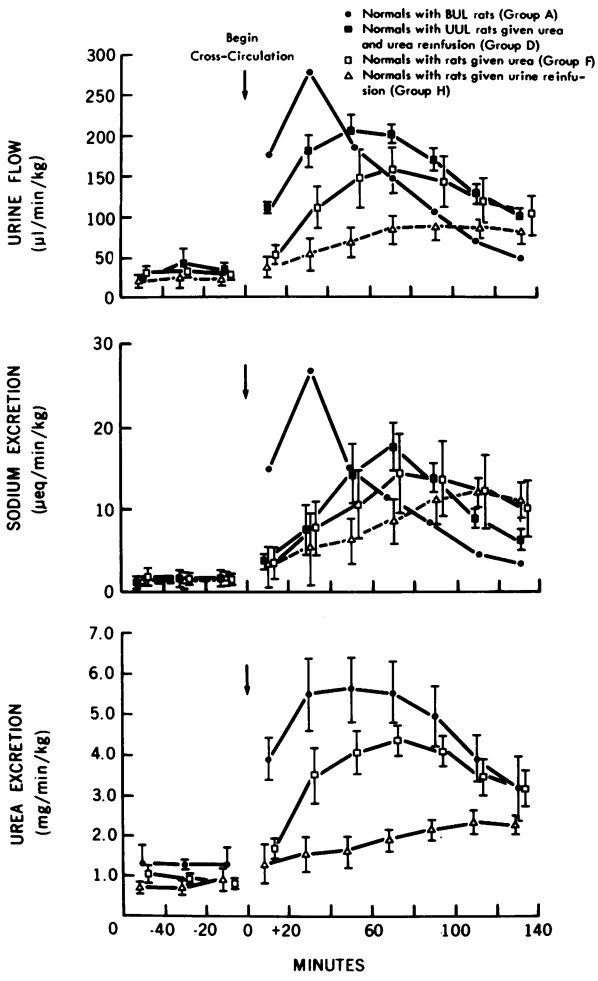

FIGURE 2 Changes in urine flow (upper panel), sodium excretion (middle panel) and urea excretion (lower panel), in normal rats undergoing cross-circulation with donor rats having UUL for 24 h and urea infusion and intravenous urine reinfusion from the contralateral kidney for 1 h (■, group D), donor rats given an acute urea load (□, group F), and donor rats given total urine reinfusion for 3 h (△, group H). Results in group A with bilateral ureteral ligation are shown for comparison (—●—).

TABLE V

The Role of Various Factors in the Natriuresis and Diuresis Produced in Normal Rats by Cross-Circulation with BUL Rats

	Time of cross-circulation							
	Control	20 min	40 min	60 min	80 min	100 min	120 min	140 min
D. Normals with UUL rats loaded with urea, and with urine of contralateral kidney reinfused i.v. (n = 6)								
C_{IN}, *ml/min/kg body wt*	8.9±0.3	—	+0.3±0.5	+0.8±0.5	+0.6±0.4	−0.02±0.4	−1.1±0.5	−1.3±0.6
Urine flow, *µl/min/kg body wt*	33.4±11.0	+79±15*	+148±22*	+173±22*	+170±15*	+138±14*	+95±13*	+60±15‡
Sodium excretion, *µeq/min/kg body wt*	1.6±0.7	+2.2±1.1	+6.1±3.3	+12.7±4.8‡	+15.9±3.2*	+12.2±1.4*	+7.2±0.8*	+4.7±1.2‡
FENa, (×100), %	0.15±0.07	—	+0.41±0.23‡	+0.83±0.29*	+1.13±0.22*	+0.92±0.09*	+0.62±0.06*	+0.43±0.10*
U_{osm}, *mosmol/kg*	1,370±229	−483±242	−608±218‡	−586±224‡	−480±241	−410±249	−367±256	−261±256
T_cH_2O, *µl/min/kg body wt*	74.6±12.9	+119±13*	+173±16*	+221±22*	+285±33*	+255±20*	+194±15*	+167±17*
BUN, *mg/100 ml*	149±8¶	—	—	—	—	—	—	66±3‖
Hct, %	49.3±0.6	−5.0±1.0*	—	−3.5±1.5	—	−3.6±0.8*	—	−5.3±0.9*
Blood pressure, *mm Hg*	123±5	−1±2	—	−2±2	—	−5±4	—	−8±6
E. Urea-loaded normal rats with BUL rats (n = 6)								
C_{IN}, *ml/min/kg body wt*	7.9±0.3	—	−1.1±1.4	−0.4±1.2	−2.2±0.7‡	−1.9±0.8‡	−2.9±0.5*	−2.8±0.6*
Urine flow, *µl/min/kg body wt*	347±64	−131±31*	−165±55‡	−174±70	−196±71‡	−220±66‡	−244±64‡	−255±65‡
Sodium excretion, *µeq/min/kg body wt*	12.2±4.1	−5.7±4.0	−5.7±4.0	−5.6±5.0	−6.2±4.0	−7.4±4.6	−8.4±4.6	−9.5±4.3
FENa (×100), %	1.1±0.35	—	−0.41±0.29	−0.51±0.37	−0.46±0.38	−0.53±0.38	−0.52±0.41	−0.70±0.37
U_{osm}, *mosmol/kg*	693±86	91±27‡	±73±61	+78±86	+100±80	+178±57‡	+204±53‡	+221±58‡
T_cH_2O, *µl/min/kg body wt*	—	—	—	—	—	—	—	—
BUN, *mg/100 ml*	131±14‖	—	—	—	—	—	—	118±8‖
Hct, %	46±0.7	−5.3±1.5‡	−3.6±2.0	−3.6±2.0	—	−4.3±1.5‡	—	−3.5±1.9
Blood pressure, *mm Hg*	116±5	4±4	+2±4	+2±4	—	−8±7	—	−14±8
F. Normals with rats given an acute urea load (n = 5)								
C_{IN}, *ml/min/kg body wt*	7.6±1.0	—	+0.4±1.3	−0.6±0.5	−0.8±0.5	−0.5±1.2	−0.8±1.4	+1.6±1.4
Urine flow, *µl/min kg body wt*	31.0±7.0	+21±11	+82±29‡	+116±90‡	+127±34‡	+111±34‡	+89±33‡	+71±29
Sodium excretion, *µeq/min/kg body wt*	1.5±0.8	+2.0±1.2	+6.1±3.0	+8.9±4.1	+12.8±4.5‡	+12.0±4.3‡	+10.5±4.4	+8.5±3.6
FENa(×100), %	0.13±0.05	—	+0.61±0.29	+1.29±85	+1.70±0.85	+1.96±0.74	+1.47±0.81	+1.33±0.83
U_{osm}, *mosmol/kg*	1,116±142	−74±102	−208±177	−280±205	−278±189	−225±201	−192±183	−95±236
T_cH_2O, *µl/min/kg body wt*	68±5	—	+105±30‡	+129±25*	+157±18*	+151±20*	+117±15*	+115±29*
BUN, *mg/100 ml*	105±10¶	—	—	—	—	—	—	+72±5‖
Hct, %	44±0.4	−0.3±0.8	—	+2.1±1.8	—	+3.5±0.5*	—	+1.4±1.4
Blood pressure, *mm Hg*	109±5	+5±2	—	+9±4	—	+9±4	—	+8±4
G. Normals with UUL rats with urine of contralateral kidney reinfused i.v. (n = 5)								
GFR, *ml/min/kg body wt*	9.3±1.1	—	+2.0±1.5	+1.4±1.5	−0.19±1.3	−0.19±1.7	−1.0±1.1	−1.0±1.3
Urine flow, *µl/min/kg body wt*	22.0±5.7	+15.3±4.8‡	+46.5±18.2‡	+81.2±19.2‡	+73.6±8.2*	+48.5±6.6*	+35.7±8.9‡	+28.3±11.9
Sodium excretion, *µeq/min/kg body wt*	1.14±0.52	−0.08±0.35	+3.3±1.2‡	+8.9±2.0‡	+9.1±1.7*	+8.8±1.9*	+6.2±1.1*	+4.9±0.87
FENa (×100), %	0.08±0.03	—	+0.22±0.10	+0.59±0.16‡	+0.70±0.14*	+0.67±0.11*	+0.50±0.05*	+0.42±0.04*
U_{osm}, *mosmol/kg*	1,188±198	−269±120	+203±189	−159±249	−225±216	−58±208	+239±207	+279±262
T_cH_2O, *µl/min/kg body wt*	56±22	+53±13‡	+107±34‡	+131±19*	+107±13*	+100±30*	+115±25‡	+89±26‡
BUN, *mg/100 ml*	18±1¶	—	—	—	—	—	—	28±2‖
Hct, %	48±0.4	−1.8±1.3	—	−2.5±1.5	—	−4.7±1.7‡	—	−2.0±0.8
Blood pressure, *mm Hg*	116±4	+5±1‡	—	+3±3	—	+3±4	—	+2±2
H. Normals with rats with total intravenous urine reinfusion for 3 h (n = 4)								
C_{IN}, *ml/min/kg body wt*	7.3±0.6	—	+0.8±0.8	+1.4±0.4‡	+1.5±0.5‡	+1.6±0.9	+1.6±0.2*	+1.2±0.5
Urine flow, *µl/min/kg body wt*	25±8.8	+13±5.4	+28±12	+42±11‡	+58±8.6*	+63±8.9*	+61±5.6*	+55±9.2*
Sodium excretion, *µeq/min/kg body wt*	1.3±0.9	+1.6±0.9	+3.9±2.2	+4.9±2.2	+7.1±2.3‡	+9.7±2.3*	+10.5±0.8*	+9.7±1.8*
FENa (×100), %	0.15±0.12	—	+0.34±0.22	+0.41±0.20	+0.51±0.16‡	+0.70±0.15*	+0.81±0.10*	+0.75±0.13*
U_{osm}, *mosmol/kg*	1,287±226	−20±25	−81±65	−261±106	−331±73*	−193±77	−167±102	−82±98
T_cH_2O, *µl/min/kg body wt*	56±12	—	+52±28	+62±31	+81±32	+122±30‡	+133±28*	+131±19*
BUN, *mg/100 ml*	30±3.2¶	—	—	—	—	—	—	27±1‖
Hct, %	47.4±1.1	−0.4±2.3	—	+0±0	—	+1.5±1.4	—	−1.1±1.0
Blood pressure, *mm Hg*	118±3	−3±2	—	−2±1	—	−2±4	—	−1±3

* $P < 0.01$ or greater compared to control, values expressed as mean ±1 SEM.

‡ $P < 0.05$ compared to control.

§ C_{IN}, inulin clearance (glomerular filtration rate); FENa(×100), fractional excretion of sodium; T_cH_2O, free water reabsorption. in minutes.

‖ Recipient rat.

¶ Donor rat at start of cross-circulation.

TABLE VI

Changes in Urea Excretion in Normal Rats during Cross-Circulation with Various Donors (groups A, F, and H)

Control	20 min	40 min	60 min	80 min	100 min	120 min	140 min
				mg/min/kg body wt			
Group A: donors with BUL (n = 3)							
1.32±1.52	+2.59±0.68‡	+4.21±1.07*	+4.32±0.97*	+4.24±0.96*	+3.65±0.94‡	+2.57±0.79‡	+1.85±0.99
Group F: donors with acute urea load (n = 5)							
0.94±0.11	+0.76±0.27‡	+2.54±0.68‡	+3.15±0.54*	+3.45±0.34*	+3.17±0.40*	+2.53±0.41*	+2.22±0.95*
Group H: donors with total urine reinfusion (n = 4)							
0.78±0.20	+0.56±0.40	+0.78±0.31	+0.83±0.27‡	+1.11±0.21*	+1.38±0.19*	+1.56±0.25*	+1.50±0.20*
Comparison							
F:A	↓*	NS	NS	NS	NS	NS	NS
H:A	↓‡	↓‡	↓‡	↓‡	↓‡	NS	NS
H:F	NS	NS	↓‡	↓‡	↓*	NS	NS

* $P < 0.01$ or less compared to control or to other group, values expressed as mean ±1 SEM.

‡ $P < 0.05$ compared to control.

in the early periods after cross-circulation. The higher urea excretion rates in group A may be due to longer equilibration of urea in the donor rat during 24 h of bilateral ureteral obstruction as compared to acute urea loading. The correlation coefficient between urea and sodium excretion rates were significant in groups A and F, $r = 0.73$, $P < 0.01$ and $r = < 0.41$, $P < 0.05$, respectively. The slope of the regression lines was steeper in group A but not significantly different ($P < 0.10 > 0.05$, Fig. 3). The correlation between urea excretion and urine flow was also similar in the two groups, the regression lines being $y = 56.4\ x - 104.7$, $r = 0.83$, $P < 0.01$, and $y = 34.1\ x + 0.25$, $r = 0.64$, $P < 0.01$, (Fig. 4).

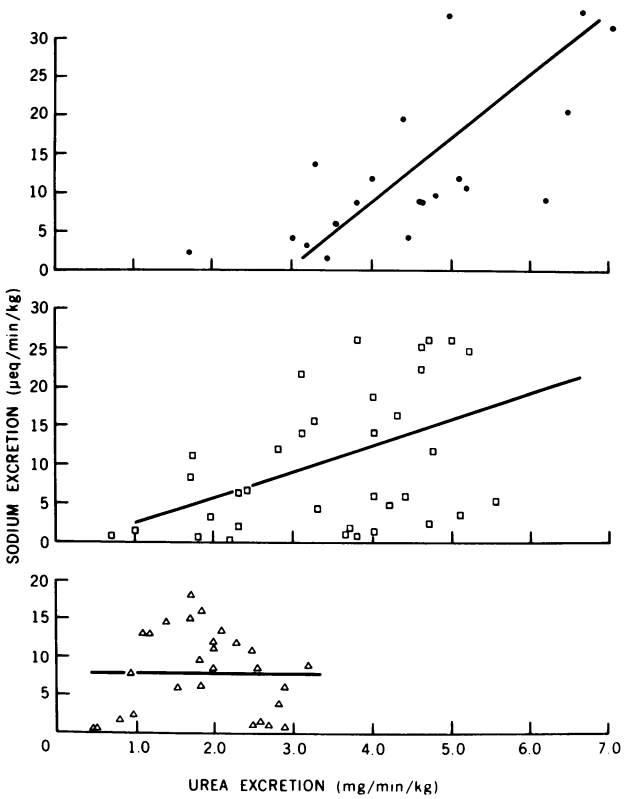

FIGURE 3 Relationship between urea excretion and sodium excretion in group A (● BUL, upper panel, $y = 7.95x - 22.8$, $r = 0.73$, $P < 0.01$), group F (□, normal donors with urea load, middle panel, $y = 2.92x + 0.04$, $r = 0.41$, $P < 0.05$), and group H (△, donors with total urine reinfusion, lower panel, $y = 0.07x + 7.9$, $r = 0.01$).

Role of other urinary natriuretic factors. To determine whether retained urine could produce a diuresis and natriuresis apart from an effect mediated by urea accumulation, five rats with 24-h UUL underwent reinfusion of urine from the contralateral normal kidney for 1 h before and during cross-circulation with normal recipients (group G, Table IV). There was a significant diuresis and natriuresis observed in the recipient rats, greater than seen with UUL donors not receiving urine reinfusion (group B), as shown in Fig. 5 and Table III, G:B). A series of experiments was also done with urine-reinfused normal donors (group H). The results were similar to those of group G, indicating that the presence of an obstructed kidney was not essential for this effect (Table IV).

BUN levels changed little in the reinfused donor rats, with an increase of 9±2 mg/100 ml and of 10±3 mg/100 ml in groups G and H, respectively. Urea excretion rates did increase significantly in recipients of group H (Table VI), although much less than in groups A and F, and thus urea could also be contributing to the natriuretic effect produced by urine reinfusion. In contrast to groups A and F, however, there was no correlation between urea excretion and sodium excretion or urine flow in group H ($r = 0.01$, $y = 0.07\ x + 7.91$ for sodium excretion, and $r = 0.09$, $y = 4.1\ x + 62.8$ for urine flow, Figs. 3 and 4). Moreover, urine flow and natriuresis

were not significantly different in groups F (urea-loaded donors) and group H, but urea excretion rates were significantly lower at several time intervals in group H (Fig. 2 and Table VI, H : F).

DISCUSSION

Postobstructive diuresis occurs after the relief of bilateral, but not unilateral, ureteral ligation in the rat (1–4). If circulating natriuretic factors are important in the phenomenon of postobstructive diuresis, such factors should be demonstrable by cross-circulation techniques (7, 8). Although it is recognized that the beginning of a cross-circulation experiment is not a steady-state situation, in this respect it is similar to the changing circumstances that occur with sudden relief of ureteral ligation. The method does allow a study of the role of blood-borne natriuretic factors, as compared to intrinsic renal damage, in the initial phase of postobstructive diuresis, as well as a study of the effect of such factors on the normal kidney of recipient animals.

The present experiments clearly indicate that circulating diuretic and natriuretic factors are present in rats with 24-h BUL but such factors could not be detected by cross-circulation techniques in animals with UUL. Previous authors have also suggested a possible role for humoral factors in postobstructive diuresis (1–3), and Yarger et al. have recently reported results that support our observations, using other techniques (11).

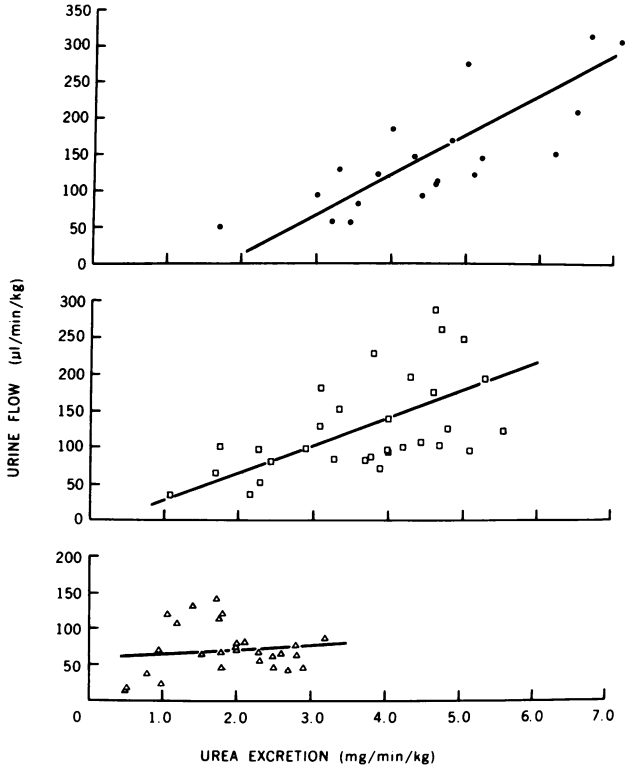

FIGURE 4 Relationship between urea excretion and urine flow in group A (●, BUL donors, upper panel, $y = 56.4\ x + 104.7$, $r = 0.83$, $P < 0.01$); group F (□, normal donors with urea load, middle panel, $y = 34.1\ x + 0.25$, $r = 0.64$, $P < 0.01$); and group H (△, donors with urine reinfusion, lower panel, $y = 4.1\ x + 62.8$, $r = 0.09$).

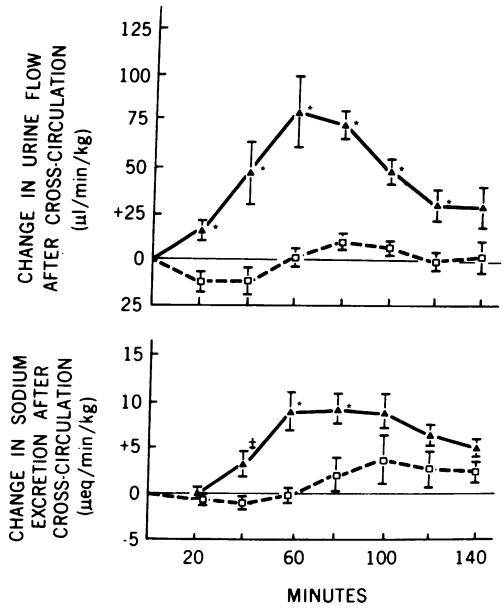

FIGURE 5 Changes in urine flow (upper panel) and sodium excretion (lower panel) in normal rats undergoing cross-circulation with donor rats having UUL for 24 h and intravenous urine reinfusion from the contralateral kidney for 1 h (▲, group G) or simply UUL (○, group B).
* Difference from group B, $P < 0.01$ or less.
‡ $P < 0.05$.

The diuresis and natriuresis occurring in normal rats during cross-circulation with BUL donors were rapid in onset, beginning in 10 min, and of large magnitude, with peak levels 10 times greater than control (Table II, Fig. 1). The diuresis was sustained for the 140 min of cross-circulation, while natriuresis returned to control levels after 80 min. This latter change may have been due to extracellular fluid volume depletion developing in the normal recipient rat. The diuresis and natriuresis were often greater than those reported in BUL rats after relief of obstruction (1-4), a difference probably due to the normal glomerular filtration rate and therefore filtered sodium load in our recipient rats, compared to the low glomerular filtration rate of BUL rats. The longer duration of postobstructive diuresis in BUL animals is probably determined by factors not studied in the present experiments, such as the more prolonged excretion of urea or possibly by other natriuretic factors, and particularly the nature and severity of the intrinsic renal damage.

The intrinsic renal defect in BUL rats undergoing postobstructive diuresis is characterized by vasodilatation (3) and decreased tubular reabsorption of salt and water in surface nephrons (1–3), while in UUL rats studied after relief of obstruction there is renal vasoconstriction (5) and enhanced tubular reabsorption (6). The present experiments suggest that these differences in renal hemodynamics and tubular reabsorption, which are the immediate cause of postobstructive diuresis, may be secondary to natriuretic and diuretic factors present in the circulation of BUL, but not UUL, rats.

The natriuretic and diuretic response seen in normal rats undergoing cross-circulation with BUL donors was not associated with a significant increase in glomerular filtration rate, which actually decreased in several animals. Since serum sodium levels also did not change, it appears that an increase in filtered sodium load was not responsible for the natriuresis and diuresis. Change in renal hemodynamics did not appear to be an important mediator of natriuresis, since renal plasma flow and arterial blood pressure were not significantly altered (Table II). The hematocrit tended to decrease with time in both groups A and B, suggesting that this change did not contribute to the natriuresis seen in group A. Thus circulating natriuretic factors in BUL rats appear to act by direct inhibition of tubular sodium and water reabsorption unrelated to obvious hemodynamic changes. Alterations in the intrarenal distribution of blood flow or glomerular filtration remain as possible additional contributing factors. However, recent studies using the ferrocyanide-microdissection method (12) and microsphere methods (3) do not indicate any consistent abnormality in these parameters after relief of BUL. No information can be obtained from the present studies concerning the site in the nephron where inhibition of tubular reabsorption occurred, but other experiments suggest that the medullary collecting duct may be the critical nephron segment affected in the postobstructive kidney (13).

The technique of cross-circulation used in our studies, in which small changes in blood volume can be immediately detected and corrected, should prevent any changes in the volume status of the donor from directly affecting the recipient animal (see Methods). Humoral natriuretic factors that may be released during sustained volume expansion (8) are not likely to be involved in the observed diuresis and natriuresis in group A, since BUL rats were deprived of fluid and lost 6-7% of body weight in the 24 h before the experiment. Circulating factors that act by inhibition of aldosterone and/or Pitressin appear to be ruled out since the administration of these hormones to normal rats, in doses sufficient to increase urine osmolality and decrease sodium excretion rate in the control period, did not prevent a marked natriuresis and diuresis during cross-circulation with BUL donor rats (group C, Table II). Thus two separate groups of experiments (A and C) indicated the presence of circulating natriuretic factors in BUL donors. Factors that act by inhibition of renal Na-K-ATPase activity were excluded by the observation of similar levels of enzyme activity in recipient rats of groups A and B, (BUL and UUL donors, Table IV), although reduced enzyme activity has been found in the postobstructive kidney 24 h after relief of obstruction (4).

Identification of the natriuretic factors present in BUL as compared to UUL donors by cross-circulation techniques is necessarily indirect, but certain conclusions can be drawn by altering the state of the donor or recipient rat. The possibilities considered include: (*a*) release of natriuretic factor(s) from the kidney by bilateral ligation, (*b*) retention of urea, or (*c*) retention of other substances normally excreted in the urine. BUL accompanied by prolonged high intrarenal pressure (1) could release renal natriuretic factors not affected by UUL. However, this possibility appears unlikely because when rats with UUL received an acute urea load and urine reinfusion (group D), they produced a diuretic-natriuretic response not significantly less than that produced by BUL donors (Fig. 2 and Table III, D:A). Similarly, normal rats given an acute urea load (group F) produced a marked response (Fig. 2).

Previous studies to evaluate the role of urea in postobstructive diuresis have been conflicting. Urea administration to rats after relief of UUL did not produce a diuresis and natriuresis comparable to that seen after relief of BUL (1, 3). However, recent evidence in which the effects of volume depletion were taken into account has suggested a possible important role for urea (11). The present experiments support this concept. When urea was given to a series of normal recipient rats before cross-circulation with BUL rats (group E), there was a gradual decline, rather than a marked increase, in salt and water excretion in the recipient rat during cross-circulation, indicating prevention of natriuresis by prior urea loading. However, this effect may have been due to progressive volume contraction in the recipient rat, resulting from urea diuresis. When the blood urea level of normal donor rats was raised by acute intravenous urea loading (group F), a marked diuresis and natriuresis occurred in normal recipient animals during cross-circulation (Table V). The natriuresis, although smaller and delayed in onset, was not significantly less in comparison with normal-BUL pairs (group A), while diuresis was significantly less only in the first two periods of cross-circulation (Fig. 2 and Table III, F:A).

The importance of urea was further indicated by the

correlation between urea excretion rate and sodium excretion and urine flow in groups A and F (Figs. 3, 4). Thus it appears that a large proportion, if not all, of the difference between the response to cross-circulation with BUL as compared to UUL rats could be accounted for by the osmotic diuretic effect of high concentrations of urea in BUL rats.

Although urea diuresis may be a sufficient explanation for the diuresis-natriuresis produced by cross-circulation with BUL rats, it is not necessarily the only explanation. Other natriuretic factors may be present in the retained urine and contribute to this phenomenon. Urine reinfusion in UUL donors (group G) resulted in a significant diuresis and natriuresis in recipient animals, which was of considerable interest since the increase in blood urea level was minimal. In contrast, no significant diuresis or natriuresis occurred when rats with UUL alone were used as donors (group B), although there was a similar slight rise in BUN (Fig. 5 and Tables II and V). Similarly, normal rats with total urine reinfusion for 3 h (group H, Table V) produced a significant diuresis and natriuresis in recipient animals nearly identical to that produced by urea-loaded animals (group F), although the urea excretion rates were much lower in the urine-reinfused group (Fig. 2, Tables V and VI). In addition there was no correlation between urea excretion rate and sodium excretion or urine flow in group H, in contrast to group F (Figs. 3, 4). These experiments suggest that circulating factors other than urea, normally excreted in the urine, could be important in postobstructive diuresis occurring after the relief of BUL. Harris and Yarger have recently provided support for the presence of natriuretic factors other than urea in reinfused urine (11). Natriuretic factor(s) have also been described in the urine (14), as well as the serum (15, 16) of patients with chronic uremia. The relationship, if any, of these factor(s) to the substances accumulating in the blood after 1-3 h of total suppression of urine output in the present experiments (groups G and H) remains to be determined.

In summary, the results indicate that natriuretic factors are present in the blood of rats with bilateral, but not with unilateral, ureteral ligation. Such factors appear to act by inhibition of tubular sodium and water reabsorption, rather than through changes in glomerular filtration or renal hemodynamics. High blood and urine urea levels appear to that account for the diuresis and natriuresis produced in normal rats by cross-circulation with BUL animals, although evidence suggesting the presence of other natriuretic factors in urine reinfused intravenously was also obtained. The data suggest that urea osmotic diuresis has an important role in the postobstructive diuresis that occurs after relief of bilateral but not unilateral ureteral ligation.

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