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Research Article

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A Natriuretic Factor in the Serum of Patients with Chronic Uremia

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ABSTRACT Sera from chronically uremic and normal individuals were subjected to gel filtration with Sephadex G-25 and the same fraction of both was infused into rats with a decreased nephron population to determine the effects on sodium excretion. Sodium excretion rate and fractional sodium excretion increased slightly with the normal fractions; but the increase in both functional parameters produced by the uremic fractions was substantially and significantly greater. The natriuresis could not be explained by associated changes in glomerular filtration rate (GFR), para-aminohippurate (PAH) clearance, filtration fraction, hematocrit, or blood pressure. The possibility thus exists that the inhibitor affected some component part of the transepithelial sodium transport system. The elution characteristics of the fraction plus certain of its physicochemical properties suggest that the inhibitor of sodium reabsorption by the rat nephron may be identical with the inhibitor of PAH uptake by kidney slices and the inhibitor of transepithelial sodium transport by the frog skin and toad bladder previously found in the serum of chronically uremic patients.

INTRODUCTION

Recent studies from this laboratory have demonstrated the existence of a low molecular weight fraction of sera from patients with far-advanced chronic renal disease that inhibits solute transport in vitro in two different systems. The active fraction, which was obtained by gel filtration, inhibited the uptake of para-aminohippurate (PAH)[1] by rabbit kidney cortical slices (1) and it decreased the short-circuit current of the frog skin and toad bladder (2). The comparable fraction of normal sera inhibited PAH uptake and decreased short-circuit current infrequently; the difference between the effects of normal vs. uremic fractions was highly significant for both systems. Because PAH uptake by kidney slices is a sodium-dependent function which is inhibited by inhibition of sodium transport[2] and the short-circuit current across the anuran membrane is a measure of transepithelial sodium transport, the foregoing studies point to the presence of a circulating inhibitor of sodium transport in patients with chronic uremia. In the present studies, we have examined the effects of the same gel filtration fraction of uremic serum on sodium excretion by the rat. Control studies were performed using the serum fraction from normal subjects or equal volumes of isotonic saline.

METHODS

Blood was obtained from the radial artery or antecubital veins of patients with far-advanced chronic renal disease ranging in age from 17 to 62 yr. The endogenous creatinine clearance was less than 12 ml/min in all patients and averaged 5.5 ml/min for the group. None of the patients had congestive heart failure, none was nephrotic, none was on a salt intake in excess of 8–10 g/day and all were judged to be in sodium balance on clinical grounds. None of the patients was being treated with chronic hemodialysis. A detailed drug history was obtained on each patient and only five of the patients were on either diuretics or digitalis glycosides;[3] no correlation emerged between the experimental results and the drug intake.

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[1] *Abbreviations used in this paper:* FE_{Na}, fractional sodium excretion; GFR, glomerular filtration rate; PAH, para-aminohippurate; U_KV, potassium excretion rate; $U_{Na}V$, sodium excretion rate.

[2] PAH uptake by rabbit kidney cortical slices is inhibited by ouabain, ethacrynic acid, furosemide, and by the absence of sodium in the incubation medium, Klahr, S., and N. S. Bricker. Unpublished observations.

[3] Patients 2, 10, 14, 23, and 31 of Table V.

For comparison with the studies performed with the blood from uremic patients, blood samples were obtained from 18 normal subjects (physicians and laboratory personnel) ranging in age from 22 to 42 yr and subsisting on a salt intake of 12 g/day or less. 27 samples were obtained from subjects maintained either on an ad lib. salt intake or a 12 g daily salt intake and 10 samples were obtained from subjects maintained on a 1 g salt intake for 3 days. No statistically significant difference was observed between these groups and the results were pooled for the control data.

The serum samples were prepared and fractionated by column chromatography through Sephadex G-25 in a manner identical with that described previously (2). 25 ml of serum was applied to individual 2.5 by 95-cm columns and eluted with a solution of 10 mM ammonium acetate (pH 6.8). The effluent solution was divided into fractions as previously described and the fraction containing the inhibitor of PAH uptake by kidney slices and sodium transport by the frog skin was used in the present studies. This fraction which appears in the eluate after the principal peaks of sodium, potassium, and urea was lyophilized. The dried extract from 25 ml of original serum was very small in quantity and was adherent to the walls of the flask after lyophilization. The flask was carefully rinsed with 2.5 ml of distilled water. The resulting solution, whether it was obtained from a uremic patient or from a normal subject, typically contained less than 6 mEq/liter of sodium, 1 mEq/liter of potassium, 10 mEq/liter of ammonium and 6 mg/100 ml of urea (1 mM). The fractions were stored at $-80°C$ until the day of assay. Just before injection the sodium and potassium concentrations were adjusted to 140 and 4 mEq/liter respectively and the pH was brought to 7.40.

The majority of assays were performed without the investigator knowing whether the sample being tested was from a uremic patient, a normal subject, or was isotonic saline. In most instances, assays were performed using a single fraction from a single patient or normal subject. However, several experiments including some of the special experiments which required two or more assays with the same material were performed using pooled samples from more than one patient. The latter experiments included: assays in which uremic and nonuremic fractions were administered sequentially to the same animal; examination of the effect of concentrating the fraction 50-fold (rather than the usual 10-fold); examination of the effects on the fraction of incubation with pronase or chymotrypsin; examination of the effects of extraction with isobutanol; and examination of the effects of alkalinization to pH 10.5 and heating. The more concentrated preparations were obtained by pooling several fractions, relyophilizing them, and then dissolving them in a reduced volume of water. Incubation with pronase and chymotrypsin was performed for 45–60 min at room temperature and at a pH of 7.4 using 1 mg of enzyme/ml of fraction. At the completion of the incubation, the samples were boiled for 10 min to destroy the enzyme using a glass tear to prevent loss of fluid by evaporation. The isobutanol extractions were performed at room temperature by adding 3 ml of isobutanol to 2 ml of fraction after acidification with 6 N HCl to pH 1–1.5. Two extractions were performed on each sample. Mixing was accomplished with a Vortex mixer for 45–60 sec and after centrifugation, the organic and aqueous phases were separated. Both were evaporated to dryness in a water bath at 50°C under a stream of 100% nitrogen and the dried samples were reconstituted to the initial volume of 2 ml with distilled water. Both organic and aqueous phases were tested in the assay system. Alkalinization of the fractions to a pH of 10.5 was accomplished using 1 M NaOH. The fractions were incubated at this pH for 45–60 min, boiled for 10 min, and then restored to pH 7.4 with HCl. In all of the foregoing experiments, the sodium and potassium concentrations and the pH of the treated fractions were adjusted before infusion in a routine fashion.

The assays were performed on female Sprague-Dawley rats of the Holtzman strain weighing from 200 to 300 g. Because of evidence in both man (3) and dog (4) suggesting that the sensitivity of the nephrons to a given natriuretic stimulus increases as the nephron population diminishes, the model chosen for these studies was the "stage III" remnant rat (5). In an initial operation, one kidney was partially infarcted by ligating several of the second and third order branches of the renal artery. A week later, the remaining kidney was removed. The animals were allowed to recover from the second surgical procedure for at least a week. At the time of study glomerular filtration rate (GFR) averaged 0.83 ml/min. The occasional values which deviated appreciably from this mean presumably reflect the variation in the degree of infarction and/or in the degree of compensatory hypertrophy of the remnant organs. The rats were caged individually and were fed a standard Purina laboratory rat chow and water ad lib. During the 2–3 day period preceding each assay, 0.2% NaCl was substituted for the tap water. The mean blood pressure of 43 consecutive animals (measured during the performance of the assay) was 180 mm Hg ±22 (SD). There was no difference in blood pressure between the animals used to assay the uremic fractions and those used for the normal fractions, and no change in blood pressure occurred after infusion of either uremic or normal fractions.

On the day of assay, the rats were anesthetized lightly with ether and one jugular vein and one femoral artery were cannulated. A silastic catheter (I.D. 0.025, O.D. 0.047 inches) was introduced into the urinary bladder and was maintained in place by suturing an attached piece of tape to the urethral orifice. With catheters in place, the rats were placed into a plastic restraining device (5) and a recovery period of 60–90 min was allowed for them to awaken from the anesthetic and for the urine flow rate to stabilize. No fluid replacement for surgical losses or for urine flow during the immediate postoperative recovery period was administered. A priming dose of radioactive inulin (0.3–0.4 μCi) or chemical inulin (22–30 mg) then was administered in a volume of 0.4–0.6 ml, and a sustaining solution of the same material was infused in isotonic saline at a rate of 22 μl/min using a Harvard pump (model 975, Harvard Apparatus Co., Inc., Millis, Mass.). In some experiments radioactive PAH was included in the infusion in order to measure PAH clearance. When PAH-^{14}C was used, inulin-^{3}H rather than inulin-^{14}C was employed. The concentrations of radioactive inulin and PAH in the infusate were adjusted so that the counting rate in the plasma would be at least 20 times background. In initial experiments no other solution was administered. In later experiments, if the rate of urine flow during the equilibration period exceeded the rate of the sustaining infusion by more than 30–40 μl/min, a second infusion of isotonic saline was administered at a rate equal to the difference. The infusion rates were then maintained constant during the entire experiment. All infusions were delivered through the jugular venous catheter.

After an equilibration period of 80±20 min, several control clearance periods ranging from 10 to 20 min in duration were obtained. When two consecutive clearance periods

were obtained with less than 10% variation in urine flow rate, the control phase was terminated and the test solution was administered either intravenously or intra-arterially into the femoral artery[4] in a volume of no greater than 1 ml. (In experiments in which the more concentrated fraction of uremic serum was employed, a total volume of as little as 0.2 ml was employed.) The test solutions were infused at a rate of 100 μl/min. The test solutions employed were: (*a*) the serum fraction from uremic patients; (*b*) the comparable serum fraction from normal subjects; and (*c*) isotonic saline. Clearance periods of either 10 or 20 min in duration were then continued for a period of at least 60 min. When 20-min periods were employed, the first experimental period included the 10 min of infusion of the fraction. When 10-min periods were obtained, the experimental collections were started at the completion of the infusion of the test solution. At the end of each urine collection period, the urinary bladder was gently massaged for 1 min to facilitate bladder emptying. If, at any time, evidence of intermittent obstruction to urine flow was observed, any leakage of urine around the catheter was detected during the abdominal massage, or gross hematuria occurred, the experiment was terminated. This happened in less than 15% of experiments.

Blood samples were collected every 20 min from the femoral arterial catheter or occasionally from the tail when the arterial catheter became occluded. The blood was allowed to flow directly into heparinized microhematocrit tubes. The hematocrit was determined on each sample and the plasma was separated for the determination of inulin and, when indicated, PAH. Sodium and potassium concentrations were determined at the beginning, midpoint and end of each experiment. Because these values remained quite constant, the mean value of the three determinations was used for the calculation of the filtered load of sodium. A Donnan correction factor of 0.95 was employed.

The following prospective criteria were established for the acceptability of an assay: (*a*) that sodium excretion rates ($U_{Na}V$) average more than 1 and less than 12 μEq/min during the control clearance periods; and (*b*) that there be no more than 20% change in $U_{Na}V$ in either direction between the penultimate and the final control period. 12 experiments were discarded because they did not satisfy the last criteria. In all studies, the values for GFR, absolute sodium excretion rate, fractional sodium excretion (FE_{Na}), and potassium excretion rate (U_KV) after injection of the test solution were compared with the mean values for the last two control periods. The experimental results were collected from three to five consecutive collection periods. The data were analyzed by comparing the control values with both the mean and peak values for the experimental periods obtained during a 50–60 interval after administration of the test solution.

Chemical inulin concentration in plasma and urine was measured using the anthrone method (6). For the determination of radioactive inulin and PAH in urine and plasma samples, 25–50-μl samples of either undiluted urine or plasma were pipetted into glass counting vials containing 10–15 ml of Bray's solution (7). The samples were counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3214, Packard Instrument Co., Inc., Downers Grove, Ill.). At least 10,000 counts were obtained for each sample. When ^{14}C and ^{3}H nuclides were present in the same samples, the relative counts due to each isotope were determined with appropriate mixtures of radioactive standards to which 25–50 μl of "cold" urine or plasma were added. Sodium and potassium were measured using a flame photometer (model 43, Instrumentation Laboratory, Inc., Lexington, Mass.) and the pH of the fractions during their preparation for infusion was measured with a glass electrode. The variation for sets of measurements is expressed as the standard error of the mean. Statistical analysis was performed using Student's *t* test and significance in the text is expressed as the 2P value.

[4] No difference in results was found between the two routes of administration.

RESULTS

Two individual studies demonstrating the effects of the serum fractions from a uremic patient and a normal subject on glomerular filtration rate, urine volume, sodium, and potassium excretion of assay animals are shown in Table I. Table IA illustrates the effects of the fraction from a chronically uremic patient. After the surgical procedures had been completed, a period of approximately 3 hr was allowed for the animal to recover from the anesthetic and to achieve a relatively constant rate of urine flow. During the last two control clearance periods, GFR averaged 0.94 ml/min, urine flow 73 μl/min, and $U_{Na}V$ 7.8 μEq/ min. The fraction obtained from 10 ml of uremic serum then was infused intra-arterially in a volume of 1 ml over a 10 min interval at the rate of 100 μl/min. During the hour after the start of the infusion, GFR did not change in any consistent direction and urine volume increased modestly. $U_{Na}V$ increased during the first experimental clearance period, reached a peak of 10.4 μEq/min during the third experimental period, and decreased to the control level during the fifth period. FE_{Na} rose from a mean value of 5.9% during the last two control periods to a peak value of 8.4% during the third experimental period and then decreased to approximate the control value. U_KV did not increase after administration of the serum fraction and the hematocrit remained constant.

Table IB depicts the effects of the serum fraction from a normal subject. GFR in the assay animal averaged 1.37 ml/min and $U_{Na}V$ 7.2 μEq/min during the last two control periods. At the end of the control periods, the fraction was infused intra-arterially over a 10 min interval and observations were made during four consecutive 20-min clearance periods. GFR changed little during the experimental periods and $U_{Na}V$ decreased by a mean 0.5 μEq/min. FE_{Na} changed from a mean control value of 3.7% to a mean value of 3.5% after administration of the fraction. There was a slight decrease in U_KV and the hematocrit remained constant.

Representative experiments depicting the effects of normal and uremic fractions in rats with lower GFRs are shown in Table II. After administration of the fraction from a uremic patient (Table IIA) mean and peak changes were: GFR, + 0.03 and + 0.08 ml/min; $U_{Na}V$, + 1.8 and + 3.1 μEq/min; and FE_{Na} + 1.9 and + 2.9%.

TABLE I

Effects of Uremic and Normal Serum Fractions

Subject studied	Clearance period	Time	GFR	FL_{Na}	V	$U_{Na}V$	FE_{Na}	U_KV	Hematocrit
		min	*ml/min*	*μEq/min*	*μl/min*	*μEq/min*	%	*μEq/min*	%
(A) Uremic patient									
		−170	Rat positioned in animal holder with catheters in place in femoral artery, jugular vein, and bladder						
		−86	Priming solution containing 31 mg inulin in 0.6 ml isotonic saline						
		−84	Sustaining solution containing 1.3 g inulin in 100 ml isotonic saline at 22 μl/min						
	1	0–15	0.87	118.1	71	7.18	6.08	1.98	40
	2	15–30	0.95	132.5	76	7.95	6.00	1.97	39
	3	30–45	0.93	130.8	69	7.57	5.79	1.73	39
		45–55	1 ml serum fraction from a uremic patient infused intra-arterially at 100 μl/min						
	4	45–65	0.96	134.2	83	9.48	7.06	1.84	40
	5	65–85	0.83	114.3	78	8.89	7.78	1.63	40
	6	85–105	0.93	123.5	85	10.40	8.38	1.82	39
	7	105–125	0.92	122.8	77	9.46	7.70	1.80	39
	8	125–145	0.90	121.5	61	7.59	6.24	1.50	40
(B) Normal subject									
		−189	Rat positioned in animal holder with catheters in place in femoral artery, jugular vein, and bladder						
		−92	Priming solution containing 27 mg inulin in 0.5 ml isotonic saline						
		−94	Sustaining solution containing 3.0 g inulin in 100 ml isotonic saline at 22 μl/min						
	1	0–20	1.39	196.3	58	6.47	3.30	3.15	34
	2	20–40	1.37	194.3	53	7.36	3.79	2.96	34
	3	40–60	1.37	190.8	50	6.96	3.65	2.63	34
		60–70	1 ml serum fraction from a normal subject infused intra-arterially at 100 μl/min						
	4	60–80	1.34	188.3	43	6.45	3.43	2.31	34
	5	80–100	1.40	196.4	46	6.88	3.50	2.38	34
	6	100–120	1.39	192.2	43	6.62	3.44	2.48	34
	7	120–140	1.32	180.8	48	6.50	3.59	2.42	34

Abbreviations: FL_{Na}, filtered load of sodium = GFR × plasma Na concentration × 0.95; V, urine flow rate; $U_{Na}V$, sodium excretion rate; FE_{Na}, fractional excretion of Na = $U_{Na}V/FL_{Na}$; U_KV, potassium excretion rate.
No additional infusion of saline was given in these experiments.

In the second experiment (Table IIB), where comparable control values were obtained, mean and peak changes were: GFR, + 0.06 and + 0.09 ml/min; $U_{Na}V$, + 0.8 and + 1.2 μEq/min; and FE_{Na} + 0.4 and + 1.2%. In both experiments U_KV and hematocrits remained constant.

A representative experiment demonstrating the effects of 1 ml of isotonic saline infused at 100 μl/min on GFR, urine volume, sodium, and potassium excretion is presented in Table III. During the 62 min preinfusion control periods GFR was stable and averaged 1.34 ml/min for the last two control periods. $U_{Na}V$ averaged 4.9 μEq/min and FE_{Na} 2.6% during the last two control periods. After infusion of saline, GFR increased by a mean of 0.03 and a peak of 0.08 ml/min; $U_{Na}V$ increased by a mean of 0.7 and a peak of 0.9 μEq/min and FE_{Na} increased by a mean of 0.2 and a peak of 0.4%. U_KV decreased slightly and hematocrit remained constant.

The results of 42 assays in which the serum fraction from patients with chronic uremia were tested, 37 assays in which the test solution consisted of the serum fraction from normal subjects, and 10 experiments performed with isotonic saline are shown in Tables IV, V, and VI respectively. The mean and peak values for the postinjection experimental periods are compared with the mean values for the last two preinjection control periods and the differences are shown for GFR, $U_{Na}V$, and FE_{Na}. In Table VII, the data are analyzed statisti-

cally. The change in GFR after both normal and uremic fractions was small and inconsistent and there was no significant difference between the two groups. $U_{Na}V$ increased by a mean of 0.61±0.19 μEq/min and a peak of 1.39±0.25 μEq/min after administration of the normal fractions and by a mean of 1.50±0.17 μEq/min and a peak of 2.52±0.27 μEq/min after administration of the uremic fractions. The differences for both mean and peak values are statistically significant ($P < 0.005$). Mean and peak FE_{Na} also increased modestly after infusion of the normal fractions and more markedly after administration of the uremic fractions and the differences between the two groups are statistically significant ($P < 0.025$). For none of these functional parameters was there any significant difference between the response to isotonic saline and normal serum fractions.

TABLE II

Effects of Uremic and Normal Serum Fractions in Rats with Low Glomerular Filtration Rate

Subject studied	Clearance period	Time	GFR	FL_{Na}	V	$U_{Na}V$	FE_{Na}	U_KV	Hematocrit
		min	*ml/min*	*μEq/min*	*μl/min*	*μEq/min*	%	*μEq/min*	%
(A) Uremic patient									
		−165	Rat positioned in animal holder with catheters in place in femoral artery, jugular vein, and bladder.						
		−102	Priming solution containing 0.35 μCi inulin-^{14}C in 0.6 ml saline						
		−100	Sustaining solution containing 25 μCi inulin-^{14}C in 100 ml isotonic saline at 20 μl/min						
		−55	Additional isotonic saline infusion at 20 μl/min						
	1	0–20	0.53	73.5	43	2.49	3.39	2.23	38
	2	20–30	0.66	91.5	52	3.58	3.91	2.49	
	3	30–40	0.60	83.2	48	3.66	4.40	2.21	38
	4	40–50	0.55	76.3	49	3.62	4.75	2.15	
	5	50–60	0.66	91.5	56	4.16	4.54	2.28	38
		60–70	1 ml serum fraction from a uremic patient infused intravenously at 100 μl/min						
	6	70–80	0.60	83.2	60	5.20	6.25	2.27	
	7	80–90	0.63	87.4	60	4.58	5.24	2.11	37
	8	90–100	0.63	87.4	60	5.31	6.08	2.27	
	9	100–110	0.69	95.7	75	7.01	7.32	2.38	37
	10	110–120	0.67	92.9	71	7.01	7.54	2.29	
	11	120–130	0.57	79.1	57	5.28	6.68	1.93	37
(B) Normal subject									
		−150	Rat positioned in animal holder with catheters in place in femoral artery, jugular vein, and bladder						
		−93	Priming solution containing 0.35 μCi inulin-^{14}C in 0.6 ml saline						
		−90	Sustaining solution containing 25 μCi inulin-^{14}C in 100 ml isotonic saline at 20 μl/min						
		−73	Additional isotonic saline infusion at 20 μl/min						
	1	0–20	0.50	66.9	43	4.28	6.40	1.41	30
	2	20–30	0.49	65.6	44	4.41	6.72	1.40	
	3	30–40	0.49	65.6	43	4.48	6.83	1.31	30
	4	40–50	0.45	60.3	37	3.92	6.50	1.26	
	5	50–60	0.50	66.9	37	4.04	6.04	1.39	30
		60–70	1 ml serum fraction from a normal subject infused intravenously at 100 μl/min						
	6	70–80	0.56	74.9	40	4.41	5.89	1.33	30
	7	80–90	0.57	76.3	42	4.70	6.16	1.52	
	8	90–100	0.53	70.9	44	5.22	7.36	1.52	30
	9	100–110	0.57	76.3	44	5.16	6.72	1.44	
	10	110–120	0.49	65.6	40	4.68	7.13	1.28	30
	11	120–130	0.48	64.3	41	4.85	7.54	1.26	

See Table I for definition of abbreviations.

TABLE III
Effects of 1 ml Isotonic Saline

Clearance period	Time	GFR	FL_{Na}	V	$U_{Na}V$	FE_{Na}	U_KV	Hematocrit
	min	*ml/min*	*μEq/min*	*μl/min*	*μEq/min*	*%*	*μEq/min*	*%*
	−180	Rat positioned in animal holder with catheters in place in femoral artery, jugular vein, and bladder						
	−114	Priming solution containing 23 mg inulin in 0.5 ml isotonic saline						
	−112	Sustaining solution containing 3.1 g inulin in 100 ml isotonic saline at 22 μl/min						
1	0–21	1.32	187.4	48	5.62	3.00	1.84	43
2	21–39	1.30	186.2	33	4.71	2.53	1.48	43
3	39–62	1.37	198.7	32	5.08	2.60	1.62	42
	62–72	1 ml isotonic saline infused intra-arterially at 100 μl/min						
4	62–82	1.42	207.9	42	5.65	2.71	1.37	42
5	82–102	1.37	201.1	34	5.32	2.65	1.06	42
6	102–122	1.38	194.2	35	5.75	2.96	1.11	42

See Table I for definitions of abbreviations.

Fig. 1 depicts the average values for the mean and the peak changes (expressed as a percentage of the control values) for absolute and fractional sodium excretion for all three groups. After infusion of isotonic saline (10 experiments), no change occurred in mean values for $U_{Na}V$ and FE_{Na}. Peak values were 16.5±9.5 and 18.1±11.7% greater than control values. After infusion of normal serum fractions (37 experiments), mean values for $U_{Na}V$ and FE_{Na} increased by 19.9±5.7 and 19.2±5.1% respectively. Peak values increased by 38.6±8.2 and 36.7±6.1%. None of these values differs significantly from those observed after infusion of isotonic saline. By contrast, with the uremic fractions (42 experiments) the increase in mean $U_{Na}V$ was 45.8±8.3% and in peak $U_{Na}V$ 80.4±14.4%. Both values differ significantly from those observed with the normal fractions ($P < 0.02$). The mean and peak increases in FE_{Na} were 43.0±8.5 and 71.2±13.6% respectively, and both values again are significantly greater than those observed with the normal fractions ($P < 0.025$).

Table VIII presents the fluid balance (infusion rate(s) minus urine flow) during the control and post-infusion experimental periods in the three groups of assay rats. Urine flow rates during the control periods were comparable in all three groups. All three groups were in slightly negative water balance during both the control and experimental periods; but it is of note that the rats receiving the uremic fraction had the greatest degree of negative balance. Thus, the greater degree of natriuresis observed in the latter group occurred despite net loss of total body water and presumably of extracellular fluid.

The effects of the uremic fractions on sodium excretion did not appear to be related to the volume of fluid in which the fraction was infused. Thus, similar changes in $U_{Na}V$ were observed when the same quantity of uremic fraction was dissolved in 0.2 ml as when the volume of diluent was 1.0 ml. Three experiments (11, 12, and 20), in which 0.2 ml of a 50-fold concentrated material rather than 1 ml of a 10-fold concentrate was infused, are included in Table IV.

In Table IX, eight experiments are shown in which both normal and uremic test solutions were administered sequentially during the course of the same experiment. In the first four experiments, the uremic fraction was administered first and three clearance periods were obtained. Thereafter, the normal material consisting of normal serum fraction in three and isotonic saline in one was administered and three additional clearance periods were obtained. In the other four experiments, the sequence was reversed with the normal fraction (three experiments) or isotonic saline (one experiment) being administered first and the uremic fraction being administered second. In all experiments in which the uremic fraction was administered first, $U_{Na}V$ and FE_{Na} decreased after administration of the nonuremic material. In contrast, in all experiments in which the nonuremic solution was administered first, $U_{Na}V$ and FE_{Na} increased after infusion of the uremic fraction.

The results of 10 experiments in which both PAH clearance and inulin clearance were measured are shown in Table X. In nine of these experiments, the serum fraction from uremic patients was administered and in one a normal fraction was administered. In seven of the nine experiments with the uremic fraction, a substantial natriuresis ensued; however, in the

TABLE IV

Effects of the Serum Fraction from Patients with Chronic Uremia on Glomerular Filtration Rate and Sodium Excretion

Experiment No.	Control periods			Experimental periods					
				ΔGFR		$\Delta U_{Na}V$		ΔFE_{Na}	
	GFR	$U_{Na}V$	FE_{Na}	Mean	Peak	Mean	Peak	Mean	Peak
	ml/min	*μEq/min*	%						
1	0.72	7.04	6.83	−0.01	+0.04	+2.62	+4.01	+2.19	+3.47
2	0.94	7.76	5.89	−0.03	+0.02	+1.81	+2.59	+1.85	+2.49
3	1.16	2.89	1.98	+0.01	+0.03	+3.41	+5.19	+3.04	+3.43
4	0.15	4.17	19.88	−0.01	0.00	+1.21	+1.28	+7.02	+8.42
5	1.06	1.84	1.34	+0.13	+0.19	+0.77	+0.80	+0.20	+0.28
6	2.24	5.75	1.90	−0.28	+0.01	+1.64	+3.41	+0.74	+1.08
7	0.98	2.08	1.54	+0.02	+0.24	+1.29	+1.79	+0.57	+0.73
8	2.32	1.21	0.35	+0.21	+0.28	+3.47	+6.41	+1.07	+1.84
9	0.93	1.40	1.11	+0.06	+0.19	+0.60	+0.78	+0.42	+0.66
10	1.20	1.08	0.71	+0.11	+0.24	+0.19	+0.34	+0.34	+0.85
11	0.65	6.13	7.34	0.00	+0.01	+1.55	+2.01	+1.63	+2.84
12	0.87	1.70	1.53	+0.12	+0.15	+3.20	+5.38	+2.52	+3.85
13	1.12	7.52	5.25	+0.06	+0.14	+2.83	+3.14	+1.63	+2.39
14	0.83	3.67	3.17	−0.03	−0.03	+1.14	+1.29	+1.10	+1.29
15	0.46	1.45	2.54	+0.01	+0.09	+0.62	+0.76	+0.93	+1.18
16	0.88	10.30	8.59	+0.13	+0.16	+1.77	+2.88	+0.14	+0.66
17	0.95	3.68	3.08	+0.06	+0.14	+1.76	+2.42	+1.03	+1.48
18	0.32	3.17	7.62	+0.02	+0.14	+1.28	+2.65	+2.11	+4.23
19	1.54	10.73	5.60	−0.06	+0.04	+2.84	+5.41	+1.69	+2.51
20	0.86	4.37	3.95	+0.02	+0.08	+1.23	+1.76	+0.89	+1.45
21	1.01	4.94	3.51	+0.03	+0.20	+1.99	+3.00	+1.27	+2.25
22	0.27	1.84	5.31	+0.03	+0.04	+0.87	+1.03	+1.46	+1.64
23	0.73	8.99	9.39	−0.04	−0.02	−0.58	+0.56	−0.17	+1.41
24	0.78	3.69	3.65	0.00	+0.10	+1.87	+3.22	+1.95	+3.75
25	0.67	3.07	3.47	−0.05	+0.10	−0.50	−0.07	−0.18	+0.14
26	0.52	8.75	13.29	+0.04	+0.10	+1.27	+2.96	+0.49	+1.46
27	0.49	4.32	6.29	−0.03	+0.14	+3.44	+8.08	+5.51	+10.26
28	0.55	1.89	2.61	−0.03	−0.01	−0.39	+0.38	−0.47	+0.44
29	0.39	2.62	5.27	+0.08	+0.33	+1.98	+3.88	+1.83	+2.19
30	0.29	1.73	4.72	+0.08	+0.18	−0.01	+0.64	−0.73	+0.22
31	0.46	1.80	3.08	+0.09	+0.25	+2.18	+2.96	+2.17	+4.82
32	1.27	3.04	2.08	−0.07	+0.15	+2.27	+2.83	+1.61	+2.08
33	0.90	1.95	1.67	+0.09	+0.29	+0.51	+1.40	+0.22	+0.49
34	0.40	4.31	3.17	+0.02	+0.04	+2.59	+3.76	+1.87	+2.69
35	0.39	3.62	7.00	−0.03	+0.02	+0.12	+0.83	+0.29	+2.31
36	0.93	4.72	3.74	−0.01	+0.16	+1.64	+1.72	+1.39	+2.02
37	0.80	3.94	3.85	+0.14	+0.36	+1.97	+3.17	+0.95	+2.12
38	0.42	3.14	5.69	−0.05	−0.03	+0.09	+1.91	+1.49	+5.38
39	0.60	5.72	7.31	−0.01	+0.02	+2.65	+3.74	+4.25	+6.22
40	0.87	2.39	3.07	0.00	+0.24	+0.16	+0.58	+0.07	+0.31
41	0.61	3.94	4.65	+0.03	+0.08	+1.88	+3.07	+2.23	+2.89
42	1.51	6.76	3.35	+0.01	+0.14	+1.00	+2.08	+0.48	+0.65
Mean	0.84	4.17	4.67	+0.02	+0.12	+1.52	+2.52	+1.37	+2.40
±SE	0.07	0.39	0.55	0.01	0.01	0.17	0.27	0.23	0.33

The serum fraction used corresponded to 10 ml original serum for all experiments. The fraction was infused at a rate of 100 μl/min in a volume of 0.2 ml in three experiments (11, 12, 20) and 1 ml in all other experiments.

The mean and peak values for GFR, $U_{Na}V$, and FE_{Na} for the clearance periods obtained over the 50–60 min interval after infusion of the fractions were compared with the mean values obtained during the last two control clearance periods. The mean values under "experimental periods" thus represent the differences in GFR, $U_{Na}V$, and FE_{Na} between the mean experimental and the control values. The peak values under "experimental periods" represent the differences in GFR, $U_{Na}V$, or FE_{Na} between the single experimental period with the greatest value for each parameter. A negative "peak value" thus indicates that all values during the experimental periods were lower than the mean control value. The values for ΔGFR, $\Delta U_{Na}V$, and ΔFE_{Na} are expressed in milliliters per minute, microequivalents per minute, and per cent respectively.

other two there was no natriuretic response and in the experiment with the normal fraction there was no natriuretic response. PAH clearance and filtration fraction varied in a random manner which bore no relationship to the change or lack of change in sodium excretion.

TABLE V

Effects of the Serum Fraction from Normal Subjects on Glomerular Filtration Rate and Sodium Excretion

Experiment No.	Control periods			Experimental periods					
				ΔGFR		$\Delta U_{Na}V$		ΔFE_{Na}	
	GFR	$U_{Na}V$	FE_{Na}	Mean	Peak	Mean	Peak	Mean	Peak
	ml/min	*μEq/min*	%						
				1 g NaCl diet					
1	0.60	2.19	2.76	+0.03	+0.11	−0.17	+0.14	−0.31	+0.04
2	0.82	7.63	8.09	+0.03	+0.07	+1.07	+1.49	+0.78	+2.46
3	1.30	6.55	3.81	−0.02	+0.04	+1.13	+2.15	+0.33	+1.25
4	0.95	3.35	2.66	+0.01	+0.08	+0.41	+0.75	+0.31	+0.41
5	0.83	4.62	4.13	−0.11	−0.09	+0.77	+1.08	+1.38	+1.76
6	0.91	11.29	9.17	0.00	+0.09	−1.21	+0.10	−0.96	+0.33
7	1.07	1.71	1.20	0.00	+0.29	+0.51	+0.71	+0.40	+0.79
8	0.87	4.46	4.30	−0.05	+0.03	+0.21	+0.60	+0.47	+0.94
9	0.52	4.51	6.33	0.00	+0.01	+0.93	+2.12	+1.89	+2.66
10	0.56	5.93	7.68	−0.01	+0.03	+0.19	+2.14	+1.42	+2.23
11	1.42	5.61	2.93	−0.21	−0.08	−1.40	−0.59	−0.36	−0.09
				Random NaCl diet					
12	1.27	4.86	2.80	−0.03	+0.01	+1.06	+2.01	+0.64	+1.16
13	1.37	7.16	3.72	0.00	+0.03	−0.66	−0.29	−0.26	−0.22
14	0.58	2.34	2.88	−0.01	+0.05	+0.26	+0.84	+0.56	+0.69
15	1.41	3.27	1.80	−0.02	+0.11	−0.27	−0.04	−0.13	−0.10
16	1.22	7.83	4.71	−0.15	−0.09	+1.35	+1.87	+2.01	+2.65
17	0.55	4.01	5.43	0.00	+0.04	+0.77	+1.21	+0.95	+1.83
18	0.56	6.58	9.37	−0.06	−0.03	−0.03	+0.18	+1.04	+1.39
19	1.10	7.23	5.18	−0.13	−0.03	−1.03	−0.25	−0.42	+0.08
20	0.58	3.74	5.18	+0.09	+0.21	+1.72	+2.71	+1.24	+2.24
21	0.86	4.68	4.44	−0.08	0.00	+1.18	+2.74	+1.50	+2.70
22	1.15	2.90	2.10	−0.20	−0.05	+0.47	+2.44	+0.75	+2.06
23	0.84	3.78	3.28	+0.06	+0.09	+1.36	+2.10	+0.91	+1.99
24	0.22	2.01	7.21	+0.01	+0.03	+0.57	+0.85	+1.55	+1.98
				12 g NaCl diet					
25	0.32	2.95	7.37	+0.01	+0.17	+1.07	+1.68	+1.95	+5.24
26	0.41	5.17	9.22	+0.03	+0.06	+0.59	+0.93	+0.20	+0.90
27	0.38	2.16	4.21	+0.01	+0.06	+0.52	+0.91	+0.88	+1.62
28	0.48	3.98	6.27	+0.05	+0.09	+0.85	+1.24	+0.38	+1.09
29	0.97	2.12	1.69	−0.14	+0.06	+1.37	+2.05	+1.82	+2.78
30	1.31	4.60	2.57	+0.07	+0.28	+0.68	+1.60	+0.16	+0.49
31	1.18	7.85	4.72	+0.48	+0.73	+1.65	+2.46	−0.05	+1.98
32	0.76	2.96	3.01	+0.11	+0.18	+5.93	+8.72	+4.36	+4.38
33	0.21	10.63	37.30	+0.02	+0.04	−0.36	+1.04	−4.54	+1.62
34	0.16	2.58	12.10	0.00	+0.04	+0.26	+0.92	+0.94	+2.95
35	0.90	3.96	3.34	0.00	+0.06	+0.41	+0.56	+0.36	+0.50
36	0.73	2.26	2.43	+0.03	+0.09	+0.24	+0.67	+0.09	+0.34
37	0.99	2.41	1.85	−0.10	−0.01	+0.53	+1.51	+0.63	+1.16
Mean	0.82	4.65	5.60	−0.01	+0.08	+0.61	+1.39	+0.62	+1.52
±SE	0.06	0.39	0.98	0.02	0.02	0.19	0.25	0.21	0.20

Results are expressed as in Table IV.

1 ml of serum fraction equivalent to 10 ml original serum was infused at a rate of 100 μl/min in all assays.

TABLE VI

Effects of 1 ml Isotonic Saline on Glomerular Filtration Rate and Sodium Excretion

Experiment No.	Control periods			Experimental periods					
				ΔGFR		$\Delta U_{Na}V$		ΔFE_{Na}	
	GFR	$U_{Na}V$	FE_{Na}	Mean	Peak	Mean	Peak	Mean	Peak
	ml/min	*μEq/min*	%						
1	1.22	5.45	3.31	−0.03	−0.02	−0.64	−0.32	−0.30	−0.18
2	1.34	4.90	2.57	+0.06	+0.08	+0.69	+0.91	+0.20	+0.39
3	0.14	3.51	15.82	+0.02	+0.02	+0.65	+0.92	+3.04	+5.25
4	1.30	11.16	6.48	−0.10	−0.01	−2.41	+0.46	−0.73	+0.57
5	0.61	2.35	2.85	−0.11	+0.06	+0.11	+1.69	+0.67	+2.77
6	0.60	3.17	4.10	+0.39	+0.65	−0.67	−0.17	−2.19	−1.93
7	0.87	1.64	1.40	−0.07	−0.02	−0.57	−0.43	−0.45	−0.31
8	0.78	5.13	4.69	−0.10	−0.05	+0.43	+0.74	+1.15	+1.46
9	0.62	4.59	5.31	+0.06	+0.12	+1.12	+1.47	+0.67	+1.78
10	0.66	4.94	5.50	−0.01	+0.03	+1.17	+1.57	+1.40	+1.50
Mean	0.81	4.66	5.20	+0.01	+0.09	−0.01	+0.68	+0.35	+1.13
±SE	0.12	0.82	1.28	0.05	0.06	0.34	0.24	0.45	0.62

Results are expressed as in Table IV.

1 ml of isotonic saline was infused at a rate of 100 μl/min in all assays.

The effects of incubation of uremic fraction with pronase and chymotrypsin are shown in Table XI. Five experiments are shown for each enzyme. With pronase, control experiments were performed with four of the fractions and all produced some natriuresis. In three of these, the same fractions still produced natriuresis after treatment with pronase, while in one (experiment 4) sodium excretion decreased after administration of the pronase-treated fraction. In the fifth study, no control experiment was performed; but the pronase-treated fraction produced a marked natriuretic response.

TABLE VII

Statistical Analysis for the Mean and Peak Changes in GFR, $U_{Na}V$, and FE_{Na} Observed during the Experimental Periods with the Serum Fraction from Chronically Uremic Patients vs. Normal Subjects

		t	*P*
ΔGFR	Mean	1.43	NS
	Peak	1.68	NS
$\Delta U_{Na}V$	Mean	3.43	<0.001
	Peak	3.07	<0.005
ΔFE_{Na}	Mean	2.36	<0.025
	Peak	2.29	<0.055

Mean base line values for GFR, $U_{Na}V$, and FE_{Na} of the control periods were not statistically different between the group of rats injected with serum fractions from patients with chronic uremia vs. the group of rats injected with serum fractions from normal subjects.

The results using chymotrypsin were virtually identical with those with pronase. In four of the five studies, natriuresis was produced after injection of the chymotrypsin-treated fractions, while in the fifth experiment sodium excretion decreased.

Table XII depicts the effects of extraction of uremic fractions with isobutanol and of incubation at pH 10.5. Four experiments are shown after isobutanol extraction. In two, both aqueous and organic phases were tested and in each the natriuresis produced by the aqueous phase was appreciable while that produced by the organic phase was small in magnitude. In the third experiment, the untreated fraction was tested and then the aqueous phase was examined after isobutanol extraction. The increase in sodium excretion was comparable in both experiments. In the fourth study a control experiment was done first and then isobutanol extraction was performed and the organic phase was tested. Natriuresis was produced by the untreated fraction but sodium excretion decreased after administration of the organic phase of the extracted fraction. One fraction (not shown in the Table) which did not produce natriuresis in the untreated state also was extracted with isobutanol and the aqueous phase was tested. In the control experiment $\Delta U_{Na}V$ averaged + 0.19 μEq/min and ΔFE_{Na} averaged + 0.30%. With the aqueous phase of the same fraction, $\Delta U_{Na}V$ averaged − 0.06 μEq/min and ΔFE_{Na} − 0.26%. Thus, the isobutanol extraction per se does not appear to render the aqueous phase natriuretic.

In the experiments in which the natriuretic fractions

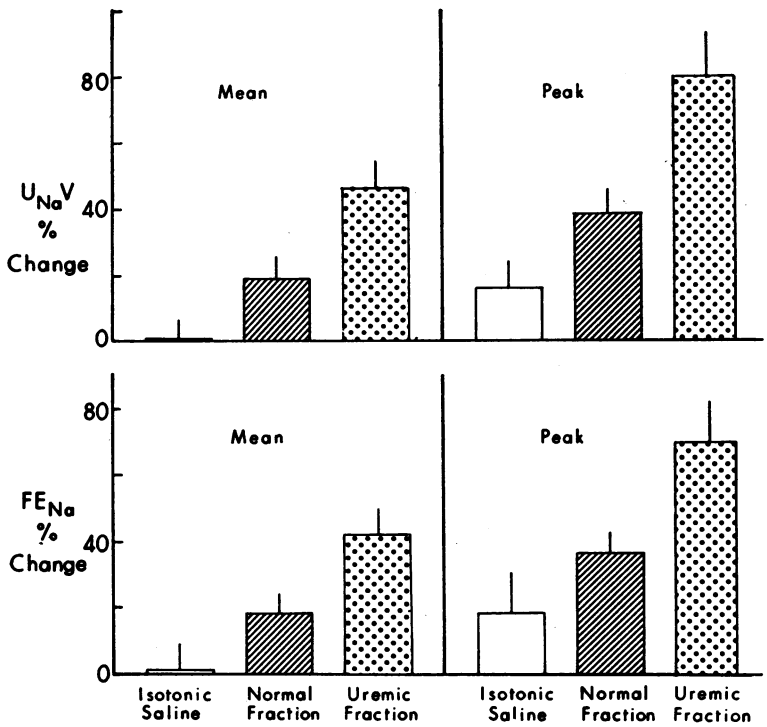

FIGURE 1 Average per cent changes ±SE in mean and peak sodium excretion rate (top panel) and mean and peak fractional sodium excretion (lower panel) obtained during the experimental periods, after infusion of 1 ml isotonic saline (10 experiments), 1 ml serum fraction from normal subjects (37 experiments), and 1 ml serum fraction from patients with chronic uremia (42 experiments). The results were calculated as in Table IV.

were incubated at an alkaline pH and then boiled, the natriuretic effect was abolished or markedly decreased. The mean change in $U_{Na}V$ with the unmodified fractions in the control experiments was + 1.96 μEq/min and the mean change in FE_{Na} was + 1.34%. After alkalinization the mean change in $U_{Na}V$ was + 0.27 μEq/min and FE_{Na} decreased by 0.14% (Table XII).

DISCUSSION

The present studies were designed to determine whether the same gel filtration fraction of uremic serum that previously has been shown to inhibit PAH uptake by rabbit kidney cortical slices (1) and sodium transport by frog skin and toad bladder (2) would prove to be natriuretic in the rat. Because fractional sodium reab-

TABLE VIII

Fluid Balance during the Control and the Experimental Periods for the Different Groups of Assay Rats

Group of assay rats	Infusion rate	Control periods		Experimental periods	
		Urine flow	Balance	Urine flow	Balance
	μl/min	*μl/min*	*μl/min*	*μl/min*	*μl/min*
Isotonic saline	30.6±4.6	40.5±6.6	−9.9±6.1	38.7±6.0	− 8.1±6.6
Normal fraction	40.2±2.3	41.1±2.1	−0.9±2.3	43.4±2.6	− 3.2±2.2
Uremic fraction	38.7±2.4	46.0±3.2	−7.3±2.8	51.9±3.3	− 13.2±3.1

Fluid balance represents the difference between the infusion rate minus the urine flow rate.
Control and experimental periods are defined in Table IV.
Values presented correspond to the same control and experimental periods used in Tables IV, V, and VI.

TABLE IX

Effects of the Sequential Administration of Uremic Fraction and either Normal Fraction or Isotonic Saline on $U_{Na}V$ and FE_{Na}

Experiment No.	$U_{Na}V$	FE_{Na}	$U_{Na}V$	FE_{Na}	$U_{Na}V$	FE_{Na}
	μEq/min	%	*μEq/min*	%	*μEq/min*	%
	(A) Uremic fractions		(B) Normal fraction or isotonic saline		Δ (A vs. B)	
1	9.02	6.97	6.60	5.76	−2.42	−1.21
2	10.11	9.71	7.06	7.13	−3.05	−2.58
3	8.22	6.06	5.14	5.73	−3.08	−0.33
4	1.64	1.40	1.04	0.96	−0.60	−0.44
Mean					−2.29	−1.14
	(A) Normal fraction or isotonic saline		(B) Uremic fractions		Δ (A vs. B)	
5	4.17	19.89	5.36	26.52	+1.19	+6.63
6	5.43	4.52	8.26	6.99	+2.83	+2.47
7	8.28	4.56	14.87	8.09	+6.59	+3.53
8	6.02	3.55	6.25	4.21	+0.23	+0.66
Mean					+2.73	+3.32

In experiments one to four, 1 ml of uremic fraction was injected and three clearance periods were collected; thereafter, 1 ml of normal fraction (experiments 1–3) or isotonic saline (experiment 4) was infused and three additional clearance periods were obtained. The sequence of the injections was reversed in experiments 5–8: 1 ml of the normal fraction (experiments 5–7) or isotonic saline (experiment 8) was administered first followed by the infusion of 1 ml of uremic fraction.

Values for set A represent the average of the two last clearance periods collected immediately before the second infusion. Values for set B are the average of the clearance periods collected during 60 min after the second infusion.

sorption decreases throughout the natural history of chronic renal disease, if salt intake stays constant, the possibility exists that a circulating factor with natriuretic activity could contribute to the regulated increase in sodium excretion per nephron that accompanies nephron loss. An associated, and equally important, question concerns the possibility that a circulating substance that inhibits sodium or sodium-dependent transport mechanisms in several different systems could affect extrarenal sodium transport in uremic man and thereby contribute to certain of the adverse effects of the uremic state.

The assay was performed by infusing the equivalent of 10 ml of original uremic serum in a volume of infusate of 1 ml or less into rats weighing from 200 to 300 g. Thus the maximum amount of inhibitor harvested would equal that contained in the original 10 ml volume of serum; but this would presuppose complete recovery with no loss in the fractionation and preparative procedures. If the volume of distribution of the material is that of the extracellular space of the animals, which in a 250 g rat would approximate 50 ml, the amount of natriuretic material administered should have increased the concentration in the recipient animals by no more than 20% of the concentration present in the serum of the donor patient. The fraction from uremic patients proved to be natriuretic with the mean increase in sodium excretion over a 50–60 min period averaging 1.5 μEq/min or 90 μEq/hr and the peak increase in sodium excretion averaging 2.5 μEq/min. Fractional sodium

TABLE X

Correspondence between Natriuresis and Changes in Filtration Fraction

Experiment No.	Control periods				Experimental periods			
	GFR	C_{PAH}	Filtration fraction	$U_{Na}V$	ΔGFR	ΔC_{PAH}	ΔFiltration fraction	Δ$U_{Na}V$
	ml/min	*ml/min*		*μEq/min*	*ml/min*	*ml/min*		*μEq/min*
1	1.27	2.25	0.56	3.04	−0.07	−0.17	+0.01	+2.29
2	0.46	0.83	0.55	1.80	+0.09	+0.16	−0.02	+2.18
3	0.39	0.98	0.40	2.62	+0.08	+0.01	+0.24	+1.98
4	0.29	0.50	0.57	1.73	+0.08	+0.07	+0.06	−0.73
5	0.49	0.99	0.50	4.32	−0.03	+0.11	−0.08	+5.51
6	0.52	0.99	0.52	8.75	+0.04	−0.04	+0.05	+1.27
7	2.24	7.58	0.30	5.75	−0.28	−0.54	0.00	+1.64
8	0.98	2.63	0.38	2.08	+0.02	+0.32	−0.04	+1.29
9	1.20	2.19	0.55	1.08	+0.11	+0.42	−0.04	+0.19
10	1.41	4.09	0.35	3.27	+0.02	+0.09	−0.01	−0.27

GFR, clearance of inulin; C_{PAH}, clearance of PAH; filtration fraction, ratio of GFR/C_{PAH}; $U_{Na}V$, sodium excretion rate.

Serum fractions from uremic patients were used in all experiments except experiment 10 which was performed with a fraction obtained from a normal subject.

The data are presented as in Table IV and the mean changes are shown for the experimental periods.

excretion also increased by an average of 1.4% and a peak of 2.4%. When the comparable fraction of serum from normal subjects was administered, some degree of natriuresis was observed in group data; but the response was significantly greater with the uremic fractions than with the normal fractions. When 1 ml of isotonic saline was infused under identical experimental conditions, virtually no change in sodium excretion resulted. Moreover, the response produced by the normal fractions was not significantly different from that produced with the saline.

The natriuretic response typically began within 10–20 min of administration of the fraction and reached a peak within 40–60 min. When the fraction was concentrated by relyophilization, natriuresis was observed with as little as 0.2 ml of material equivalent to 10 ml of original serum. Moreover, when both uremic fractions and normal fractions or isotonic saline were administered to the same assay animal in random sequence, sodium excretion rate invariably was greater with the uremic fraction than with the normal fraction or isotonic saline.

The reason for using the rat with a reduced nephron population as the assay animal requires some discussion. Theoretically, if the circulating inhibitor in the uremic patients is a natriuretic hormone, or even if it is a toxic substance retained in the blood in uremia, the assay animals should have an increase in the endogenous concentration of the factor. Thus, the response to additional factor might be decreased. However, there is considerable evidence to support the view that the sensitivity of the control system governing sodium excretion increases markedly as the nephron population decreases. For example, in patients subjected to extracellular fluid volume expansion the increase in fractional sodium excretion varies inversely with GFR (3). Furthermore, in dogs with one remnant kidney given a fixed sodium load by one of several different routes, the hourly excretion of sodium over a 5 hr period is actually greater by the remnant kidney alone (the opposite kidney having been removed) than by both the remnant and contralateral kidney in control studies (4). Finally, if dietary salt intake is increased by any given amount, the increment in fractional sodium excretion varies inversely

TABLE XI

Effects of Uremic Fraction on Sodium Excretion before and after Incubation with Pronase and Chymotrypsin

Experiment No.		Control periods		Experimental periods	
		$U_{Na}V$	FE_{Na}	$\Delta U_{Na}V$	ΔFE_{Na}
		μEq/min	*%*	*μEq/min*	*%*
Incubation with pronase					
1	Control experiment	1.15	0.74	+2.35	+2.09
	Pronase	3.97	1.44	+1.02	+0.30
2	Control experiment	1.65	1.23	+0.96	+0.40
	Pronase	3.98	1.40	+2.56	+1.09
3	Control experiment	4.31	3.17	+2.59	+1.87
	Pronase	4.58	2.99	+5.84	+3.35
4	Control experiment	3.69	3.65	+1.87	+1.95
	Pronase	6.08	5.45	−1.54	−1.01
5	Pronase	7.78	3.82	+4.28	+1.30
Incubation with chymotrypsin					
1	Control experiment	4.31	3.17	+2.59	+1.87
	Chymotrypsin	4.73	6.74	+2.47	+1.39
2	Control experiment	3.68	3.08	+1.76	+1.03
	Chymotrypsin	6.08	4.34	+5.22	+2.25
3	Control experiment	3.98	—	+1.66	—
	Chymotrypsin	4.68	4.00	+1.69	+0.72
4	Control experiment	1.15	0.74	+2.35	+2.09
	Chymotrypsin	3.50	2.28	+1.23	+0.47
5	Control experiment	3.69	3.65	+1.87	+1.95
	Chymotrypsin	1.32	2.61	−0.74	−1.19

See Table I for definition of abbreviations.
The data are presented as in Table IV and the mean changes are shown for the experimental periods.

TABLE XII

The Influence of Isobutanol Extraction or Alkalinization on the Natriuretic Effects of the Serum Fraction from Uremic Patients

Experiment No.		Control periods		Experimental periods	
		$U_{Na}V$	FE_{Na}	$\Delta U_{Na}V$	ΔFE_{Na}
		μEq/min	*%*	*μEq/min*	*%*
Extraction with isobutanol					
1	Aqueous phase	7.85	8.49	+1.27	+1.23
	Organic phase	2.55	1.26	+0.65	+0.19
2	Aqueous phase	11.10	5.29	+2.29	+0.34
	Organic phase	11.95	16.0	+0.56	−0.25
3	Control experiment	10.30	8.58	+1.77	+0.14
	Aqueous phase	5.72	3.40	+2.32	+1.33
4	Control experiment	7.52	5.25	+2.83	+1.63
	Organic phase	7.15	2.84	−0.53	−0.25
Alkalinization to pH 10.5					
1	Control experiment	4.34	8.55	+1.04	+1.08
	Alkalinization	2.51	2.34	−0.07	+0.06
2	Control experiment	6.13	7.34	+1.55	+1.63
	Alkalinization	2.68	2.55	−0.26	−0.21
3	Control experiment	3.68	3.08	+1.76	+1.03
	Alkalinization	1.42	3.27	+0.14	+0.08
4	Control experiment	7.85	5.58	+2.86	+1.26
	Alkalinization	4.84	3.27	+1.26	+0.64

See Table I for definition of abbreviations.
The data are presented as in Table IV and the mean changes are shown for the experimental periods.

TABLE XIII

Persistence of Activity of the Serum Fraction from Uremic Patients in Three Assay Systems

Experimental condition	Rabbit kidney PAH uptake	Frog skin short-circuit current	Rat sodium excretion
Heat (98°C)	+	+	+
Storage (−80°C)	+	+	+
Chymotrypsin digestion	+	+	+
Pronase digestion	+	+	+

with GFR; for example it is 64 times as great in a patient with a GFR of 2 ml/min as in a normal subject with a GFR of 120 ml/min (3). Thus, the smaller the number of nephrons contributing to sodium excretion, the greater must be the response of each nephron to a given load of sodium. One possible element in this increased responsiveness could be an enhanced sensitivity of the nephrons to a circulating natriuretic factor.

The natriuretic response is not explicable on the basis of changes in either hematocrit (8, 9) or blood pressure (10). Moreover, the increase in sodium excretion rate occurred in the presence of an unchanged or decreased GFR as well as an increased GFR, and there was no correlation between the change in GFR and the change in sodium excretion. Finally neither changes in PAH clearance nor filtration fractions (11, 12) bore any relationship to the changes in sodium excretion rates.[5] The basis for the natriuresis, therefore, does not seem to reside in an alteration in the hemodynamic or physical factors (13) that we have been able to measure and, if the natriuretic material is similar to the material which inhibited sodium transport in the anuran membrane, a more likely explanation is that a component step involved in transepithelial sodium transport was affected.

In analyzing the studies done with normal serum fractions, no difference was observed with samples obtained from subjects on a 1 and 12 g salt diet. This could indicate that the factor found in uremic patients is not present or has no physiological significance in normal subjects. Alternatively, if the factor is a natriuretic hormone which is present in the normal state, the activity in subjects on a 12 g salt diet might be too low to be detectable with the present bioassay system. In possible support of the latter view is the fact that in a patient with a GFR of 6 ml/min (the average GFR in the patients studied) on a 7 g salt diet, the requirements for sodium excretion *per nephron* are equivalent to those that would be obtained in a normal subject (GFR 120 ml/min) ingesting over 120 g of salt per day.

The evidence that the natriuretic material is the same as the fraction of uremic serum previously shown to inhibit sodium transport by frog skin and toad bladder and to inhibit PAH uptake by kidney slices is quite substantial. In all three systems, the inhibitor was obtained from a Sephadex G-25 fraction of serum from patients with chronic uremia and the elution characteristics were the same in each instance. The inhibitor thus was contained in the same fraction. For all three assay systems, the inhibitor or inhibitors resisted boiling, freezing, and digestion with pronase and chymotrypsin (Table XIII). Furthermore, extraction of the fraction with an organic solvent was ineffective in removing the inhibitor of PAH uptake by kidney slices (14) and of sodium reabsorption by rat tubules. Using ultrafiltration techniques evidence has been presented which suggests that the inhibitor of sodium transport by the frog skin may have a molecular weight of about 500–1000 (2); similar data have not been obtained yet with the rat assay. The present studies suggest that the inhibitor of sodium transport by the nephron is insensitive to incubation at very low pHs, while treatment with alkali at pH 10.5 destroys the activity. Comparable data are not yet available for the other two systems.

Studies currently are in progress to characterize further the chemical nature of the natriuretic material and to define its biologic properties as well as its site of action in the nephron using micropuncture techniques.

[5] In these experiments we have assumed that the serum fraction had no effect on the renal extraction of PAH in vivo. This may not necessarily be valid, however, in view of the known effects of the uremic fraction on PAH uptake by rabbit kidney cortical slices in vitro (1). The results, therefore, do not rigorously exclude the contribution of a change in filtration fraction to the genesis of the natriuresis. However, this explanation appears unlikely in view of the inhibition of sodium transport by the fraction in the frog skin and toad bladder (2). Moreover, if the fraction did decrease PAH extraction, thereby obscuring a rise in C_{PAH}, the inhibition of PAH transport would have to have been just enough to offset precisely the true increase in renal plasma flow produced by the fraction.

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REFERENCES

1. Bricker, N. S., S. Klahr, M. Purkerson, R. G. Schultze, L. V. Avioli, and S. J. Birge. 1968. In vitro assay for a humoral substance present during volume expansion and uraemia. *Nature (London).* **219**: 1058.
2. Bourgoignie, J. J., S. Klahr, and N. S. Bricker. 1971. Inhibition of transepithelial sodium transport in the frog skin by a low molecular weight fraction of uremic serum. *J. Clin. Invest.* **50**: 303.
3. Slatopolsky, E., I. O. Elkan, C. Weerts, and N. S. Bricker. 1968. Studies on the characteristics of the control system governing sodium excretion in uremic man. *J. Clin. Invest.* **47**: 521.
4. Schultze, R. G., H. S. Shapiro, and N. S. Bricker. 1969. Studies on the control of sodium excretion in experimental uremia. *J. Clin. Invest.* **48**: 869.
5. Shankel, S. W., A. M. Robson, and N. S. Bricker. 1967. On the mechanism of the splay in the glucose titration curve in advanced experimental renal disease in the rat. *J. Clin. Invest.* **46**: 164.
6. Fuhr, J., J. Kaczmarczyk, and C. D. Kruttgen. 1955. A

simple colorimetric method for inulin determinations for kidney clearance examinations in the metabolically healthy and in diabetes. *Klin. Wochenschr.* **33**: 729.
7. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**: 279.
8. Schrier, R. W., and L. E. Earley. 1970. Effects of hematocrit on renal hemodynamics and sodium excretion in hydropenic and volume-expanded dogs. *J. Clin. Invest.* **49**: 1656.
9. Burke, T. J., R. R. Robinson, and J. R. Clapp. 1971. Effect of arterial hematocrit on sodium reabsorption by the proximal tubule. *Amer. J. Physiol.* **220**: 1536.
10. Koch, K. M., H. S. Aynedjian, and N. Bank. 1968. The effect of acute hypertension on sodium reabsorption by the proximal tubule. *J. Clin. Invest.* **47**: 1696.
11. Lewy, J. E., and E. E. Windhager. 1968. Peritubular control of proximal tubular fluid reabsorption in rat kidney. *Amer. J. Physiol.* **214**: 943.
12. Brenner, B. M., K. H. Falchuk, R. I. Keimowitz, and R. W. Berliner. 1969. The relationship between peritubular capillary protein concentrations and fluid reabsorption by the renal proximal tubule. *J. Clin. Invest.* **48**: 1519.
13. Daugharty, T. M., L. J. Belleau, J. A. Martino, and L. E. Earley. 1968. Interrelationship of physical factors affecting sodium reabsorption in the dog. *Amer. J. Physiol.* **215**: 1442.
14. Klahr, S., J. Bourgoignie, C. L. Miller, H. Lubowitz, and N. S. Bricker. 1969. Studies in search of a natriuretic hormone in uremic patients. *Proc. Int. Cong. Nephrol. 4th 1969.* **2**: 88.