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Research Article

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The Presence of a Natriuretic Factor in Urine of Patients with Chronic Uremia

THE ABSENCE OF THE FACTOR IN NEPHROTIC UREMIC PATIENTS

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ABSTRACT A gel filtration fraction of serum from chronically uremic patients has been shown previously to produce natriuresis in the rat. In the present studies, the same fraction from urine of uremic patients and normal subjects was studied for its natriuretic activity. Urine samples were obtained from 17 chronically uremic patients (mean glomerular filtration rate [GFR], 8.7 ml/min; mean fractional sodium excretion [FE_{Na}], 5.7%), and 14 normal subjects. The fraction from the uremic patients produced a significant increase in absolute sodium excretion ($U_{Na}V$) and FE_{Na} ; the fraction from normal subjects had no statistically significant effect on either $U_{Na}V$ or FE_{Na} ; and the difference between the response to the uremic vs. normal fractions was highly significant for both parameters of sodium excretion. When a more concentrated urine fraction from uremic patients was administered, a striking natriuresis was observed with values for FE_{Na} rising to levels as high as 12%. Studies also were performed on eight patients with far advanced chronic renal insufficiency and the nephrotic syndrome. The serum fraction was studied in each of these patients and the urine fraction in three. For the group, $U_{Na}V$ in the assay rats decreased by 0.87 μ eq/min and FE_{Na} decreased by 1.35% after infusion of the serum fraction. These results differ significantly from those of patients with chronic uremia without the nephrotic syndrome. The data are consistent with the

view that the increased activity of the natriuretic factor in the serum of chronically uremic patients is not due to failure of excretion; rather it relates either to an increased rate of production and/or a decreased rate of degradation. The data also show that the inhibitor is detectable when FE_{Na} is increased, but not when uremia is associated with a sodium-retaining state.

INTRODUCTION

The inhibitor of sodium transport which has been found in the serum of patients with chronic uremia has been presumed to be a component part of the control system regulating sodium excretion, and to play a role in the genesis of the progressive natriuresis per nephron that occurs in patients with advancing chronic renal disease (1). However, the natriuretic factor could also be a nonspecific toxic substance, which is retained in the blood of uremic patients because of failure of excretion, and which bears no relation to the dictates of sodium balance. In the latter event, the rate of excretion of the factor in the urine should be no greater in uremic patients than in normal subjects, and the appearance of the inhibitor in serum and urine of uremic patients should be independent of the concurrent patterns of sodium excretion.

In the present studies, urine from chronically uremic patients and from normal subjects was examined for the presence of the natriuretic factor. In addition, assays for the presence of the factor were performed on urine and/or serum fractions from eight patients with advanced chronic renal disease and uremia who were nephrotic and edematous.

Parts of this work have been published in abstracted forms (7, 8).

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METHODS

Assays of urine from uremic patients. Urine samples were collected quantitatively, generally for periods of 24 h in glass or plastic containers maintained at 4°C. The samples were frozen as soon as the collections were completed. Within 24–72 h of collection, the total volume was concentrated 10–15-fold in a stainless steel container by lyophilization (model 5012 freeze-dryer, Refrigeration for Science, Inc., Island Park, N. Y.) and stored in plastic bottles at –80°C in an ultracold cabinet (model UC 105, Kelvinator Commercial Products, Inc., Manitowoc, Wis.). Then samples of the concentrate were thawed at room temperature, centrifuged at 4°C to discard any precipitate, and fractionated by column chromatography in a manner identical to that described previously for the fractionation of serum samples (2). In brief, 25-ml aliquots of the concentrated urines, equivalent to 5-h samples of original urine, were applied to individual 2.5 × 95-cm columns packed with Sephadex G-25 (fine grade, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). Elution was carried out at 4°C by gravity at a rate of 55–65 ml/h with a solution of 10 mM ammonium acetate at pH 6.8. The effluent solution was collected overnight in 18 × 150-mm glass tubes using an automated fraction collector (model 7000 Ultrorac, LKB Producter AB, Stockholm, Sweden). On the basis of the ultraviolet absorption at 280 nm (LKB Uvicord) and of the electrical conductivity (model 5300B Conductolyzer with 5312B Conductivity cell, LKB Producter AB) tracings, the effluent solution and the contents of the tubes were pooled into several different fractions. The fraction containing the principal peaks of sodium, potassium, urea, and creatinine was discarded. As in the case with the serum samples, of several fractions eluted before and after the salt peak, natriuretic activity was contained only in the fraction appearing immediately after the salt peak (3). Only this portion of the effluent solution was then routinely prepared by pooling the contents of 10 consecutive tubes (120 ml total) starting with the tube in which the specific conductance of the eluate had returned to base-line values and lyophilizing to dryness overnight in a glass container.

The dried eluate was then carefully dissolved in 2.5 ml of distilled water, transferred into a screw cap glass or polyethylene counting vial, and stored at –80°C until the day of assay. Each milliliter of this solution was equivalent to the volume of original urine excreted in approximately 2 h. Storage of the concentrated urine samples or of the final fraction for periods of up to several months was found to have no influence on the natriuretic activity of the material.

The standard fractions, whether obtained from uremic patients or normal subjects, typically contained less than 10 meq/liter of sodium and 1 meq/liter of potassium. Differences were not observed between fractions from uremic patients and normal subjects for ammonium, urea, and protein concentrations. The mean values were: ammonium, 24.4 ± 3.7 meq/liter; urea, 15.8 ± 2.6 mg/100 ml; and protein, 2.0 ± 0.6 mg/ml.

The day of assay the sample was thawed, adjusted to a pH of 7.4, and to sodium and potassium concentrations of 140 and 4 meq/liter, respectively. All samples were prepared in a separate laboratory and the investigator performing the bioassay had no knowledge of the nature of the sample being tested.

The assays for natriuretic activity were performed on female Sprague-Dawley rats weighing from 180 to 270 g.

The assay rats had a reduced glomerular filtration rate (GFR) and were given 0.2% NaCl to drink for 2 days before performance of an assay. The rationale for using this model relates to the observation that, although the natriuretic factor from human urine has been found to produce a natriuretic response in normal hydropenic rats, the sensitivity of salt-loaded rats with a reduced nephron population to the factor appears increased substantially (4). The details of the assay procedure have been described previously (3). In brief, between 8:00 and 9:00 a.m. the rats were anesthetized lightly with ether to permit the insertion of catheters into the urinary bladder, one femoral artery, and a jugular vein. The rats were then placed in a plastic restraining device and allowed to recover from the anesthesia. No fluid replacement for surgical losses or for urine flow during the first 30 min period of recovery was provided. Between 10:00 and 11:00 a.m. a priming dose of [¹⁴C]inulin (New England Nuclear, Boston, Mass.) was administered in a volume of 0.4–0.6 ml and a sustaining solution of radioactive inulin in isotonic saline was infused at a rate of 20 μl/min. As in the previous study with serum fractions, if the rate of urine flow during the equilibration period exceeded the rate of the sustaining infusion by more than 25 μl/min, a second infusion of isotonic saline was administered at a rate equal to the difference and the second infusion rate was maintained constant throughout the experiment (3). After approximately 2 h, clearance periods were initiated for determination of absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion¹ and GFR.

At least six control clearance periods ranging from 10 to 20 min in duration were obtained. After the fourth period, the control phase was terminated as soon as two consecutive clearance periods were obtained in which urine flow rate varied by less than 10%. The test solution consisting of either a urine fraction from a uremic patient or the comparable urine fraction from a normal subject was administered intravenously, generally between 1:00 and 2:00 p.m., in a volume of no greater than 1 ml at a rate of 100 μl/min. With the exception of the dose-response studies described in the text, the amount of the fraction administered was harvested from the volume of original urine excreted in 126 ± 15 min for the uremic subjects and 115 ± 10 min for the normal subjects. These two values do not differ significantly. At the completion of the infusion, clearance periods of 10 min duration were resumed and continued for 60 min.

Arterial blood samples were collected during each clearance period for the determination of hematocrit and plasma inulin activity. Sodium and potassium concentrations were determined three times during each experiment (3). In representative experiments, arterial blood pressure of the assay animal was measured during and after infusion of the urine fraction. As in the previous experiments with the serum fractions, no change in blood pressure was detected either in the animals receiving the uremic fractions or in those receiving the normal fractions.

If evidence of intermittent obstruction to urine flow, leakage of urine around the catheter, or gross hematuria occurred, the experiment was terminated. The same criteria were employed in these studies as in the previous studies for the rejection of a completed experiment (3). These included: (a) $U_{Na}V$ in the assay rat of less than 1 or greater than 12 μeq/min during the control clearance peri-

¹Abbreviations used in this paper: FE_{Na} , fractional sodium excretion; $U_{Na}V$, absolute sodium excretion.

ods; or (b) a change in $U_{Na}V$ of greater than 20% between the penultimate and the final control clearance period. Five experiments were discarded because of these criteria. As in our previous study with the serum fractions, in all experiments the values for GFR, $U_{Na}V$, and FE_{Na} during the last two control periods were averaged and compared with the mean of the six consecutive 10-min clearance periods obtained after infusion of the test solution (3).

The first part of the study involved the comparison of urine fractions from chronically uremic patients with those from normal subjects. Urines were obtained from 17 patients with far advanced chronic renal disease. Endogenous creatinine clearance values were less than 20 ml/min in all patients and the average was 8.7 ± 1.4 ml/min; the mean value for blood urea nitrogen (BUN) was 107 ± 7 mg/100 ml; and FE_{Na} averaged $5.7 \pm 0.8\%$. Clinically, all of the patients, with one exception discussed below, were in external sodium balance. None of the uremic patients had congestive heart failure and none were on chronic dialysis.

For comparison, urines were obtained from 14 normal subjects (physicians and laboratory personnel). All were on an ad lib. salt intake. For several uremic patients and normal subjects, more than one study was performed using the same or different urine samples collected under the same clinical conditions.

One patient (no. 14, Table II) with a GFR of approximately 4 ml/min was studied under two very different clinical circumstances. He was admitted to the hospital after a prolonged period of anorexia, nausea, and vomiting. The first urine sample was collected when the patient was volume depleted, and had a FE_{Na} of 1.2%. Thereafter extracellular fluid volume was restored to normal and the

patient was maintained on a 5-g salt diet. Urine was then recollected 30 days later, when FE_{Na} was 8.3%. The fractions from both urine samples were assayed.

To study the dose-response relationships, uremic or normal fractions obtained from several columns were pooled, relyphilized to dryness, and diluted in a volume of distilled water which provided either a 5- or a 10-fold increase in concentration over the standard preparation. The assays were performed by first infusing 0.1 ml (of the 10-fold concentrate) or 0.2 ml (of the 5-fold concentrate). 1 h later, after the completion of six 10-min clearance periods, 1 ml of the same preparation was administered and six additional periods were obtained.

Studies on nephrotic patients. These studies consisted of examination of the serum fraction from eight patients with far advanced chronic renal disease (mean GFR, 4.3 ± 0.6 ml/min) who were nephrotic. All were markedly edematous, had a serum albumin of 3 g/100 ml or less, and were excreting greater than 5 g of protein/day. The urine fraction was also assayed in three of these patients. The preparation and bioassay of the serum fraction were performed in accordance with the methods and techniques previously described (3). The patients in both the non-nephrotic uremic and nephrotic uremic groups who were receiving either digitalis glycosides or a diuretic preparation are designated in Tables II and IV. 11 of the 17 nonnephrotic uremic patients and 5 of the 8 nephrotic patients received neither type of medication. As in the previous studies with the serum factor (2, 3), there was no correlation between the history of drug ingestion and the experimental results.

Radioactive inulin was determined in 25–50- μ l samples of either urine or plasma diluted in 10 ml of Aquasol solution (New England Nuclear). The samples were counted

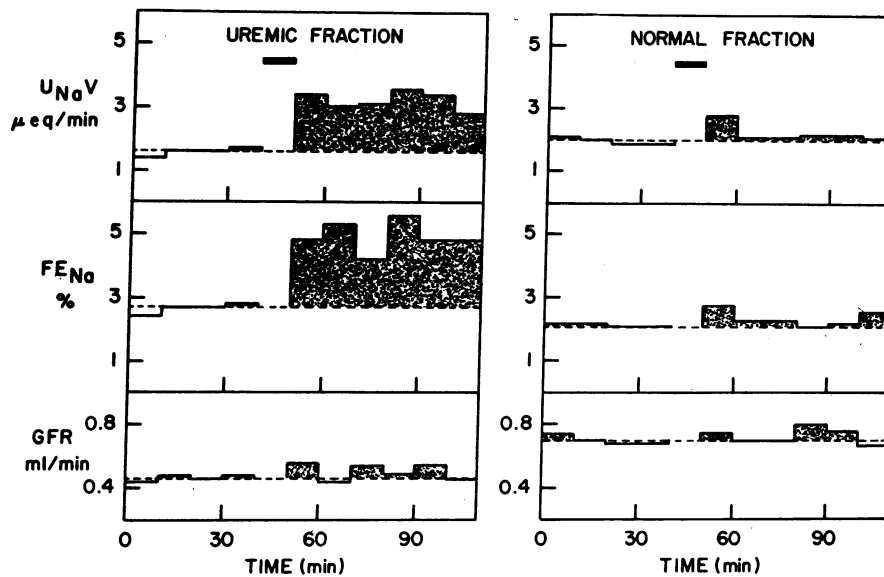


FIGURE 1 Effects of uremic vs. normal urine fraction on $U_{Na}V$, FE_{Na} , and GFR. The dotted line indicates the mean base-line control values observed before infusion of the fraction. In each experiment the infusion rate of fluid during the control and the experimental periods was 20 μ l/min. In the animal which received the uremic fraction, urine flow rates during the control periods averaged 31 μ l/min and increased by a mean of 12 μ l/min after infusion of the fraction. In the animal which received the normal fraction urine flow rate averaged 19 μ l/min during the control periods and 21 μ l/min during the experimental periods.

TABLE I
Effects of the Urine Fraction from Normal Subjects on
Glomerular Filtration Rate and Sodium Excretion

Subject no.	Control periods			Experimental periods		
	GFR	U _{Na} V	FE _{Na}	ΔGFR	ΔU _{Na} V	ΔFE _{Na}
	ml/min	μeq/min	%	ml/min	μeq/min	%
1	0.74	6.66	6.02	+0.01	-0.62	-0.70
2	0.83	7.91	7.22	-0.12	+1.10	+2.40
3	0.61	4.48	4.93	-0.03	-0.02	+0.24
4	0.78	6.25	6.53	-0.19	-0.70	+0.81
5	0.88	4.97	4.14	+0.13	-0.06	-0.60
6	0.66	1.79	2.03	+0.05	+0.45	+0.34
7	1.41	8.17	4.19	+0.04	-0.14	-0.22
8	1.07	2.74	2.24	-0.09	+0.70	+0.82
9	1.20	2.51	1.84	+0.02	+0.76	+0.20
10a	0.49	5.13	8.07	-0.08	+1.46	+4.45
b	1.33	4.85	3.38	-0.05	+1.21	+0.89
11a	2.02	6.41	2.25	-0.42	-0.32	+0.44
b	1.31	5.59	3.19	+0.04	+2.86	+1.21
12a	0.98	8.08	5.80	+0.24	+2.99	+0.54
b	0.41	3.51	6.05	-0.02	+2.88	+5.24
13a	0.34	1.30	3.39	-0.04	-0.64	-1.41
b	0.91	3.66	2.96	-0.14	-1.70	-1.06
14a	1.62	5.14	2.45	-0.28	-1.16	-0.12
b	1.21	2.74	2.24	-0.09	+0.70	+0.82
Mean	0.99	4.92	4.19	-0.05	+0.53	+0.77
±	0.10	0.47	0.44	0.03	0.31	0.39

The mean values for U_{Na}V and FE_{Na} for the clearance periods obtained over the 60 min interval after infusion of the fractions were compared with the mean values obtained during the last two control clearance periods. The values under "experimental periods" thus represent the difference in GFR, U_{Na}V, and FE_{Na} between the mean experimental and the control values.

in a Tri-Carb liquid scintillation spectrometer (model 3330, Packard Instrument Co., Inc., Downers Grove, Ill.). Sodium and potassium were measured using a flame photometer (model 143, 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.

RESULTS

Assays of urine from nonnephrotic uremic patients.
Two representative experiments are shown in Fig. 1 demonstrating the effects of the infusion of a urine fraction from a uremic patient, and from a normal subject. Values are shown for U_{Na}V, FE_{Na}, and GFR. With the uremic fraction, U_{Na}V increased from the control level during the first postinfusion period and values remained above the control level throughout the six periods of observation. FE_{Na} followed a similar pattern. The mean increments in U_{Na}V and FE_{Na} were 1.53 μeq/min and 2.09%, respectively. GFR showed no significant change. With the administration of the urine fraction from the normal subject, an increase in U_{Na}V and FE_{Na}

was noted during the first postinfusion period only. Thereafter values returned towards the baseline and the comparison of the mean postinfusion values with the control values showed an increase of 0.45 μeq/min for U_{Na}V and 0.34% for FE_{Na}.

The results of 19 assays in which the urine fractions from 14 normal subjects were tested and 28 assays in which the test solution consisted of urine fractions from 17 patients with chronic uremia are shown in Tables I and II, respectively. The change in GFR after both normal and uremic fractions was small, inconsistent in direction, and not statistically significant; nor was there a significant difference between the change in GFR between the two groups. U_{Na}V increased by a mean of 0.53

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

Patient no.	Control periods			Experimental periods		
	GFR	U _{Na} V	FE _{Na}	ΔGFR	ΔU _{Na} V	ΔFE _{Na}
	ml/min	μeq/min	%	ml/min	μeq/min	%
1a	0.50	2.80	4.21	+0.02	+1.93	+2.56
b	0.52	2.43	3.24	+0.02	+2.80	+3.66
c	0.58	4.51	5.18	+0.06	+1.81	+1.35
d	0.80	2.30	2.19	-0.02	+0.48	+0.90
e	0.66	3.18	3.54	+0.08	+1.37	+0.94
2a	0.58	5.15	6.58	+0.04	+3.37	+3.71
b	0.50	2.41	3.62	+0.06	+1.42	+1.49
3a	0.97	4.12	3.26	-0.08	+4.90	+4.58
b	1.24	4.12	2.77	+0.07	+1.82	+1.06
4a	0.36	9.27	21.91	0	+3.12	+7.97
b	1.96	8.57	3.25	-0.23	+0.63	+0.75
5a	0.41	5.09	9.01	-0.09	-0.04	+2.46
b	1.87	6.97	2.97	+0.05	+2.75	+1.01
c	1.56	3.54	2.01	-0.42	+1.80	+2.17
6	0.59	6.81	8.49	+0.09	+2.06	+1.31
7	0.41	4.12	7.81	-0.05	+0.64	+2.63
8	0.78	4.25	4.23	+0.02	+1.28	+1.19
9	0.47	1.69	2.86	+0.04	+1.53	+2.09
10a	0.91	4.03	3.32	-0.03	+1.44	+1.44
b	1.11	6.49	4.06	-0.08	+2.32	+1.87
11	0.45	1.88	3.07	-0.08	-0.03	+1.79
12	1.49	9.07	4.67	-0.06	-0.47	-0.08
13	1.08	8.59	5.38	-0.04	+0.40	+0.40
14a*	1.50	6.57	3.24	-0.16	-1.39	-0.40
b	0.47	2.39	4.32	+0.05	+1.54	+1.55
15	1.15	3.94	2.80	-0.25	+2.72	+2.92
16	0.46	5.30	8.87	+0.01	+0.50	+0.77
17	0.79	7.04	7.17	0	+1.56	+1.67
Mean	0.84	4.82	5.21	-0.03	+1.62	+2.01
±SE	0.09	0.44	0.76	0.02	0.23	0.31

Results are expressed as in Table I.

* Sample 14a is not included in the statistical analysis (see Methods). One patient (7) was receiving 500 mg hydrochlorothiazide daily for hypertension and five patients (6, 9, 10, 11, and 13) were receiving a maintenance dose of a digitalic preparation. The other 13 patients received neither medication.

$\pm 0.31 \mu\text{eq}/\text{min}$ and FE_{Na} increased by a mean of $0.77 \pm 0.39\%$ after administration of the normal fraction. Neither change differs significantly from zero. After administration of the uremic fraction, U_{NaV} increased by a mean of $1.62 \pm 0.23 \mu\text{eq}/\text{min}$ and FE_{Na} increased by a mean of $2.01 \pm 0.31\%$. Both increases differ significantly from zero ($P < 0.001$). The difference between the two groups is also significant for U_{NaV} ($P < 0.01$) and FE_{Na} ($P < 0.02$). If all of the results from each individual patient who was studied more than once, whether they represent replicate studies of the same urine sample or studies of different urine samples collected under similar clinical conditions are entered as a single value, the difference between the two groups remains highly significant for both U_{NaV} ($P < 0.01$) and for FE_{Na} ($P < 0.005$).

Fig. 2 depicts the mean changes in U_{NaV} and FE_{Na} , expressed as a percentage of control values, after infusion of the urine fractions from normal subjects and patients with chronic uremia. With the urine fractions from the normal subjects, insignificant increases in U_{NaV} and FE_{Na} (10.4 ± 7.3 and $13.7 \pm 7.0\%$, respectively) were observed. After infusion of the urine fractions from the uremic patients, U_{NaV} increased by a mean of $40.5 \pm 6.2\%$ and FE_{Na} increased by a mean of $46.8 \pm 6.9\%$. Both of these changes differ significantly from zero ($P < 0.001$) and are significantly different from the changes observed after infusion of the fractions from normal subjects ($P < 0.005$ for both U_{NaV} and FE_{Na}).

Fig. 3 shows mean changes in U_{NaV} , FE_{Na} , and GFR for each of the six postinfusion clearance periods. The values are expressed as a percentage of the respective control values and, for each period, the response to the normal fractions is compared with that to the uremic fractions. With the normal fractions, a significant increase in U_{NaV} and FE_{Na} was observed during the first postinfusion period only, concomitant with a small, al-

beit insignificant, increase in GFR. In the succeeding periods U_{NaV} and FE_{Na} did not differ significantly from the control values. In contrast, after infusion of the uremic fractions, U_{NaV} and FE_{Na} were significantly greater than the control values in each of the six clearance periods ($P < 0.001$ for all periods). Moreover, with the exception of the first period for U_{NaV} , and periods 1 and 2 for FE_{Na} , the differences between the two groups were significant. In none of the periods did the values for GFR differ significantly between the two groups.

The sequential assays on a patient studied first with extracellular fluid volume depletion and again after volume repletion are included in Table II as experiment 14a and b. In the initial assay, both U_{NaV} and FE_{Na} decreased slightly. In the second assay, the changes in U_{NaV} and FE_{Na} averaged $+1.54 \mu\text{eq}/\text{min}$ and $+1.55\%$, respectively.

The difference between the response to the uremic fractions and normal fractions could not be related to a difference in net fluid balance during the course of the experiments. The data referable to external fluid balance in both groups of assay rats are shown in Table III. The total infusion rates were closely comparable in the two groups. However, in the rats receiving the normal fractions, urine flow rates were essentially equal to the infusion rates whereas, in the rats receiving the uremic fractions, urine flow rates exceeded the infusion rates before and after administration of the test solution. Thus, the natriuresis occurred with the uremic fractions despite negative fluid balance in the assay rats.

Dose-response experiments using normal and uremic fractions are shown in Fig. 4. With the uremic fraction, the initial dose, equivalent to 49 min of urine, was administered in a volume of 0.1 ml. U_{NaV} during the six postinfusion clearance periods increased by a mean of $1.82 \mu\text{eq}/\text{min}$ over the control value. 1 ml of the same concentrate (equivalent to the urine excreted in 490

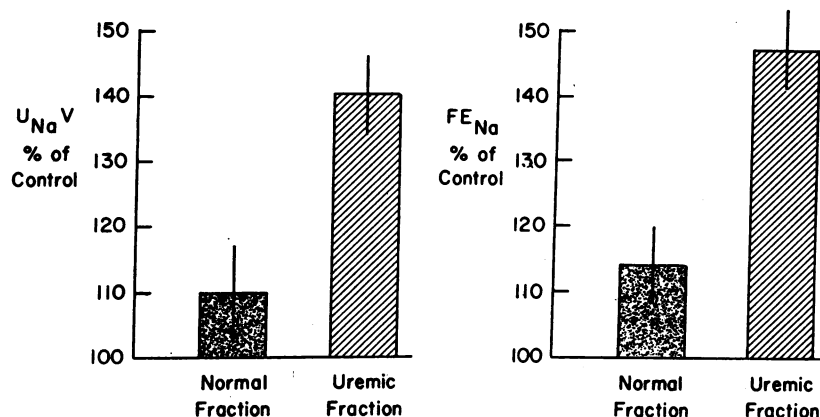


FIGURE 2 Mean changes, $\pm\text{SE}$, in U_{NaV} and FE_{Na} expressed as percent of control values, after infusion of urine fractions from normal subjects and from chronically uremic patients.

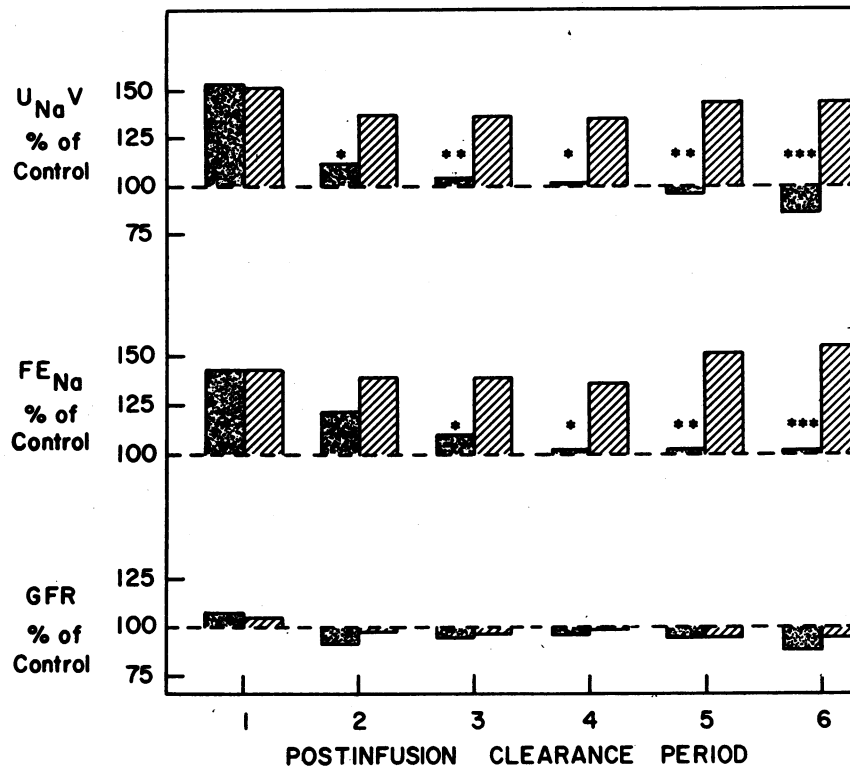


FIGURE 3 Mean changes in $U_{Na}V$, FE_{Na} , and GFR, expressed as percent of control values, for each of the six clearance periods after infusion of urine fractions from normal subjects (stippled bars) and from chronically uremic patients (hatched bars). Significant differences between the responses to the normal fractions and the uremic fractions are indicated as (*) for $P < 0.02$, (**) for $P < 0.005$, and (***) for $P < 0.001$. Each clearance period was of 10 min duration.

min) was then administered. $U_{Na}V$ rose to a peak of $19 \mu\text{eq}/\text{min}$ in the second post-infusion period. Values then fell sharply presumably because no fluid replacement was given and the rat became markedly volume depleted and sustained a substantial fall in GFR. The administration of 0.2 ml of urine fraction from a normal subject (obtained from the urine excreted in 93 min) produced

no increase in $U_{Na}V$. With the injection of 1 ml of the same fraction (equivalent to urine excreted during 465 min), $U_{Na}V$ increased from a mean value of $3.6 \mu\text{eq}/\text{min}$ during the control periods to a peak of $7.1 \mu\text{eq}/\text{min}$ during the second postinfusion period.

Changes in FE_{Na} for these experiments are also shown in Fig. 4. With the uremic fraction FE_{Na} in-

TABLE III
External Fluid Balance during the Control and the Experimental Periods for the Two Groups of Assay Rats

Group of assay rats	Infusion rate	Control periods		Experimental periods	
		Urine flow	Balance	Urine flow	Balance
	$\mu\text{l}/\text{min}$	$\mu\text{l}/\text{min}$	$\mu\text{l}/\text{min}$	$\mu\text{l}/\text{min}$	$\mu\text{l}/\text{min}$
Normal fraction	47.8 ± 2.4	46.0 ± 4.1	$+1.8 \pm 3.2$	47.6 ± 4.3	0.2 ± 4.1
Uremic fraction	50.0 ± 2.3	57.1 ± 4.0	-7.1 ± 4.5	64.7 ± 4.1	-14.7 ± 4.0

Fluid balance represents the difference between the infusion rate and the urine flow rate.

Control and experimental periods are defined in Table I.

The values correspond to those for the same control and experimental periods presented in Tables I and II.

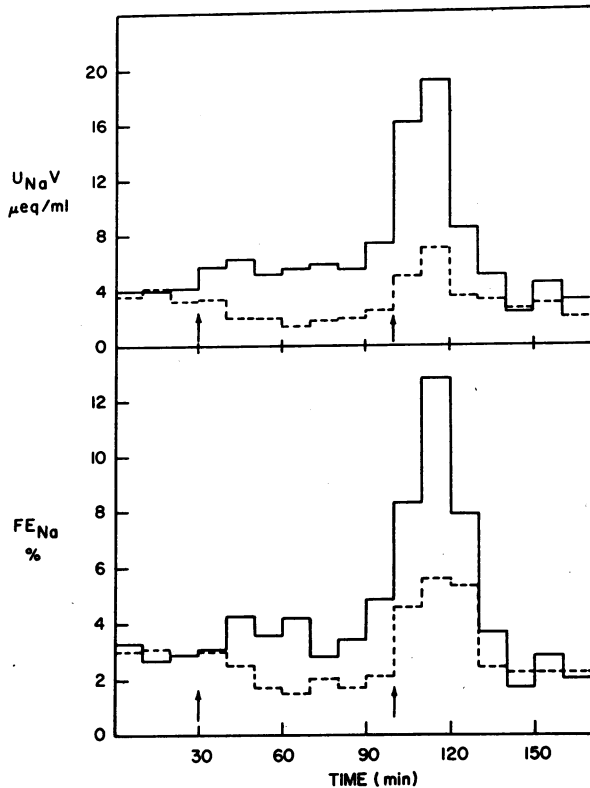


FIGURE 4 Dose-response experiments with urine fractions from a uremic patient (solid line) and a normal subject (broken line). Absolute values for $U_{Na}V$ (upper panel) and FE_{Na} (lower panel) are shown. The initial dose (first arrow) was equivalent to 43 min of urine for the uremic sample and 95 min of urine for the normal sample. At the second arrow, the doses infused were equivalent to 465 and 490 min of urine for the uremic and the normal sample, respectively.

creased from 2.8% to mean values of 3.8% after the first infusion and 5.2% after the second infusion. The peak value for FE_{Na} was 12.8%. With the normal fraction mean FE_{Na} values changed from 2.9 to 1.9% and then to 3.4%. The peak value for FE_{Na} after the second infusion was 5.6%. The mean values for FE_{Na} in four dose-response experiments using uremic fractions were 2.9% (control), 3.5% (first injection), and 4.9% (second injection).

Studies on nephrotic patients. Table IV presents the results of 12 experiments from the eight patients with the nephrotic syndrome. Serum fractions were infused in nine studies and urine fractions in three. $U_{Na}V$ and FE_{Na} did not increase significantly in 10 studies using the fractions from 7 of the 8 patients. The serum fraction from the eighth patient increased $U_{Na}V$ by a mean of 1.85 $\mu eq/min$ and FE_{Na} by a mean of 1.94%. The urine fraction from the same patient also produced an in-

TABLE IV
Effects of Serum and Urine Fractions from Patients with Chronic Uremia and the Nephrotic Syndrome on Glomerular Filtration Rate and Sodium Excretion

Patient no.	Control periods			Experimental periods		
	GFR	$U_{Na}V$	FE_{Na}	ΔGFR	$\Delta U_{Na}V$	ΔFE_{Na}
	ml/min	$\mu eq/min$	%	ml/min	$\mu eq/min$	%
1 Serum	0.83	7.54	6.33	+0.13	+0.79	-0.54
2 Serum	0.46	2.25	3.64	0.00	-0.80	-0.80
3 Serum	0.35	2.97	6.59	-0.03	-1.06	-1.99
4 Serum	0.52	10.19	14.75	-0.10	-6.03	-7.29
5 Serum	0.54	7.70	10.50	-0.03	-0.24	+0.23
6a Serum	0.55	5.41	8.04	+0.11	-1.45	-2.86
b Serum	0.46	2.63	4.30	-0.02	-0.19	-0.10
Urine	0.84	1.37	1.23	-0.13	+0.51	+0.71
7 Serum	0.68	5.78	6.15	-0.03	-0.73	-0.77
Urine	1.29	7.41	4.35	0.00	-2.17	-1.30
8 Serum	0.68	6.81	7.92	+0.01	+1.85	+1.94
Urine	1.51	11.59	6.26	-0.14	+1.80	+1.78

Results are expressed as in Table I.

Therapy included furosemide in one patient (4) and furosemide and digitalis in two patients (1 and 8). The other five patients received neither medication.

crease in $U_{Na}V$ and FE_{Na} in the assay rat. This patient was seen as an outpatient 3 wk after his urine was collected. During this interval he had undergone a spontaneous diuresis and lost 3 kg of body weight.

Fig. 5 depicts the average values for the mean changes in $U_{Na}V$ and FE_{Na} , previously reported (3), for the serum fractions from nonedematous chronically uremic patients in external sodium balance. The results are compared with those for the same serum fraction from the eight uremic patients with the nephrotic syndrome. The mean value for GFR in the natriuretic uremic patients

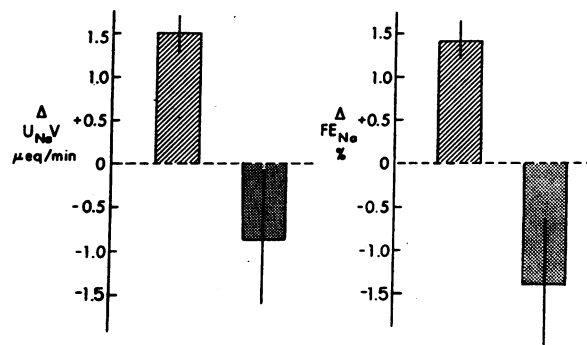


FIGURE 5 Mean changes, $\pm SE$, in $U_{Na}V$ and FE_{Na} produced by serum fractions from patients with chronic uremia in the absence (hatched bars) and in the presence (dotted bars) of the nephrotic syndrome. The results of 42 assays, in which the serum fraction from nonedematous patients with chronic uremia was tested, have been previously reported (3).

was 5.5 ml/min in comparison to the mean value for the nephrotic patients of 4.3 ml/min. Values for BUN for the two groups were similar. The mean increase in $U_{Na}V$ with serum fractions from the nonnephrotic chronically uremic patients was 1.52 $\mu\text{eq}/\text{min}$. The mean value for the nephrotic uremic patients was $-0.87 \mu\text{eq}/\text{min}$. The values for FE_{Na} for the same groups were +1.37 and -1.35% , respectively. The differences between the two groups of chronically uremic patients are highly significant, both for changes in $U_{Na}V$ ($P < 0.001$) and FE_{Na} ($P < 0.001$).

DISCUSSION

The present experiments were performed in an effort to develop additional information about the significance of the circulating inhibitor of sodium transport that has been found in the serum of patients with chronic uremia. The rationale for seeking a natriuretic factor in uremic patients relates to the fact that a progressive rise in FE_{Na} (and sodium excretion per nephron) typically occurs in patients with chronic renal disease as GFR falls. At very low levels of GFR, FE_{Na} values may exceed 30–40% in patients who ingest only an average amount of salt (5). Thus, it was postulated, that if a natriuretic hormone contributes to the modulation of sodium excretion in health (where values for FE_{Na} typically are less than 1%), the activity of the hormone might be markedly increased in patients with chronic uremia (1). But the demonstration of a circulating factor with natriuretic activity does not, per se, establish either that the inhibitor is a physiologic substance or that it contributes in a regulated manner to the modulation of sodium excretion in uremia. For example, the inhibitory factor could be one of the multitude of substances retained in the blood of chronically uremic patients due to the failure of its excretion. Though it could still contribute to the natriuresis per nephron under these circumstances, this effect would be a fortuitous one.

The approximate molecular weight of the serum natriuretic factor was estimated to be between 500 and 3,000 the basis of its ultrafiltration characteristics (2). Thus, the factor could be filterable and might be excreted in the urine.

The present data document the presence of a natriuretic factor in the urine of patients with chronic uremia who were in external sodium balance on an unrestricted salt intake and who had appropriately high rates of FE_{Na} . The degree of natriuresis produced in the assay rats using the amount of fraction harvested from an average of a 2-h urine sample was equivalent to that obtained from 10 ml of uremic serum.

In contrast, the same gel filtration fraction of urine from normal individuals failed to produce a significant increase in either $U_{Na}V$ or FE_{Na} in the assay rats. More-

over, for both parameters of sodium excretion, the differences between normal and uremic fractions were highly significant.

Several points of evidence suggest that the inhibitor obtained in urine may be identical to that observed in serum. Both active fractions have the same elution characteristics in the gel filtration system employed, appearing after the major peaks of sodium, potassium, urea, and creatinine. Both are heat stable and resist refrigeration at -80°C . Both inhibit transcellular sodium transport by the urinary bladder of the toad (2, 6), when added to the serosal surface but neither affects sodium transport when added to the mucosal surface (2, 6). Finally, both are inactivated by leucine amino peptidase (7, 8).

Thus, the elevated level of the natriuretic factor in the serum of chronically uremic patients exists in the face of excretion rates in the urine which are also increased in relation to normal subjects. This observation is inconsistent with the thesis that the material is retained in the blood due to failure of its excretion. Rather, it indicates that the natriuretic factor is produced in greater quantities in the uremic patients and/or that its rate of inactivation is diminished.

The second major question which was addressed in these studies concerns the relationship between the patterns of sodium excretion in chronic uremia and the activity of the natriuretic factor. If the factor is unrelated to the control system for the regulation of sodium excretion, its presence in serum and its excretion in urine of chronically uremic patients should be independent of the intensity of the natriuretic state. The availability of a group of eight patients with advanced uremia who had the nephrotic syndrome and were in a sodium-retaining state provided a unique opportunity to examine this question. The mean GFR in these patients was 4.3 ml/min and the mean BUN was 136 mg/100 ml. Despite the high grade uremia, no natriuretic activity could be demonstrated in the fractions from seven of the eight patients with the nephrotic syndrome. In the one patient with positive serum and urine assays a spontaneous natriuresis of major proportion took place shortly after the urine sample was collected.² The observations made with the two urine fractions from the uremic patient who was studied first with a FE_{Na} of 1.2% and again, 30 days later, with a FE_{Na} of 8.3% lend further support to the view that there is a relationship between the activity of the natriuretic factor and the concurrent patterns of so-

² Since these studies were completed a second patient with the nephrotic syndrome and a GFR of 15 ml/min has been observed to develop a spontaneous diuresis with the loss of 10 kg of edema fluid. In the midst of his natriuretic state, his urine was assayed and found to be strongly positive.

dium excretion. Finally, in studies by Schmidt, Bourgoignie, and Bricker (9), which will be reported separately, it was found that dogs subjected to experimental reduction of renal mass demonstrated the presence of the circulating inhibitor of sodium transport at low GFR if sodium intake was maintained constant throughout; however, if sodium intake was decreased in exact proportion to the decrement in GFR, such that FENa remained at less than 1% throughout, no natriuretic activity could be detected.

The composite data are consistent with the view that the circulating inhibitor of sodium transport present in chronically uremic patients cannot be explained by a failure of excretion. Rather it appears to be produced in increased amounts and/or to be degraded at a decreased rate. The inhibitor also seems to appear in the serum and urine only when uremic patients exhibit an increased rate of sodium excretion per nephron and it is not detectable when uremia is associated with a sodium-retaining state.

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