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Evidence from Cross Circulation Studies for a Humoral Mechanism In the Natriuresis of Saline Loading. (30965)

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Sodium excretion by the kidney is dependent upon both the amount of sodium filtered at the glomerulus and renal tubular reabsorption. Although a rise in the filtered load of sodium (F_{Na}) plays an important role in the natriuresis resulting from saline loading, experiments by de Wardener *et al*(1) demonstrated that an increase in sodium excretion occurs even when the glomerular filtration rate (GFR) is reduced. This dissociation between urinary sodium excretion and filtered sodium load was subsequently confirmed by Levinsky and Lalone(2). Rector and coworkers(3) provided indirect evidence that decreased proximal tubular reabsorption of sodium occurs during saline loading. Direct confirmation of decreased proximal reabsorption of fluid in saline diuresis was recently reported by Dirks, Cirksena and Berliner(4) using micropuncture methods in the dog.

It has been suggested from cross circulation experiments by de Wardener and associates(1,5) and from studies in an isolated kidney preparation by Lichardus and Pearce(6) that a humoral sodium excreting factor contributes to the natriuresis of saline loading but the evidence presented failed to exclude the possibility that an increase in GFR produced the increase in sodium excretion.

In the present study, blood was cross circulated from saline loaded donor dogs through normal recipients in which a reduction in GFR was produced by aortic constriction immediately above both renal arteries. In 9 of 12 experiments an increase in sodium excretion occurred in the recipient in the presence of a decreased filtered sodium, a finding which suggests a decrease in fractional renal tubular reabsorption by a humoral mechanism.

Material and methods. The experiments were performed in 15-25 kg female mongrel dogs. Blood from each pair of dogs was cross matched to insure compatibility. All dogs were fed a synthetic diet containing 60 mEq of sodium and 18 mEq of potassium per day. The last feeding was 20-24 hours before the experiment but water was allowed *ad libitum*. The donor dogs received 15 mg of desoxycorticosterone acetate in oil intramuscularly per day for 2 days prior and 30-60 minutes before the experiment was begun. The animals were anesthetized with sodium pentobarbital 30 mg/kg intravenously and endotracheal intubation performed.

Two series of animals were studied. In Series I blood was cross circulated from normal non-saline loaded donors through normal recipients. In Series II the blood was cross circulated from saline loaded donors. Saline loading of the donor was accomplished by infusing 1 liter of normal saline intravenously as rapidly as possible and continu-

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ing the saline infusion at approximately 9 ml/min. In Series I two 30-minute control periods of observation, four 30-minute periods during cross circulation and two 30-minute recovery periods were made. In Series II, after a rate of urine flow in the donor of at least 5 ml/min was accomplished, studies were conducted during 2 control periods in both dogs. Subsequently, observations were made for one or two 30-minute periods during cross circulation after which the aorta was constricted above both renal arteries to reduce the GFR and 2-4 additional 30-minute periods of study were conducted. After cessation of cross circulation recovery observations were made. No renal clearance determinations were performed during the 15 minutes after the commencement and end of cross circulation to allow washout of the urinary dead space. The antihistaminic compound, diphenhydramine hydrochloride, Benadryl (50 mg) was given intramuscularly 15-40 minutes before the beginning of the control observations to all dogs in Series I and to 3 pairs of animals in Series II to prevent non-specific blood reactions(7).

Blood was cross circulated between the recipient and donor at a rate of 70-80 cc/min through polyvinyl tubing from femoral artery to artery and returned from femoral vein to vein. The rate and constancy of transfer of blood between the dogs was maintained by a precalibrated Harvard peristaltic pump, Model No. 500-1200, and by using a crossover in the tubing. By this maneuver blood was alternated through either side of the pump every 10 minutes and the direction of flow reversed every 10 minutes. Thus, minor imbalances on either side of the pump were corrected. Arterial pressures were recorded continuously from the brachial artery of the donor and the brachial and femoral arteries of the recipient by Statham pressure transducers and a Sanborn recording system.

Aortic constriction was produced above the renal arteries in the recipient animal by tightening a ligature which had been placed around the aorta 60-90 minutes before the commencement of the control periods. The aorta was constricted until the femoral artery pressure had fallen to 80-110 mm Hg.

Renal clearances of creatinine (Cr) and para-aminohippurate (PAH) were measured in the recipient by standard techniques. Donor and recipient animals were given priming and sustaining solutions of Cr and PAH in normal saline at approximately 0.5 cc/min; this was sufficient to produce and maintain a plasma concentration of Cr between 20-30 mg% and PAH between 1-2 mg%. Blood was collected into heparinized syringes at the midpoint of each urinary collection period and analyzed for hematocrit, plasma sodium, Cr and PAH. Plasma and urinary electrolytes were measured by flame photometry. Filtered sodium was calculated from C_{Cr} in ml/min $\times$ plasma sodium in μEq/ml; a Donnan factor of 0.95 was used.

Results. Series I non-saline loaded donors vs *normal recipients (N = 8 pairs).* The results of changes observed in the recipient animals are presented in Table I. Renal sodium excretion ($U_{Na}V$), renal potassium excretion (U_KV), F_{Na}, C_{Cr}, C_{PAH}, urine flow (UV), plasma sodium concentration and body weight were not significantly altered. Arterial pressure declined slightly in some donor and recipient dogs but for the group the change was not significant.

Series II saline loaded donors vs *normal recipients (N = 12 pairs).* During the first two cross circulation periods before aortic constriction, there was a rise in urinary sodium excretion for the group of recipient dogs from a mean control level of 27 μEq/min to 98 and 75 μEq/min ($P<0.01$ and $P<0.02$, Fig. 1) without a significant change of filtered sodium (Fig. 2); also, the plasma sodium concentration was unaltered. Potassium excretion and urine flow were increased in the recipients during the first 2 periods of cross circulation. Average values for renal sodium excretion in the donors were 528 and 408 μEq/min during these first 2 cross circulation periods.

To evaluate further the relationship between filtered sodium and renal sodium excretion, the increase in sodium excretion was plotted against the decrease in filtered sodium for all experimental periods in which filtered sodium was less than the lowest control value for each individual experiment. Such a de-

TABLE I. Effects of Cross Circulation of Blood from Non-Saline Loaded Donors to Normal Recipient Dogs (Series I, N = 8).

	Changes in recipient															
	Control periods				Experimental periods							Recovery periods				
	1		2		3		4		5		6	7		8		
C_{Cr} (ml/min)	57.2	± 7.7	56.1	± 8.8	55.3	±10.9	55.8	± 8.6	50.5	± 7.3	46.1	± 6.1	43.8	± 5.7	43.6	± 4.6
P_{Na} (mEq/l)	146	± 1.4	146	± 1.1	146	± .7	146	± .8	147	± 1.3	147	± 1.4	147	± 1.0	147	± 1.4
F_{Na} (mEq/min)	7.91	± 1.04	7.78	± 1.17	7.66	± 1.82	7.78	± 1.17	7.08	± 1.16	6.38	± .76	5.59	± .74	6.10	± .64
$U_{Na}V$ (μEq/min)	15	± 6	10	± 2	24	±12	26	±12	38	±20	46	±27	26	± 5	30	± 4
U_KV (μEq/min)	19	± 6	18	± 4	30	± 5	32	± 4	33	± 6	28	± 8	26	± 5	30	± 4
UV (ml/min)	.34	± .08	.31	± .04	.33	± .04	.39	± .06	.60	± .6	.68	± .6	.43	± .12	.41	± .08
MAP† (mm Hg)	140	± 5	141	± 6	141	± 6	136	± 6	132	± 7	137	± 5	137	± 6	138	± 5
Body wt (kg)	16.20*														16.20*	

Values are means ± S.E. * Range of change in weight of dogs was −.45 to +.35 kg. † MAP = Mean arterial blood pressure.
C_{Cr}—Creatinine clearance. P_{Na}—Plasma sodium. F_{Na}—Filtered sodium. $U_{Na}V$—Urinary sodium excretion. U_KV—Urinary potassium excretion. UV —Urinary volume.

crease in filtered sodium was present during 25 periods in 9 experiments (Fig. 3). Eighteen of these 25 values for filtered sodium were at least 5% lower than the average control value for filtered sodium. The increase in sodium excretion for these 25 periods was from an average control value of 30 to 64 μEq/min ($P<.001$) while the associated fall in filtered sodium was from 9.47 mEq/min to 7.84 mEq/min ($P<.01$) (Fig. 4).

Discussion. In cross circulation experiments with a design similar to that in the present study, de Wardener and coworkers (1) found an increase in sodium excretion in a recipient dog receiving blood from a saline loaded donor, but there was an associated rise in GFR. More recently, McDonald and de Wardener(5) perfused isolated dog kidneys with blood from saline loaded donors; again sodium excretion increased. Although this rise did not correlate with a change in GFR or filtered sodium, small increases in these functions were not excluded as the mechanism for the natriuresis. Similarly, Lichardus and Pearce(6) reported that natriuresis was induced in an isolated perfused kidney preparation but the increase in sodium excretion was small and often occurred with a rise in the filtered sodium.

In the present experiments there was a significant rise in urinary sodium excretion in the recipient dog during cross circulation with blood from a saline loaded donor. This rise persisted despite substantial reductions in GFR brought about by aortic constriction. The increased sodium excretion reflects a decrease in the fractional tubular reabsorption of sodium. The data suggest that a humoral mechanism in addition to increased GFR is responsible for the natriuresis of saline loading.

The magnitude of the rise in sodium excretion in the recipients of the present study is similar to that reported by de Wardener *et al*(1). In both these and de Wardener's experiments, the increase in renal sodium excretion in the recipient was much less than the natriuresis in the donor. As pointed out by Levinsky and Lalone(2) an increase in F_{Na} accounts for a substantial part of the natriuresis of saline loading. In the present

study, GFR and F_{Na} in the recipient were not detectably altered during the first 2 cross circulation periods. Under these circumstances a smaller natriuresis would be expected than that occurring with an increased F_{Na} superimposed upon a humoral factor. The other important consideration in evaluating the magnitude of the natriuresis is the plasma level of the humoral agent in the recipient; this is dependent upon the bio-

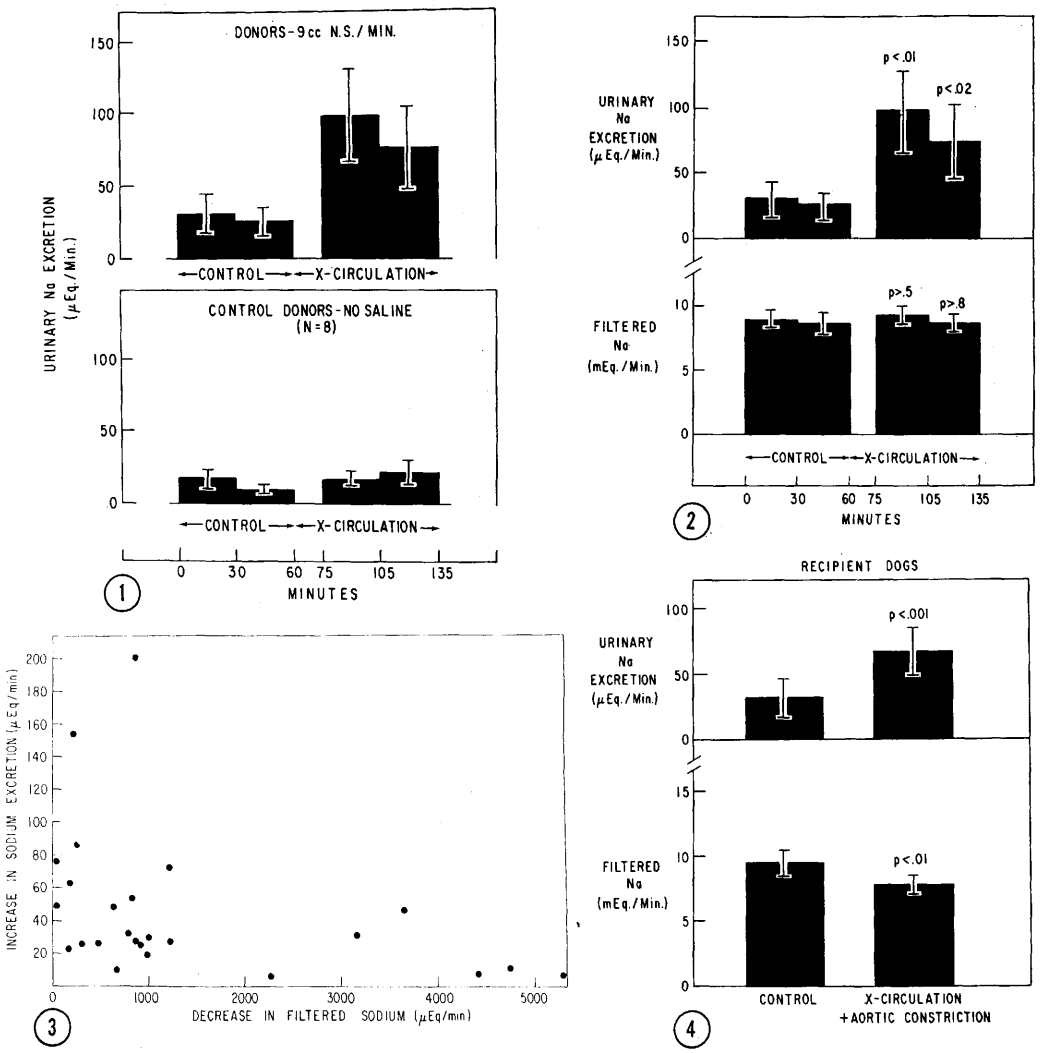

FIG. 1. In upper part of figure, data are presented for recipient dogs of Series II for control periods and first 2 periods during cross circulation before aortic constriction. Data for the 2 control periods and first cross circulation period are average values for 12 dogs. Value for the second cross circulation period is the average for 9 dogs. In the other 3 animals aortic constriction was applied after only one 30 min. period of cross circulation. Lower chart shows values for corresponding periods in recipient dogs cross circulated with blood from non-saline loaded donors, Series I. Deviations are standard errors of means.

FIG. 2. Relation of changes in renal sodium excretion to filtered sodium in recipient dogs of Series II. Deviations are standard errors of means.

FIG. 3. Relationship between increase in renal sodium excretion and decrease in filtered sodium in recipient dogs of Series II during cross circulation. Only those values in which the filtered sodium was less than lowest control value for each individual experiment are plotted.

FIG. 4. Urinary sodium excretion and filtered sodium for those 25 cross circulation periods plotted in Fig. 3 compared to mean control values for corresponding experiments. Standard errors of means are presented.

logical half-life ($T_{½}$) of the substance and rate of transfer of blood from donor to recipient. With the present rate of cross circulation of only 75 cc/min, if the $T_{½}$ is short a high equilibrated concentration of the substance would never be attained in the recipient because the substance would be destroyed too rapidly. Under these circumstances, the increase in renal sodium excretion in the recipient would not be expected to be great.

Validation of the cross circulation technique was established by circulating blood from non-saline loaded donors through normal recipient dogs. By means of the cross-over design and reversal of the direction of blood flow any minor imbalances in the rate and magnitude of pumping were corrected. The adequacy of this technique is demonstrated by the constancy of the body weights in both donor and recipient dogs in Series I. Blood pressure was recorded continuously in both donor and recipient and at a rate of cross circulation of 75 cc/min, mechanical failure to circulate blood was apparent within seconds. It is noteworthy that renal sodium excretion, urine flow, GFR and filtered sodium were unchanged during the control cross circulation series.

The present observations indicate that the decrease in fractional renal tubular reabsorption of sodium during saline loading cannot be due to either direct nervous influences upon the kidney or to some primary alteration in intrarenal function but must result from a change in the blood either physical or humoral. Evidence from other studies suggests that the increased sodium excretion of saline loading is not secondary to alterations in hematocrit, plasma proteins, hemodilution or to small changes in plasma sodium concentration(2,5,9,10). Hemodilution with resultant intercompartmental fluid shifts may have led to a fall in the recipient's blood volume but this would favor an antinatriuresis rather than the observed natriuresis. Also, the humoral mechanism of saline loading does not appear to be mediated by increased amounts of endogenous antidiuretic hormone (ADH). ADH was not administered in the present study but other workers(1,2,4) have given a large dose of vasopressin before and during saline loading and observed a natriuresis in the presence of decreased F_{Na}. The increase in sodium excretion cannot be related to changes in mineralocorticoids since in this study and in observations of others (1-4) excess quantities of salt retaining steroid were administered. Also, the rapid onset of the natriuresis excludes aldosterone.

As in previous studies(1-7,11,12) the present data do not define the primary action of the humoral agent. It is possible that saline loading led to a decrease in plasma level of a sodium-retaining factor in the donor and that cross-circulated donor blood decreased the plasma level of the same sodium-retaining factor in the recipient. A more direct mechanism would be for saline loading to produce a sodium-excreting substance in the donor which decreased the renal tubular transport of sodium in the recipient.

Summary. Blood was cross circulated from saline loaded donor dogs given excess mineralocorticoid to normal recipient animals. A significant increase in urinary sodium excretion occurred in the recipients and this persisted despite a reduction in GFR produced by suprarenal aortic constriction. This decrease in fractional renal tubular sodium reabsorption is consistent with the action of a humoral factor.

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Anticoagulant from the Sea Anemone *Rhodactis howesii.** (30966)

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The toxic extracts from the sea anemone *Rhodactis howesii* seem to affect the central nervous system of vertebrates(1,2). As a side effect, acute hemorrhages occur in cats, rabbits and mice when the crude extracts are given intravenously. This side effect suggested that an anticoagulant and/or vasculotoxic factor may be present in the extracts. Experiments designed to test the first possibility demonstrated occurrence of an anticoagulant in *R. howesii.*

Material and methods. The anemones, collected in Samoa and stored frozen, were thawed and homogenized at 5°C with 4 times their volume of water. The homogenate was centrifuged at 10,000 *g* for 10 minutes. The supernatant was dialyzed successively against 0.15 M sodium chloride adjusted with HCl to pH 2.0, then 0.15 M sodium chloride adjusted with NaOH to pH 10.5, and finally 0.15 M sodium chloride with 0.01 M sodium phosphate of pH 7.0. The non-dialyzable solution was frozen and thawed. An insoluble fraction which appeared after the thawing was removed by centrifugation. The supernatant was used for the experiments.

To estimate the organic matter in this solution aliquots were exhaustively dialyzed against distilled deionized water, then freeze-dried and ashed. They averaged 7 mg of combustible material per ml. This weight is the estimated total organic matter per ml of extract.

The anticoagulant properties of the anemone extracts were studied by the plasma recalcification method. Citrated, outdated human plasma obtained from a blood bank was used. The reactions were done in 8 mm I.D. glass tubes. The reaction mixture consisted of 200 μl plasma and 600 μl buffer containing 0.15 M sodium chloride and 0.01 M sodium phosphate at pH 7.0 per tube. For fastest coagulation at least 8 μl of 0.4 M calcium chloride was necessary, but 10 μl of that solution was used routinely. In the experimental tubes the anemone extract was added before the calcium chloride solution. Dilution of the plasma was kept constant by reducing the amount of buffer by the amount of extract to be added. The tubes with the reaction mixture were incubated at 25° to 26°C and checked for clotting every minute for the first half hour and every 5 minutes thereafter. The time elapsed from the addition of the calcium chloride until clotting occurred was recorded. Controls which contained no anemone extract were run with each series. The clotting time of the controls varied from 8 to 11 minutes depending on the series. The anticoagulant potency was estimated from the ratio of the mean clotting time of the sample with anemone extract to the mean clotting time of the controls. Duplicate samples were run for each condition and showed acceptable agreement.

Results. The anemone extract effect on plasma clotting time and how it compares with heparin is shown in Table I. The extract of *R. howesii* has anticoagulant effect such that about a 10-times prolongation of the clotting time is caused by 700 μg of dry anemone material as compared with 3.3 μg of heparin. The dose-effect relation of anemone extract does not significantly differ from that of heparin.

Some of the properties of the extract were then studied with respect to the anticoagulant factor. The extract did not digest gela-

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