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Research Article

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ABSTRACT A natriuretic and diuretic response to whole blood infusion in the rat, exaggerated and sustained by intravenous reinfusion of excreted urine, was shown to be associated with increased glomerular filtration and reduced tubular reabsorption. Cross-circulation of animals so responding (donor rats) with isovolemic recipients led to a modest natriuretic and diuretic response in the latter, not accounted for by altered physical composition of the blood nor by observed changes in filtration rate or arterial blood pressure. The recipient natriuresis was unchanged when nephrectomized donors were used and it occurred in experiments in which donor urine was simultaneously replaced by intravenously infused Ringer-Locke solution; the natriuretic property of the cross-circulating blood could therefore not have been due to reinfusion of urinary constituents, nor to accumulation of metabolites, nor to a factor of renal origin. A recipient natriuresis was also observed when the expanded and urine reinfused donor had been acutely adrenalectomized, ruling out an altered secretion of adrenal cortical or medullary hormones as a principal cause of this natriuresis; the data, however, do not exclude participation of reduced aldosterone secretion in the normal effector mechanism. In control experiments in which whole blood was exchanged for donor blood, a small delayed natriuresis did occur in the recipient; this could be completely prevented by administration of aldosterone. In similar exchange experiments with adrenalectomized donors, a small natriuresis developed in the recipient before blood administration but declined afterwards. These minor natriuretic effects probably resulted from altered mineralocorticoid content of the cross-circulating blood due to factors other than blood volume change. The larger natriuretic response seen in all recipients when the donor was volume expanded must have been due largely to a humoral natriuretic factor of other than renal or adrenal origin.

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INTRODUCTION

Various effector mechanisms have been proposed to account for the renal natriuretic response to blood volume expansion. There is convincing evidence that decrease in peritubular capillary oncotic pressure, such as may result from plasma dilution or decreased filtration fraction, can diminish uptake of tubular reabsorbate (1). This change, associated with increase in peritubular capillary blood pressure, is advanced as a possible basis of "volume natriuresis" (2, 3). On the other hand, the possibility of a humoral natriuretic effector has been extensively explored (4–10), but the natural occurrence and physiological importance of such a "natriuretic hormone" has not been convincingly demonstrated for reasons outlined in recent commentaries (11, 12). In particular, some earlier reports (6–8) of a humoral natriuretic factor remain questionable because the blood volume expansion techniques involved alterations in blood composition which are now known to affect the kidney directly (13–16). Furthermore, the renal reaction to injection of blood from expanded animals into bioassay animals has been small and inconsistent (17, 18) or absent (19, 20). The first of these major obstacles to accepting the existence of a humoral effector of natriuresis could be removed by demonstrating transfer of natriuresis in a bioassay procedure where vascular expansion occurred without alteration in blood composition. Two studies with positive results which approach or meet this requirement are available (21, 22). The second difficulty, of inconsistently positive findings, could be resolved if the humoral activity were shown to be normally complementary to some other renal effector mechanism.

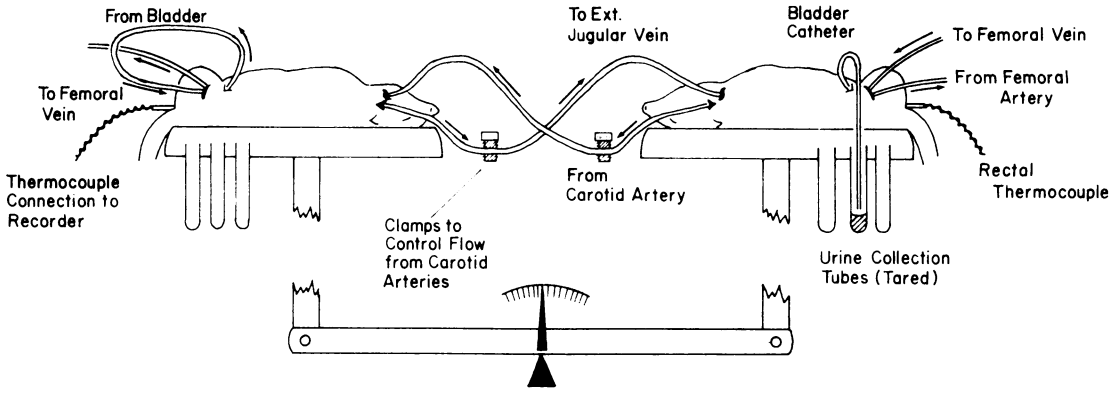

FIGURE 1 Elements of technique of cross-circulation used.

We have reported (20) that cross-circulation of two rats, one of which is undergoing natriuresis and diuresis after whole blood infusion, fails to reveal a humoral causation of the renal response by a similar alteration in renal function of the recipient animal. It was concluded that either a humoral natriuretic factor does not exist in the rat, or that its action can only be expressed in the presence of some other change resulting from vascular expansion. Only if the latter is provided, or if the hormone is circulating in greatly increased amounts, might its physiological action, or even presence, be demonstrable.

In the course of micropuncture experiments, in which a sustained state of volume expansion was a requirement, the technique of intravenous reinfusion of formed urine was employed in the rat (23). Such "reinfused" animals showed exaggerated renal responses to blood infusion, and these, as well as successful transfer of a natriuretic effect by cross-circulating reinfused rats with normal recipients, will be described.

METHODS

Male Sprague-Dawley rats, ranging in weight from 194 to 397 g and anesthetized with Inactin[1] (100 mg/kg), were used in the eight series of experiments. Series I consisted of five single animals infused with whole rat blood and reinfused with their own urine for 2 hr, after which urine reinfusion was discontinued but urine collections continued for 70 min. Series II–VIII were cross-circulation experiments, identified in Table I by treatment of the donor rat; the recipient rats were normal in all these series. Identical protocol was used throughout series II–VIII, except for deliberate deviations to be described. Cross-circulation was commenced after completion of the surgical preparations; the initial weight of the recipient, including secreted urine, was kept constant thereafter as described below. After 40 min, "control period" observations were made for 1 hr and blood was then infused into the donor and urine reinfusion instituted; observations continued over the next 2 hr ("post-infusion" period).

[1] Sodium ethyl-(L-methylpropyl)-malonyl-thiourea supplied by Promonta, Hamburg.

In series III, VII, and VIII the renal vessels in the donor rat were ligated, with or without excision of the kidneys, through a midline laparotomy which was then closed. In series V and VI, the adrenal glands of the donor were excised through lumbar incisions, the procedure completed about 30 min before cross-circulation. Series II and IV were parallel, except that in series IV the donor urine flow simultaneously displaced an equal volume of Ringer-Locke solution from a separate reservoir into the donor circulation; the urine was collected but not reinfused. Series III and VII were parallel, except that during whole blood infusion in series VII, the donor was simultaneously hemorrhaged at the same rate; this "exchange" procedure allowed addition of homologous blood to the crossed-circulation without vascular expansion of either partner. Series V and VI were similarly parallel, except for the absence of vascular expansion in series VI. Series VII and VIII were also parallel exchange experiments, except that the animals of the latter series received aldosterone and vasopressin in the continuous infusion.

Preparation and procedures. In all animals, after tracheal cannulation, a femoral artery and vein were cannulated for blood pressure recording and blood sampling and for infusions, respectively. A carotid artery and jugular vein were cannulated with PE90 polyethylene tubing for the cross-circulation procedure depicted in Fig. 1. In the donors of series III, VII, and VIII the renal vessels were ligated; in all other animals the bladder was cannulated, care being taken to reduce dead space in the bladder stump by ligaturing close to the ureters. In donor rats and those of series I a second femoral vein was cannulated for urine reinfusion. The method of cross-circulation was similar to that described earlier (24) except that free flow was allowed through the crossed arteriovenous shunts. Rats of each pair were placed on two pans of a simple balance set in equilibrium initially; after opening the shunts any change in weight of either rat due to unequal volume flow rate in the shunts was corrected by appropriate adjustment of the arterial catheter clamps; the balance was sensitive enough to register two scale divisions for a weight difference of 0.4 g. Momentary deflections occurred during an experiment, but these rarely exceeded the equivalent of 0.5 ml of blood volume change in any one rat. Urine was collected into tared polyethylene tubes attached to the balance pans and, during blood infusion to the donor rat, an equal volume of saline was simultaneously delivered into a container on the side of the recipient rat. The latter animal was thus kept isovolemic, except for the urine secreted. Blood for

TABLE I

Identification of Series by Treatment of Donor Rat

Series	Experiments	Preparation	Blood used for	Donor urine
II	7	Normal	Expansion (Inf)	Reinfused
III	5	Nephrectomized (Nx)	Expansion	—
IV	7	Normal	Expansion	Replaced with Ringer solution
V	10	Adrenalectomized (Adrx)	Expansion	Reinfused
VI	10	Adrenalectomized	Exchange (Exch)	Reinfused
VII	5	Nephrectomized	Exchange	—
VIII	5	Nephrectomized +Aldosterone + ADH	Exchange	—

infusion was obtained immediately before use by decapitation of an anesthetized normal rat; the amount infused was equal to 33% of the blood volume (estimated as 7% of the body weight) of the rat to be expanded. Each animal received an initial dose of 1500 IU/kg heparin and a priming dose of 1 ml Ringer-Locke solution containing 3 μCi of inulin-^{3}H; this was followed by a continuous intravenous infusion of 1.25 ml/hr of Ringer-Locke solution containing

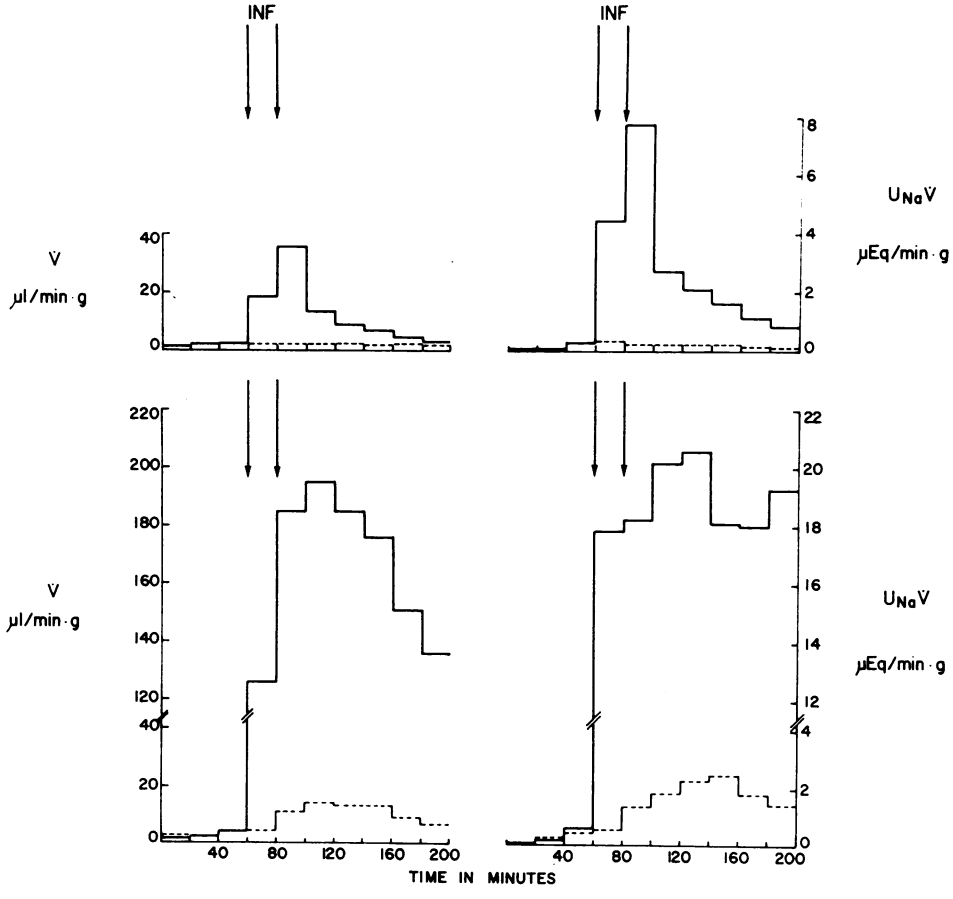

FIGURE 2 Donor and recipient responses to simple (top) or sustained (bottom) blood volume expansion. Averaged data for water (V) and total sodium ($U_{Na}V$) excretion before and after infusion of whole blood (Inf) into the donor (solid lines) of cross-circulated pairs of normal rats; the data for the isovolemic recipient are shown by interrupted lines. The upper two panels show data from simple blood infusion experiments reported earlier to compare with data from series II (lower two panels) in which blood volume expansion was sustained by urine reinfusion. Values are expressed per gram kidney weight.

TABLE II

Direct and Transferred Effects of Blood Infusion,

Series		Animal	Mean body wt.	Mean kidney wt.	n		$\dot{V}$		$U_{Na}\dot{V}$	
					Actual	Reported	Control	Post-inf	Control	Post-inf
			g	*g*			*μl/min·g*		*μEq/min·g*	
I	Inf	Single	308	2.85	5	5	3.8±2.1*	*217.8±7.3*‡	0.10±0.06	*18.5±4.3*
II	Inf	Donor	325	2.91	10	7	2.8±0.5	*171.3±8.6*	0.30±0.10	*19.0±3.0*
IV	Inf + Ringer-Locke	Donor	233	2.58	7	7	1.4±0.2	*118.7±25.9*	0.05±0.001	*16.2±3.5*
II	Inf	Recipient	326	2.64	(10	7)	3.3±0.3	*11.9±2.5*	0.37±0.09	*2.00±0.46*
III	Nx + Inf	Recipient	299	2.39	9	5	3.4±0.3	*13.1±1.9*	0.28±0.09	*1.68±0.47*
IV	Inf + Ringer-Locke	Recipient	231	2.35	(7	7)	1.7±0.2	*15.0±3.2*	0.10±0.03	*2.86±0.78*
V	Adrx + Inf	Recipient	276	2.69	12	10	4.6±0.8	*9.5±1.5*	0.20±0.06	*1.33±0.24*
VI	Adrx + Exch	Recipient	305	2.68	12	10	3.4±0.5	4.4±0.6	0.17±0.03	0.38±0.10
VII	Nx + Exch	Recipient	311	2.69	8	5	2.7±0.4	4.4±0.7	0.20±0.13	0.50±0.15
VIII	Nx + Exch +Aldosterone	Recipient	298	2.82	7	5	3.2±0.6	3.2±0.4	0.21±0.07	0.26±0.11

Abbreviations: ABP, arterial blood pressure; GFR, glomerular filtration rate represented by inulin-^{3}H clearance; TRF_{Na}, tubular rejection fraction for sodium or percentage of filtered sodium appearing in the urine; $U_{Na}\dot{V}$, total sodium excretion; $\dot{V}$ urine volume.

* Values ±standard error of the mean.

‡ Italics denote significant difference ($P < 0.05$) from corresponding control value with each series.

3.3 μCi of inulin-^{3}H. In series VIII, 13 μg/kg per ml of D-aldosterone and 1 mU/kg per ml of vasopressin (Pitressin, Parke, Davis & Company, Detroit, Mich.) were added to both priming dose and constant infusion.

In series IV only, donor urine was collected into an air-tight volumetric tube connected by tubing to a second tube containing Ringer-Locke solution; this reservoir was also stoppered except for a connection between an intake needle below the fluid surface and the femoral vein catheter of the donor rat. A minimum outflow pressure was arranged so that entry of urine into the first tube automatically displaced into the animal an equal amount of Ringer-Locke solution (NaCl, 130 mEq; KCl, 5 mEq; $CaCl_2$, 5.3 mEq; $NaHCO_3$, 20 mEq/liter). An exchange of protein-free electrolyte solution for protein-free urine was thus achieved, permitting no change in the fluid volume of the rat but allowing the removal of products excreted in the urine.

Determinations. In series II–VIII, determinations were made at 20-min intervals; the 1st hr included three control periods and was followed by blood infusion or exchange of the donor rat during the fourth period with simultaneous urine reinfusion where relevant; observations then continued over the remaining six periods. In series I only, urine reinfusion was discontinued at the end of the ninth period but urine collections and determinations were made over a further 70 min at shorter intervals as depicted in Fig. 3. Urine was collected continuously over each period except where it was reinfused and here 30-sec samples were obtained at the end of the period by briefly interrupting the reinfusion; the choice of this time for sampling led to some overestimate of the total excretion over a period if a function were increasing. In reinfusion experiments, plasma inulin specific activity rose in both donor and recipient rats. The changing plasma level was compensated for in recipient rats by averaging the values bracketing a urine collection period; in donor rats plasma and urine sampling were closely consecutive. Urinary and plasma sodium concentrations were measured by flame photometry from samples of collected urine or from plasma obtained from blood samples of 0.1 ml collected into microhematocrit tubes from the femoral arterial catheter; this volume of withdrawn blood was replaced with homologous donor blood in all rats except in the nephrectomized donors where serial samples were not taken. Plasma potassium concentrations were also determined on selected blood samples, two before and two after infusion. Arterial blood pressure was monitored with a strain gauge connected to the femoral arterial catheter. Rectal temperature of the animals was kept close to 37.5°C with a heat lamp. At the conclusion of experiments a post-mortem examination was carried out and the kidneys removed for weighing; a loss of 2 ml or more of blood into the abdominal cavity (from operative incisions) was considered grounds for exclusion of experimental data. As shown in Table II, such blood loss occurred more commonly in acutely nephrectomized donors. Plotted data are *collection period* averages for all animals in a series. Statistical evaluation of selected data was done on the mean values for the control period (of 1 hr) and of the post-infusion period (of 2 hr), subjected to the unpaired *t* test for significant differences (Table II). The paired *t* test was also used in evaluating the less obvious differences in the data of series VII and VIII. Correlation coefficients were calculated between absolute period values for selected variables, and the changes from control in these values after blood infusion, for the individual recipient rats of series II, V, and VI.

RESULTS

In comparison with the responses to simple vascular expansion as reported earlier (20) and reproduced in Fig. 2, rats in which expansion was maintained by urine reinfusion exhibited a greatly enhanced diuresis

GFR		TRF_{Na}		Mean ABP		Hematocrit		Plasma Na	
Control	Post-inf	Control	Post-inf	Control	Post-inf	Control	Post-inf	Control	Post-inf
ml/min·g		%		*mm Hg*		%		*mEq/liter*	
0.91±0.02	*2.06±0.04*	0.09±0.01	*5.95±0.34*	133±3	130±6	47.2±0.9	*53.5±0.7*	142.3±1.5	142.4±1.2
1.15±0.04	*2.30±0.14*	0.19±0.09	*6.01±0.96*	112±2	*126±3*	47.1±1.1	*50.9±0.7*	141.7±1.1	142.1±1.3
0.98±0.03	*1.35±0.04*	0.03±0.00	*8.47±1.71*	111±5	120±5	43.3±0.6	*46.4±0.5*	144.8±0.9	143.5±0.8
1.12±0.04	1.14±0.08	0.29±0.08	*1.14±0.22*	117±6	117±6	47.1±1.0	*50.8±0.7*	143.7±1.8	142.8±1.3
1.15±0.04	1.18±0.07	0.19±0.06	*1.00±0.22*	122±4	122±4	44.7±0.6	*47.0±1.2*	142.7±0.7	142.8±1.2
1.12±0.06	1.24±0.04	0.07±0.02	*1.56±0.40*	108±4	107±2	43.1±0.7	*46.3±0.6*	144.8±0.7	143.7±1.1
1.18±0.04	1.09±0.03	0.12±0.03	*0.86±0.17*	105±2	103±2	44.0±0.7	*46.9±0.6*	142.4±0.8	141.3±0.7
1.24±0.05	*1.04±0.02*	0.09±0.01	*0.27±0.07*	112±5	109±5	45.0±0.5	44.5±0.4	143±0.7	142.5±0.5
0.97±0.11	1.07±0.10	0.13±0.06	0.31±0.09	115±4	*102±4*	47.5±0.9	*44.1±0.5*	143±1.0	142.4±0.9
0.88±0.03	0.85±0.04	0.18±0.06	0.21±0.08	112±3	102±6	45.5±1.4	*40.9±1.8*	143.1±0.4	142.2±0.5

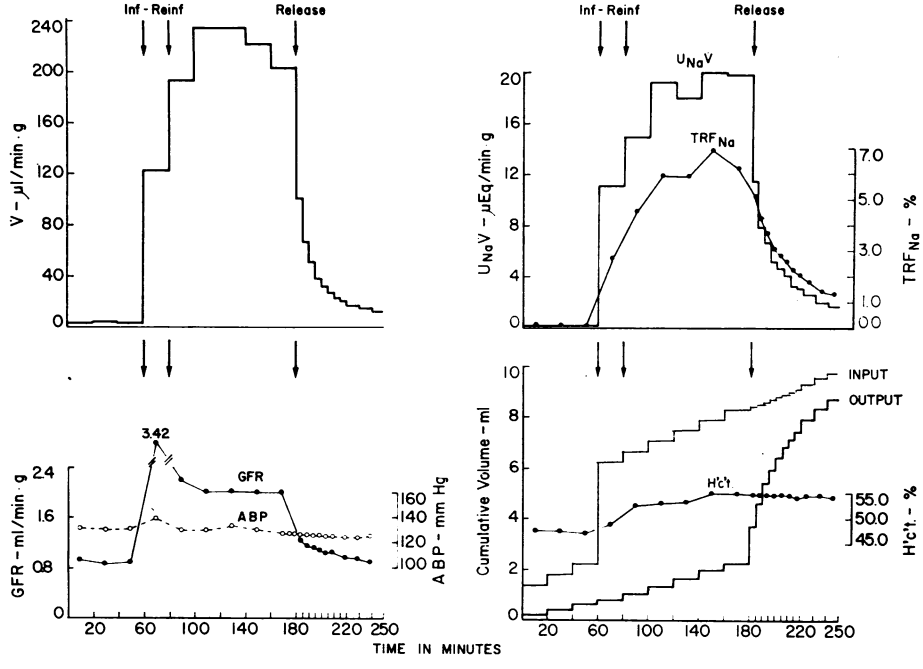

FIGURE 3 Renal and other responses to blood infusion with urine reinfusion. Averaged changes in normal rats (series I) in which urine reinfusion followed intravenous whole blood infusion (Inf-Reinf) for 2 hr after which reinfusion of urine was discontinued (Release). $\dot{V}$, urine volume; $U_{Na}V$, total sodium excretion; TRF_{Na}, tubular rejection fraction of filtered sodium; GFR, glomerular filtration rate; ABP, arterial blood pressure. The cumulative totals of fluid input and output (including loss in blood samples) are plotted at lower right.

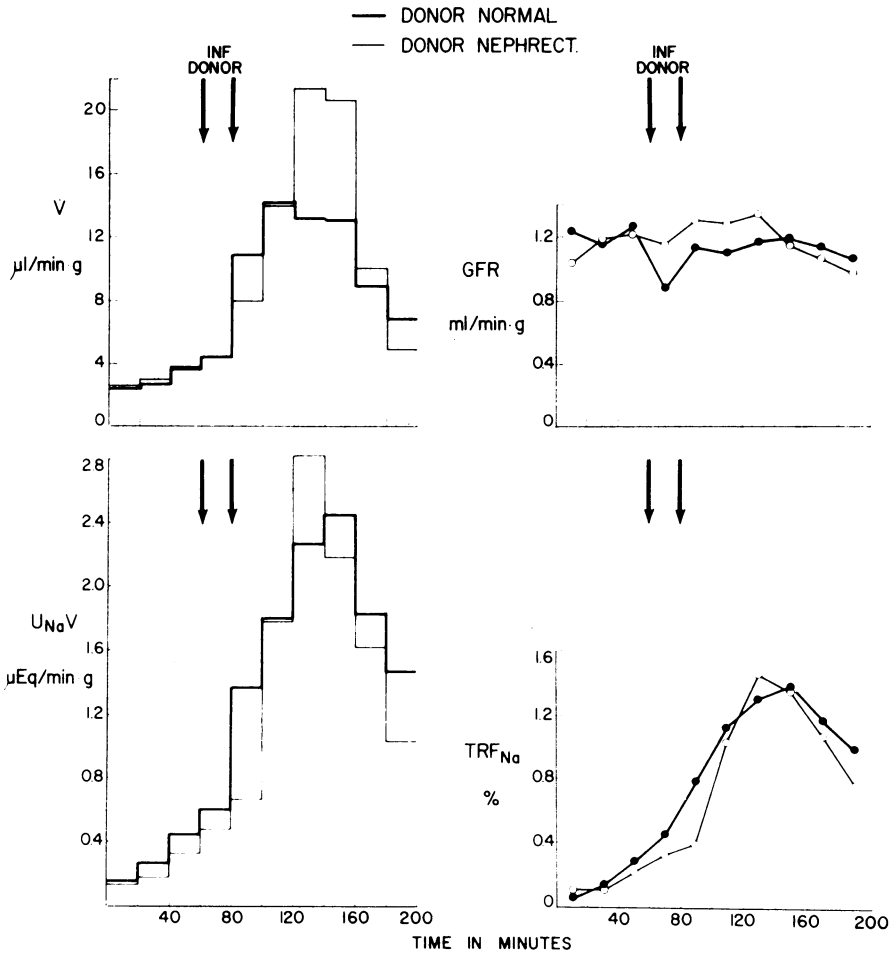

FIGURE 4 Averaged changes in renal function of recipient rats of cross-circulated pairs of series II (normal infused and urine reinfused donors) and of series III (nephrectomized infused donors). Abbreviations as for Fig. 3.

and natriuresis. There is evidence, from the urine-replacement experiments of series IV (Table II), that the initial expulsion of urine stored by slight distension of the bladder stump during interruption of the reinfusion line did not lead to any significant overestimation of the absolute values for renal excretion during the reinfusion period. The renal response, studied in detail in series I rats (in which the reinfusion was eventually terminated) was of a magnitude such that the volume of blood infused was usually equalled by the urine flow measured over the first or second postinfusion period. As seen in Fig. 3, the increased excretion reached a peak within 1 hr of the start of vascular expansion and was maintained with little decrement until reinfusion was stopped. The response was associated with a pronounced increase in GFR and TRF_{Na}; at the cessation of reinfusion, the increased levels of excretion fell at a progressively decreasing rate in parallel with a fall in GFR until at 70 min after the end of the reinfusion the GFR had returned to normal. At this time, however, TRF_{Na} still exceeded 1.0%, a value 10-fold greater than during control periods. The natriuresis was therefore accounted for both by increased tubular load and by inhibition of sodium reabsorption. Blood infusion resulted in some progressive increase in hematocrit and a temporary small elevation in mean arterial blood pressure. During the rapid excretion of water after the end of reinfusion, the hematocrit remained relatively constant; this could imply an extravascular source of the excreted water or a concomitant sequestration of red blood cells. Also, after cessation of urine reinfusion, the increased rate of urine formation had almost returned to control values by the end of the experiment at which time there remained only a small positive fluid balance similar to that measured during the control periods.

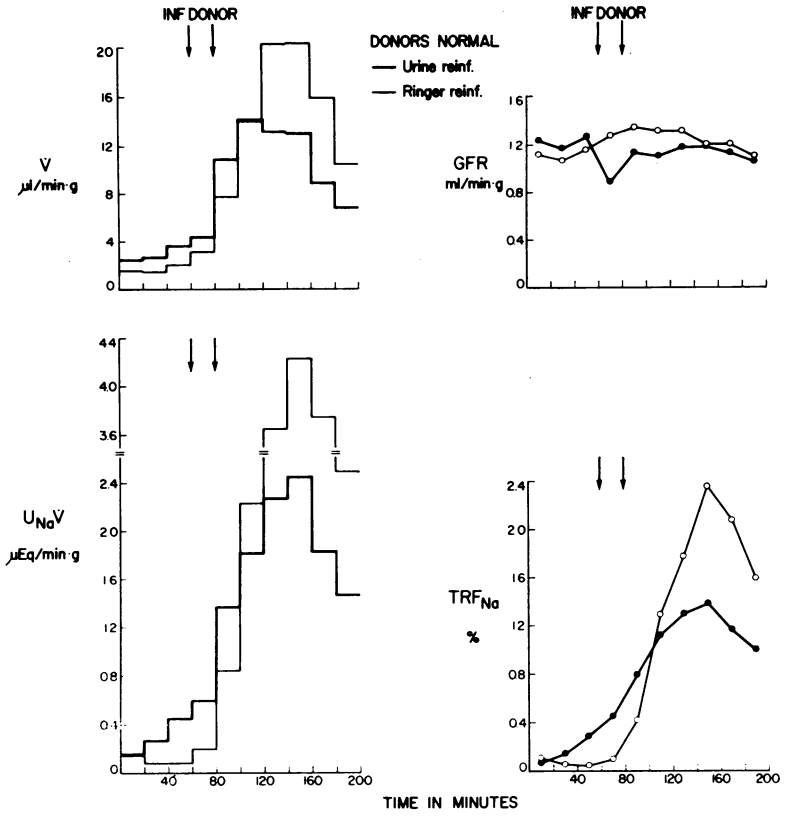

FIGURE 5 Averaged changes in renal function of recipient rats of cross-circulated pairs of series II (normal infused and urine reinfused donors) and of series IV (infused donors with Ringer-Locke replacement of excreted urine). Abbreviations as for Fig. 3.

In series II experiments, the recipient rats, cross-circulated with expanded urine-reinfused donors, exhibited the gradual development of a modest natriuresis and diuresis. This is shown in Fig. 2 and, with expanded scales, in Fig. 4. In this and other series, there was some increase in urinary excretion during the control periods. This was probably related to the initial priming infusions of saline; as seen in Fig. 2, this trend was short-lived in the recipients of blood from simply expanded donors (20). The natriuresis in series II recipients reached a peak only after 100 min and then declined, possibly due to the ongoing reduction in the recipient's own fluid volume. The increase in sodium excretion occurred without an increase in GFR (see also Table II) and was closely followed initially by increased water excretion; the hematocrit of the recipients rose in parallel with that of the donors, but there was no change in blood pressure of the recipient animals. There were no consistent or significant changes in recipient plasma Na^+ (see Table II) or K^+ concentrations, the values for the latter averaging 4.66 mEq/liter before infusion of blood to the donor, and 4.80 afterwards, during the final period.

Series III, designed to determine whether the natriuretic factor was of renal origin, was identical to series II except that the donor animals possessed no functioning kidneys and hence were kept expanded after blood infusion by virtue of their anephric state. The renal response of the cross-circulated recipients of series III (Fig. 4, thin lines, open circles) was essentially similar to that of recipients in series II except that the diuresis was slightly greater and the peak delayed. Again the hematocrit rose in parallel with that in the donor rats, but no change in blood pressure occurred (Table II). It was therefore clear that the source of any natriuretic factor transferred was not the kidneys of the expanded animal nor could the kidneys subserve any sensory function related to this factor.

Series IV, designed to determined whether the recipient natriuresis was dependent on some constituent of the reinfused urine or due to a nonspecific effect of accumulating metabolites, again revealed a natriuresis

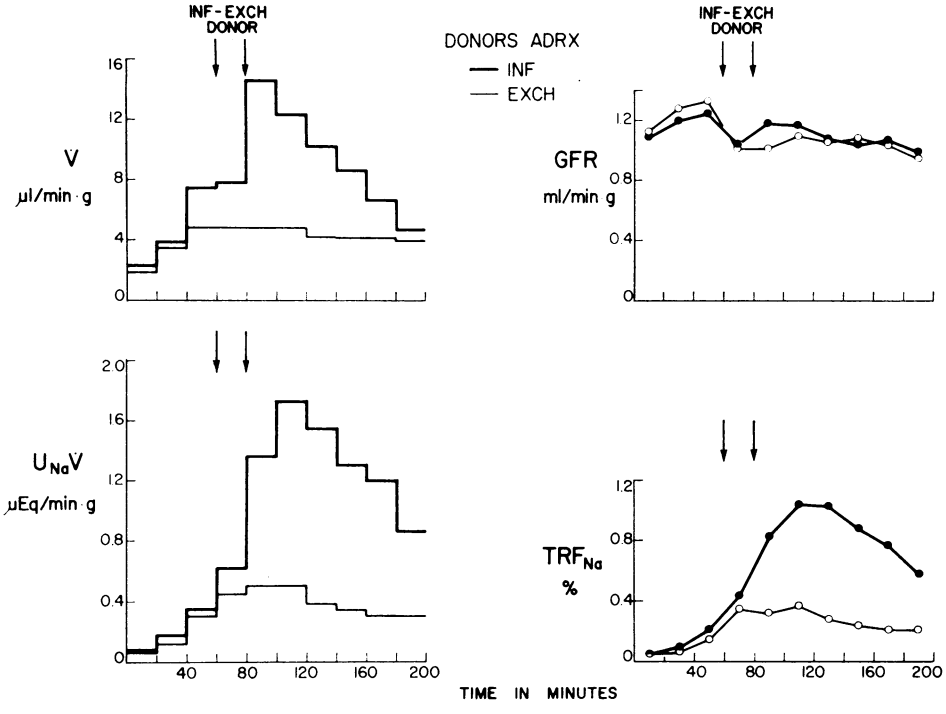

FIGURE 6 Averaged changes in renal function of recipient rats of cross-circulated pairs of series V (adrenalectomized donor infused and urine reinfused) and of series VI (adrenalectomized donors exchanged and urine reinfused).

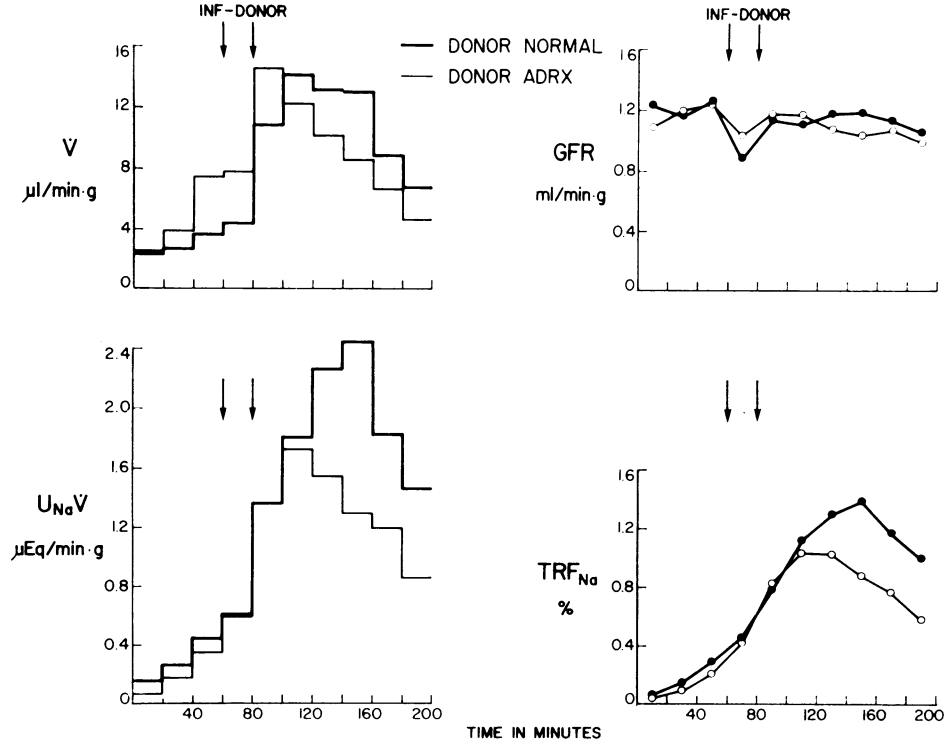

FIGURE 7 Averaged changes in renal function in recipient rats of series II (normal infused and reinfused donors) and series V (adrenalectomized infused and reinfused donors).

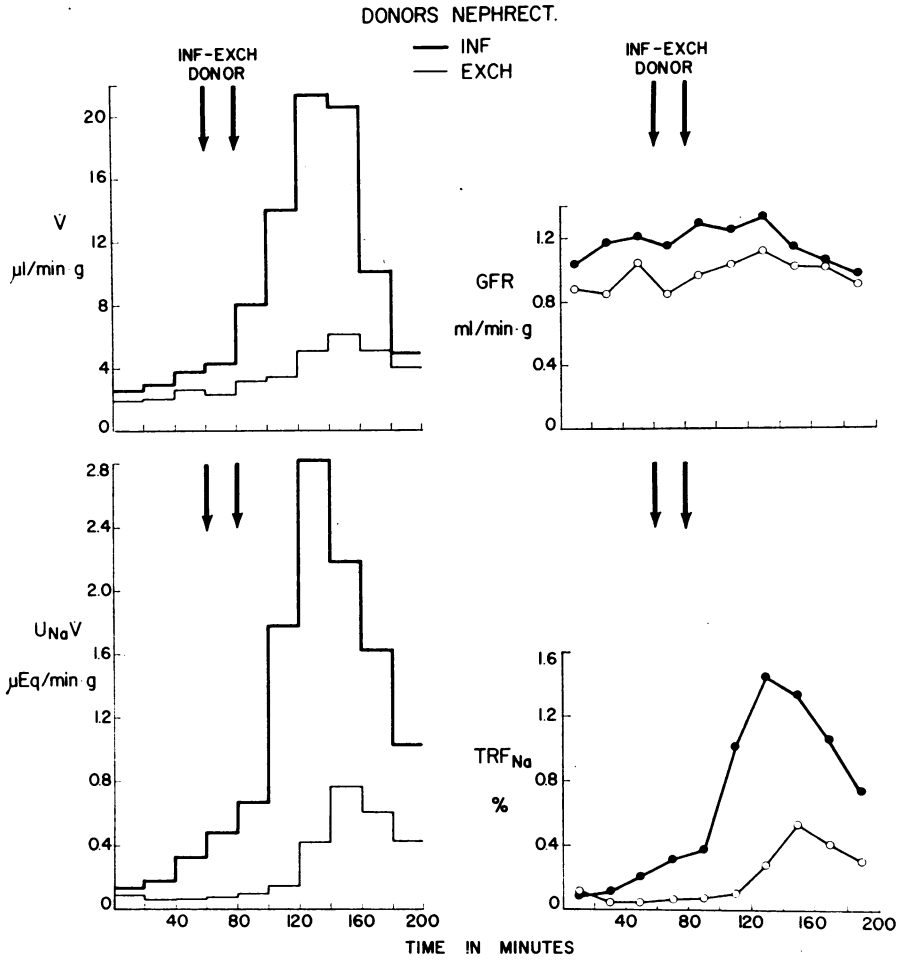

FIGURE 8 Averaged recipient responses to blood infusion to nephrectomized donors (series III, thick outlines and filled circles) compared to blood exchange in nephrectomized donors (series VII, thin outlines and open circles). There is a minimal renal response transferred from the exchanged donors.

in the recipient rats (Fig. 5, Table II). The temporal pattern of this natriuresis was similar to that seen in recipients of series II and III, but its magnitude appeared greater; this difference was not statistically significant. There was a trend toward an increase in GFR during the early postinfusion period. The natriuresis was associated with diuresis and these responses declined, as in series II and III, in spite of maintained volume expansion of the donor rat. Considerable variability was seen in recipient renal function in series IV, probably related to the replacement with isotonic solution of urine of variable tonicity. A slight consistent decrease in plasma K+ concentration occurred in these recipients from an average of 4.54 to 3.50 mEq/liter, measured in the first and last periods of the day. Hematocrit changes were similar to those seen in earlier series, indicating some hemoconcentration. The donor animals of series IV showed a greatly augmented excretion of sodium and water, not significantly different from the donor responses in series I and II (Table II); the elevation in GFR in the electrolyte-reinfused donors was significantly ($P < 0.01$) less than that seen in series II urine-reinfused donors. The pertinent finding, however, was that expansion, rather than functional anuria, was the common feature related to recipient natriuresis.

Series V and VI data, from experiments in which adrenalectomized donors were either expanded or exchanged respectively, are given in Table II and Fig. 6. The recipient rats of both series exhibited a rise in urine volume and sodium excretion before blood infusion in the donor; this trend reached a plateau in series VI whereas a superimposed natriuresis and diuresis ($P < 0.01$) followed expansion of the donor in

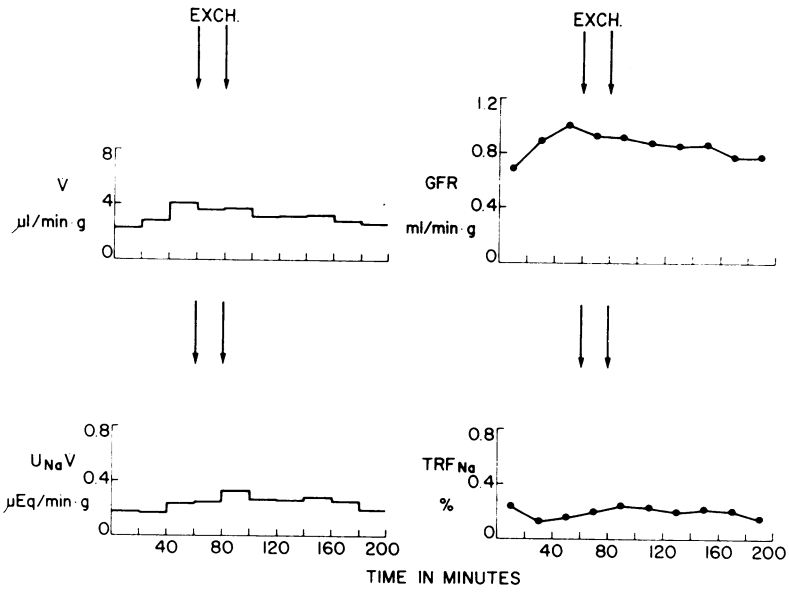

FIGURE 9 Averaged recipient responses in series VIII recipient rats, cross-circulated with nephrectomized donors which were "exchanged" with whole blood during the administration of excess aldosterone and vasopressin. No changes in renal function of these recipients is evident.

series V. The recipient natriuresis in series V resembled that seen in series II, but was somewhat smaller and reached a maximum earlier (Fig. 7); as in series II, there was no increase in GFR over control period values. The arterial blood pressure was usually lower in adrenalectomized donors, particularly when then were exchanged rather than expanded. The diuresis in recipient rats of series V was poorly sustained as it usually was in the donor; this feature of the volume response in acutely adrenalectomized rats has been observed in this laboratory in other experiments without urine reinfusion. Acute adrenalectomy did not, however, alter the essential features of the natriuretic activity in the blood of the expanded animal.

Series VII data, from experiments in which a nephrectomized donor rat was exchanged, reveal the late development of a small natriuresis and diuresis, poorly related to the slight rise in GFR, as seen in Fig. 8. None of these changes proved to be significant statistically (Table II). The possibility arose, nevertheless, that these trends were due to dilution of circulating aldosterone and vasopressin, which might be expected to be higher in experimental than in blood donor animals. (It is noteworthy that similar trends were not seen in series VI exchange experiments with adrenalectomized donors.) This reasoning led to series VIII experiments in which the level of these two hormones in the cross-circulating blood was exogenously elevated; the data (Fig. 9, Table II) show that blood exchange in the donor then had no effect on renal excretion in the recipient. The decline in arterial pressure and in hematocrit were, however, quite similar in recipients of series VII and VIII.

Correlation of variables in recipient rats where reinfused donors were normal or adrenalectomized. Period values for individual rats of three selected series (II, V, and VI) for two parameters of natriuresis ($U_{Na}V$ and TRF_{Na}) were related to corresponding period values for ABP, and for GFR in the case of $U_{Na}V$. This was done both for absolute levels and for ratios of individual postinfusion values to an averaged control value (t/c) in each animal. Table III gives the correlation coefficients obtained with their significance.

There were significant but low correlations between sodium excretion and absolute blood pressure levels in series II and VI but not V; a correlation between (t/c) ($U_{Na}V$) and (t/c) (ABP) was also seen in series II and V but not in VI. The low values for r indicate that although the initial and subsequent level of arterial pressure positively influences recipient natriuresis, some other factor is the principal cause of the increased sodium excretion. In series II the arterial pressure fell as often as it rose and in series V the pressure usually fell; in both series the recipient natriuretic responses tended to be greater when the absolute arterial pressure was initially higher and when it declined less after expansion of the donor.

In all correlations but one, the GFR was not significantly related to sodium excretion in the recipient; the one exception (series II) yielded an r value of

TABLE III

Coefficients of Correlation between Period Values in Individual Recipient Rats

Series	$U_{Na}\dot{V}$	TRF_{Na}	vs.	$\frac{t}{c}(U_{Na}\dot{V})$*	$\frac{t}{c}(TRF_{Na})$	vs.
II	*0.3750*‡	*0.3612*	ABP	*0.4605*	*0.4390*	$\frac{t}{c}$(ABP)
(n = 60)			n = 42			
	0.2042	—	GFR	*0.3221*	—	$\frac{t}{c}$(GFR)
V	0.0871	0.1109	ABP	*0.3304*	*0.3498*	$\frac{t}{c}$(ABP)
(n = 90)			n = 60			
	0.0989	—	GFR	0.0244	—	$\frac{t}{c}$(GFR)
VI	*0.275*	*0.230*	ABP	0.228	0.204	$\frac{t}{c}$(ABP)
(n = 90)			n = 60			
	0.126	—	GFR	0.054	—	$\frac{t}{c}$(GFR)

* Ratio between period value after infusion and average control period value.

‡ Italics indicate $P < 0.05$.

0.3221 between (t/c)($U_{Na}\dot{V}$) and (t/c)(GFR), implying that about 10% of the rise in sodium excretion could be accounted for by rise in GFR. Again, however, the natriuresis was accompanied by a fall in GFR more often than with a rise.

DISCUSSION

The volume diuresis and natriuresis resulting from sustained blood volume expansion, associated with significant increase in GFR as well as reduced tubular reabsorption, was greatly augmented over the reaction to simple expansion. The blood of the expanded reinfused animal underwent an alteration, not related to plasma sodium or potassium levels, which caused natriuresis in an isovolemic cross-circulated recipient rat. This alteration was not due to a factor elaborated by the kidneys, as the transfer of natriuresis occurred similarly in recipients paired with nephrectomized donors. It was not the result of accumulation of products normally excreted by the kidney, as natriuresis occurred in the recipient when the expanded donor was permitted to excrete freely, volume expansion being maintained by reinfusion of electrolyte solution. Furthermore, no natriuresis occurred in the recipients of nephrectomized but unexpanded donors (also treated with aldosterone and Pitressin) which would be accumulating metabolites.

As nonspecific metabolite levels did not play an essential role in the recipient renal response, it may be concluded, from comparison of recipient responses to expansion of the donor with those after exchange, that increase in extracellular fluid volume was required to yield the natriuretic change in cross-circulating blood. One obvious change in the character of this blood, in experiments with vascular expansion, was the rise in hematocrit which occurred equally, of course, in both donor and recipient animals. This change is most readily accounted for by hemoconcentration associated with increased outward capillary filtration and it is reasonable to assume that plasma protein concentration also rose. The latter change has been shown to increase proximal tubular reabsorption (1) and an increase in red cell concentration has been reported to decrease renal excretion of water and sodium in the rat (16). There is no reason, then, to attribute to hematocrit elevation a direct effect on the kidney leading to the natriuresis observed in recipient rats as the opposite change might have been expected. A more serious objection, however, could be that the probable elevation of protein concentration led to expansion of the recipient by shift of interstitial fluid into its vascular compartment. If such an effect were large enough to produce a volume response in the recipient, this should have occurred in the simple expansion and cross-circulation experiments reported earlier (20). In the recipients of these experiments, the averaged hematocrit rise (n = 13) was from 45.7 to 51.1%, but no natriuresis or diuresis resulted.

Hemodynamic alterations in the recipient kidneys could have occurred, but not in response to change in arterial perfusion pressure as this showed no systematic rise. The possibility that increase in central venous pressure occurred in the recipient rats cannot be directly excluded as this was not measured; such an increase could be expected if the blood flow increased from the donor to the jugular vein catheter of the recipient. However,

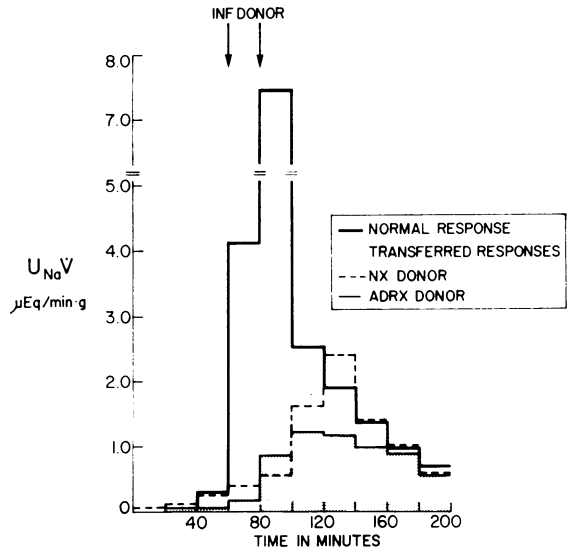

FIGURE 10 Natriuretic response to simple vascular expansion compared to natriuretic responses in recipient rats cross-circulated with donors subjected to sustained vascular expansion. Averaged data in each case derived by subtracting responses to exchange from responses to infusion. Thick outline, intact donor rats cross-circulated (12); dotted line, recipient rats cross-circulated with nephrectomized donors (series II–VII); thin outline, recipient rats cross-circulated with adrenalectomized donors (series V–VI). The stippled response is believed to be due to a humoral factor of non-adrenal origin.

as the recipient arterial pressure did not rise, there was no physical basis for increased flow from the recipient to the donor animal through a shunt with fixed resistance. In maintaining the isogravimetric conditions, flow from donor to recipient must also have been kept constant (by constriction of the arterial line as the donors' arterial pressure rose). There is thus no basis for a direct effect on venous pressure of the recipient animal. Furthermore, if the change in recipient kidney function had been related to a direct effect of vascular pressure changes in the donor, it is improbable that such a change would develop gradually over more than 1 hr. Hence, it seems safe to conclude that even if the recipient natriuresis should prove to have been a consequence of altered renal hemodynamics, this effect was of humoral causation.

Series V and VI were designed to examine whether reduced secretion rates of adrenal cortical or medullary hormones, known to regulate tubular sodium reabsorption or renal hemodynamics, could have accounted for the recipient natriuresis. The sustained expansion of an adrenalectomized donor led to a natriuresis in the recipient resembling that seen in earlier series with donors having intact adrenals; it differed only in reaching a slightly lower peak value slightly earlier. As in series II, the recipient natriuresis occurred without a mean increase in GFR or in ABP. The response to expansion of the adrenalectomized donor rat (data not included) was somewhat smaller than that seen in the donors of series II or III, both the natriuresis and diuresis being depressed. This was probably related to the hypodynamic-state of the cardiovascular system, known to develop very soon after total adrenalectomy (25) and evidenced by a relative arterial hypotension in these experiments. In another unpublished study, we have observed that central venous pressure is also initially low in acutely adrenalectomized rats and rises only transiently on vascular expansion with whole blood. The reduced recipient natriuresis in series V experiments is not therefore unexpected, if it is related to the response of the donor. Assuming that this natriuresis is due to a factor also operating to cause recipient natriuresis when the donor rats are intact, such a factor cannot be related to adrenal gland secretions. Our findings confirm the observations of Tobian, Coffee, and McCrea (21) and the conclusions of other workers (22, 26) that a humoral natriuretic factor exists which is not of adrenal or renal origin.

The results of the exchange experiments, with or without aldosterone and Pitressin treatment (series VIII and VII) suggest that a late and small component of the natriuresis observed, whenever blood was infused, was attributable to dilution of existing levels of aldosterone. The magnitude of this response was comparable to that seen in series VI, before exchange, when an acutely adrenalectomized rat was cross-circulated with a normal one. These effects may be regarded as artefacts of the experimental design, but must, nevertheless, be taken into consideration in assessing the true magnitude of the natriuretic effect due to expansion itself. On the other hand, our data do not exclude the possibility that expansion leads to inhibition of aldosterone secretion and that this, as well as an additional natriuretic factor, exert a combined humoral influence on sodium excretion. Indeed, comparison of the recipient responses shown in Fig. 7 could support this view. The recipient natriuresis demonstrated when adrenalectomized donor rats were expanded with aldosterone-containing blood, still implies that a major part of the humoral component of this response is due to an extra-adrenal factor.

The recipient natriuresis appears to depend on altered tubular sodium handling; the poor correlation with changes in filtered load and in arterial pressure in the recipient implies that these were not primary causes of the recipient response. Our results do not permit speculation on the mechanism of action or source of the natriuretic factor. They may suggest, however, that it is not normally excreted and concentrated in the urine, as the transferred effect was even greater when the donor urine was replaced with electrolyte solution rather than rein-

fused. The factor may therefore be different from the natriuretic activity recently revealed by bioassays of human urine (9, 10).

The results of the present experiments are not necessarily at variance with our earlier negative findings (20). A humoral component of the natriuretic effector mechanism apparently can be revealed with sustained blood volume expansion but not with simple expansion of the donor of a cross-circulated pair. We suggest that the rapid correction of the simple expansion (by the renal response) may prevent the humoral component from reaching a level sufficient to manifest a significant effect in a recipient animal lacking other consequences of vascular expansion. It is possible, as suggested by Lichardus and Ponec (27), who also observed recipient natriuresis using urine reinfused donors, that concentration of plasma protein during the associated diuresis may have cancelled out a positive response in the recipient. Sustained expansion, judging by the resultant rise in hematocrit, must also have lead to concentration of protein. In addition, however, it may allow accumulation of a humoral factor such that there is a slowly developing and modest natriuresis in the recipient in spite of plasma protein concentration.

Finally, the physiological importance of this humoral component of volume natriuresis should be assessed. As it has not been demonstrable, by arterio-arterial or arteriovenous cross-circulation, when the donor rat was simply expanded, the prompt excretion by such animals of the extracellular fluid volume increment may depend mainly on other than humoral mechanisms. The natriuretic response to simple expansion (ref. 12, normal rats but cross-circulated with a partner) may be compared to the recipient natriuresis in series II (normal donors) and series V (adrenalectomized donors); values for $U_{Na}V$, adjusted by subtraction of values obtained in parallel exchange experiments, are plotted in Fig. 10. The humorally mediated natriuresis in series II and V recipients amounts to an appreciable fraction of the total response in the simply expanded animals; indeed, the magnitudes overlap during the 2nd postinfusion hr. Although the initial stimulus of vascular expansion was comparable in the simply expanded rat and in the donors of series II and V, this stimulus was, of course, short lived in the first group and prolonged in the latter two. The duration of the stimulus could be related to the accumulation of a natriuretic factor, possibly accounting for the failure to demonstrate it in recipients cross-circulated with simply expanded donors. Alternatively, the factor could act synergistically with other effector mechanisms and thus be effective in these circumstances at much lower levels than would be required in unexpanded recipients. Finally, it is possible that the changed blood activity observed in these experiments was a reduced level of an antinatriuretic factor (4, 26). If this were true, elevated levels might have far greater physiological effects than reduced levels and could be an important determinant of the sodium retention of disease states.

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