Inhibition of Transepithelial Sodium Transport in the Frog Skin by a Low Molecular Weight Fraction of Uremic Serum

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ABSTRACT An inhibitor of transepithelial sodium transport was found in a low molecular weight fraction obtained from serum of patients with far advanced chronic renal disease. In 18 nondialyzed patients, the mean inhibition of short circuit current (SCC) was 24.9 ±2.2% (SE). With a comparable fraction from 11 normal subjects, SCC decreased by only 5.3 ±1.5%. There was significantly greater inhibition with the serum fractions of patients with end stage renal disease being maintained on chronic hemodialysis than in the normal control group; but the degree of inhibition in the dialyzed population was significantly less than that observed in the nondialyzed chronically uremic patients. The inhibition of SCC produced by the serum fractions of a group of seven patients with acute renal failure was not significantly different from the control group despite the presence of high grade uremia in the former. The inhibitory fraction has characteristics identical with the uremic serum fraction which previously has been shown to inhibit *p*-aminohippurate (PAH) uptake by rabbit kidney cortical slices. With gel filtration through Sephadex G-25, the active fraction appears after the major peaks of substances as small as urea and sodium; hence it may have been retarded on the column. But its ultrafiltration characteristics suggest that its molecular weight may be less than 1000. The inhibitory capability was not destroyed by boiling, freezing, or digestion with chymotrypsin or pronase. Neither methylguanidine nor guanidinosuccinic acid in concentrations well above those present in the serum of uremic patients inhibited sodium transport in the frog skin. The data suggest that there is an inhibitor of sodium transport in the serum of patients with chronic uremia. The role of this material in the regulation of sodium excretion in uremia as well as its possible role as a uremic toxin are subjects of both theoretical and practical interest.

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INTRODUCTION

The maintenance of sodium balance in patients with chronic renal disease is accomplished by means of a progressive increase in the absolute rate of sodium excretion per nephron as the nephron population and the glomerular filtration rate (GFR) diminish. This natriuresis per nephron may be effected in part by an increase in GFR per nephron; but the major factor is a progressive fall in the fraction of filtered sodium reabsorbed (1, 2). Thus, natriuretic forces capable of diminishing fractional sodium reabsorption must mount in intensity throughout the natural history of chronic renal disease. But the natriuresis per nephron seen with nephron reduction can occur appropriately without a decrease in mineralocorticoid hormone activity (3). Nor does the natriuresis which occurs in dogs subjected to experimental reduction of their nephron population appear to correlate with changes in cardiac output, mean arterial blood pressure, peripheral resistance, or filtration fractions.[1] Thus, if there is a natriuretic hormone which contributes normally to the regulation of sodium excretion (4), its activity might be very high in the blood of patients with advanced chronic renal disease. We recently have described a low molecular weight fraction of uremic serum which inhibits the uptake of *p*-aminohippurate (PAH) by rabbit kidney cortical slices in vitro (5). Because PAH uptake by kidney slices is a sodium-dependent function, these observations could provide in-

[1] Taggart, D., and N. S. Bricker. Unpublished observations.

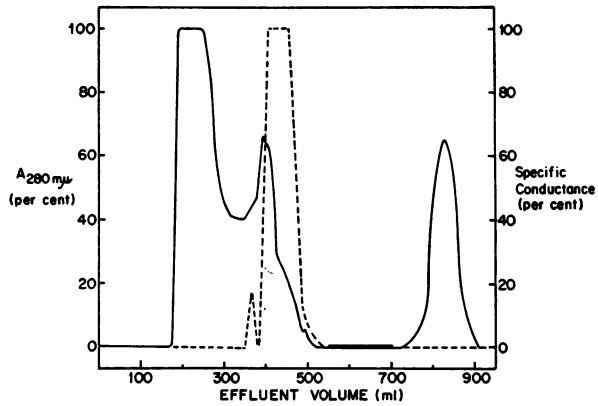

FIGURE 1 Elution pattern of human serum (25 ml) obtained with a 10 mM ammonium acetate solution using a 95 × 2.5 cm column packed with Sephadex G-25. The absorbance at 280 mμ (left abscissa) and the specific conductance (right abscissa) of the effluent solution are indicated by solid and broken lines, respectively. The fraction of serum routinely lyophilized and used for assay is depicted by the heavy line.

ferential evidence for the presence of an inhibitor of sodium transport in uremic serum. The present studies were undertaken to examine the effects of the same serum fraction from uremic patients and the comparable serum fraction from normal subjects on transepithelial sodium transport by the isolated frog skin.

METHODS

Blood was collected from 11 normal subjects and from 18 patients with chronic progressive renal disease. GFR in the patients ranged from 2 to 15 ml/min. They were maintained on a 60–100 mEq/day sodium intake, and none had been dialyzed. Additional studies were performed with blood obtained from 7 patients with acute renal failure and 13 patients with chronic renal disease who were being treated with chronic hemodialysis. Three of the latter group were anephric. 13 of the 31 patients with chronic uremia were normotensive, and the rest had hypertension of varying degrees of severity. The history of drug intake, with particular respect to digitalis glycosides and diuretic agents, was recorded in each patient, but the majority of patients was on neither type of medication and no correlation between the experimental results and the drug history emerged.

Whole blood was collected from the brachial vein or the radial artery and allowed to clot at room temperature. All of the following procedures then were performed at 4°C. Blood samples were centrifuged at 2000 rpm for 20 min, the serum was separated, filtered through a coarse inert nylon netting,[2] and one or two 25-ml aliquots were applied to individual 95 × 2.5 cm columns packed with Sephadex G-25.[2] The columns were eluted with a solution of 10 mM ammonium acetate. 900 ml of effluent solution was collected from each column in 12-ml quantities, using an automatic fraction collector.[3] The ultraviolet absorption at 280 mμ and the electrical conductivity of the effluent solution in each tube were recorded continuously during the fractionation procedure.[3] On the basis of the UV absorption pattern and the specific conductance tracing, the effluent solution was separated into several different fractions (Fig. 1). Two fractions, eluted from 150 to 350 ml and from 350 to 550 ml of effluent solution, contained the principal protein and salt peaks, respectively. These two fractions were discarded. The fraction eluted between about 550 and 700 ml of effluent solution was found to affect sodium transport by the frog skin. This fraction (see Fig. 1) appeared immediately after the major peaks of sodium, potassium, calcium, urea, and creatinine. There was virtually no recorded absorption at 280 mμ and the specific conductance fell strikingly in the two tubes just preceding appearance of this active fraction. In the initial studies the contents of the tubes corresponding to 750 to 900 ml of effluent solution were also pooled and lyophilized for subsequent testing. After it became apparent that changes in the short circuit current (SCC) of the frog skin were induced by a single fraction, only this portion of effluent solution was lyophilized[4] routinely. This is the same fraction that has previously been shown to inhibit the uptake of PAH by rabbit kidney cortical slices (5). The lyophilized powder from the fraction, which had been obtained from an initial volume of 25 ml of serum, was dissolved in 2.5 ml of distilled water and stored at −80°C until it was used.

The activity of the serum fractions was assayed at room temperature using the isolated bladder of the toad (*Bufo marinus*) or the isolated ventral skin of the frog (*Rana pipiens*).[5] The majority of experiments was performed using the frog skin and, unless otherwise indicated in the text, the data refer to assays performed on frog skin. The skins or bladders were dissected free and mounted as diaphragms between the two halves of conventional Lucite transport chambers. The exposed surface area was 2 cm². Both surfaces of the membranes were bathed by 4 ml of anuran Ringer's solution which had the following composition: as the chloride salts, 110 mM sodium, 2.5 mM potassium, 2 mM magnesium, and 1.5 mM calcium; the glucose concentration was 10 mmoles/liter. The solutions were gassed with compressed air and the pH was maintained at 7.8 ±0.2 U using either 2.5 mM sodium bicarbonate or 2.5 mM Tris-chloride. The agar bridges connecting the interior of the chamber to the external electrodes were prepared using the same Ringer's solutions. Transepithelial sodium transport was equated with the short circuit current (6). Short circuiting was maintained continuously manually except for 15 sec during the measurement of the open circuited potential difference every 7–10 min. After mounting the skins or bladders, an equilibration period of at least 90 min was allowed before studies were initiated. No membrane was employed for assay unless the short circuit current and potential difference values had been stable for 30 min and unless the short circuit current exceeded 30 μa/2 cm².

For performance of the assay, 500 μl of the concentrated fraction were mixed with an equal volume of anuran Ringer's solution. The ionic composition of the mixture then was adjusted so that the sodium and potassium concentrations, pH, and osmolality were the same as that of the unmodified Ringer's solution. The ammonium concentration also was measured routinely and only those samples which contained less than 4 mmoles/liter of ammonium (equivalent to less than 0.4 mmoles/liter in the test system) were used. Samples of higher ammonium concentration were rejected because low concentrations of ammonium have been shown to

[2] Pharmacia Fine Chemicals, Uppsala, Sweden.
[3] LKB, Produkter AB, Stockholm, Sweden.
[4] Freeze-Mobile Model, Virtis Co., Gardiner, N. Y.
[5] Lemberger Company, Oshkosh, Wis.

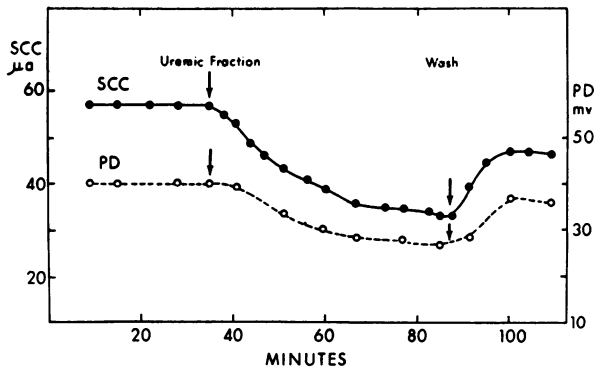

FIGURE 2 Effect of the low molecular weight fraction from uremic serum on short circuit current (SCC) and potential difference (PD) across the isolated frog skin.

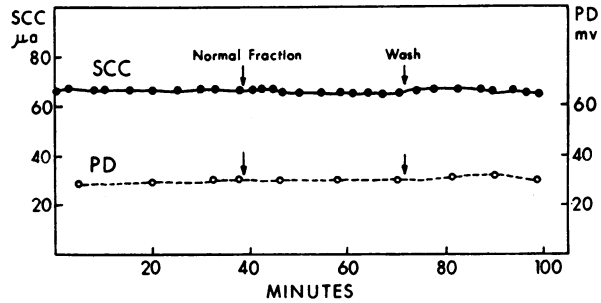

FIGURE 3 Effect of the low molecular weight fraction from normal serum on short circuit current (SCC) and potential difference (PD) across the isolated frog skin.

inhibit sodium transport by the frog skin (7, 8). The calcium concentration of the fractions was never high enough to lead to an increase in the final concentration of calcium in the test system. The assay was performed by removing 800 μl of the 4 ml volume of Ringer's solution bathing the inside surface of the skin (serosal surface of the toad bladder) and replacing this by an equal volume of the 1 : 1 dilution of the fraction.[6] Thus the fraction, which had been concentrated tenfold after lyophilization was diluted 1 : 10 for assay. The concentration of an inhibitor, therefore, could not have exceeded the in vivo concentration and only if there were no loss of inhibitor in the preparative procedures could it have equalled the in vivo concentration.

After adding the fraction, the short circuit current and potential difference were measured for a 30 min period and the values at the end of this interval were compared with the steady-state control values. The data are expressed as percentage changes from the control. In all but two assays, two separate samples of each fraction were tested using frog skin preparations or toad bladders from different animals; the results of the two tests were averaged.

Several additional groups of experiments were performed. In one set the inhibitory capacity of the fraction from normal and uremic subjects was measured before and after ultrafiltration through a Diaflo UM 2 membrane.[7] The UM 2 membrane is designed to retain substances with an average molecular weight greater than about 1000. Ultrafiltration was performed with nitrogen gas at a pressure of 20 lbs. per square inch (p.s.i.). Fractions also were tested before and after boiling for 10 min and before and after incubation with pronase and chymotrypsin.[8] For the latter studies, the concentrated fraction was incubated with pronase or chymotrypsin (1 mg/ml) for 30–60 min at 37°C at pH 7.4. At the end of the incubation the mixture was boiled for 10 min in a test tube covered with a glass tear to prevent loss by evaporation. Control observations for these studies were obtained by using the same fractions processed in the same manner; but either the pronase or chymotrypsin was omitted or the enzymes were previously inactivated by boiling. A group of experiments was also performed to determine the effects of methylguanidine[9] and guanidinosuccinic acid[10] on the short circuit current of the isolated frog skin.

Finally a series of experiments was performed in which whole serum from uremic patients and normal subjects was ultrafiltered through Diaflo XM 50 or UM 10 membranes. These membranes have an average molecular weight rejection of 50,000 and 10,000, respectively. The ultrafiltrates were diluted so that their sodium concentration would equal that of the anuran Ringer's solution. At the end of the equilibration period, the Ringer's solutions bathing both surfaces of the frog skin were removed by gravity and replaced by 4-ml quantities of ultrafiltrate.

Sodium and potassium were determined by flame photometry, calcium by atomic absorption spectrophotometry, BUN by the method of Marsh, Fingerhut, and Miller (9) adapted to the Technicon AutoAnalyzer, osmolality with a Fiske osmometer, and pH with a glass electrode. Ammonium was measured by the microdiffusion method of Conway (10). PAH uptake by rabbit kidney cortical slices was measured as previously reported (5). 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.[11]

RESULTS

A representative experiment depicting the effects of a fraction from a chronically uremic nondialyzed patient is shown in Fig. 2. Following addition of the fraction to the Ringer's solution bathing the inside surface of the frog skin, both the short circuit current and the potential difference decreased. The inhibition typically was delayed for 5–10 min and progressed during the ensuing 20–30 min. When the Ringer's solution containing the fraction was removed and replaced with fresh Ringer, values for both short circuit current and potential difference increased toward the control level. Fig. 3 depicts the ef-

[6] The serum fractions were systematically added to the inside or serosal surface of the membranes, since in pilot experiments, the fraction from uremic patients decreased short circuit current in the toad bladder by an average of 35% (−30 to −44%) when added to the serosal solution, but when added to the mucosal solution, the current changed by a mean of +2.5% (−8 to +16%).

[7] Amicon Corporation, Lexington, Mass.

[8] Sigma Chemical Co., St. Louis, Mo.

[9] K and K Labs. Inc., Plainview, N. Y.

[10] Mann Research Laboratories, Division of Becton, Dickinson and Co., New York.

[11] Scientific Tables, 1962. Documenta Geigy. Geigy Pharmaceuticals. Division of Geigy Chemical Corp., Ardsley, N. Y. 6th edition, 32.

fects of the comparable serum fraction from a normal subject. There was no change in either short circuit or potential difference. With the exception of the first two fractions, which could not be tested in the present assay system, none of the other fractions of serum inhibited short circuit.

Table I presents the data from the 11 normal subjects and 18 nondialyzed patients with chronic uremia. The average value for GFR in the uremic patients was 5.5 ml/minute (range 2.0–15) and the mean BUN was 140 mg/100 ml (range 58–267). The mean inhibition of short circuit current with the normal serum fractions was 5.3 ±1.5%. In contrast, the samples from the 18 chronically uremic patients produced a mean decrease in short circuit current of 24.9 ±2.2%. This value differs significantly from the value obtained for the group of normal

TABLE I

Effects of a Comparable Fraction of Serum Obtained from Normal Subjects and from Patients with Chronic Uremia on Short Circuit Current and Potential Difference across the Frog Skin

	Control		Experimental		Change in	Recovery		Change in
	SCC	PD	SCC	PD	SCC	SCC	PD	SCC
	μamp	*mv*	*μamp*	*mv*	*% of control*	*μamp*	*mv*	*% of experimenta*
Normal subjects								
1.	53	18	54	20	+1.9	57	—	+5.5
2.	43	15	44	16	+1.0	48	19	+9.9
3.	54	15	50	17	−7.4	55	17	+10.0
4.	44	23	42	23	−4.5	46	24	+9.5
5.	68	36	66	38	−2.9	67	37	+1.5
6.	47	35	42	31	−10.6	44	—	+4.7
7.	67	44	66	45	−1.5	70	48	+4.5
8.	59	42	57	42	−3.4	63	48	+10.5
9.	122	70	109	67	−10.7	104	64	+4.6
10.	87	48	75	47	−13.8	78	50	+4.0
11.	31	24	29	23	−6.5	—	—	—
Mean	61.4	33.6	57.6	33.5	−5.3	63.2	38.4	+5.6
±SE	7.6	5.1	6.5	4.8	1.5	5.7	6.0	1.5
Uremic subjects								
1.	92	37	56	26	−39.1	76	36	+35.7
2.	61	38	53	35	−13.1	58	39	+9.4
3.	73	37	53	30	−27.4	63	—	+18.8
4.	71	56	51	40	−28.2	80	51	+56.8
5.	62	39	41	30	−33.9	50	31	+21.9
6.	53	20	40	16	−24.5	49	20	+22.5
7.	69	75	52	61	−24.6	60	76	+15.4
8.	65	42	61	37	−6.2	84	—	+37.7
9.	74	45	63	48	−14.9	82	52	+30.1
10.	84	50	56	50	−33.3	61	52	+8.9
11.	123	44	104	41	−15.5	108	—	+3.8
12.	77	27	56	27	−27.3	—	—	—
13.	146	75	115	75	−21.2	—	—	—
14.	97	73	69	67	−28.9	102	76	+47.8
15.	78	41	67	38	−14.1	—	—	—
16.	41	31	28	23	−31.7	31	25	+10.7
17.	56	14	41	11	−26.8	—	—	—
18.	85	37	52	32	−38.8	71	40	+36.5
Mean	78.2	43.4	58.8	38.1	−24.9	69.6	45.3	+25.4
±SE	5.9	4.1	5.0	4.0	2.2	5.6	5.6	4.2

SCC = short circuit current; PD = potential difference. Experimental SCC and PD represent the values 30 min after addition of the serum fraction to the Ringer's solution bathing the inside surface of the skin. Recovery SCC and PD represent the values 30 min after removal of the serum fraction and replacement of fresh Ringer's solution on both sides of the skin.

subjects ($P < 0.001$). Following removal of the fraction from the chronically uremic patients and the addition of fresh Ringer's solution, short circuit current recovered towards the control value in a highly consistent fashion. When the normal fraction was replaced, small changes in short circuit current were observed. The difference in recovery of the short circuit current between the normal and the uremic groups was highly significant ($P < 0.001$).

Table II presents the results of 11 studies in which the inhibitory capacity of the uremic fraction was measured before and after ultrafiltration through a Diaflo UM 2 membrane. Each of 11 fractions inhibited short circuit current by at least 16% before ultrafiltration. The mean inhibition was 22.3%. With 10 of the 11 fractions inhibition persisted after ultrafiltration and the mean decrease in short circuit current produced by the ultrafiltrates was 21.1%. When normal sera were studied before and after ultrafiltration, no inhibition was observed under either circumstance. Of interest is the fact that when whole uremic serum, rather than the gel filtration fraction, was ultrafiltered, no inhibition was obtained with the ultrafiltrates from membranes having molecular weight exclusions of 10,000 and 50,000 (see Table II).

TABLE II

Effect of Ultrafiltrates of the Serum Fraction and Whole Serum Obtained from Uremic Patients on Short-Circuit Current across the Frog Skin

	Ultrafiltration of serum fraction UM 2 membrane	
No.	Before ultrafiltration	After ultrafiltration
1.	−23	−25
2.	−17	−21
3.	−27	−18
4.	−29	−26
5.	−17	−14
6.	−19	−17
7.	−16	−16
8.	−16	−2
9.	−20	−46
10.	−30	−19
11.	−31	−28
Mean	−22.3	−21.1

	Ultrafiltration of whole serum		
No.	XM 50 membrane	No.	UM 10 membrane
1.	−10	1.	−33
2.	+24	2.	+12
3.	+18	3.	+19
4.	+5	4.	+58
5.	0	5.	+15
6.	+4	6.	+20
Mean	+6.8		+15.1

Results are expressed as the per cent change of control short circuit current observed 30 min after addition of the ultrafiltrate. In the experiments with the serum fraction (top part), 800–1000 μl of Ringer's solution bathing the inside surface of the skin was replaced by an identical volume of ultrafiltrate of concentrated fraction. Different samples of whole serum were used on the XM 50 and UM 10 membranes (bottom part). In these experiments, the Ringer's solution bathing the inside and outside surfaces of the skin was replaced by 4 ml of ultrafiltrate.

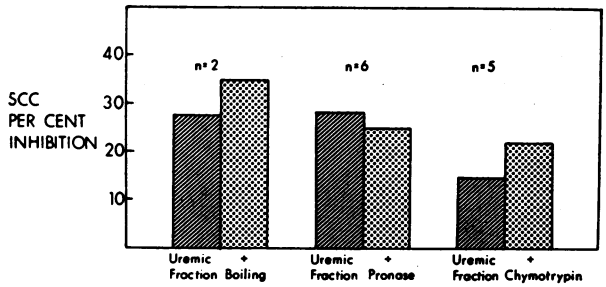

FIGURE 4 Inhibition of short circuit current (SCC) produced by the low molecular weight fraction of uremic serum before and after boiling and before and after incubation with pronase or chymotrypsin.

Certain additional characteristics of the inhibitory factor are presented in Fig. 4. Inhibition of sodium transport persisted after boiling the fraction for 10 min. It also persisted after digestion with pronase and subsequent boiling and after digestion with chymotrypsin and subsequent boiling. The inhibitory activity was not affected by freezing of the fraction for periods of up to 4 wk; it also could be obtained from sera which were stored at −80°C for periods as long as 10 wk before fractionation through Sephadex.

Recent interest has been devoted to two compounds which are retained in the serum of uremic patients and which are believed to represent uremic toxins. One is methylguanidine (11), the other guanidinosuccinic acid (12). To examine the possible relevance of each of these compounds to the present observations, their effects on the short circuit current of the isolated frog skin were measured, using a protocol identical with that employed for the assay of the serum fractions. The results are presented in Table III. After obtaining control observations, methylguanidine was added to the Ringer's solution bathing the inside surface of the skin in a final concentration of 1 mmole/liter. The mean change in short circuit current for six experiments was −4% and the maximum decrease observed was 9%. Guanidinosuccinic acid also was added to the inside solution in a final concentration of 1 mmole/liter. The mean change in short circuit current for five experiments was −2.6%.

TABLE III

A. Effects of 1 mM Methylguanidine on Short Circuit Current and Potential Difference across the Frog Skin

Control		Experimental		Change in
SCC	PD	SCC	PD	SCC
μamp	*mv*	*μamp*	*mv*	%
44	34	42	33	−5
49	59	47	59	−4
42	45	38	42	−9
56	23	53	22	−4
50	36	52	36	+4
82	21	76	18	−6
		Mean change		−4

B. Effects of 1 mM Guanidinosuccinic Acid on Short Circuit Current and Potential Difference across the Frog Skin

Control		Experimental		Change in
SCC	PD	SCC	PD	SCC
μamp	*mv*	*μamp*	*mv*	%
30	29	29	29	−3
30	22	30	22	0
52	17	49	16	−5
65	17	60	16	−8
64	33	66	34	+3
		Mean change		+2.6

Values increased in three, decreased in one, and remained unchanged in one experiment.

The results of studies performed with the serum fractions from seven patients with acute renal failure are shown in Table IV. Five of the patients had acute tubular necrosis, one bilateral cortical necrosis, and one bilateral renal infarction. Five of the patients were anuric or oliguric and two had nonoliguric renal failure. Three of the patients had been dialyzed, four had not. At the time the blood for assay was obtained, the average value for the BUN was 127 mg/100 ml (range 92–165), a value not significantly different from that from the 18 patients with chronic renal disease shown in Table I. Of interest is the fact that, in contrast to the data presented in Table I, the serum fractions from only two of the seven patients produced substantial inhibition of short circuit current. Both of these patients were nonoliguric at the time of blood sampling. Neither had been dialyzed. Of the remaining five patients, three had been dialyzed, two had not.

16 samples were obtained from 13 patients with chronic uremia who were being maintained on chronic hemodialysis. Blood was obtained just before beginning dialysis and thus, usually, 72 hr after the previous dialysis. The average BUN was 79 mg/100 ml (range 35–104). Inhibition of short circuit current averaged 16.1 ±2.1%. 3 of the 13 patients were anephric. The serum fraction from two of the anephric patients inhibited the short circuit current by 16 and 23%, respectively. The sample from the third anephric patient decreased the short circuit current by 6%.

The composite data for the normal subjects, the patients with acute renal failure and the two groups of patients with chronic renal failure are shown in Fig. 5. The mean inhibtion produced by the fractions from seven patients with acute renal failure was 6.8 ±3.0%, a value not significantly different from the value of 5.3 ±1.5% observed for normal subjects. The inhibition produced by the fractions from the 13 uremic patients on chronic hemodialysis (16.1 ±2.1%) is significantly different from that for the normal subjects ($P < 0.01$) and for the subjects with acute renal failure ($P < 0.05$). It is also significantly lower than the value of 24.9 ±2.2% for the nondialyzed chronic uremic patients ($P < 0.005$).

As has been noted above, the same fraction from uremic serum which inhibits sodium transport by the frog skin and toad bladder has previously been found to inhibit PAH uptake by rabbit kidney cortical slices. Moreover the PAH inhibitor also is resistant to boiling, freezing, and digestion with pronase and chymotrypsin. In Table III, nine experiments are shown in which the same fractions and ultrafiltrates were tested both on sodium transport by the anuran membrane and on PAH uptake by rabbit kidney cortical slices. Three of the assays were performed using material from normal subjects, three from nondialyzed chronic uremic patients, and three from dialyzed chronic uremic patients. For the entire group, the correspondence between the two assay systems is good.

TABLE IV

Patients with Acute Renal Failure

No.	Diagnosis	Duration	BUN*	Interval since last dialysis	Change in SCC
		days	*mg/100 ml*	*days*	%
1.	Nonoliguric ATN	6	130	—	−16
2.	ATN	14	92	4	−8
3.	ATN	7	102	3	+2
4.	ATN	6	142	—	+1.5
5.	Nonoliguric ATN	6	148	—	−22
6.	Bilateral renal infarction	18	165	—	−5
7.	Acute cortical necrosis	30	108	7	+2

ATB = acute tubular necrosis.

* Blood urea nitrogen determined within 24 hr of blood collected for assay.

DISCUSSION

The present data demonstrate the existence of a substance (or perhaps substances) in the serum of patients with chronic uremia which inhibits transepithelial sodium transport by the isolated frog skin. The active material was extracted from uremic serum via molecular sieving using Sephadex G-25, and it appeared in a low molecular weight fraction. When this fraction was added to the Ringer's solution bathing the inside surface (i.e. blood side) of the isolated frog skin in a concentration which could not have exceeded the in vivo level in the donor uremic patients, short circuit current fell predictably. After a lag period of approximately 5–10 min, short circuit current began to decrease and the decrement persisted for 25–30 min. When the Ringer's solution containing the inhibitory fraction was removed and replaced with fresh anuran Ringer, short circuit current returned towards the control level. The potential difference across the skin fell and subsequently rose in a manner which was qualitatively similar to the changes in the short circuit current.

Certain of the characteristics of the inhibitory material are apparent in the present studies. The serum fraction containing the inhibitor was eluted from Sephadex G-25 after the major peaks of sodium, potassium, creatinine, and urea. It seems very unlikely, however, that the molecular weight of the inhibitor could be less than that of the sodium ion; hence, the inhibitory compound probably was retarded on the Sephadex column. The studies performed with Diaflo UM 2 membranes suggest that the molecular weight is low, possibly less than 1000; at least the inhibitor passed freely through a filter designed to prevent the filtration of molecules with an average molecular weight greater than approximately 1000.[12] The inhibitor resisted boiling and freezing. It also resisted digestion by two agents, pronase and chymotrypsin, which destroy certain peptide compounds. Nevertheless, the failure of these two enzymes to inactivate the inhibitor does not exclude the possibility that it might be a low molecular weight peptide. The studies simply do not provide any support for this possibility.

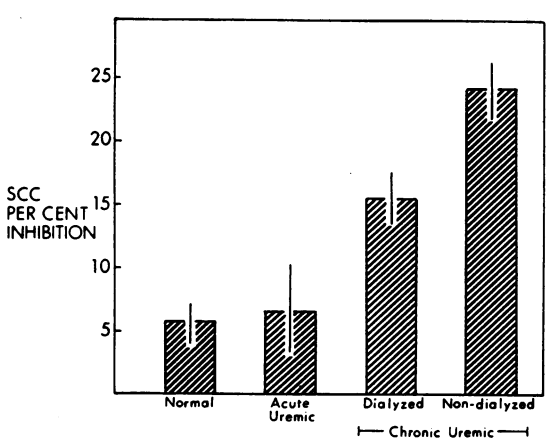

FIGURE 5 Mean inhibition ±SE of short circuit current (SCC) produced by comparable fractions of serum from normal subjects, patients with acute renal failure and patients with chronic renal failure on chronic hemodialysis, and nondialyzed patients with chronic renal failure.

TABLE V

Comparison Between the Effects of the Same Serum Fraction on Short Circuit Current across an Anuran Membrane and on p-Aminohippurate Uptake by Rabbit Kidney Cortical Slices

No.	Change in SCC	Change in PAH uptake
Normal subjects		
1.	0	+8
2.	0	0
3.	−11	+10
Nondialyzed chronic uremic patients		
4.	−49	−43
5.	−39	−59
6.	−25	−45
Dialyzed chronic uremic patients		
7.	−10	−25
8.	−14	−27
9.	−36	−29

Results are expressed as the per cent change of control short circuit current (see Methods) and of control PAH uptake (uptake of PAH by kidney slices incubated in Ringer's solution to which the serum fraction was not added).
The isolated toad bladder was used for samples 1–4, and the frog skin for the remaining samples.

The inhibitor of sodium transport by the frog skin appears to have certain of the same characteristics as the substance obtained from uremic serum that inhibits PAH uptake by rabbit kidney cortical slices (13). Both inhibitors were obtained from the serum of patients with chronic uremia; both appeared in the same low molecular weight fraction following filtration through Sephadex G-25; and both had the same elution characteristics. Both inhibitors resisted boiling, freezing, and digestion with chymotrypsin and pronase. Moreover, when the same serum fractions or ultrafiltrates were tested in the two assay systems, the effects observed were comparable. Thus the inhibitor obtained from uremic serum may be active in at least two different systems involving the transport of two different solute species.

[12] Buckalew, Martinez, and Green (19) recently have cited as a personal communication the fact that compounds with molecular weights as high as 3000 and with the appropriate configuration may pass through the UM 2 membrane.

The relationship of our material to the humoral inhibitor(s) of sodium transport described by other groups of investigators in the blood of nonuremic animals and man (14–20) remains to be determined. Cort and associates have described observations which they believe demonstrate the presence of an inhibitor of sodium transport in the serum following carotid artery occlusion in cats (14, 15) and acute volume expansion with isosmotic isoncotic dextran infusions in cows (16) or physiologic saline in dogs (17). Their substance inhibited short circuit current in the frog skin and was natriuretic in the rat, although the quantitative aspect of their data is not apparent in their publications. Unlike our material, the factor with a molecular weight said to be from 800 to 1000, did not appear in mixed venous blood and its activity was inhibited by chymotrypsin as well as by trypsin and aminopeptidase.

Sealey, Kirshman, and Laragh (18) have obtained an extract from plasma and urine of saline-loaded man and sheep that is natriuretic in rats with hereditary diabetes insipidus. Their substance appears to have a molecular weight in excess of 5000 and possibly in excess of 50,000 and thus would appear to be fundamentally different from the present inhibitor. However, the possibility exists that the inhibitory substance of Sealey et al. (18) could be the same as the present one if it circulates in vivo bound to a larger molecular weight compound. Some indirect evidence in support of the possibility of binding of the present inhibitor arises from the studies presented in Table II involving ultrafiltration of whole uremic serum. When whole serum rather than the gel filtration fraction was subjected to ultrafiltration using Diaflo XM 50 and UM 10 membranes, with an average molecular weight rejection of 50,000 and 10,000, respectively, the ultrafiltrates failed to inhibit transepithelial sodium transport by the frog skin. Thus if the inhibitor is protein bound, it would appear to be separated in the process of gel filtration through Sephadex G-25 but would remain bound when subjected to ultrafiltration at a pressure of 20 p.s.i.

Recently, Buckalew, Martinez, and Green have described a circulating inhibitor of sodium transport by the isolated toad bladder in dogs with extracellular fluid volume expansion imposed either by acute saline loading (19) or long-term administration of mineralocorticoid hormones (20). The substance was obtained from plasma following ultrafiltration through Diaflo UM 10 and UM 2 membranes which, as indicated above have a molecular weight cut-off of approximataely 10,000 and 1000, respectively. The inhibitor also was obtained in the dialysate following dialysis with a Klung dialyzer. On the basis of the Diaflo experiments, the molecular weight of this inhibitor would appear to correspond closely to that of the inhibitor obtained in the present studies from uremic serum and so also would its capacity to inhibit transepithelial sodium transport by an anuran membrane. However, there is a difference which would appear to be of a substantive nature. In the former studies the inhibitor could be obtained in the ultrafiltrate with UM 10 and UM 2 Diaflo membranes; whereas in the present studies, when whole serum was used the inhibition was not observed with the filtrate of UM 10 or XM 50 membranes. Thus none of the inhibitors of sodium transport found in nonuremic animals or man corresponds precisely to the inhibitor described in the present studies and the exact relationship between the uremic inhibitor and the circulating substances described in volume expansion requires further exploration.

It is of interest that inhibition was produced by the serum fractions from only two of seven patients with acute renal failure despite the fact that all seven had high-grade uremia. Thus, whatever the nature of the substance, it apparently takes time to appear in detectable activity in the serum. It is also of interest that the only two patients who manifested inhibition in their serum fraction were nonoliguric at the time blood was obtained. Additional studies are obviously necessary to examine the consistency of this relationship. Inhibition was obtained in chronic uremic patients who were maintained on chronic dialysis, but the mean inhibition for group data was significantly less than that for the nondialyzed chronic patients. To date, studies have not been performed in which blood obtained before and immediately after hemodialysis was compared. The fact that the inhibitor was present in two of three anephric patients would indicate that it is not synthesized by the kidneys.

It is not yet clear whether the substance described in these studies is a hormone or a metabolic product retained in uremia. However, if it is a hormone, it seems likely that the hormonal activity increases as part of the adaptation in the sodium control system which leads to a progressive increase in sodium excretion rate per nephron as the nephron population diminishes. Moreover, if the inhibitor, whatever its nature, is present in sufficiently high concentration in uremia to affect solute transport in extrarenal systems, it could ultimately contribute importantly to certain of the symptoms and signs of the uremic state.

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