The Effects of the Natriuretic Factor from Uremic Urine on Sodium Transport, Water and Electrolyte Content, and Pyruvate Oxidation by the Isolated Toad Bladder

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ABSTRACT The urine of patients with chronic uremia contains a gel filtration fraction that is natriuretic in the rat. The effects of this fraction on the isolated urinary bladder of the toad were examined in the present studies. When added to the serosal surface of the bladder, a significant and substantial fall in short-circuit current and potential difference was observed. The changes began after a lag period of at least 10 min and continued over a period of 60 min. The decrease in short-circuit current at the end of 1 h averaged 44%. The same fraction from the urine of normal subjects produced no significant change in either short-circuit current or potential difference. When the isolated epithelial cells from the toad bladder were incubated in the presence of the inhibitor, intracellular sodium content increased significantly. There was no change in intracellular water content; hence the intracellular concentration of sodium increased by a mean of 7 meq/liter. The changes in intracellular potassium content and concentration were not statistically significant. When the isolated epithelia were incubated with the uremic factor, there was also a significant decrease in pyruvate utilization in relation to cells from paired hemibladders incubated in the absence of the fraction. The fraction from normal subjects produced no change in either intracellular sodium content or pyruvate oxidation.

The results suggest that the inhibitor acts from the serosal surface, inhibits sodium transport across the serosal barrier, and produces a decrease in substrate utilization in association with the change in transepithelial sodium transport.

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INTRODUCTION

The effector element of the biologic control system which regulates the renal excretion of sodium is believed to be multifaceted and to have several component parts (1–3). Among these is a postulated natriuretic hormone which theoretically could play a central role in modulating the rate of sodium excretion.

In previous studies, we have described an inhibitor of sodium transport in the serum of patients with advanced chronic renal disease (4, 5). This serum factor was found to inhibit sodium transport by the isolated frog skin and toad bladder (4) and to produce natriuresis in rats with a reduced nephron population (5). More recently, the same gel filtration fraction obtained from urine of patients with chronic uremia has been found to possess natriuretic activity in the rat (6).

In the present studies, attempts have been made to derive information about the site and mechanism of action of the urinary inhibitor using the isolated bladder of the toad. The toad bladder lends itself well to this type of study for several reasons. First, the serum factor inhibits transepithelial sodium transport by the anuran bladder, and the urine factor, if it is the same substance, should have similar inhibitory capabilities. Second, isolated epithelial cells removed from the intact toad bladder have been used recently to investigate the site of action of inhibitors of sodium transport (7). Inhibitors that act at the serosal surface of the epithelia (e.g. ouabain) increase intracellular sodium content, while those acting at the mucosal surface (e.g., amiloride) have the opposite effect (7). Third, the isolated

epithelial cell preparation allows for evaluation of the effects of agents which influence sodium transport on substrate utilization (7). In the intact toad bladder, transepithelial sodium transport is the major endergonic biologic process and the rate of oxidation of pyruvate closely parallels the rate of active sodium transport. Vasopressin, which increases transepithelial sodium transport, increases pyruvate oxidation by the isolated epithelial cells of the toad bladder; whereas substances which decrease transepithelial sodium transport diminish pyruvate oxidation whether they act at the mucosal or the serosal surface of the cells (7).

METHODS

Timed urine collections were obtained from 15 patients with far advanced chronic renal failure of diverse etiology and 11 normal subjects. The endogenous creatinine clearance was less than 20 ml/min in all patients. Clinically all were in external sodium balance. No patient was edematous and none were taking diuretics. Four patients who were receiving maintenance doses of digitalis are designated in the Tables. As in the previous studies (4–6), there was no correlation between the history of drug ingestion and the experimental results.

The urine samples from the uremic patients and from the normal subjects were prepared in an identical manner and fractionated by column chromatography. The details about the fractionation procedure are contained in an associated manuscript (6). In brief, 25-ml aliquots of concentrated urine were applied to 2.5 × 95-cm columns packed with Sephadex G-25 and eluted at 4°C with 10 mM ammonium acetate at pH 6.8. The fraction employed in these studies appeared in the eluate immediately after the fraction containing the principal peaks of sodium, potassium, urea, and creatinine. The fraction was lyophilized to dryness, redissolved in a total volume of 2.5 ml of distilled water, and stored at −80°C until the day of study. Just before testing, the pH of each fraction was titrated to 8.0 and the sodium and potassium concentrations were adjusted to 110 and 2.5 meq/liter, respectively. Assuming no loss in the preparative procedures, each milliliter of the final solution contained the amount of inhibitor excreted in approximately 2 h (6). All samples were prepared in a separate laboratory and for all three types of studies to be described below, the investigator was unaware of the nature of the samples being tested.

Sodium transport by the intact isolated toad bladder. Transepithelial sodium transport was measured as the short-circuit current (SCC) [1] (4). The urinary bladder of Bufo-Marinus (National Reagents Co., Bridgeport, Conn.) was freed by dissection and mounted as a diaphragm between the two halves of a conventional 2-cm^2 Lucite transport chamber. Both surfaces of the bladder were bathed with 5 ml of Ringer's solution having the following composition in millimoles: NaCl, 110; KCl, 2.5; $NaHCO_3$, 2.5; $CaCl_2$, 1.0; dextrose, 10. Gassing was accomplished with compressed air. The pH of the aerated Ringer's was 8.0±0.2 U. Short-circuiting was maintained continuously except for 15-s intervals every 10 min when the open-circuited potential difference (PD) was recorded. After mounting the bladders, a control period of at least 60 min was allowed for the SCC and PD values to stabilize. No bladder was used unless both the SCC and PD remained stable for the last 30 min of the control period. At the end of the control period (t_0), 500 μl of Ringer's solution was removed from both hemichambers and replaced by 500 μl of the unknown fraction on one side and 500 μl of fresh Ringer's on the other side. After adding the fraction, SCC and PD were monitored for 60 min. In several experiments, at the end of this period of time, the bathing solutions from both chambers were removed and replaced with fresh Ringer's. The data for SCC and PD are expressed as the ratio of the values observed at specific times after addition of the fraction to the control value.

Most of the fractions from both normal and uremic urine contained ammonium remaining from the preparative chromatographic extraction. Because of the known inhibitory effect of ammonium on the SCC of the toad bladder (8), the ammonium was completely removed from each sample before testing by evaporation at pH 10 under a stream of nitrogen gas at 4°C for 3.5 h. The ammonium concentration of the fractions was measured routinely, and none of the samples employed in the studies of SCC contained any detectable amount.

Studies of intracellular fluid volume and sodium and potassium content. Isolated epithelial cells from Bufo Marinus were prepared by a method similar to that described by Gatzy and Berndt (9). The toads were doubly pithed and were perfused for 5–10 min with anuran Ringer's solution via the heart until there was no visible blood in the capillaries of the urinary bladder. Then the bladders were excised, washed in Ringer's for 30 min, and incubated in 5 ml of Ringer's solution containing 0.5 mg/ml of crude collagenase (Worthington Biochemical Corp., Freehold, N. J.) for 90 min at room temperature in a Dubnoff metabolic shaker. Afterwards they were transferred to fresh Ringer's and incubated for an additional 30 min to remove the collagenase. Finally the bladders were placed (mucosal side up) on a piece of Parafilm (Marathon Rubber Products Co., Wausau, Wis.) and the mucosal surface was scraped once lightly with the edge of a glass microscope slide to harvest the epithelial cells. The cells from four bladders were used in each pair of flasks (one experimental, one control) to obtain a minimum of 5 mg of dry tissue weight per incubation flask.

The incubation solution consisted of 2 ml of Ringer containing 0.2 μCi of [^{14}C]inulin (New England Nuclear, Boston, Mass.). After an equilibration period of 60 min, 200 μl of urine fraction from either a normal or uremic subject was added to each experimental flask, and 200 μl of Ringer's was added to the control flasks. The incubations were continued for 45 min. The contents of the flasks then were centrifuged for 3 min at 10,000 *g* at room temperature and the supernatant fluid was decanted. The cells were blotted quickly with filter paper to remove the excess Ringer and transferred with fine forceps to tared tubes. Wet weights were obtained. The cells were dried for 15 h at 80°C and reweighed to obtain the dry tissue weight and the water weight. Then 1 ml of 0.75 N nitric acid was added to the dry tissue and extraction of electrolytes and inulin was carried out by repeated mechanical inversion for 48 h. 0.1-ml aliquots of the supernate and the cell extract were added to 10 ml of Aquasol (New England Nuclear) and the ^{14}C activity was counted. The Na and K concentrations of both the supernatant solution and the cell extract were also measured.

Each urine fraction was added to three separate experimental flasks and triplicate control flasks were run simul-

[1] *Abbreviations used in this paper:* SCC, short-circuit current. PD, potential difference.

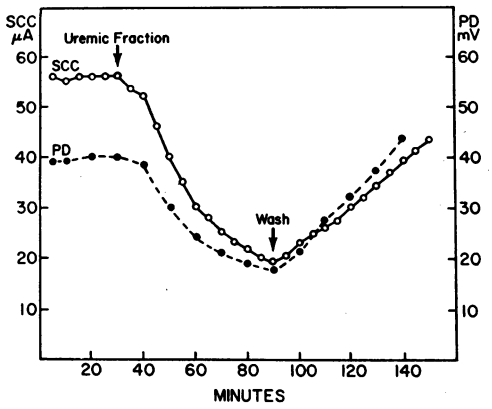

FIGURE 1 Effects of a urine fraction from a patient with chronic uremia on SCC and PD across the isolated toad bladder. The urine fraction was added to the Ringer's solution bathing the serosal surface of the bladder.

taneously. The results from the three experimental flasks were averaged and compared with the mean value for the three control flasks.

To determine whether ammonium influences the intracellular water and electrolyte content of the epithelial cells, and thus, whether it would be necessary to free these samples of ammonium, experiments were also performed using NH_4Cl in Ringer's solution. The procedures followed were the same as those in the experiments with urine fractions and the cells in the experimental flasks were incubated in the presence of 2 mM NH_4Cl for 45 min.

The extracellular fluid volume was estimated using the [^{14}C]inulin concentration of the Ringer's and the total inulin content of the cell extracts. Intracellular water was assumed to represent the difference between the total water content of the extract and the calculated volume of extracellular water. Intracellular fluid sodium and potassium contents were determined from the difference between the total content in the cell extracts and the content in the extracellular phase of the extracts. The electrolyte concentrations of intracellular fluid were calculated by dividing the intracellular cation content (in microequivalents) by intracellular water (in milliliters).

Measurement of pyruvate oxidation. The isolated mucosal cells were prepared for these studies in the same manner as for the studies on intracellular water and electrolytes. The epithelial cells were placed in 2 ml of Ringer's solution in plastic vials. Each urine fraction (200 μl) was added to two different vials and the cells from the paired hemibladders were incubated in two control vials to which 200 μl of Ringer's was added. Both experimental and control flasks were shaken in a Dubnoff metabolic shaker at room temperature for 45 min, after which 100 μl of 20 mM pyruvate containing 0.5 μCi of [2-^{14}C]pyruvate (New England Nuclear) was added to each flask. Incubations were continued for an additional 60 min. Then the vials were sealed and 100 μl of 10 N H_2SO_4 was injected through the plastic vial into the incubation medium. The $^{14}CO_2$ released from [^{14}C]bicarbonate (which in turn was derived from the oxidation of pyruvate by the epithelial cells), was absorbed in a center well which contained 1 ml of Hyamine hydroxide (Sigma Chemical Co., St. Louis, Mo.). 30 min after adding the H_2SO_4, the vials were opened, the Hyamine hydroxide was collected, and the center well was washed twice with 1 ml of methanol. The washed volumes were added to the Hyamine and a 100-μl aliquot of the Hyamine-methanol mixture was added to 10 ml of Aquasol for counting the ^{14}C activity. A standard containing [^{14}C]pyruvate in Ringer's solution without epithelial cells was used to determine the activity of pyruvate in the experimental and control flasks. Quenching was determined for each sample and appropriate corrections were made. After completion of the studies the epithelial cells were dried for 15 h at 80°C to determine the dry tissue weight.

To test the viability of the isolated epithelial cell preparations, separate experiments were performed using vasopressin and ouabain instead of urine fractions. In the former, aqueous Pitressin (Parke, Davis & Company, Detroit, Mich.) was added to the experimental vials (in a final concentration of 100 mU/ml incubate) at the same time the pyruvate was added. In the control flasks containing the cells from the paired hemibladders, no Pitressin was added. The incubations were continued for 60 min, H_2SO_4 was added, and the same procedure described above was followed. In the ouabain experiments, the cells were preincubated with (experimental) and without (control) the cardiac glycoside (final concentration 10^{-8} M) for 45 min before the addition of the [^{14}C]pyruvate. The incubations then were continued in the manner described for the urine fractions.

An additional series of experiments was performed in which ammonium chloride, in a final concentration of 5 mM, was added to the experimental flasks. A 45-min period of preincubation was employed before adding the [^{14}C]-pyruvate.

Ammonium was determined using the microdiffusion method of Conway (10). Sodium and potassium were measured with a flame photometer with an extended range for low sodium concentrations (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). ^{14}C activity was counted with a Liquid Scintillation Spectrometer (model 3330, Packard Instrument Co., Inc., Downers Grove, Ill.). Results are expressed as the mean ±1 SE. Statistical analysis was performed using Student's *t* test with the level of significance expressed as the *2P* value.

RESULTS

Bioelectric phenomena. A representative study showing the effects of a urine fraction from a chronically uremic patient on SCC and PD of the toad bladder is shown in Fig. 1. After a 30-min period of observation, during which stable SCC and PD values were obtained, 500 μl of the fraction was added to the Ringer's solution bathing the serosal surface of the bladder. SCC and PD decreased progressively over the 60 min period of observation. The solutions bathing both surfaces of the bladder were then removed and replaced with fresh Ringer's solution. SCC and PD increased towards the control levels.

The results of experiments in which the urine fractions from seven chronically uremic patients and six normal subjects were added to the serosal surface of the bladder are shown in Tables I and II. The values for SCC are shown in Table I. Six of the seven uremic fractions inhibited SCC. As in the previously reported studies with the serum fraction from uremic patients (4), the onset

of the decrease in SCC was delayed generally for at least 10 min after addition of the fraction. However, in contrast to the pattern observed with the serum fractions where the inhibition appeared to level off within 30 min, the inhibition produced by the urine fraction progressed over the entire period of 60 min. The maximum degree of inhibition observed was 85% (exp. 5) and the mean inhibition for the seven experiments was 44%. In the six experiments in which the urine fraction from normal subjects was assayed, stimulation of SCC occurred in four and in the other two limited inhibition was observed (16 and 13% respectively). For the group, the SCC value at the end of 60 min was 110±9% of the control value. The inhibition observed with the uremic fractions was highly significant. The change observed with the normal fractions was not significant and the difference between the response to the uremic and the normal fractions was significant at 30, 40, 50, and 60 min.

The data for PD across the bladder in the same 13 experiments are shown in Table II. The results generally paralleled those observed for SCC.

Fig. 2 compares the effects of adding the urine fractions from uremic patients to the mucosal vs. the serosal side of the toad bladder. In these experiments, fresh aliquots of four uremic fractions, known to inhibit SCC and PD (nos. 1, 2, 5, and 7 of Table I), were used. Each fraction was first added to the Ringer's solution bathing the mucosal surface of a bladder and SCC and PD were followed for 60 min. The mucosal solution was then removed and transferred to the serosal side of another membrane; values for SCC and PD were followed for an additional 60 min. With the mucosal ex-

TABLE I

Effect of Urine Fractions from Patients with Chronic Uremia and Normal Subjects on SCC across the Isolated Toad Bladder

Patient no.	SCC_0	SCC_t/SCC_0						
		0	10	20	30	40	50	60
	μA							
Uremic patients								
1	59	1.00	0.93	0.93	0.81	0.74	0.71	0.68
2	72	1.00	1.02	1.04	0.94	0.85	0.79	0.70
3*	112	1.00	1.01	0.94	0.89	0.79	0.73	0.66
4*	44	1.00	1.02	0.86	0.71	0.50	0.33	0.36
5	80	1.00	0.78	0.51	0.31	0.24	0.18	0.15
6	222	1.00	1.09	1.09	1.04	1.05	1.03	1.03
7	56	1.00	0.93	0.71	0.54	0.44	0.39	0.34
Mean		1.00	0.97	0.87	0.75	0.66	0.60	0.56
±SE		0.00	0.04	0.08	0.10	0.11	0.11	0.11
P		NS	NS	NS	<0.05	<0.02	<0.02	<0.01
Normal subjects								
1	115	1.00	1.10	1.25	1.32	1.35	1.38	1.40
2	116	1.00	1.16	1.22	1.18	1.18	1.18	1.16
3	172	1.00	1.06	0.99	0.96	0.90	0.87	0.84
4	78	1.00	0.78	0.83	0.85	0.89	0.87	0.87
5	145	1.00	1.26	1.27	1.21	1.21	1.21	1.12
6	23	1.00	0.96	1.00	1.09	1.13	1.17	1.21
Mean		1.00	1.06	1.09	1.10	1.11	1.11	1.10
±SE		0.00	0.07	0.07	0.07	0.08	0.08	0.09
P		NS	NS	NS	NS	NS	NS	NS
Difference uremic vs. normal								
P	NS	NS	NS	NS	<0.02	<0.01	<0.005	<0.005

Results are expressed as the ratio of SCC values observed at specific times (SCC_t), in minutes, after addition of the urine fraction to the Ringer's solution bathing the serosal surface of the bladder to the control value observed at time zero (SCC_0). Absolute values in microamperes are also given for SCC_0.

* Indicates patients on maintenance digitalis therapy.

TABLE II

Effects of Urine Fractions from Patients with Chronic Uremia and Normal Subjects on Potential Difference across the Isolated Toad Bladder

Patient no.	PD_0	PD_t/PD_0						
		0	10	20	30	40	50	60
	mV							
Uremic patients								
1	11	1.00	1.00	0.91	0.82	0.73	0.73	0.64
2	29	1.00	1.03	1.03	0.86	0.79	0.76	0.72
3*	54	1.00	1.00	0.98	0.88	0.83	0.80	0.75
4*	21	1.00	1.09	0.91	0.71	0.38	0.33	0.28
5	61	1.00	0.80	0.52	0.34	0.28	0.21	0.16
6	92	1.00	1.00	1.00	1.01	1.01	1.01	1.01
7	40	1.00	0.95	0.75	0.60	0.52	0.47	0.45
Mean		1.00	0.98	0.87	0.75	0.65	0.62	0.58
±SE		0.00	0.03	0.07	0.08	0.10	0.11	0.11
P		NS	NS	NS	<0.025	<0.02	<0.02	<0.01
Normal subjects								
1	27	1.00	1.15	1.29	1.37	1.44	1.51	1.55
2	49	1.00	1.08	1.12	1.12	1.12	1.14	1.14
3	46	1.00	1.06	1.04	1.09	1.07	1.04	1.04
4	36	1.00	0.72	0.86	0.92	0.92	0.92	0.94
5	51	1.00	1.14	1.17	1.19	1.19	1.19	1.19
6	8	1.00	1.00	1.13	1.25	1.25	1.37	1.37
Mean		1.00	1.03	1.10	1.16	1.17	1.20	1.21
±SE		0.00	0.06	0.06	0.06	0.07	0.09	0.09
P		NS	NS	NS	NS	NS	NS	NS
Difference uremic vs. normal								
P	NS	NS	NS	NS	<0.005	<0.005	<0.005	<0.005

Results are expressed as the ratio of potential difference values observed at specific times (PD_t) after addition of the urine fraction to the Ringer's solution bathing the serosal surface of the bladder to the control value observed at time zero (PD_0). Absolute values, in millivolts, are also given for PD_0.

* Indicates patients on maintenance digitalis therapy.

posure, no inhibition of SCC or PD occurred. However, the same solutions, added to the serosal surface produced marked inhibition.

Intracellular water and electrolyte contents. The results of exposing isolated epithelial cells to the urine fractions from chronically uremic patients are shown in Table III. There was no significant change in intracellular water content. However, intracellular sodium content increased in 16 of the 18 experiments and for the group the increment ws highly significant. Intracellular sodium concentration increased in all 18 experiments and the mean increase for the group was 7 meq/liter. The changes in intracellular potassium content and concentration were inconsistent in direction and were not significant.

The results of seven experiments in which the urine fractions from normal subjects were employed are shown in Table IV. No change occurred in intracellular water content. In contrast to the experiments using the uremic fractions, there also was no significant change in intracellular sodium content. Intracellular sodium concentration showed no consistent change and both potassium content and concentration also were unchanged.

The ammonium concentration in the Ringer's solution averaged 1.6±0.4 meq/liter in the experiments in which uremic fractions were used and 2.4±0.8 meq/liter in the experiments in which normal fractions were used. Table V depicts the effects of 2 mM ammonium chloride in the absence of a urine fraction on intracellular water, sodium, and potassium. There were no significant

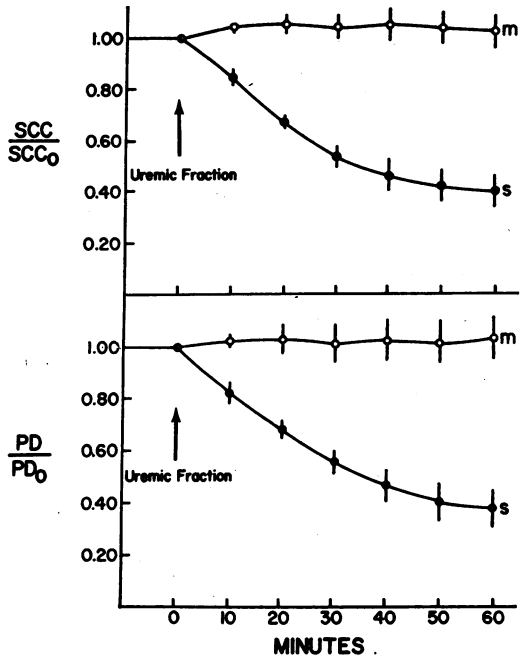

FIGURE 2 Mean changes, ±SE, in SCC and PD across the isolated toad bladder during exposure to urine fractions from patients with chronic uremia. Each urine fraction was added first to the Ringer's solution bathing the mucosal surface (m) of a bladder and SCC and PD were followed for 60 min. The mucosal solution containing the fraction was then removed and transferred to the serosal surface (s) of another bladder with stable values for SCC and PD. SCC and PD were again followed for 60 min. Results are expressed as in Tables I and II.

changes in any of the parameters measured including intracellular sodium content.

A summary of the changes in intracellular sodium content and concentration is shown in Fig. 3. The changes observed with the uremic fractions were significantly different ($P < 0.005$) from those observed with either the normal fraction or 2 mM ammonium chloride for both sodium content and concentration.

Pyruvate oxidation. The effects of urine fractions from seven uremic patients and seven normal subjects on pyruvate oxidation are shown in Table VI. In all experiments, the rate of pyruvate oxidation was depressed in the flasks containing the uremic fraction and, for the group, the decrease averaged 27±7% ($P < 0.01$). For the experiments in which the urine fraction from normal subjects was employed, the mean difference in pyruvate oxidation between experimental and control flasks was + 4%, a value which is not significantly different from zero.

The control experiments using ammonium chloride, ouabain, and vasopressin are shown in Table VII. With 5 mM ammonium chloride, a concentration which exceeded the mean value in the Ringer's solutions containing both the uremic fractions (1.76 mM) and the normal fractions (1.67 mM), there was no significant change in pyruvate oxidation. With ouabain there was a 47% decrease in pyruvate oxidation, and with vasopressin there was a 67% increase in pyruvate oxidation.

DISCUSSION

The transcellular transport of sodium by the isolated toad bladder is an active, energy-dependent process. The present data demonstrate that patients with chronic uremia and an acquired natriuresis per nephron excrete a factor in their urine that inhibits sodium transport by the toad bladder. This factor has characteristics very similar to the inhibitor of sodium transport previously demonstrated in the serum of chronically uremic patients. Thus, the location of the active fraction based on the conductivity tracings of the eluate in the gel filtration system employed is the same for both the serum and urine. Both the serum and urine fractions are natriuretic in the rat (5, 6). Both fractions inhibit SCC in the toad bladder when added to the serosal surface but not when added to the mucosal surface (4); with both fractions there is a delay in the onset of the inhibition after addition of the fraction; and with both serum and urine fractions, the inhibition is reversible after replacement of the serosal solution with fresh Ringer's (4). Both serum and urine inhibitors resist freezing and boiling, and both are inactivated by leucine amino peptidase (11, 12). The urine fraction produced more marked and more sustained inhibition of SCC than did the serum fraction, but the equivalence of dosage is not established.

The same gel filtration fraction obtained from the urine of normal subjects did not inhibit SCC; nor does

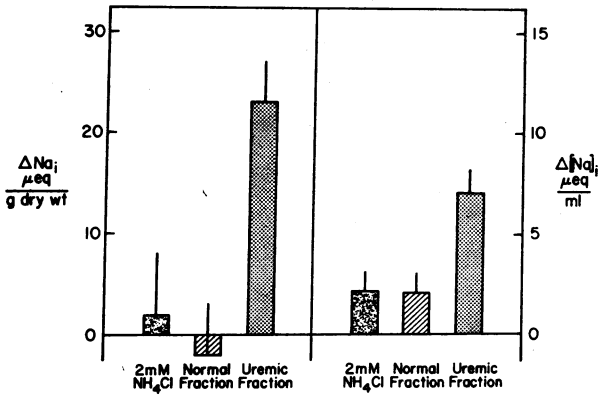

FIGURE 3 Mean changes, ±SE, in estimated intracellular sodium content (ΔNa_i) and intracellular sodium concentration ($\Delta[Na]_i$) of isolated toad bladder epithelial cells incubated in the presence of 2 mM NH_4Cl, urine fraction from normal subjects, and urine fraction from uremic patients.

TABLE III

Changes in Intracellular Water, Sodium, and Potassium of Isolated Toad Bladder Epithelial Cells in the Presence of Uremic Urine Fraction

Patient no.	H_2O_i		Na_i		K_i		$(Na)_i$		$(K)_i$	
	C	ΔExp.	C	ΔExp	C	ΔExp	C	ΔExp	C	ΔExp
	ml/g dry wt		*μeq/g dry wt*		*μeq/g dry wt*		*μeq/ml*		*μeq/ml*	
1*a*	3.07	−0.09	233	+33	329	−51	75	+12	107	−14
1*b*	3.35	−0.07	120	+20	353	+42	36	+7	107	+15
2*a**	3.54	+0.19	108	+60	416	−7	31	+14	118	−8
2*b*	3.91	−0.38	204	+44	437	−33	52	+11	112	−10
3*a*	3.03	+0.16	112	+45	400	−24	38	+6	132	−29
3*b*	3.62	−0.40	134	+12	403	−53	37	+7	112	−3
4	3.53	−0.34	202	−5	474	−6	57	+4	134	+13
5*a**	3.52	−0.02	218	+11	458	−7	71	+3	135	−2
5*b*	3.57	+0.01	172	+22	345	+14	47	+6	97	+4
6	3.60	−0.40	190	+14	397	−38	53	+12	110	+3
7*	3.55	−0.44	182	−3	395	+6	51	+7	112	+19
8	3.96	−0.17	198	+29	425	+6	50	+10	108	+6
9	3.45	−0.02	230	+15	379	+4	67	+4	111	0
10*	3.52	+0.04	158	+14	410	−7	45	+5	117	−1
11*a*	3.60	−0.05	144	+25	384	−16	40	+7	107	−3
11*b*	3.63	+0.10	250	+20	374	+16	69	+4	103	+2
12	3.24	−0.03	241	+28	382	−4	75	+10	119	+1
13	3.21	+0.01	148	+21	377	−6	46	+5	118	−3
Mean	3.49	−0.08	180	+23	397	−9	52	+7	114	−1
±SE		0.06		4		6		1		3
P		NS		<0.001		NS		<0.001		NS

Changes (ΔExp) represent the difference in intracellular water content, H_2O_i, intracellular sodium, Na_i and potassium, K_i content; and intracellular sodium, $(Na)_i$ and potassium, $(K)_i$ concentration between the mean experimental and the mean control (C) values, each obtained from triplicate studies run simultaneously.

* Indicates patients on maintenance digitalis therapy.

the normal urine fraction produce natriuresis in the rat (6). Similar observations have been made using the serum fraction from normal individuals (4–5).

When isolated epithelial cells from the toad bladder were exposed to the inhibitor of sodium transport, there was a significant increase in intracellular sodium content, an observation which is consistent with a serosal site of action (7). Conceptually, the movement of sodium ions across the epithelial cells of the toad bladder involves two major steps. The first is the entry across the mucosal barrier; the second is the movement across the serosal barrier. The latter is thermodynamically "uphill" and is generally considered to represent active transport against an electrochemical gradient. Amiloride, an inhibitor of sodium transport which has a mucosal site of action (13), produces a fall in intracellular sodium content (7). Ouabain, on the other hand, is presumed to act at the serosal surface (14); and this agent produces an increase in intracellular sodium content (7). The increase in intracellular sodium content with the uremic fraction was less marked than that observed with 10^{-4} M ouabain by Handler, Preston, and Orloff (7); however, the degree of inhibition of SCC was also less (14). The urine fraction from normal subjects produced no change in intracellular sodium content.

TABLE IV

Changes in Intracellular Water, Sodium, and Potassium of Isolated Toad Bladder Epithelial Cells in the Presence of Normal Urine Fraction

Subject no.	H_2O_i		Na_i		K_i		$(Na)_i$		$(K)_i$	
	C	ΔExp	C	ΔExp	C	ΔExp	C	ΔExp	C	ΔExp
	ml/g dry wt		*μeq/g dry wt*		*μeq/g dry wt*		*μeq/ml*		*μeq/ml*	
1	2.21	+0.06	121	−15	395	−21	56	−7	181	−11
2	3.45	+0.07	166	+1	379	+8	48	−1	110	+1
3	3.30	−0.32	219	−7	428	−14	68	+3	133	−5
4	3.74	−0.13	235	+13	476	−19	63	+6	127	0
5	3.14	−0.01	260	−18	403	−32	84	+3	131	−12
6	3.20	−0.11	234	+15	470	−15	73	+7	116	−1
7	3.18	−0.11	159	−3	427	+6	50	+1	134	+1
Mean	3.17	−0.08	199	−2	411	−12	63	+2	133	−4
±SE		0.05		5		6		2		2
P		NS		NS		NS		NS		NS

Results are expressed as in Table III.

The increase in sodium content in the epithelial cells observed with the uremic fraction was not associated with a statistically significant change in intracellular water content; nor was there a significant decrease in intracellular potassium content, a change that is observed with ouabain (7). The explanation for the failure of intracellular water content to increase and/or for intracellular potassium content to decrease is not apparent, and future studies will be required to clarify this apparent paradox.

It is of considerable interest that the inhibitor of sodium transport also had an effect on substrate utilization by the isolated epithelial cells. Thus, pyruvate oxidation was decreased by a mean of 27% in relation to the rate of oxidation of epithelia from paired hemibladders incubated simultaneously but not exposed to the uremic fraction. Ouabain (10^{-3} M) decreased pyruvate oxidation by 47%, an observation consistent with the greater inhibition of SCC and greater increase in intracellular sodium content. Vasopressin produced an increase in pyruvate oxidation of 67%. The latter studies were performed to establish the fact that the method for measuring pyruvate oxidation was operative under the conditions employed in our laboratory. In addition, the data suggest that the inhibitor of sodium transport was not heavily contaminated with vasopressin. The effects on SCC and intracellular sodium content carry the same connotation.

Whether the primary effects of the inhibitor are on metabolism or on some step in the transport sequence

TABLE V

Changes in Intracellular Water, Sodium, and Potassium in Isolated Toad Bladder Epithelial Cells in the Presence of 2 mM NH_4Cl

Experiment no.	H_2O_i		Na_i		K_i		$(Na)_i$		$(K)_i$	
	C	ΔExp	C	ΔExp	C	ΔExp	C	ΔExp	C	ΔExp
	ml/g dry wt		*μeq/g dry wt*		*μeq/g dry wt*		*μeq/ml*		*μeq/ml*	
1	3.44	−0.11	213	+26	433	+14	62	+10	126	+8
2	3.29	−0.05	150	+4	498	−53	46	+2	152	−15
3	2.59	+0.03	174	−5	436	−19	67	−2	168	−9
4	2.69	−0.16	203	−12	409	−4	76	+3	152	+15
5	2.90	−0.16	151	+7	443	−14	52	+6	153	+4
6	3.14	+0.14	193	−8	404	+16	62	−6	129	−1
Mean	3.01	−0.05	181	+2	437	−10	60	+2	145	0
±SE		0.05		6		10		2		4
P		NS		NS		NS		NS		NS

Results are expressed as in Table III.

TABLE VI

Changes in Pyruvate Oxidation in Isolated Toad Bladder Epithelial Cells after Exposure to Urine Fractions from Patients with Chronic Uremia and Normal Subjects

Patient no.	Control	ΔExperimental	Mean change
	nmol/g dry wt/h	*nmol/g dry wt/h*	*%*
Uremic patients			
1	3,061	−160	−9
	2,339	−249	
2	2,521	−770	−24
	1,448	−226	
3*	1,335	−338	−14
	3,810	−99	
4*	4,165	−2767	−58
	1,858	−921	
5	1,862	−527	−47
	2,190	−1448	
6	2,009	−842	−20
	2,413	+44	
7	3,489	−391	−18
	1,835	−459	
Mean	2,453	−654	−27
±SE		194	7
P		<0.005	<0.01
Normal subjects			
1	4,477	+454	−6
	2,833	−618	
2	1,112	−369	+11
	933	+503	
3	1,291	+365	−8
	3,048	−1302	
4	1,865	−461	−19
	1,456	−170	
5	2,498	+737	+17
	2,101	+82	
6	1,730	−159	+15
	1,786	+671	
7	3,208	+628	+15
	3,543	+331	
Mean	2,277	+49	+4
±SE		158	5
P		NS	NS

Individual studies are shown and the results are expressed as the difference (ΔExperimental) in pyruvate utilization between the control flask containing epithelial cells without the urine fraction and the experimental flask containing epithelial cells from the paired hemibladders with the urine fraction. The mean change, in percent, represents the average percent change observed in two studies run simultaneously with the same urine fraction on cells from different bladders.

* Indicates patients on maintenance digitalis therapy.

across the serosal barrier of the epithelial cells is not evident on the basis of the present observations. Future studies on the effects of the inhibitor on Na-K-ATPase activity and on intermediates of glycolytic and oxidative metabolism will be required to clarify this question.

TABLE VII

Changes in Pyruvate Oxidation in Isolated Toad Bladder Epithelial Cells in the Presence of 5 mM NH_4Cl, 1 mM Ouabain, and 100 mU/ml Vasopressin

Experiment no.	Control	Δ Experimental	Change
	nmol/g dry wt/h	*nmol/g dry wt/h*	*%*
NH_4Cl, 5 mM			
1	3,039	+205	+7
2	2,725	−159	−6
3	2,739	−87	−3
4	2,832	+562	+20
5	2,146	+254	+12
6	2,756	−340	−12
7	3,559	−562	−16
8	4,525	+753	+17
9	3,259	+143	+4
10	2,612	−62	−2
11	4,773	+369	+8
12	3,416	−951	−28
Mean	3,198	+10	0
±SE		138	4
P		NS	NS
Ouabain, 1 mM			
1	3,215	−1525	−47
2	4,121	−2694	−65
3	2,165	−479	−22
4	3,409	−1457	−43
5	2,467	−1175	−48
6	3,450	−1847	−54
Mean	3,138	−1529	−47
±SE		300	6
P		<0.005	<0.001
Vasopressin, 100 mU/ml			
1	1,285	+828	+64
2	2,834	+1452	+51
3	3,212	+1768	+55
4	1,990	+1496	+75
5	2,445	+1680	+69
6	2,115	+1869	+88
Mean	2,314	+1516	+67
±SE		152	6
P		<0.001	<0.001

Results are expressed as in Table VI.

If one can extrapolate from the present studies on the isolated toad bladder to the mammalian kidney, the inhibitor of sodium transport should act from the peritubular surface of the nephron and should exercise at least part of its effect on the distal segments of the nephron. Previous studies using the recollection micropuncture technique (15) have shown that there is a proximal effect; but it is likely that if the natriuretic factor is a fine modulator of sodium excretion, its physiologic effects must extend to the distal tubule and/or collecting duct. The present data also would suggest that the action of the inhibitor may be attended by an increase in intracellular sodium content and by a decrease in substrate utilization.

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REFERENCES

1. Earley, L. E., and T. M. Daugharty. 1969. Sodium metabolism. *N. Engl. J. Med.* **281**: 72.
2. Schrier, R. W., and H. E. De Wardener. 1971. Tubular reabsorption of sodium ion: influence of factors other than aldosterone and glomerular filtration rate. *N. Engl. J. Med.* **285**: 1231.
3. Bricker, N. S. 1967. The control of sodium excretion with normal and reduced nephron populations. The pre-eminence of third factor. *Am. J. Med.* **43**: 313.
4. Bourgoignie, 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 urine. *J. Clin. Invest.* **50**: 303.
5. Bourgoignie, J. J., K. H. Hwang, C. Espinel, S. Klahr, and N. S. Bricker. 1972. A natriuretic factor in the serum of patients with chronic uremia. *J. Clin. Invest.* **51**: 1514.
6. Bourgoignie, J. J., K. H. Hwang, E. Ipakchi, and N. S. Bricker. 1974. The presence of a natriuretic factor in urine of patients with chronic uremia. The absence of the factor in nephrotic uremic patients. *J. Clin. Invest.* **53**: 1559.
7. Handler, J. S., A. S. Preston, and J. Orloff. 1972. Effect of ADH, aldosterone, ouabain, and amiloride on toad bladder epithelial cells. *Am. J. Physiol.* **222**: 1071.
8. Guggenheim, S. J., J. Bourgoignie, and S. Klahr. 1971. Inhibition by ammonium of sodium transport across isolated toad bladder. *Am. J. Physiol.* **220**: 1651.
9. Gatzy, J. T., and W. O. Berndt. 1968. Isolated epithelial cells of the toad bladder. Their preparation, oxygen consumption, and electrolyte content. *J. Gen. Physiol.* **51**: 770.
10. Conway, E. J. 1950. Ammonia. Biologic determinations. *In* Microdiffusion Analysis and Volumetric Error. Crosby, Lockwood & Son Ltd., London. 3rd edition. 108.
11. Bourgoignie, J., R. W. Schmidt, K. Hwang, T. Nawar, S. Klahr, L. Chase, and N. S. Bricker. 1972. On the nature of the circulating natriuretic factor in chronic uremia. *J. Clin. Invest.* **51**: 12*a*. (Abstr.)
12. Ipakchi, E., J. Bourgoignie, K. Hwang, and N. S. Bricker. 1973. On the apparent increase in production of natriuretic factor in chronic uremia. *J. Clin. Invest.* **52**: 42*a*. (Abstr.)
13. Bentley, P. J. 1968. Amiloride: A potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (Lond.)*. **195**: 317.
14. Herrera, F. C. 1968. Action of ouabain on bioelectric properties and ion content in toad urinary bladder. *Am. J. Physiol.* **215**: 183.
15. Weber, H., J. J. Bourgoignie, and N. S. Bricker. 1974. The effects of the natriuretic fraction from human uremic serum on proximal tubular sodium reabsorption in the rat. *Am. J. Physiol.* **226**: 419.