On the Influence of the Natriuretic Factor from Patients with Chronic Uremia on the Bioelectric Properties and Sodium Transport of the Isolated Mammalian Collecting Tubule

LEON G. FINE, JACQUES J. BOURGOIGNIE, KUO H. HWANG, and NEAL S. BRICKER

From the Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT A gel filtration fraction of urine from patients with chronic renal disease (natriuretic factor) has been shown previously to cause natriuresis in rats and to inhibit sodium transport in the isolated toad bladder. The effect of this fraction on transtubular potential difference and sodium transport was examined on the isolated perfused cortical collecting tubule of the rabbit. A rapid inhibition of potential difference from -22.5 mV to -12 mV ($P < 0.001$) was observed when the fraction was applied to the peritubular surface. This effect was accompanied by a decrease in net sodium flux from 6.29 to 3.21 pmol/cm per s ($P < 0.001$). Unidirectional fluxes using isotopic sodium revealed that the inhibition of net sodium transport was due to a decrease in flux from the lumen to the peritubular surface, i.e., an inhibition of active sodium transport. There was no change in sodium flux in the reverse direction. These changes were all rapidly reversed by removal of the fraction from the peritubular surface. The addition of the fraction to the lumen had no effect on potential difference or net sodium flux. Control studies using the same fraction from the urine of normal subjects had no effect on any of the parameters studies. Where both a uremic and a normal fraction were sequentially applied to the peritubular surface of the same tubule, inhibition of potential difference was obtained only with the former.

In the light of evidence implicating the collecting duct as the site of final regulation of sodium excretion by the nephron and the recent isolation of an identical urine fraction from normal animals, the data are consistent with the view that the natriuretic factor may be biologically important in the regulation of sodium balance via it's regulatory role in active sodium transport in the collecting tubule.

INTRODUCTION

Among the factors responsible for sodium homeostasis in man is a postulated humoral agent whose role is thought to be the modification of sodium excretion by the kidney according to the dictates of external sodium balance. The results of a series of studies from our laboratory in both patients (1, 2) and experimental animals (3) with chronic renal failure and in normal animals subjected to mineralocorticoid "escape" (4), have implicated a natriuretic factor as a key element in the control system regulating sodium excretion. Recollection micropuncture studies have demonstrated a proximal tubular site of action of this factor (5), however, in light of recent evidence implicating the collecting duct as the final site of regulation of sodium excretion in experimental animals (6) the possibility was considered that the natriuretic factor, if biologically important should have as its major site of action the terminal part of the nephron. The fact that the factor inhibited sodium transport by the toad bladder (7), a structure with many similarities to the distal nephron, seemed to support the possibility that the factor would inhibit distal sodium transport. The present studies thus were performed to examine the effect of the natriuretic factor from the urine of patients with chronic renal failure on transtubular potential difference and sodium transport across the isolated collecting tubule of the rabbit nephron.

The present address of the authors is Department of Medicine, University of Miami School of Medicine, P.O. Box 520875, Biscayne Annex, Miami, Fla. 33152.

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METHODS

Preparation of urine fractions. 12–24 h timed urine collections were obtained from patients with advanced chronic renal failure of various etiologies and from normal subjects. Endogenous creatinine clearance was less than 15 ml/min in all of the patients. All of the subjects whether uremic or normal were on an unrestricted salt intake and all were judged on clinical grounds to be in external sodium balance. None of the uremic patients was being maintained with chronic hemodialysis. The technique of processing the urine samples has been described previously (1, 2). All of the fractions employed in the present studies were pretested using the standard bioassay in the salt-loaded uremic rat with a single remnant kidney (1, 2) or the isolated toad bladder assay (7). According to the criteria previously established for these bioassays all of the uremic fractions were found to produce natriuresis or to inhibit short circuit current in the toad bladder and all of the normal fractions were shown to be non-natriuretic and without effect on toad bladder.

Since ammonium has been demonstrated to have an inhibitory effect on toad bladder sodium transport (8), all fractions were rendered ammonium free by adjusting pH to 10.0 and subsequent lyophilization. No ammonium was detected by the Conway method (9) following this procedure. The lyophylate was reconstituted to its original volume by addition of distilled water and pH was adjusted to 7.4. This reconstituted fraction contained no calcium or magnesium and sodium and potassium concentrations were less than 30 meq/liter and 1.5 meq/liter, respectively. The dissolved fraction was mixed with both the bath solution (*vide infra*) and the perfusion solution (*vide infra*) in a dilution of 1:2. The ionic composition of the diluted fractions was then adjusted to that of the control bath or perfusate. This was achieved by measuring the deficit of each cation and adding appropriate amounts of 1–5 M solutions of each cation with its anions in the same proportions as in the control solutions. Sodium and potassium concentrations were measured with a model 143 flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.) calcium and magnesium concentrations with a model 107 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) and osmolality with a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Mass.). Final bath and perfusate osmolalities were identical for each experiment. The total amount of the fraction present in the bath during an experiment was derived from a 1-h urine collection.

Perfusion of isolated collecting tubules. The technique of isolated tubule perfusion has been developed and described by Burg and co-workers (10) and the methods used in the present study follow those previously described.

Female New Zealand white rabbits 1.5–2.5 kg in weight maintained on standard rabbit chow were killed by a blow on the head and the left kidney rapidly removed. A 1–3 mm thick cross section was transferred to a dish containing chilled bath medium (*vide infra*) which was bubbled with 95% O_2 and 5% CO_2. Segments of collecting tubules 0.7–3.2 mm in length were dissected from the cortex. The tubules were transferred to a bath chamber mounted on an inverted Nikon microscope and viewed at magnifications of × 40–× 400. The tubules characteristically had a shaggy appearance on the peritubular surface, an irregular luminal surface with well-defined single cell outlines. In all cases the tubule was carefully inspected under high magnification for evidence of breaks in the basement membrane.

The bath (vol, 1.2 ml) had the following composition (in millimoles per liter) NaCl 115, KCl 5, $NaHCO_3$ 25, Na acetate 10, NaH_2PO_4 1.2, Mg SO_4 1.2, $CaCl_2$ 1.0, dextrose 5.5 and 5% vol/vol rabbit serum (Microbiological Associates, Bethesda, Md.). Urea was added to the bath in a concentration ranging from 1–5 mM to adjust osmolality to that of the fractoin to be tested. There was no correlation between the results of the present studies and the amount of urea added. pH of the bath solution was maintained at 7.4 by bubbling with 95% O_2 and 5% CO_2 and temperature was controlled at 37°C using a YSI temperature controller and model 421 thermistor probe. (Yellow Springs Instrument Co., Yellow Springs, Ohio). Since the continuous bubbling of the bath at 37°C was found to cause significant evaporation, osmolality of the bath was controlled by one of two methods. In approximately 50% of the experiments the bath solution was completely changed every 10 min throughout the experiment; in the other 50% of experiments the bath was recirculated at a rate of approximately 12 ml/h by means of an LKB 10200 Perpex peristaltic pump (LKB Produkter Bromma 1, Sweden) through silicone rubber tubing (vol, 1 ml) and a siliconized model D10-4184 conductivity cell connected to a SM 5 Solu meter (Beckman Instruments, Inc., Cedar Grove, N. J.). Conductivity was monitored continuously and was maintained constant by adjusting the delivery of deionized water into the bath by means of a Sage syringe pump, model 305 (Sage Instruments, Cambridge, Mass.). Once the correct delivery rate of water into the system had been obtained and stable conductivity readings obtained, the circulation of the bath was stopped and the addition of water at a fixed rate continued throughout the experiment. Sampling of the bath at 30-min intervals revealed variations in osmolality of less than 3 mosmol/kg H_2O with this method.

Tubules were perfused through concentric glass pipets and both ends of the tubule insulated with Sylgard 184 (Dow Corning Corp., Midland, Mich.). Perfusion rate (±5 nl/min) was regulated by adjusting the height of the column of perfusion fluid; perfusion pressure remained constant throughout the experiment. Timed collections of fluid emerging from the tubular lumen were made under water-saturated mineral oil in a precalibrated constriction pipet. The perfusate contained (in millimoles per liter) NaCl 150, K_2HPO_4 2.5 $CaCl_2$ 1.0 Mg SO_4; pH was titrated 7.4 with HCl. [^{14}C]Inulin (50 μCi/ml) was added to the perfusate as an impermeant volume marker to detect leakage of tubular fluid into the bath. Urea (1–5 mM) was added to the perfusate to adjust osmolality to that of the experimental solution and to maintain equality between the osmolalities of bath and perfusate throughout the experiment.

Electrical measurements. The perfusion pipet which was advanced into the lumen of the tubule served as a luminal electrode and was connected to a calomel half-cell through a 0.16-M NaCl—4% agar bridge. The calomel half-cell was connected to the input of an impedence converter consisting of a junction field-effect transistor with an input impedence of 1,000 MΩ and an output impedence of less than 5 kΩ. The signal was displayed on a Grass model 7 polygraph with a model 7P1 A low level DC preamplifier (Grass Instrument Co., Quincy, Mass.). The circuit was completed through a 0.16-M NaCl—4% agar bridge connecting the bath to a reference calomel half-cell connected to ground through a model 101 precision millivolt reference source (W-P Instruments, Hamden, Conn.) which was used to calibrate the system and to null any small potential difference due to assymmetry of electrodes. Transtubular potential

differences (PD) [1] stabilized at 120–210 min. PD measurements were recorded continuously throughout each experiment.

12 fractions from uremic patients were tested by addition of the fraction to the bath; in six of these experiments, after its removal from the bath and return of PD to control values the fraction was added to the bath a second time. In 6 of the 12 experiments the fraction was subsequently added to the perfusate. Five fractions from normal subjects were similarly tested being added first to the bath and then to the perfusate. In 14 of the above 18 experiments sodium fluxes were measured either chemically (protocol I; *vide infra*) or isotopically (protocol II; *vide infra*). The studies in protocol I were performed in a randomized manner without the investigator knowing the nature of the fraction being tested until the completion of the experiment.

An additional series of randomized experiments was performed using coded fractions from six uremic and six normal subjects. In each experiment both a uremic and a normal fraction were sequentially tested on the same tubule by the addition of each fraction to the bath. In two of these experiments fractions were also added to the perfusate.

The PD values during the control and experimental periods were expressed as follows. During a 15-min period preceding the addition of the fraction to bath or lumen PD was required to vary by no more than 3 mV. The "precontrol" PD was the value obtained at the midpoint of this 15 min period. After addition of the fraction, "experimental" PD was likewise recorded at the midpoint of a 15-min period during which PD had remained stable within a range of 3 mV. Upon replacement of the control solution and return of PD towards control values a "postcontrol" value was recorded at the midpoint of the earliest 15 min period during which PD was stable to within 3 mV.

Sodium fluxes and net fluid absorption. After a $2\frac{1}{2}$–$3\frac{1}{2}$ h period of stabilization during which the tubule was perfused with control solution, one of two experimental protocols was followed. In both protocols the administration of the fraction is referred to as the experimental period and in all cases was preceded by a precontrol period and followed by a postcontrol period. PD was monitored throughout the experiments.

Protocol I—net sodium fluxes (eight experiments). Three accurately timed control collections (approximately 10 min) of tubular fluid were made. The fraction to be tested (four normal, four uremic) was then added to the bath and upon achievement of a new steady-state PD (approximately 7–10 min) three further collections made. The bath was then replaced by the control solution and three further collections made. In seven of these experiments the same fraction was then added to the perfusate and following a 10-min period of equilibration three tubular fluid collections made. The experiment was concluded by returning to the control perfusate during which three final collections were made.

One sample from each experimental period was pipetted directly into liquid scintillation fluid and total counts per minute measured with a Packard Tricarb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) for the calculation of net fluid reabsorption. Perfusion rate (V_o) was calculated as: $V_o = [^{14}C]_t/[^{14}C]_o T$ where $[^{14}C]_t$ is the total amount of [^{14}C]inulin collected, $[^{14}C]_o$ the concentration of isotope in the perfusion solution, and T the time. Collection rate (V_L) was measured by timed collections of perfusate into a calibrated constriction pipet. Net fluid absorption is thus $(V_o - V_L)/L$ where L is the length of the tubule. Tubule length was measured using a calibrated reticle in the ocular of the microscope and was taken as the distance between the two Sylgard seals. The two remaining samples from each experimental period were analyzed for sodium concentration using a helium-glow photometer (American Instrument Co., Silver Spring, Md.). Assuming zero net fluid movement (see Results) net flux of the ion (Ji) is $Ji = V_L/L(C_o - C_L)$ where V_L is the collection rate (nanoliters per second), C_o and C_L the concentrations of the ion in perfusion and collected fluids, and L the length of the tubule.

Protocol II—unidirectional fluxes of ^{22}Na (six experiments). In this protocol only uremic fractions were tested. In all experiments the measurement of lumen to bath flux preceded that of bath to lumen flux due to the difficulty to decontaminating the bath once a high concentration of isotope had been used in the bath.

After the 2–3 h stabilization period ^{22}Na (50 μCi/ml) was added to the perfusate for determination of lumen to bath flux, and 15 min allowed for equilibration. Two 10-min collections of bath fluid were then made by washing the bath with 4 ml of bath solution and collecting the total volume (4 ml). The uremic fraction was then added to the bath and two additional 10-min collections completed. After return to the control bath two further collections were made.

The tubule was then perfused for 20 min at an increased rate (±20 nl/min) with isotope free perfusate to remove all isotope from the collecting pipet. The original perfusion rate was then restored and ^{22}Na (100 μCi/ml) added to the bath for determination of bath to lumen fluxes. 15 min were allowed for equilibration, after which two timed collections of tubular fluid were made. The urine fraction containing ^{22}Na was then added to the bath and two additional collections made. Finally, the control bath containing isotope was replaced and the experiment was concluded with two timed collections of tubular fluid.

^{22}Na was counted using a Packard Gamma Counter (Packard Instrument Co., Inc.) 10-μliter samples of bath and perfusate were transferred into 1 ml of distilled water for radioactive counting; the total volume of the bath collections (4 ml) was counted and tubular fluid samples were pipetted into 1 ml of distilled water and counted.

Lumen to bath flux of Na was calculated as: $JNa_{lb} = (Na)_o Na_b^*/(Na^*)_o L.T$, where $(Na)_o$ is the concentration of sodium and $(Na^*)_o$ the concentration of isotope in the perfusate, Na^*_b the total amount of isotope collected in the bath, L, the length of the tubule, and T the duration of the collection. Na^*_b was less than 2% of the amount of isotope perfused in all cases.

Bath to lumen fluxes of ^{22}Na were calculated as: $JNa_{lb} = (Na)_b Na^*_L/Na^*)_b L.T$, where $(Na)_b$ and $(Na^*)_b$ are the concentrations of sodium and isotope in the bath and Na^*_L the total isotope in the collected tubular fluid. Since less than 2% of the isotope perfused entered the bath, the error in the above calculation due to the change in specific activity of the tubular fluid was small (11).

Tubular leakage. To detect leakage of tubular fluid two 15-min collections of bath fluid were made by exchanging the bath with four times its total volume. In protocol I [^{14}C]inulin was present in the perfusate throughout the experiment; in protocol II inulin was present in the perfusate during the 2-h stabilization period only and was removed before determination of ^{22}Na fluxes. "Leak" of tubular fluid was calculated as a percentage of perfusion rate. If the leak exceeded 1% (approximately 0.025 nl/mm per min) the experiment was discarded.

[1] *Abbreviation used in this paper:* PD, potential difference.

Statistics. Results are expressed as means±SE. Values obtained during the experimental periods were compared with a "mean control" value which was calculated as: (Precontrol + Postcontrol)/2. Comparisons between "mean control" and experimental periods were made using a paired *t*-test. For experiments in which the effect of two different fractions was tested on the same tubule, the change produced by each (ΔPD) was compared using a paired *t*-test. Significance is expressed as the 2P value. Approximately 60% of all experiments were discontinued or excluded due to the presence of tubular leakage or to instability of the transtubular PD during the initial equilibration period.

RESULTS

Net fluid absorption. This was measured in eight tubules studied in protocol I and was 0.01±0.01 nl/mm per min which is not significantly different from zero. Collection rate was 99.50±62% of perfusion rate. Neither the normal fraction (four tubules) nor the uremic fraction (four tubules) caused any change in fluid absorption whether added to the bath or to the perfusate. The mean collection rate in all 17 tubules studied was 5.18± 0.57 nl/min.

Transtubular PD. In Fig. 1 the changes in transtubular PD as a function of time are shown for 12 experiments in which individual uremic fractions were added to the peritubular surface (bath) of each tubule. A stable precontrol PD was achieved within 2½–3½ h of perfusion. The addition of the uremic fraction resulted in a rapid decrease in intraluminal negativity in 10 experiments, the mean time required to achieve a new stable PD being 7.0±1.6 min. These effects of the uremic fractions on PD are depicted graphically in Fig. 2. Precontrol PD of 23.9±4.7 mV, (lumen negative) was not significantly different from the mean postcontrol value of 20.8±4.7 mV. The overall mean control PD was − 22.5±4.7 mV. After addition of the uremic fraction to the bath mean PD fell to − 12±4.5 mV. Intraluminal negativity thus decreased by 10.3±2.0 mV ($P <$ 0.001). In two experiments this PD was oriented lumen-positive. In six tubules the fraction was added to the bath a second time (Fig. 1). Intraluminal negativity decreased to the same extent as was observed during the first addition of the fraction, there being no significant difference in the magnitude of the PD change between the first (ΔPD 9.9 mV) and the second addition (ΔPD 8.5 mV) of the fraction.

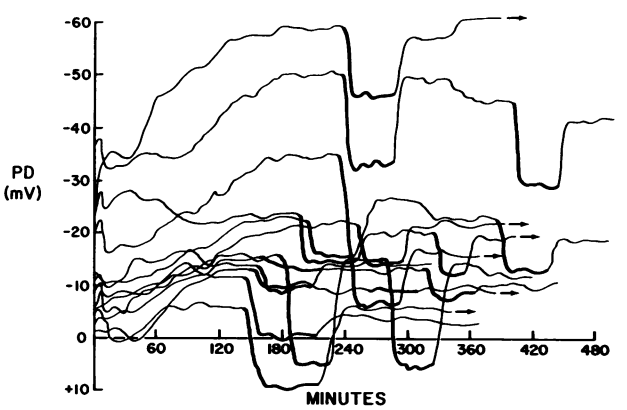

FIGURE 1 Time course of transepithelial PD of isolated cortical collecting tubules showing the effect of uremic urine fractions. Stable PD recordings were obtained after 2–3 h of perfusion. Heavy lines depict the period during which the fractions were present in the bath (peritubular surface). In six experiments the same fraction was applied twice to the same tubule. Arrows indicate those experiments in which the fraction was subsequently added to the perfusate. PD is oriented lumen negative.

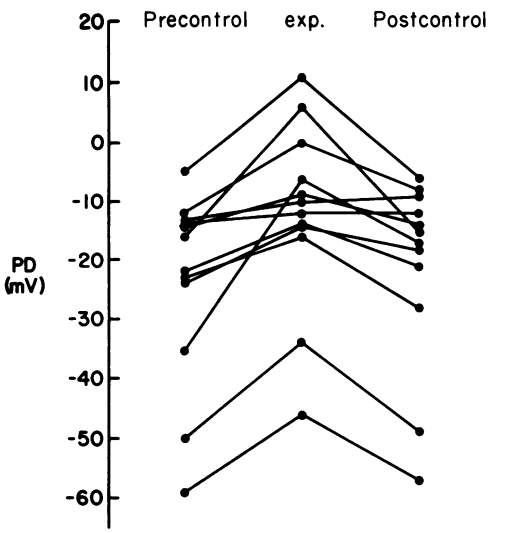

FIGURE 2 Effect of the application of uremic fractions to the peritubular surface on transtubular PD of the cortical collecting tubule. The values obtained during application of the fraction are compared with the preceding and succeeding control periods. PD is oriented lumen negative.

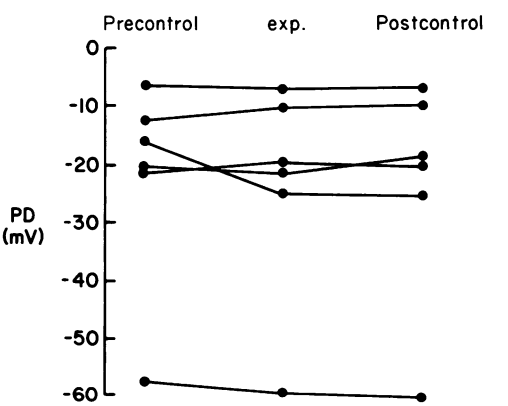

FIGURE 3 Effect of the application of the uremic fraction to the luminal surface on the transtubular PD of the cortical collecting tubule. The values obtained during the administration of the fraction are compared with the preceding and succeeding control periods. PD is oriented lumen negative.

TABLE I

Effect of Urine Fractions from Uremic and Normal Subjects on Net Sodium Flux (JNa) of Isolated Cortical Collecting Tubules

	Fraction in bath				Fraction in perfusate			
	Control		Δ Experimental		Control		Δ Experimental	
	JNa	PD	ΔJNa	ΔPD	JNa	PD	ΔJNa	ΔPD
	pmol/cm per s	*mv*	*pmol/cm per s*	*mv*	*pmol/cm per s*	*mv*	*pmol/cm per s*	*mv*
Uremic fractions								
	6.00	−58.0	−3.01	−12.0	5.46	−60.5	−0.16	−1.5
	7.20	−21.5	−6.46	−6.0	8.90	−19.5	−0.12	−1.5
	2.47	−13.0	−1.62	−1.0	2.99	−14.0	−0.36	−3
	3.91	−27.0	−3.37	−6	—		—	
	*4.45	−25.5	−1.64	−9.5	—		—	
	*7.05	−20.5	−4.16	−6.5	—		—	
	*4.58	−49.5	−1.48	−15.5	—		—	
	*9.18	−14	−4.74	−16.5	—		—	
	*12.13	−11.5	−1.24	−1.5	—		—	
	*5.99	−8	−3.21	−8	—		—	
Mean±SE	6.29±0.89		−3.09±0.53		5.78±1.71		−0.21±0.07	
Normal Fractions								
	5.40	−21.5	−0.53	+9.5	7.36	−17.5	+1.32	+1.5
	10.32	−20.0	+0.79	+2.0	9.65	−18.5	+0.50	+0.5
	4.89	−22.0	+0.10	−2.0	4.54	−12	−0.27	−2
	3.99	−20.0	−0.77	0	3.43	−19.5	−0.14	+0.5
Mean±SE	6.15±1.42		−0.10±0.35		6.25±1.40		+0.42±0.34	

Net sodium fluxes are from lumen to bath. PD is oriented lumen negative. Negative ΔPD refers to a decrease in luminal negativity.
* Denotes experiments in which net flux was calculated from the difference between unidirectional fluxes shown in Table II. In all other experiments fluxes were measured chemically.

In six tubules, the uremic fraction was added to the perfusate (Fig. 3). The mean change in PD was − 1.5± 0.8 mV which was not significantly different from the mean control value.

PD measurements were also made in five tubules after addition of normal fractions sequentially to both bath and perfusate (Fig. 4). No significant change in PD occurred whether the fraction was added to the bath (ΔPD = + 1.9±2.0 mV) or to the perfusate (ΔPD = + 0.3±0.6 mV).

In six additional experiments both a normal and a uremic fraction were tested sequentially by their addition to the bath (Fig. 5) of the same tubule. None of the normal fractions effected a change in PD (ΔPD = 1.2±0.8 mV) whereas five of the six uremic fractions led to a rapid decrease in luminal negativity (ΔPD 9.2±2.3 mV). The changes induced by the uremic fractions were significantly different from those produced by the normal fractions ($P < 0.02$). In three experiments the procedure was repeated twice on the same tubule with reproducible results. In the two experiments in which the fractions were added to the perfusate no change in PD occurred.

Net sodium fluxes. Table I presents the composite data for the effects of uremic and normal urine fractions on net sodium flux across the cortical collecting tubule. The mean control value was 6.29±0.89 pmol/cm per s, net flux being from lumen to bath. Addition of the uremic fraction to the bath (10 tubules) decreased net sodium flux to 3.21±1.08 ($P < 0.001$). This was accompanied by a parallel reduction in transepithelial PD. Addition of the same uremic fractions to the perfusate in three tubules had no significant effect on net sodium flux.

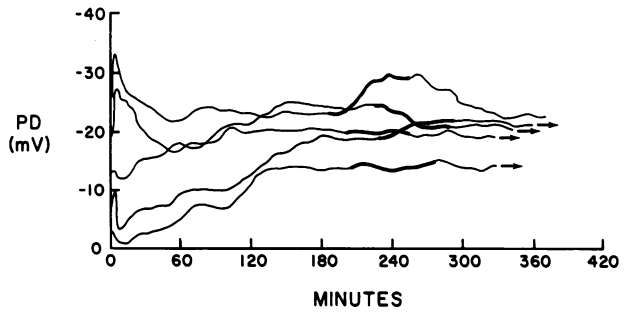

FIGURE 4 The effect of urine fractions from normal subjects on transepithelial PD of isolated cortical collecting tubules. Heavy lines depict the period during which the fractions were present in the bath (peritubular surface). Arrows indicate experiments in which the fractions were subsequently added to the perfusate. PD is oriented lumen negative.

The effects of the non-natriuretic fractions from normal subjects were tested in four tubules. In each experiment the fraction was added first to the bath and then to the perfusate (Table I). With neither maneuver was there a significant change in net sodium flux.

Unidirectional sodium fluxes. The effects of uremic fractions on unidirectional sodium fluxes are presented in Table II. In five out of six experiments there was a decrease in lumen to bath flux of at least 20%. The mean control value for lumen to bath flux was 8.92±1.27 pmol/cm per s ($P < 0.02$). The mean value after addition of the uremic fraction was 6.31±1.68 pmol/cm per s ($P < 0.02$). The mean control value for bath to lumen flux was 1.06±0.06 pmol/cm per s and after addition of the uremic fraction was 1.24±0.19 pmol/cm per s. This change was not significant.

Morphology. When viewed at magnifications up to × 400 no obvious changes in cell size or morphology were observed in the presence of the uremic or the normal fractions.

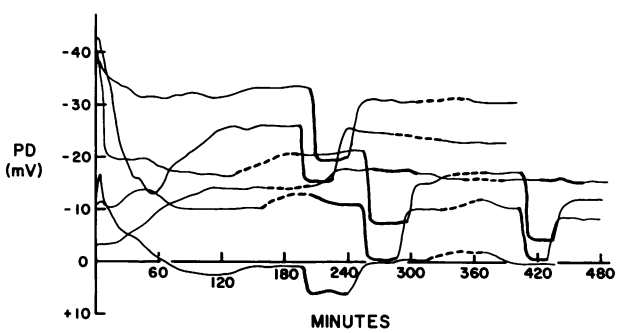

FIGURE 5 Effect of urine fractions from uremic (heavy lines) and normal (broken lines) subjects on transepithelial PD of isolated cortical collecting tubules when added to the bath (peritubular surface). Both a normal and a uremic fraction were tested on each tubule. In three experiments the procedure was repeated twice. PD is oriented lumen negative.

TABLE II

Effects of Fractions from Uremic Subjects on Unidirectional Sodium Fluxes. Fractions were Applied to the Peritubular Surface (Bath) only

	Lumen to Bath		Bath to Lumen	
	Mean control	Δ	Mean control	Δ
	pmol/cm per s	*pmol/cm per s*	*pmol/cm per s*	*pmol/cm per s*
	5.28	−1.46	0.84	+0.18
	8.15	−4.26	1.10	−0.10
	5.52	−1.28	0.94	+0.20
	10.31	−4.79	1.13	−0.01
	13.41	−0.51	1.28	+0.74
	7.08	−2.19	1.09	+0.02
Mean±SE	8.92±1.27	−2.58±0.71	1.06±0.06	+0.17±0.12
P	<0.02		NS	

DISCUSSION

The results of the present studies amplify and extend the evidence implicating the role of a humoral factor in the biologic control system in uremia. The gel-filtration fractions of urine from patients with chronic uremia used in the present experiments, were all pretested and found to produce natriuresis in rats or to inhibit short-circuit current in the toad bladder while the control fractions from normal subjects were without effect. Our previous studies have documented the increased production of a natriuretic factor in uremia and have shown that its presence in animals with experimental renal failure is determined by the requirements for external sodium balance (2). Recent studies have also demonstrated the presence of a natriuretic factor in the urine of normal dogs subject to mineralocorticoid "escape" and have shown that this factor shares all of the biochemical properties previously delineated for the "uremic" factor (4).

The cumulative evidence is thus highly suggestive of a physiological role of a natriuretic factor in the maintenance of sodium balance and suggests that the increased production of such a factor in uremia is an adaptation in response to the requirement for increased sodium excretion per nephron which accompanies loss of nephrons in chronic renal disease.

Studies on the isolated toad bladder have shown that the natriuretic factor acts from the serosal surface and inhibits transepithelial transport by reducing sodium movement across the serosal barrier (7). By extrapolation to the mammalian nephron it was thus predicted that the factor should act on distal nephron sites and

that its receptor sites should be on the peritubular surface.

The present studies were designed to evaluate the mechanism of action of the natriuretic factor on the mammalian nephron using the technique of in vitro perfusion of isolated rabbit cortical collecting tubules. The results clearly demonstrate that the natriuretic factor does act on the collecting tubule and that this action combined with its previously demonstrated inhibition of proximal tubular sodium transport (5) may serve to modulate sodium excretion by the kidney. The decrease in transtubular PD which was observed in 10 out of 12 uremic fractions tested correlated with a decrease in net sodium transport. The onset of inhibition of PD was rapid (within 1 min) and readily reversible by removal of the factor. Furthermore, when the same factor was applied twice to the same tubule the resulting decrease in PD was reproducible for each fraction tested. The rapidity of this effect seems to be compatible with the requirements for the action of a sensitive modulator of sodium excretion and suggest that the action of the factor is directly on the epithelial membrane rather than via induction of protein synthesis or changes in intracellular metabolism. A similarly rapid natriuretic effect has likewise been observed in in vivo studies (12).

The possibility that the unresponsiveness of the tubule to the normal fractions was due to a reduced sensitivity of the particular tubule under study was examined by comparing the effects of both uremic and a normal fraction tested sequentially on the same tubule. No false positive responses in PD were encountered with the normal fraction attesting both to its inactivity and to the constancy of the ionic composition of the solutions used and the stability of the experimental conditions. In contrast five of the six uremic fractions resulted in a rapid fall in PD when added to the bath. Since these experiments were randomized, the order in which the two fractions from each pair was tested varied from experiment to experiment and could not have affected the results.

Since inhibition occurred only when the fraction was added to the peritubular bathing medium, it is likely that the receptor sites are confined to the peritubular surface of the nephron; the present studies do not, however, show whether it is the entry step across the luminal membrane or the exit step across the peritubular membrane which is affected. Studies on the toad bladder (7) suggest the latter alternative. The inhibition by the uremic fraction of the negative transtubular potential does not clarify this issue since a similar inhibition has been demonstrated with both ouabain (13, 14) which inhibits active sodium extrusion from the cell and amiloride (14, 15) which decreases luminal membrane permeability and limits the entry of sodium into the cell. In both cases net sodium transport is diminished with a reduction in potential.

The decrease in net sodium transport in the present studies was due entirely to a decrease in sodium flux from the luminal to the peritubular surface of the nephron as shown by unidirectional tracer flux measurements since there was no increase in sodium flux from the peritubular to luminal surface. Since transport of sodium from the lumen is active and is opposed by an electrical gradient (13) it may be concluded that the fraction inhibits active sodium transport.

Potassium fluxes were not systematically examined in the present studies, although preliminary evidence has indicated that changes in net flux are relatively small (16) and are probably related to the action of the factor in decreasing intraluminal negativity, and consequently the electrochemical gradient for potassium secretion. The positively oriented PD observed in two experiments in the present study may have reflected the predominance of net potassium over net sodium flux but the magnitude of the PD was such that it could similarly have been generated by H^+ secretion alone (15). More extensive studies are required to fully evaluate these relationships.

Studies of sodium fluxes in the isolated cortical collecting tubule are limited to three previous studies (11, 15, 17), only two of which (15, 17) were conducted at 37°C, and it is relevant to comment on the control values obtained in the present studies. The mean PD of -22 mV observed in the present studies is slightly lower than that reported in previous studies at 37°C (15, 17). Stoner et al. reported mean net sodium flux of 8.7 pmol/cm per s (15) and Boudry et al. obtained a value of 8.2 pmol/cm per s (17). The value obtained in the present study was 6.3 pmol cm per s. This was due to a slightly lower rate of lumen-to-bath flux in the present study. The perfusion rate used in the present studies (5 nl/min) was however, lower than that used in previous studies. A recent micropuncture study (18) has shown that the single nephron filtration rate in the rabbit is approximately 20 μl/min; thus the slow perfusion rate used conforms with the physiological rate of flow through this nephron segment in vivo.

Future studies on the cellular action of the natriuretic factor will require purification and quantitation of the factor. The availability of the purified molecule will allow for a quantitative evaluation of its natriuretic effect and further sophistication in the evaluation of its mechanism of action.

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