Evidence for a Humoral Mechanism in Volume Expansion Natriuresis

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ABSTRACT The role of a humoral mechanism in the natriuresis induced by volume expansion was evaluated using an isolated dog kidney perfused by a second dog which had been pretreated with desoxycorticosterone acetate (DOCA). Expansion of the perfusion dog with an equilibrated volume of blood from a reservoir, resulted in an increase in $U_{Na}V$ (sodium excretion) from 153.6 ±27.9 (SEM) to 345.5 ±57.8 μEq/min, $P < 0.001$. FE_{Na} (fractional sodium excretion) increased from 3.4 ±0.6 to 8.1 ±1.2%, $P < 0.01$. The natriuresis occurred in the face of a significant decrease in C_{IN}, RBF, and renal arterial pressure, and in the absence of any change in plasma protein concentration or packed cell volume. In a control group of experiments, sodium excretion did not change when the perfusion dog was not volume expanded, although C_{IN} (inulin clearance) and RBF (renal blood flow) decreased to the same degree as in the expanded group. These data support the conclusion that volume expansion of the perfusion dog either stimulated the release of a natriuretic factor or suppressed the release of an antinatriuretic factor which was manifested by an increase in sodium excretion in the isolated kidney.

INTRODUCTION

The role of a natriuretic hormone in the renal regulation of sodium excretion remains a subject of interest and controversy to renal physiologists. Although several laboratories (1–7) have reported the presence of a factor in blood or urine which exhibits natriuretic activity when tested in various biological assay systems, it remains to be established that this factor plays a physiologic role in regulating sodium excretion in the intact animal.

Other investigators have employed techniques involving cross circulation (8–12) or perfusing an isolated kidney (13, 14) to study this question, but the results have been variable and at times conflicting. In two studies (11, 12), the authors concluded that the evidence did not support a humoral mechanism. In other studies (9, 10) in which a significant natriuresis was observed the influence of compositional changes in the blood, physical factors, or neurogenic stimuli was not adequately excluded. Moreover, there appears to be some conflict concerning how the humoral factor exerts its natriuretic effect. In several studies (8, 13, 14), the natriuresis paralleled an increase in renal blood flow and glomerular filtration rate suggesting that the factor promoted a natriuresis through a vasodilating effect on the kidney. However, this conclusion is difficult to reconcile with those studies purporting to show a direct effect of this factor on sodium transport in isolated membranes (2, 3, 5) or tubule fragments (4, 7).

In this paper we report studies of volume expansion natriuresis using an isolated kidney preparation in which it was possible to exclude compositional, physical, and neurogenic factors from contributing to the response.

METHODS

Experiments were performed on mongrel dogs weighing 15–30 kg. One dog served as the kidney donor; the second dog was used to perfuse the isolated kidney. The donor animals were fed a standard kennel ration whereas the perfusion dog was pretreated with 10 mg of desoxycorticosterone acetate (DOCA; Organon Inc., West Orange, N. J.) in oil given intramuscularly each day for an average of 11

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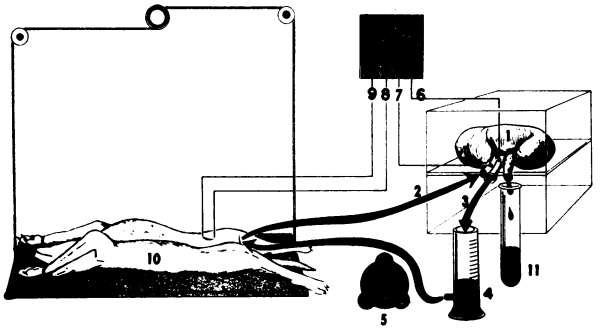

FIGURE 1 Diagram of the isolated kidney preparation. The isolated kidney (1) is placed in a constant temperature-humidity chamber where it is perfused with blood from the femoral artery (2) of a second dog (10). Renal venous blood (3) flows by gravity into a reservoir (4) from which it is pumped (5) to the femoral vein of the perfusion animal. The perfusion animal (10) rests on an adjustable platform and by raising or lowering the platform with respect to the isolated kidney, renal arterial pressure in the isolated kidney can be regulated. Pressure in the femoral artery (9) and vein (8) and renal artery (7) and vein (6) are monitored with pressure transducers. Urine (11) is collected from a catheter secured in the ureter.

days including the morning of the study. In addition, the daily diet of these animals was supplemented with 75–150 mEq of NaCl and 40–80 mEq of KCl. On the morning of the study, the animals were anesthetized with either sodium pentobarbital or sodium pentothal, 30 mg/kg, given intravenously with supplemental doses as required. An endotracheal tube was inserted and respirations were regulated with a Harvard respirator adjusted to maintain the arterial pH between 7.35 and 7.45.

Preparation of the isolated kidney. The donor kidney was mobilized through a midline abdominal incision by carefully dissecting the perirenal fat and connective tissue and freeing the renal artery and vein from the hilum to the aorta and inferior vena cava respectively. The ureter was dissected free and cannulated with a small polyethylene catheter. Systemic anticoagulation was produced in both animals by administering heparin, 2 mg/kg, intravenously after which the renal artery and vein of the donor kidney were clamped and severed. A plastic cannula was secured in the renal artery and perfusion of the kidney was accomplished with blood delivered from the femoral artery of the perfusion animal through wide bore silastic tubing. A second catheter was secured in the renal vein and returned venous blood by free flow to a polyethylene reservoir (1300 ml capacity) from which it was pumped (Holter roller pump, model RE 161; Extracorporeal Medical Specialties Inc., Mt. Laurel Township, N. J.) to the femoral vein of the perfusion animal at a rate adjusted to maintain the blood level in the reservoir constant. The isolated kidney was then placed in a constant temperature-humidity chamber maintained at 38°C and 100% humidity. The ischemia time, defined as the time from clamping the renal artery to the time when blood flow was reestablished, averaged 2 min and 20 sec and never exceeded 3 min. The perfusion animal rested on an adjustable platform and by raising or lowering the platform a hydrostatic pressure equal to the difference between the height of the animal and the isolated kidney could be added to or subtracted from the femoral arterial pressure supplied to the kidney. In this manner it was possible to maintain renal arterial pressure constant in the face of changes in the perfusion animal's systemic arterial pressure. Renal venous pressure was set by adjusting the level of the venous outflow tubing. A diagram of the isolated kidney preparation is shown in Fig. 1.

Experimental protocol. In all experiments, the reservoir was filled with 5% albumin in 0.9% saline in an amount equal to 35 ml/kg plus 200 ml. The latter volume represents the basal volume maintained in the reservoir after volume expansion experiments. After perfusion of the isolated kidney was established, the perfusion animal received a priming dose of inulin followed by a constant infusion of inulin in 0.9% saline at 1.0 ml/min. Aqueous Pitressin (Parke, Davis & Company, Detroit, Mich.) was added to the infusion to deliver 0.5 mU/kg per min. A minimum of 60 min was allowed for equilibration between the perfusion animal's blood and the volume in the reservoir and for stabilization of kidney function. In pilot studies, it was determined that sodium excretion in the isolated kidney had usually stabilized by 60 and almost always by 90 min from the time blood flow had been reestablished, and that this point could be identified when two consecutive 15-min urine collections varied by less than 10%. Accordingly, the first period which satisfied this condition was taken as the control period. In 10 experiments from group I and 11 experiments from group II, the control urine collection was started within 90 min of reestablishing renal blood flow.

Group I consisted of 11 experiments in which the function of the isolated kidney was observed with respect to time. In nine experiments, observations were recorded during five consecutive 30-min periods. Renal arterial pressure was maintained constant at about 115 mm Hg throughout the experiment; renal venous pressure was set at about 5 mm Hg and once set was not altered during the remainder of the experiment.

Group II consisted of 14 experiments. After kidney function had stabilized as defined above, either a single 30-min control urine or two 15-min control urines were collected after which the perfusion animal was expanded with the equilibrated solution from the reservoir amounting to 35 ml/kg over a 30-min period. In two experiments (No. 9, 12) a series of control urines were collected to clearly demonstrate that a steady state existed before volume expansion. The volume expansion stimulus was maintained by replacing urine losses with equal volumes of 0.9% saline; in several experiments (No. 11–14) urine losses were replaced with hypotonic saline (100–120 mEq/liter). In general the natriuretic response in the isolated kidney was evident within 30 min and maximal 60–90 min after initiating volume expansion of the perfusion animal. Accordingly, the urine collected during the latter interval, either a single 30-min collection or two 15-min collections, was used as the experimental period. During the control period, renal arterial pressure was maintained at about 115 mm Hg but during the period of volume expansion, it was maintained at about 5 mm Hg below the control level.

Data collection. Renal arterial and venous pressures in the isolated kidney were monitored with Statham pressure transducers, model 23 AA, (Statham Instruments, Inc.) connected to the respective catheters by means of a t-tube positioned about 8 cm from the kidney. The perfusion dog's systemic-arterial and venous pressures were monitored from catheters secured in a femoral artery and vein. All recordings were made on an 8-channel Beckman Dynograph recorder (Beckman Instruments, Inc.).

Urine from the isolated kidney was collected from a fine polyethylene catheter inserted in the ureter; urine from the perfusion dog was collected from a Foley catheter inserted in the bladder. Arterial blood samples were withdrawn from the femoral arterial catheter at the midpoint of each urine collection.

All blood and urine samples were analyzed for sodium, potassium, and inulin. In addition, packed cell volume (PCV)[1] and plasma protein concentration was determined on all blood samples. Sodium and potassium were measured with an Instrumentation Laboratories flamephotometer. Inulin was measured by the method of Schreiner (15) and plasma protein by the method of Gornall, Bardawill, and David (16). PCV was determined using a microhematocrit centrifuge. Renal blood flow (RBF) was measured directly by timing the flow from the renal vein into a graduated cylinder. Filtration fraction (FF) was determined from the formula $FF = C_{IN}/RPF$ when RPF equals renal plasma flow and is calculated according to the formula $RPF = RBF \times (1\text{–}0.95\ PCV)$. Renal vascular resistance (RVR) was calculated according to the formula $RVR = (P_{RA} - P_{RV})/RBF$ expressed in peripheral resistance units (PRU) of millimeters of mercury per milliliters per minute. At the conclusion of each study, the isolated kidney was drained of blood, stripped of its capsule, and weighed.

Student's *t* test was employed in the statistical analysis of paired data within each group and mean data between groups (17).

RESULTS

The data in the text and figures are expressed as the mean ±SEM.

Group I—control group. Function of the isolated kidney with respect to time is illustrated in Fig. 2. During five consecutive 30-min periods, absolute sodium excretion ($U_{Na}V$), fractional sodium excretion (FE_{Na}), and C_{IN} did not change significantly from the initial period although a decline in both $U_{Na}V$ and C_{IN} was evident by the fifth period suggesting that with time a further decrease in these parameters would have occurred. A significant decrease in RBF was evident by the second period and by the fifth period had declined to 74 ±3% of the level in period 1. The fall in RBF was associated with a reciprocal increase in renal vascular resistance (RVR). Details of an experiment from group I are given in Table I.

Group II—experimental group. Details of two experiments are given in Tables II and III. In the latter experiment, volume expansion was performed only after a steady state had been clearly documented. Table IV summarizes the data from all experiments in group II. $U_{Na}V$ increased from 153.6 ±27.9 μEq/min during control to 345.5 ±57.8 μEq/min during volume expansion,

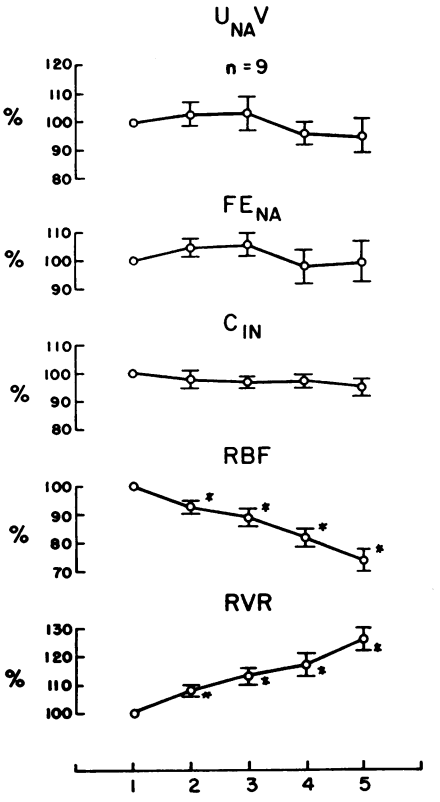

FIGURE 2 Stability of function in the isolated kidney in group I over 5 consecutive 30-min periods. The data are expressed as the per cent variation (mean ±SEM) from the first period which was taken to be 100%. $U_{Na}V$ equals absolute sodium excretion, FE_{Na} equals fractional excretion of sodium, C_{IN} equals inulin clearance, RBF equals renal blood flow, RVR equals renal vascular resistance, * means significantly different from period 1, $P < 0.01$.

$P < 0.001$). P_{Na} changed in a variable manner in individual experiments but there was no change in the mean for the group. In addition, there was no relation between the change in P_{Na} and the magnitude of the natriuresis. Filtered sodium (F_{Na}) decreased in most experiments with a significant decrease in F_{Na} occurring for the group, $P < 0.01$). The decrease in F_{Na} was due primarily to the fall in C_{IN} from 30.7 ±1.6 ml/min during control to 28.6 ±1.9 ml/min during the experimental period. RBF fell in all experiments with a mean decrease of 50 ml/min for the group, $P < 0.001$, associated with a significant increase in RVR, $P < 0.005$, and filtration fraction, $P < 0.05$. Renal arterial pressure (P_{RA}) measured 114 ±1 mm Hg during control and was purposely reduced to 109 ±1 mm Hg during volume expansion to exclude changes in this variable from influencing the response. Renal arterial pulse pressure changed in a variable manner but without a significant change in the mean for the group. Pulse pressure measured 32 ±2

[1] *Abbreviations used in this paper:* DOCA, desoxycorticosterone; FE_{Na}, fractional sodium excretion; FF, filtration fraction; F_{Na}, filtered sodium; PCV, packed cell volume; P_{Na}, plasma sodium; P_{RA}, renal arterial pressure; PRU, peripheral resistance units; RBF, renal blood flow; RPF, renal plasma flow; RVR, renal vascular resistance; $U_{Na}V$, absolute sodium excretion.

TABLE I

*Function of the Isolated Kidney with Time in the Absence of Volume Expansion in Experiment 6, Group I**

Time	$U_{Na}V$	P_{Na}	F_{Na}	FE_{Na}	C_{IN}	RBF	P_{RA}	P_{RV}	RVR	FF	PCV	Plasma protein
min	*μEq/min*	*mEq/liter*	*μEq/min*	%	*ml/min*	*ml/min*	*mm Hg*	*mm Hg*	*PRU*		%	*g/100 ml*
−120	Prepare kidney in donor dog for removal; prepare second dog for perfusing the isolated kidney.											
−3–0	Remove kidney; reestablish perfusion by connecting to femoral artery of second dog.											
0–90	Give inulin prime (1000 mg) and begin constant infusion of inulin (20 mg/min) and aqueous Pitressin (125 mU/min) in 0.9% saline at 1.0 ml/min. Allow time for equilibration of solutions and stabilization of the isolated kidney.											
90–120	58.9	155	2759	2.1	17.8	146	114	4	0.75	0.19	39	4.6
120–150	59.0	155	2868	2.1	18.5	144	110	4	0.74	0.21	39	5.0
150–180	62.1	152	2645	2.3	17.4	143	114	4	0.77	0.20	39	4.8
180–210	62.4	153	2892	2.2	18.9	127	113	2	0.87	0.24	39	4.9
210–240	63.5	152	2873	2.2	18.9	114	114	2	0.98	0.26	39	4.9

* $U_{Na}V$, sodium excretion; P_{Na}, plasma sodium; F_{Na}, filtered sodium; FE_{Na}, fractional sodium excretion; C_{IN}, inulin clearance; RBF, renal blood flow; P_{RA}, renal arterial pressure; P_{RV}, renal venous pressure; RVR, renal vascular resistance; FF, filtration fraction, PCV, packed cell volume.

mm Hg during control and 35 ±2 mm Hg during volume expansion, $P > 0.3$. There were no significant changes in packed cell volume (PCV) or plasma protein concentration.

Renal response of the perfusion dog to volume expansion. Fractional sodium excretion (FE_{Na}) in the perfusion dog increased from 2.0 ±0.6% during control to 12.5 ±2.4% during volume expansion, $P < 0.001$. The natriuresis was associated with an increase in both C_{IN} and systemic arterial pressure. C_{IN} increased from 84.3 ±5.5 to 91.5 ±4.6 ml/min, $P < 0.05$, and systemic arterial pressure increased from 111 ±5 to 149 ±5 mm Hg, $P < 0.001$. However, as can be appreciated from Fig. 3, there was no significant correlation between the magnitude of increase in FE_{Na} from the isolated kidney and that from the perfusion dog, $r = 0.126$, $P > 0.1$.

Comparison between groups I and II. To permit comparison of the two groups, the fourth period from the individual experiments in group I was identified as the experimental period since it represented the same time interval from the control period as the experimental period in group II. Table V summarizes the data from individual experiments in group I.

Fig. 4 compares the function of the isolated kidney in groups I and II, after correcting for differences in kidney weight. Control FE_{Na} was not statistically different in the two groups measuring 2.9 ±0.5% in group I and 3.4 ±0.6% in group II, $P > 0.5$. During the experimental period FE_{Na} did not change, measuring 2.7 ±0.4%, whereas after volume expansion in group II FE_{Na} increased to 8.1 ±1.2%. C_{IN} per gram kidney was the same in both groups, measuring 0.56 ±0.04 and 0.62 ±0.04 ml/min per g during the control period,

TABLE II

*Response of the Isolated Kidney to Volume Expansion in Experiment 2, Group II**

Time	$U_{Na}V$	P_{Na}	F_{Na}	FE_{Na}	C_{IN}	RBF	P_{RA}	P_{RV}	RVR	FF	PCV	Plasma protein
min	*μEq/min*	*mEq/liter*	*μEq/min*	%	*ml/min*	*ml/min*	*mm Hg*	*mm Hg*	*PRU*		%	*g/100 ml*
−120	Prepare kidney in donor dog for removal; prepare second dog for perfusing the isolated kidney.											
−2–0	Remove kidney; reestablish perfusion by connecting to femoral artery of second dog.											
0–75	Give inulin prime (840 mg); begin constant infusion of inulin (22 mg/min) and aqueous Pitressin (105 mU/min) in 0.9% saline at 1.0 ml/min. Allow time for equilibration of solutions and stabilization of the isolated kidney.											
75–90	58.0	148	4632	1.3	31.3	351	112	6	0.30	0.11	23	4.7
90–105	67.7	148	4499	1.5	30.4	331	112	6	0.32	0.12	23	4.7
105–135	Infuse equilibrated blood (35 ml/kg) from reservoir; maintain P_{RA} below control level.											
135–165	267.8	150	4905	5.5	32.7	291	110	6	0.36	0.14	24	4.8
165–180	302.5	152	4727	6.4	31.1	274	108	5	0.38	0.14	23	4.9
180–195	311.6	152	4545	6.9	29.9	268	110	5	0.38	0.14	23	4.9

* See Table I for explanation of abbreviations.

TABLE III

*Response of the Isolated Kidney to Volume Expansion after an Established Steady State in Experiment 12, Group II**

Time	$U_{Na}V$	P_{Na}	F_{Na}	FE_{Na}	C_{IN}	RBF	P_{RA}	P_{RV}	RVR	FF	PCV	Plasma protein
min	*μEq/min*	*mEq/liter*	*μEq/min*	%	*ml/min*	*ml/min*	*mm Hg*	*mm Hg*	*PRU*		%	*g/100 ml*
−120	Prepare kidney in donor dog for removal; prepare second dog for perfusing the isolated kidney.											
−2.5–0	Remove kidney; reestablish perfusion by connecting to femoral artery of second dog.											
0–60	Give inulin prime (848 mg); begin constant infusion of inulin (26 mg/min) and aqueous Pitressin (106 mU/min) in 0.9% saline at 1.0 ml/min. Allow time for equilibration of solutions and stabilization of the isolated kidney.											
60–120	132.7	148	3374	3.9	22.8	170	114	6	0.64	0.17	24	5.1
120–150	102.7	148	3345	3.1	22.6	147	112	5	0.73	0.21	28	5.4
150–180	101.1	149	3442	2.9	23.1	150	113	5	0.72	0.21	27	5.5
180–210	104.0	149	3412	3.0	22.9	147	113	5	0.73	0.21	27	5.2
210–240	111.6	149	3725	3.0	25.0	144	110	5	0.73	0.23	26	5.2
240–270	Infuse equilibrated blood (35 ml/kg) from reservoir; maintain P_{RA} below control level.											
270–300	182.5	148	3212	5.7	21.7	96	105	4	1.05	0.32	30	5.0
300–330	209.0	148	3271	6.4	22.1	95	105	4	1.06	0.33	31	4.8

* See Table I for explanation of abbreviations.

$P > 0.3$, and 0.53 ±0.03 and 0.57 ±0.04 ml/min per g during the experimental period, $P > 0.4$, in groups I and II respectively. Similarly there was no difference in RBF per gram kidney between the groups in either period. Control RBF measured 4.2 ±0.3 ml/min per g in group I and 4.7 ±0.3 ml/min per g in group II, $P > 0.2$ and fell to a similar degree during the experimental period, measuring 3.4 ±0.2 and 3.7 ±0.3 ml/min per g, $P > 0.3$, in groups I and II respectively.

Although RVR was higher in group I than in group II, this difference is more apparent than real and disappears when RVR is corrected for difference in kidney weight between the two groups. Thus, calculating RVR according to the formula $RVR = (P_{RA}-P_{RV})/RBF$ per gram kidney indicates that control RVR was 26.9 ±2.2 mm Hg/ml/min per g kidney in group I and 23.8 ±1.6 mm Hg/ml/min per g kidney in group II, $P > 0.3$. In the experimental period, RVR increased to a similar extent in both groups measuring 33.5 ±2.6 and 29.7 ±2.1 mm Hg/ml/min per g kidney, $P > 0.3$, in groups I and II respectively.

There were no significant differences between the two

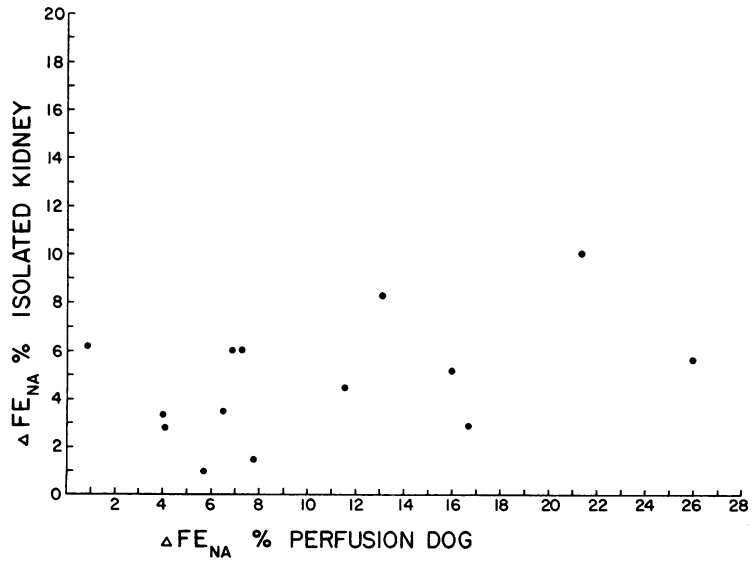

FIGURE 3 The change in fractional sodium excretion (FE_{Na}) in the isolated kidney is plotted against the simultaneous change in FE_{Na} in the perfusion dog from group II.

TABLE IV

Summary of Data from the Isolated Kidney in Group II before (C) and

Experiment	$U_{Na}V$		P_{Na}		F_{Na}		FE_{Na}		C_{IN}		RBF	
	μEq/min		*mEq/liter*		*μEq/min*		%		*ml/min*		*ml/min*	
	C	E	C	E	C	E	C	E	C	E	C	E
1	37.3	94.6	156	153	5959	5783	0.6	1.6	38.2	37.8	344	277
2	62.8	307.0	148	152	4566	4636	1.4	6.6	30.8	30.5	341	271
3	51.6	129.4	150	151	4095	4145	1.3	3.1	27.3	27.4	266	237
4	250.6	519.9	157	159	4914	4802	5.1	10.8	31.3	30.2	261	209
5	343.1	748.6	143	147	4290	4160	8.0	18.1	30.0	28.3	183	191
6	304.4	406.6	149	155	4574	4123	6.6	9.9	30.7	26.6	230	191
7	126.0	252.0	148	154	4292	3342	3.0	7.5	29.0	21.7	158	124
8	18.0	203.7	142	148	3096	3078	0.6	6.6	21.8	20.8	175	162
9	175.8	381.0	153	153	3779	3534	4.6	10.8	24.7	23.1	167	153
10	102.7	172.8	152	158	3846	3065	2.7	5.6	25.3	19.4	175	116
11	282.8	782.8	143	142	5663	5836	5.0	13.3	39.6	41.1	299	196
12	111.6	209.0	149	148	3725	3271	3.0	6.4	25.0	22.1	144	95
13	153.2	434.0	148	143	4884	4776	3.2	9.1	33.0	33.4	269	137
14	129.9	195.1	145	143	6206	5420	2.1	3.6	42.8	37.9	329	292
Mean	153.6	345.5	149	150	4563	4284	3.4	8.1	30.7	28.6	239	189
±SEM	27.9	57.8	1	1	239	257	0.6	1.2	1.6	1.9	19	17
P	<0.001		NS		<0.01		<0.01		<0.01		<0.001	

* See Table I for explanation of abbreviations.

groups in either period with regard to FF, PCV, and plasma protein concentration.

DISCUSSION

These experiments were designed to evaluate the possible role of a humoral mechanism in the natriuretic response to extracellular volume expansion using the technique of cross circulation with a completely isolated kidney.

In view of the experience of other investigators (18–20) that function in the isolated kidney may deteriorate with time accompanied by a progressive increase in sodium excretion, it was necessary to define the functional characteristics of the isolated kidney preparation employed in our experiments. Group I speaks to this point. As can be appreciated from Fig. 2, sodium excretion did not increase as a function of time but tended to decrease by the fourth and fifth period in association with a slight decline in C_{IN}. In contrast, a progressive fall in RBF was evident as early as the second period

TABLE V

Summary of Data from the Isolated Kidney in Group I

Experiment	$U_{Na}V$		P_{Na}		F_{Na}		FE_{Na}		C_{IN}		RBF	
	μEq/min		*mEq/liter*		*μEq/min*		%		*ml/min*		*ml/min*	
	C	E	C	E	C	E	C	E	C	E	C	E
1	32.8	36.3	143	145	2841	2682	1.2	1.4	19.9	18.5	171	153
2	104.5	118.4	146	150	4263	3915	2.5	3.0	29.2	26.1	157	117
3	171.1	117.7	148	151	3611	3775	4.8	3.1	24.4	25.0	139	101
4	108.0	106.7	133	131	2653	2813	4.1	3.8	20.0	21.5	127	124
5	25.7	27.4	143	139	2268	1942	1.1	1.4	15.9	14.1	135	111
6	58.9	62.4	155	153	2764	2884	2.1	2.2	17.8	18.9	146	127
7	112.6	100.6	149	149	2295	2041	4.9	4.9	15.4	13.7	143	107
8	20.8	25.7	154	155	4317	3829	0.6	0.7	23.7	24.7	187	179
9	177.3	182.0	151	152	3428	3876	5.2	4.7	22.7	25.5	194	141
10	63.1	67.0	148	151	4484	4047	1.4	1.7	30.3	26.8	269	193
11	148.0	103.9	148	149	3804	3412	3.8	3.1	25.7	22.9	190	150
Mean	93.0	86.2	147	148	3339	3201	2.9	2.7	22.3	21.6	169	137
±SEM	17.2	14.4	2	2	247	232	0.5	0.4	1.5	1.4	12	9
P	NS		NS		NS		NS		NS		<0.001	

* See Table I for explanation of abbreviations.

*after (E) Extracellular Volume Expansion of the Perfusion Dog**

P_{RA}		P_{RV}		RVR		FF		PCV		Plasma protein		Kidney weight
mm Hg		*mm Hg*		*PRU*				%		*g/100 ml*		*g*
C	E	C	E	C	E	C	E	C	E	C	E	
113	109	6	5	0.31	0.38	0.15	0.19	29	28	4.9	4.7	66
112	109	6	5	0.31	0.38	0.12	0.14	23	23	4.7	4.9	71
122	116	8	7	0.33	0.46	0.15	0.16	33	27	4.7	4.7	68
113	108	4	3	0.42	0.50	0.16	0.18	25	22	3.3	2.6	58
112	108	6	6	0.58	0.53	0.22	0.19	26	25	4.1	4.2	40
120	111	6	4	0.50	0.56	0.18	0.19	28	28	4.7	4.8	40
111	111	5	5	0.67	0.85	0.24	0.24	24	27	4.5	5.0	35
112	110	7	7	0.60	0.64	0.17	0.18	28	32	5.2	4.9	55
108	105	2	2	0.63	0.67	0.19	0.20	22	24	5.1	4.8	47
115	110	4	3	0.63	0.92	0.20	0.24	30	33	4.8	4.9	46
115	110	5	3	0.38	0.55	0.16	0.26	20	21	4.3	3.9	46
110	105	5	4	0.73	1.06	0.23	0.33	26	31	5.2	4.8	46
113	110	5	2	0.40	0.79	0.16	0.32	25	26	4.6	4.5	35
120	112	5	5	0.35	0.37	0.17	0.18	26	29	4.3	4.4	54
114	109	5.3	4.4	0.49	0.61	0.18	0.21	26	27	4.6	4.5	51
1	1	0.4	0.4	0.04	0.06	0.01	0.01	1	1	0.1	0.2	3
<0.001		<0.005		<0.005		<0.05		NS		NS		

and reflected a significant increase in RVR. Since filtration fraction also increased (Table V), part of the rise in resistance presumably occurred at the efferent arteriole. These functional characteristics are distinctly different from those reported by other investigators (18–20) who in studies of the pump perfused isolated kidney observed a progressive increase in sodium excretion in association with an increase in blood flow to supranormal levels. Based on the group I experiments, we feel confident in concluding that a significant increase in sodium excretion in the isolated kidney reflects a natriuretic intervention and not a spontaneous change.

In group II, expanding the blood volume of the perfusion dog with equilibrated blood from the reservoir resulted in a significant natriuresis in the isolated kidney (Table IV), whereas in the nonvolume expanded animals in group I no change in sodium excretion occurred over the same time interval (Table V). Since no significant differences existed between the two groups in those variables known to influence sodium excretion, we

*in which the Perfusion Dog was not Volume Expanded**

P_{RA}		P_{RV}		RVR		FF		PCV		Plasma protein		Kidney weight
mm Hg		*mm Hg*		*PRU*				%		*g/100 ml*		*g*
C	E	C	E	C	E	C	E	C	E	C	E	
120	123	5	5	0.67	0.77	0.16	0.18	31	36	4.7	5.0	64
113	112	4	3	0.69	0.93	0.25	0.29	28	25	4.9	4.5	47
113	115	4	2	0.78	1.12	0.25	0.35	32	30	4.7	4.5	35
112	111	6	6	0.83	0.85	0.21	0.23	26	26	4.6	4.4	40
109	110	7	6	0.76	0.94	0.18	0.19	36	34	4.0	3.8	30
114	113	4	2	0.75	0.87	0.19	0.24	39	39	4.6	4.9	36
114	115	4	3	0.77	1.05	0.15	0.17	28	26	5.2	4.6	34
106	105	4	3	0.54	0.57	0.23	0.20	35	34	4.5	4.8	41
108	108	3	1	0.54	0.76	0.15	0.18	22	22	4.7	5.3	47
102	98	4	3	0.36	0.49	0.20	0.18	22	22	4.7	3.8	47
115	112	6	5	0.57	0.71	0.18	0.20	25	26	5.2	5.4	35
111	111	4.6	3.5	0.66	0.82	0.20	0.22	29	29	4.7	4.6	41
1	2	0.4	0.5	0.04	0.06	0.01	0.01	2	2	0.1	0.2	3
NS		<0.001		<0.001		<0.05		NS		NS		

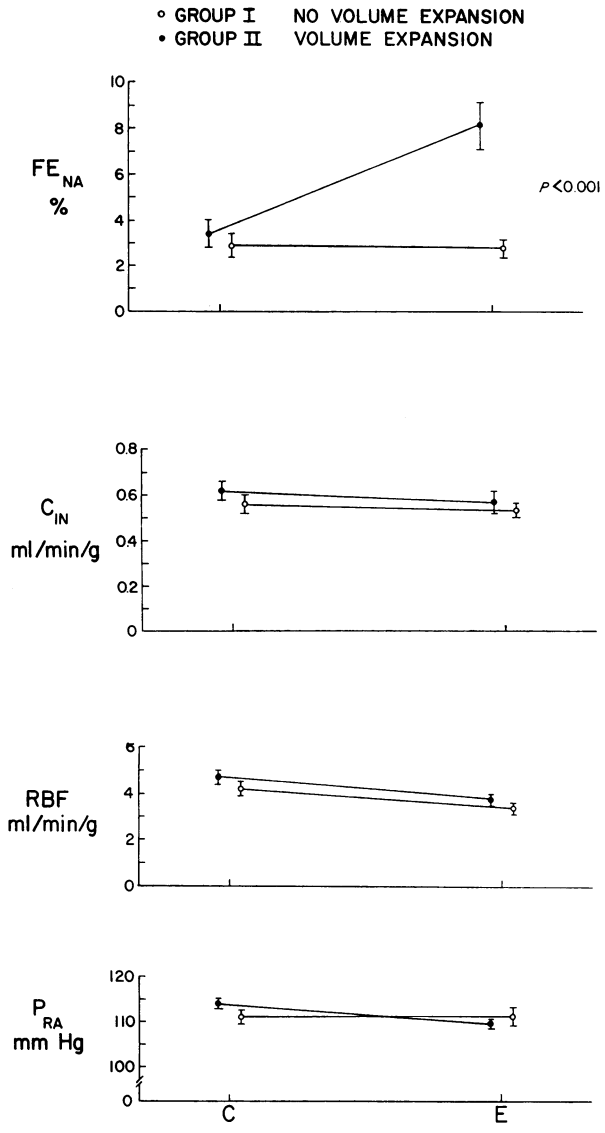

FIGURE 4 Comparison between groups I and II of function in the isolated kidney during the control (C) and experimental (E) periods. FE_{Na} equals fractional sodium excretion, C_{IN} equals inulin clearance factored by kidney weight, RBF equals renal blood flow factored by kidney weight, P_{RA} equals renal arterial pressure.

conclude that the natriuresis in the isolated kidney was a function of volume expanding the perfusion dog.

The experimental design employed in this study permitted us to either control or eliminate those variables previously implicated as possible factors contributing to the natriuresis of volume expansion. Since the kidney was completely isolated, the influence of neurogenic stimuli mediated through the renal nerves could be disregarded. The perfusion dog was preloaded with DOCA and received a continuous infusion of vasopressin which obviated a decrease in the circulating levels of these hormones as a posible factor in the natriuresis. Arterial pressure in the isolated kidney was carefully regulated and purposely reduced in group II thus eliminating this variable as a factor in the response. The problem of compositional changes in the blood including packed cell volume and plasma protein concentration was avoided by expanding with an equilibrated volume of blood. Finally, although plasma sodium concentration increased in some studies, there was no correlation between the change in plasma sodium concentration and the change in sodium excretion. Therefore, we conclude that extracellular volume expansion of the perfusion dog either stimulated the release of a natriuretic factor or suppressed the release of an antinatriuretic factor the net result of which was manifested by an increase in sodium excretion in the isolated kidney.

As illustrated in Fig. 3, the natriuresis in the perfusion dog exceeded that in the isolated kidney. The greater natriuresis probably reflects the added influence of an elevated renal-perfusion pressure and glomerular filtration rate in the perfusion dog whereas in the isolated kidney we prevented changes in these variables so that presumably only the humoral mechanism was operating. The fact that this mechanism accounted for an increase in fractional sodium excretion of 4.7% from the isolated kidney demonstrates that it could play an important physiologic role in the renal regulation of sodium balance.

Although the present experiments do not permit any definite conclusions regarding how this factor exerts its natriuretic effect, the data do warrant some comment. The increase in sodium excretion was associated with a significant decrease in the filtered load of sodium indicating that the natriuresis was primarily the result of a decrease in the tubular reabsorption of sodium which could reflect either a direct effect of this factor on tubular sodium transport or an indirect effect exerted through an alteration in intrarenal hemodynamics.

In the volume expansion studies of Lichardus and Pearce (13), Bahlman, McDonald, Ventom, and de Wardener (8), and Tobian, (14) glomerular filtration rate and renal blood flow increased significantly leading these authors to propose that the humoral factor might have mediated the natriuresis through a renal vasodilating action. Earley and Daugharty (21) have recently reviewed current concepts of how an increase in renal blood flow or increase in net filtration pressure at the peritubular capillaries might depress tubule sodium reabsorption. In the present experiments, however, a natriuresis occurred in the face of a significant decrease in renal blood flow and significant increase in renal

vascular resistance and filtration fraction, changes which according to this theory would decrease net filtration pressure and promote tubule sodium reabsorption. These observations are difficult to reconcile with a humoral factor which exerts its natriuretic effect through a vasodilating action.

A second hypothesis is that the factor caused a redistribution of renal blood flow and/or filtrate to "sodium-wasting" superficial cortical nephrons. This concept is derived in part from studies on renal blood flow distribution in dogs by Barger and colleagues (22–24) as well as the observations of Horster and Thurau (25) that redistribution of filtrate from juxtamedullary to superficial cortical nephrons occurs in rats fed a high sodium diet. However, there are conflicting reports as to whether redistribution of renal blood flow and glomerular filtrate occurs in the dog (26, 27). Nevertheless, if it is assumed, a similar mechanism does occur in the dog and would promote a natriuresis, then it would appear necessary that this factor or factors must have a highly specific vasoconstricting as well as vasodilating action on separate segments of the renal vascular tree in order to effect redistribution of filtrate to superficial nephrons at a time when RBF is decreasing.

The third alternative is that the factor exerts a direct effect on tubule sodium transport. This hypothesis would be most consistent with the findings of other investigators that extracts or dialysates of plasma from volume expanded animals exert a direct effect on sodium transport in isolated membrane systems (2–4, 7). The final answer, however, must await isolation of this factor and characterization of its effect on tubule sodium transport and intrarenal hemodynamics.

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