Fluid Secretion in Isolated Proximal Straight Renal Tubules

EFFECT OF HUMAN UREMIC SERUM

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ABSTRACT We have examined the effect of normal and uremic human sera on the transtubular flow of fluid in isolated perfused segments of rabbit proximal convoluted and straight renal tubules. Proximal convoluted and straight tubules absorbed fluid from the lumen when the external bath was normal rabbit serum. Normal human sera in the bath depressed net fluid absorption in both tubular segments, but more importantly, uremic human serum caused proximal straight tubules to secrete fluid into the lumen. Fluid secretion was also demonstrated indirectly by observing in nonperfused proximal straight, but not proximal convoluted tubules, that the normally collapsed lumens opened widely in uremic serum. Nonperfused proximal straight tubules developed expanded lumens even after a 25-fold dilution of human uremic serum with normal rabbit serum, whereas lumen expansion occurred only in undiluted normal human serum, on the average. Serum from acutely uremic rabbits possessed secretory activity but normal rabbit serum did not. The secretory effect of uremic sera in proximal straight tubules was inhibited by cooling and ouabain and probenecid. The secretory activity of uremic sera was removed by dialysis, but not by freezing or boiling. Para-aminohippurate and benzoate caused fluid secretion in proximal straight tubules but urea, creatinine, guanidinosuccinate, and urate did not. On the basis of these results, we suggest that the secretory factor in serum may be a substance or group of substances possibly related to the hippurate class of organic molecules that are accumulated to relatively high concentrations in renal failure. The secretory material in the serum of uremic patients may significantly influence the transport of salt and water in relatively intact residual nephrons.

Portions of this study were presented to the Renal and Electrolyte Section of the American Society for Clinical Investigation, Atlantic City, N. J., 29 April 1973.

Dr. Grantham is the recipient of Career Development Research Award AM 22760 from the U. S. Public Health Service.

Received for publication 17 April 1973 and in revised form 6 June 1973.

INTRODUCTION

Net fluid transport by the mammalian kidney is believed to proceed in an absorptive direction along the full extent of the nephron. There are no published accounts to indicate that inulin, the classical marker of volume absorption in micropuncture and clearance studies, ever appears in lower concentrations in urine than in serum. Consequently there has been little reason to explore the possibility that fluid secretion contributes importantly to urine formation, even when the fractional excretion of glomerular filtrate is markedly increased, as in progressive renal insufficiency (1, 2). Rather, most of the attention has been directed at identifying the factors that may inhibit transtubular solute and fluid absorption in a host of natriuretic conditions. In this climate we set out to determine the mechanism by which uremic serum inhibits salt and water absorption in specific portions of nephron isolated from the kidneys of normal rabbits. To determine net transtubular fluid transport we used a relatively new adaptation of the in vitro microperfusion method in which one end of an isolated proximal tubule was perfused with a pipette and the other end was completely occluded (3, 4). In this manner net fluid absorption could be determined simply by measuring the rate of flow of perfusate out of the perfusion pipette. To our amazement, uremic serum in the external bath not only rapidly decreased the rate of net transtubular fluid absorption of proximal straight tubules, but caused net secretion of fluid into the lumens. This surprising observation forms the basis of the present report of some

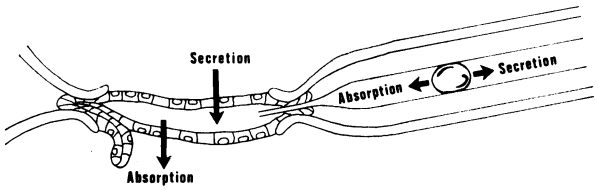

FIGURE 1 Arrangement for perfusing tubules. The perfusion pipette was connected to a reservoir to regulate hydrostatic pressure. Transtubular fluid absorption or secretion was determined from the rate of movement of the oil drop in the perfusion pipette.

of the characteristics of fluid secretion induced by uremic serum in the proximal straight tubule of the rabbit kidney.

METHODS

Kidneys were removed from young, female New Zealand white rabbits that had been anesthetized with intravenous pentobarbital. A transverse slice of kidney was placed in chilled rabbit serum (Microbiological Associates, Inc., Bethesda, Md.) and individual segments of proximal convoluted (PCT)[1] or straight tubules (PST) were dissected from the cortex with forceps as described previously (5). The tubules were transferred to an incubation chamber at room temperature above which a dissecting microscope was positioned for viewing at magnifications ranging from 10 to 100 ×. The arrangement for perfusing the proximal segments, though similar to that described previously (3, 4), was modified to simplify the measurement of net transtubular fluid absorption in the individual convoluted and straight proximal segments (Fig. 1). One end of the tubule segment was perfused with a micropipette in which the rate of perfusate flow was regulated by adjusting the height of a fluid reservoir connected to the back of the pipette, as before (3, 4). The tip of the perfusion pipette was 15 μm in outer diameter; approximately 1 mm from the tip the internal diameter of the pipette lumen was relatively constant for several millimeters (range of mean diameters, 50–100 μm). To quantify net transtubular absorption, a drop of naphtha was sucked into the tip of pipette followed by several nanoliters of the perfusate. To begin the experiment one end of the tubule was perfused for about 30 s after which the untethered end was occluded as described previously (3, 4) (Fig. 1). The hydrostatic perfusion pressure was adjusted to between 5 and 10 cm H_2O. The temperature of the serum bath was heated to 37°C and in consequence there was acceleration of net fluid absorption as indicated by the more rapid movement of the oil column toward the tubule. To quantify net fluid absorption we measured the distance (x) the oil column traveled in unit time (t) and the diameter (ID) of the pipette lumen. The dimensions of the pipette were measured using a calibrated reticle in the ocular of the microscope. Length and diameter could be determined to within ±5 μm. In the steady state, the rate of net fluid absorption by the tubule was taken to be equal to the volume of the pipette segment through which the oil column passed, and was calculated from:

$$V\ (\text{nl/min}\cdot\text{mm}) = \frac{\pi x}{tL}\left(\frac{ID}{2}\right)^2$$

where L is the length of the tubule. To quantify fluid movement in the secretory direction the same technique was used, only in this instance the oil drop moved away from the tubule.

The perfusate contained: NaCl, 125 mM; $NaHCO_3$, 25 mM; Na_2HPO_4, 1.2 mM; KCl, 5 mM; $MgSO_4$, 1.2 mM; CaCl, 1.0 mM; and glucose, 5.5 mM. In all studies the initial bath was rabbit serum that was constantly gassed with O_2 (95%) and CO_2 (5%). To minimize the effects of evaporation, the serum bath was changed every 5 min.

Studies of nonperfused tubules. The phenomenon of lumen expansion in uremic serum (to be described) subsequently formed the basis of a method to assay blood for secretory activity. In these studies, proximal straight tubules were dissected from the middle one-third of the cortex. Several bundles were dissected, each containing four to eight straight tubules. Each bundle of tubules was transferred in 15 μl of the chilled serum to an empty well in a microtiter plate (Falcon Plastics, Los Angeles, Calif.). The capacity of each well was about 40 μl. The tissue was carefully positioned in each well to permit observation of individual straight tubules. The bottom of each well was a transparent cover slip so that the tissue could be viewed with microscope from either above or below. Tubules were placed in six consecutive wells of each row. In the initial well of each row all but about 2 μl of the normal rabbit serum was decanted and the well was rinsed with an excess (ca. 40 μl) of the test serum. 5 μl of fluid in the initial well was then mixed with the 15 μl of normal serum in the second well resulting in a fourfold dilution of the test serum. 5 μl of the fourfold dilution was carried to the third well, and so on, to give a series of dilutions of 1, 4, 16, 64, 256, and 1,024. The chamber was covered and placed in an incubator at 37°C for 15 min during which time a mixture of O_2 (95%) and CO_2 (5%) flowed across the surface of the wells. Lumen expansion in the PST was used as the end point for the assay of secretory activity (See Results). The reciprocal of the maximal dilution of uremic serum to cause lumen expansion is defined as the secretory titer. The lumens of nonperfused proximal tubules normally are invisible, even at 1,000 ×. Thus, an unequivocal lumen present in any of the straight tubules in a well was taken as a positive indication of secretory activity. In each assay para-aminohippurate (PAH) was added to normal rabbit serum in a parallel group of tubules as a control.

The capacity of a number of chemical substances to cause fluid secretion (lumen expansion) was evaluated in this manner. In each case a relatively high concentration of the test agent was added to the normal rabbit serum in the first well. The test substance was then diluted serially with normal rabbit serum as described above. The following substances were tested: PAH (Eastman Kodak Co., Rochester, N. Y.).; creatinine (Fisher Scientific Co., Pittsburgh, Pa.); probenecid (Merck Sharp & Dohme, West Point, Pa); urea (Matheson Coleman & Bell, Norwood, Ohio); ouabain (Calbiochem, San Diego, Calif.); benzoic acid, guanidinosuccinic acid, lactic acid, and uric acid (Sigma Chemical Co., St. Louis, Mo.).

Studies of human serum. Samples of arterial and venous blood were drawn into vacuum tubes from patients with acute and chronic renal failure, and, after clotting and cen-

[1] *Abbreviations used in this paper:* PAH, para-aminohippurate; PCT, proximal convoluted tubule; PST, proximal straight tubule.

trifugation, the serum was decanted and stored at −10°C. Patients V. S., S. H., E. G., M. W., W. B., E. B., C. D., and L. G. had chronic renal failure requiring hemodialysis They were dialyzed for 8 h twice a week using a Travenol (Travenol Laboratories, Morton Grove, Ill.) dialysate delivery system and coil-type membranes. In this study we made no attempt to evaluate the effectiveness of different modes of dialysis. All patients in this group were ambulatory and ate a diet containing 1 g protein/kg body weight with modest K, Na, and water restriction. Samples of blood were obtained in the morning as the patient was connected to the dialysis machine. In these patients the average predialysis serum values of Na, K, Cl, and HCO_3 were 136, 4.5, 96, and 23 meq/liter, respectively. Patients G. K. and D. P. had chronic renal failure but had never been dialyzed. Patients A. L. and J. C. developed acute oliguric renal failure. Neither had chronic renal disease and both recovered life-sustaining renal function. Blood samples were obtained from six normal human volunteers after an overnight fast.

Studies of uremic rabbits. In two rabbits the ureters were ligated near the renal pelvis through a midline abdominal incision. The animals were allowed free access to food and water for the next 72 h. Blood samples were taken from ear vessels at the time of surgery and 24, 48, and 72 h postoperatively. At the time of sacrifice there were early signs of uremia; autopsy confirmed bilateral ureteral ligation.

RESULTS

Perfused proximal straight tubules. The effect of uremic serum in the bath on the net transtubular transport of fluid was examined in nine PST. Details of a representative study are shown in Fig. 2. The tubule was initially perfused with isotonic fluid at a hydrostatic pressure of 10 cm H_2O with normal rabbit serum in the external bath. Net fluid absorption was measured approximately every 2 min in the control period and averaged 0.11 nl/min·mm. The bath was exchanged with uremic serum from a patient in the chronic hemodialysis program. Within 5 min after the uremic serum had been added, net fluid transport had converted to the secretory direction. Net fluid secretion proceeded at a relatively steady rate until the human uremic serum was replaced with the normal rabbit serum. Human uremic sera were tested similarly in nine separate studies (Fig. 3). In each experiment the uremic serum caused fluid transport to move in the secretory direction and the effect was reversed to a significant extent when normal rabbit serum was placed in the bath.

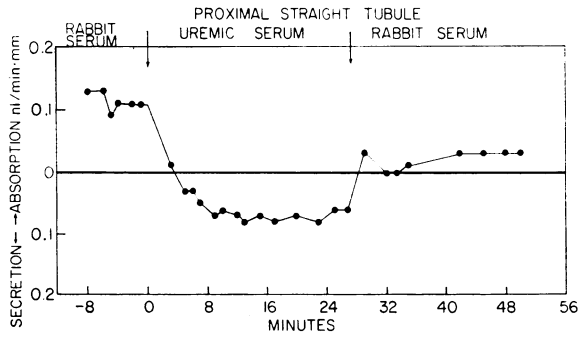

FIGURE 2 Effect of human uremic serum on net fluid transport.

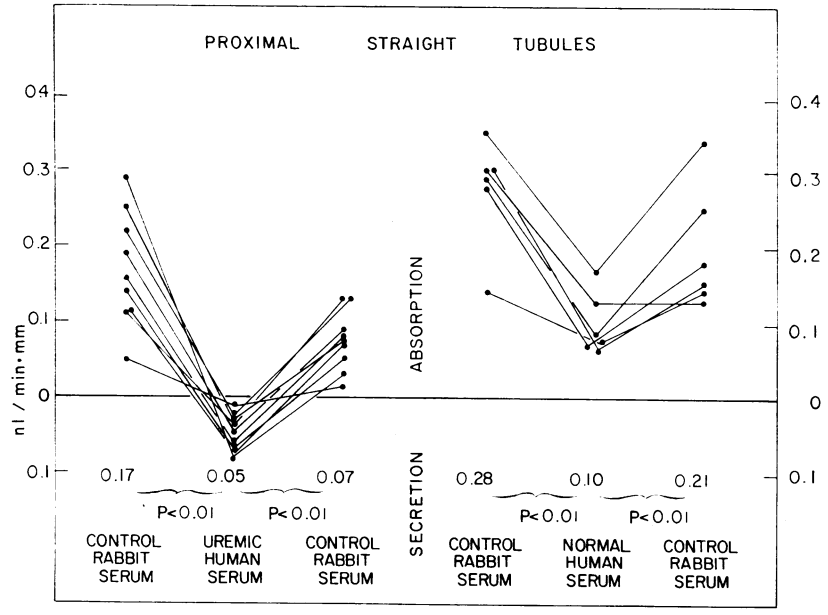

FIGURE 3 Effect of normal and uremic human sera on net fluid transport. Each point is the average of three or more measurements of fluid transport rate in the steady state. Numerical values are the average rate of fluid transport for all tubules. The levels of significance were determined by analysis of paired differences between individual tubules.

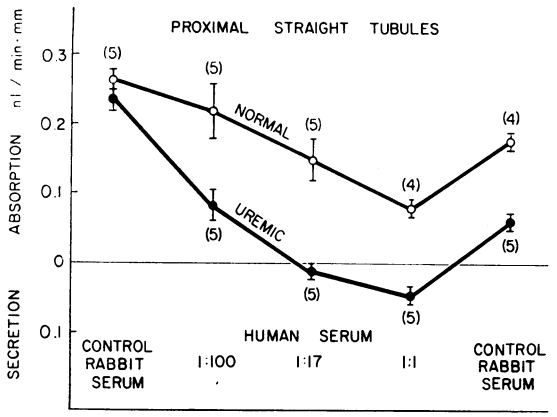

FIGURE 4 Concentration-dependence of inhibitory effect of human sera on net fluid absorption. Normal (open circles) and uremic (solid circles) human sera were diluted with normal rabbit serum. Two uremic sera and three normal sera were studied. Mean values ±1 SE. Number of experiments in parenthesis.

The effect of sera from normal humans was tested in perfused proximal tubules as shown in Fig. 3. Normal human serum caused a significant decrease in the rate of net fluid absorption in all studies, and, with one exception, the inhibitory effect was reversed upon removal of the serum. In none of these studies was net fluid secretion stimulated by normal human serum.

To determine the relative potency of the secretory factor, sera of uremic and normal humans was diluted with normal rabbit serum and placed in the bath of perfused PST as shown in Fig. 4. In these experiments net fluid absorption was strongly depressed by uremic sera that had been diluted 100-fold with rabbit serum, and further, net fluid secretion was observed in three of five studies in which the original uremic sera had been diluted 17-fold with normal rabbit serum. Net fluid secretion was detected in all five sera that had not been diluted with normal rabbit serum. In contrast, normal human serum (from R. I., D. E., and P. Q. in Fig. 7) diluted in the same fashion depressed net fluid absorption when diluted 17-fold; however, net fluid secretion was not observed even with the undiluted human serum. In all studies the effect of human sera was reversible to a significant extent.

Perfused proximal convoluted tubules. An example of the effect of human uremic serum on net fluid transport in a PCT is shown in Fig. 5. Net fluid absorption was rapidly depressed by the undiluted uremic serum but fluid secretion was not observed even after relatively long exposure. Removal of the uremic serum resulted in prompt return of net fluid absorption to the original control value in normal rabbit serum. In three studies, net fluid absorption of PCT was decreased 63% in uremic sera; net secretion was not observed. Sera from normal human volunteers caused a 40% reduction in net fluid absorption in four PCT; net secretion was not observed.

Nonperfused tubules. As a further test of the observation that uremic serum promoted net fluid secretion in perfused tubules, nonperfused PST were mounted between two holding pipettes so as to tightly occlude both ends of the fragment. The tubules were observed in the chamber at approximately 100× through the inverted light microscope. The effect of uremic serum on tubule morphology is shown in Fig. 6. In the presence of normal rabbit serum, no lumen could be distinguished, even at a magnification of 1,000×. Furthermore, electron micrographs of nonperfused proximal tubules in normal rabbit serum show the apical brush borders of opposing cells to be closely apposed (J. Bourdeau, Ph.D., and C. Ganote, M.D., personal communication). 5 min after human uremic serum was placed in the bath a lumen could be clearly discerned. After 10 min the lumen was greatly distended and could be readily seen at magnifications as low as 50×. We never observed lumen expansion in nonperfused PCT in uremic sera. The striking morphological change of nonperfused PST in uremic serum was the basis of a method for assaying a number of uremic and normal sera for secretory activity.

Prevalence of secretory activity in uremic and normal serum. The morphologic assay method was used to examine for secretory activity in the sera of a number of patients with renal failure and in normal volunteers.

We adopted the following procedure in an effort to control for differences in tissue reactivity among rabbits. We have observed that PAH causes net fluid secretion in PST which is similar in many respects to that observed with uremic serum (6). Thus in each experiment in which the secretory activity of human serum was tested, we also determined the extent to which PAH caused lumen expansion (net fluid secretion). In other

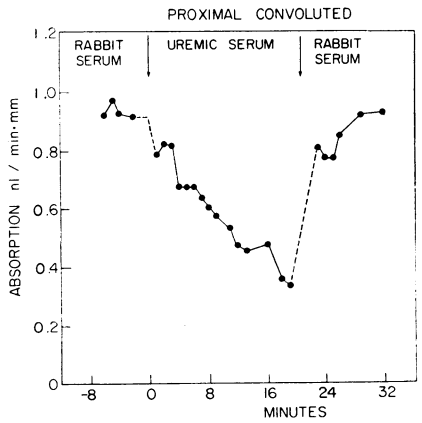

FIGURE 5 Effect of human uremic serum on net fluid transport in a PCT.

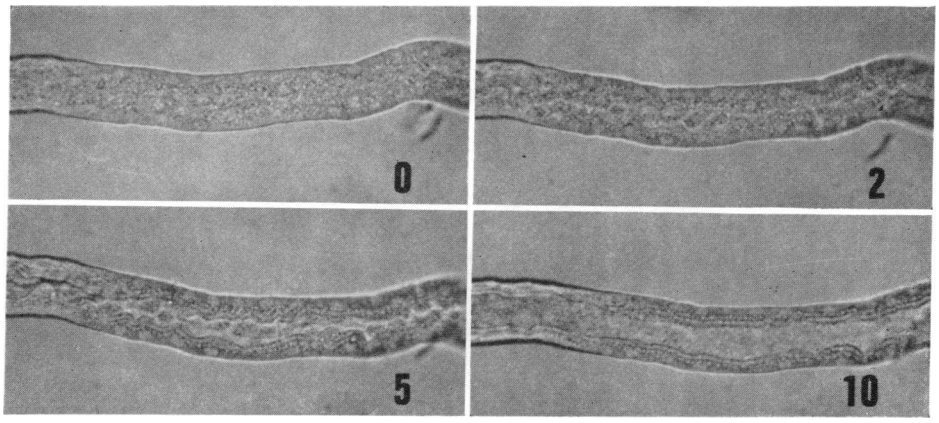

FIGURE 6 Effect of human uremic serum on morphology of a nonperfused straight tubule. The appearance of the tubule is shown in normal rabbit serum (0 time) and 2, 5, and 10 min after uremic serum was added to the bath. The lumen is markedly expanded in uremic serum. Diameter of the collapsed tubule in rabbit serum is 30 μm.

words, a PAH "titer" was determined with each analysis of uremic serum. In the PAH component of the assay the concentration of the organic acid in the initial well was 10^{-3} M. The PAH was diluted serially 1-, 4-, 16-, 64-, and 256-fold to give final concentrations of 0.25, 0.063, 0.015, and 0.004×10^{-3} M. The minimal concentration at which PAH positively caused lumen expansion was taken as the end point. The pattern of reactivity for the uremic serum and PAH was similar, i.e., when a single pool of serum was tested using 27 different rabbit kidneys 89% of the determinations fell within ±1 well dilution of the median titer for the uremic serum and 96% of the determinations fell within ±1 well dilution of the median titer for the PAH in normal rabbit serum.

In addition to providing a measure of internal control in the assays of human serum, it was possible to express the secretory activity of the original human serum in equivalents of a known secretagogue, PAH. Accordingly, in some experiments we have expressed the activity of the human serum in units of PAH activity, where 1 PAH equivalent is taken to be equal to the secretory activity of 1×10^{-6} M PAH. The PAH equivalence of human serum was obtained by multiplying the lowest concentration of PAH to produce lumen expansion times the maximal dilution at which lumen expansion was detected for the human serum. For example, human serum with a secretory activity of 1,000 PAH equivalents may be viewed as containing a concentration of secretagogue equal to the activity of 10^{-3} M PAH in normal rabbit serum.

Secretory activity was detected in the sera of patients with chronic renal failure before and after the institution of chronic hemodialysis, and in patients suffering acute oliguric renal failure (Fig. 7). In 10 patients with chronic renal failure, 39 of 43 (91%) separate determinations revealed secretory titers of 16 or greater whereas in 2 patients with acute renal failure 4 of 12 (33%) determinations revealed maximal titers of 16. In the 6 normal humans, only 2 of 33 (6%) of the determinations had titers as high as 16. It should be noted that the average control PAH "titer" was reasonably constant among the different experiments in which human sera were assayed. There appeared to be no important relationship between the secretory titer and either the sex of the patient or the serum urea nitrogen concentration. Severely uremic patients with acute renal failure appeared to have less secretory activity in their sera than patients with chronic renal failure. Viewed in another context the 10 patients with chronic renal failure had an average secretory activity in their sera equivalent to 1.1×10^{-3} M PAH whereas the activity of sera from normal controls was less than 0.054×10^{-3} M PAH.

The remarkable sensitivity of the morphologic assay of secretory activity can be appreciated by the following calculation. It is possible to clearly identify a patent lumen in a PST if the internal diameter is 10 μm. For the usual incubation period of 15 min, a fluid secretory rate of only 0.005 nl/min·mm is sufficient to cause lumen expansion that can be positively identified.

In addition, it should be noted that uremic serum and PAH did not cause lumen expansion of PST when incubated at 25°C for 15 min, whereas heating to 37°C caused prompt expansion of the lumens.

Effect of hemodialysis on serum secretory activity. To determine the dialysance of the secretory material, we determined the secretory activity of serum taken from the tubing flowing into and out of a standard hemodialysis coil (EX-03, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.) in two patients. Blood flow through

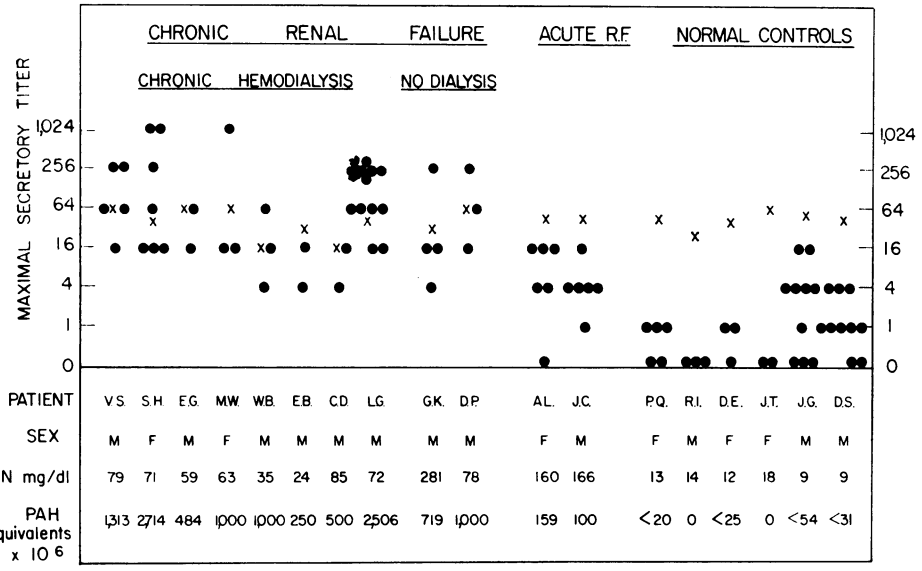

FIGURE 7 Secretory activity of uremic and normal human sera in PST. Each solid circle is the secretory titer of the patient's serum in a group of tubules taken from a single kidney. Different kidneys were used for each assay. A titer of zero means that no lumen expansion was seen in the undiluted serum. The PAH "titer" was determined with each analysis of uremic serum. The average PAH "titer" for each patient is shown by an X, and was derived from the antilog of the average logarithm of the PAH titers.

the coil was 120 ml/min. As shown in Fig. 8 the secretory activity was markedly reduced in the outflow blood indicating rapid dialysance of the secretory factor. Urea was also dialyzed from the same blood, as expected.

Secretory activity of uremic rabbit serum. It was our good fortune that normal rabbit serum contained no secretory activity; thus, normal rabbit serum could be used as diluent for the assay of secretory activity of human serum. To determine if rabbits accumulate secretory activity in the serum as a consequence of renal failure, we ligated the ureters of two animals and determined the secretory activity of the serum in the face of progressive uremia (Fig. 9). In both animals the serum urea nitrogen rose markedly after ureteral ligation. Secretory activity was detected in the serum within 24 h after ureteral ligation and increased over the next 2 days.

Nature of serum secretory factor. The morphologic assay was used to survey a number of substances to de-

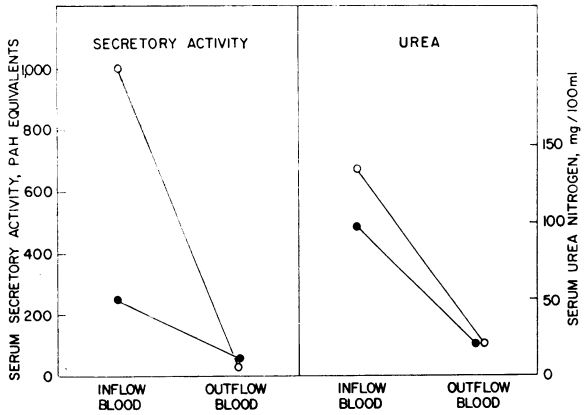

FIGURE 8 Hemodialysis of serum secretory activity in two uremic patients. Each point is the average of at least three determinations of secretory activity. Serum urea nitrogen was also determined in the same samples.

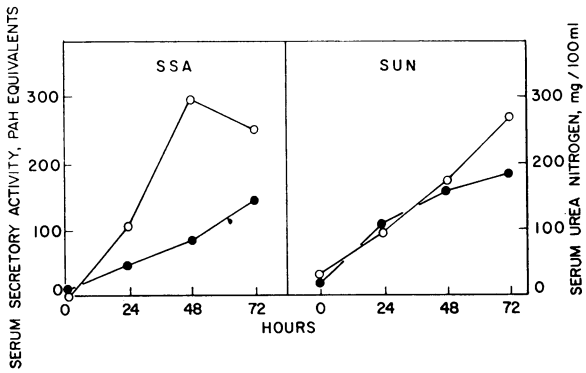

FIGURE 9 Appearance of secretory activity in the serum of uremic rabbits. Each point is the average of at least three determinations of secretory activity. Secretory activity (SSA) and serum urea nitrogen (SUN) were determined 24, 38, and 72 h after bilateral ureteral ligation in two rabbits.

TABLE I

Secretory Activity in PST of Naturally Occurring Organic Substances

Test substance	Initial concentration*	Initial PAH concentration*	Number of experiments	Secretory titer‡	
				Test substance	PAH
	mM	*mM*			
Urea	33	1	(3)	No activity	16
Creatinine	3	1	(3)	No activity	25
Urate	1	1	(3)	No activity	25
Lactate	1	1	(2)	No activity	32
Acetate	1	1	(2)	No activity	16
Benzoate	1	1	(5)	21	28
Guanidinosuccinate	1	1	(4)	No activity	32

* In each experiment two series of titers were done using PST from the same kidney. In one of the series of tubules the test substance was added to the initial well. In the other series PAH was added to the initial well. The material in each initial well was serially diluted with normal rabbit serum to a maximum of 256-fold.

‡ The end point was the maximal dilution at which lumen expansion was positively observed. "No activity" means that lumen expansion was not observed in any of the wells.

termine their capacity either to stimulate or inhibit fluid secretion in PST. Urea, creatinine, urate, lactate, acetate, and guanidinosuccinate did not cause lumen expansion over a wide range of concentrations (Table I). In contrast, benzoate, a precursor of hippurate, was as effective as PAH in causing lumen expansion.

Both ouabain and probenecid completely inhibited the secretory effect of human uremic serum (Table II).

In addition we found that heating uremic serum to 57°C for 30 min or to 90°C for 10 min had no effect on the secretory activity. The secretory activity of uremic sera was stable when stored at −10°C. Dialysis of uremic serum in a cellulose acetate membrane for 24 h in a Ringer's bath removed more than 95% of the secretory activity. Secretory activity was present in the ultrafiltrate of uremic serum after passage through an Amicon (Amicon Corp., Lexington, Mass.) PM-05 membrane (> 500 mol wt excluded). Finally, addition of PAH to uremic serum resulted in a secretory titer slightly greater than that of the PAH alone, indicating that uremic serum does not inhibit the secretory effect of PAH.

DISCUSSION

In the present studies we add to the already long list of metabolic derangements known to accompany renal failure. It was observed that a substance or group of substances in the serum of patients with acute and chronic renal failure promotes the secretion of fluid, and presumably solute, into the lumens of PST isolated from rabbit kidneys in vitro. The secretory material may be a normal derivative of metabolism as suggested by the finding that some normal humans have secretory activity in their sera, albeit at an apparent concentration that is much lower than is found in the sera of persons with acute and chronic renal failure. The secretory activity of uremic sera is heat stable, is dialyzed readily through

TABLE II

Inhibitory Effect of Probenecid and Ouabain on Fluid Secretion of PST

Inhibitor	Secretory titer*			
	Inhibitor only	PAH control (10^{-3} M)	Uremic serum	Uremic serum and inhibitor‡
Probenecid (4)§ (2.5×10^{-4} M)	0	32	16	0
Ouabain (4) (5×10^{-5} M)	0	64	32	0

* See Table I for explanation.

‡ The concentration of inhibitor was constant and the concentration of PAH and uremic serum was varied.

§ Number of experiments.

cellulose acetate or cuprophane membranes, and is ultrafiltered through a membrane that restricts the passage of molecules of a molecular weight greater than 500, findings that may be regarded as circumstantial evidence to support the possibility that the material is a water soluble substance of relatively low molecular weight. Known substances accumulated to relatively high levels in renal failure, such as urea, creatinine, and uric acid, do not cause fluid secretion in PST, and therefore may be excluded as possible contributors to the secretory effect of uremic serum in vitro. It is also apparent that the secretory phenomenon is not the consequence of a subtle interaction of factors such as hyperosmolality and acidosis, for the uremic sera was found to cause fluid secretion after extensive dilution with normal rabbit serum. Three observations support the possibility that the secretory material may be an organic acid. First, probenecid, an inhibitor of organic acid transport, strongly inhibited the secretory activity of uremic serum. Secondly, PAH and benzoic acid, a precursor of hippurate, potently stimulated net fluid secretion in PST. Thirdly, the secretory effect of uremic sera or PAH was found only in the proximal straight tubule, a segment that has been shown recently to transport PAH to a much greater extent than the convoluted portion of the proximal tubule (7). Additionally, there is evidence that organic acids, specifically of the hippurate variety, are increased in the sera of patients with renal failure (8–11).

The possible physiologic consequences of the present observations must be interpreted in the light of our method to identify and quantify the secretory activity of serum. We have shown previously (4), and in the present report that isolated tubules perfused in vitro with one end of the tubule obstructed absorb fluid at a reasonably constant rate for relatively long periods of time in a bath of normal rabbit serum. In our hands, PST in rabbit serum normally absorb fluid at a rate of about 0.2–0.4 nl/min·mm (4). Rabbits evidently possess a powerful mechanism to transport the secretory material in the PST, and yet the sera from normal animals must contain negligible quantities of material. In the present studies, human uremic serum caused the PST to secrete fluid at a rate of approximately 0.05 nl/min·mm, and while this rate of secretion may at first glance seem inconsequential, it represents a considerable reduction in the *absorptive capacity* of this segment of the nephron. In other words, by totally inhibiting fluid absorption in the PST together with the effect to diminish reabsorption in the PCT, the secretory material could have a considerable diuretic effect in the intact animal. It must be recalled, however, that the present studies were performed in obstructed nephrons only, and we do not as yet have quantitative data which bears on the effect of uremic serum or PAH in tubular segments at "normal" rates of perfusion.

We have only a rudimentary understanding of the mechanism of net fluid secretion induced by uremic sera. It seems clear that net fluid secretion is "uphill" by virtue of the fact that fluid steadily accumulates in the lumen against an imposed hydrostatic pressure of 10 cm H_2O in the perfusion pipette. The pronounced effect of temperature on the rate of fluid secretion suggests that metabolism is importantly coupled to the secretory process. Ouabain, an inhibitor of net fluid absorption and sodium transport in proximal tubules (4, 12), strongly inhibits the secretory effect of uremic serum. As to whether ouabain interferes specifically with sodium transport in the secretory direction, or produces its effect as a consequence of a secondary change in the intracellular environment of the proximal tubule remains to be determined. We cannot exclude the possibility that uremic serum inhibits an absorptive salt pump, leaving an occult secretory pump unopposed, as has been postulated for the effect of cholera toxin in the gut (13). However, the similarity of the effects of probenecid and ouabain on the secretory activity of uremic serum, together with the physiochemical characteristics of the secretory factor, is more supportive of the view that fluid secretion in uremic serum is probably coupled to the transport of an organic molecule, in a manner similar to that observed for PAH (6).

There is abundant evidence to indicate that uremic serum inhibits organic acid transport in kidney slices and suspensions (11, 14–17). In most of the experiments it was observed that uremic serum inhibited the uptake of PAH into the cortical cells. Of importance in this regard is the fact that normal human serum also inhibited PAH uptake in slices but to a lesser extent than uremic serum (17). It seems plausible to consider that the substances responsible for the inhibitory effect of human sera on PAH uptake are the same as the secretory material encountered in the present studies. We may consider, however, that uremic serum, rather than depressing cellular transport of kidney cells in vitro, contributes a substrate which, like PAH, is taken up into the cells. In the present studies we found that addition of uremic serum did not depress the secretory activity of PAH. Thus, in the final analysis, the total uptake of organic acid in the presence of uremic serum, if indeed the secretory factor is an organic acid, may be normal or even increased rather than decreased.

Operating on the assumption that the secretory material resembles a hippurate, we have estimated the apparent concentration of the secretory material in uremic and normal human serum. If the secretory material is a

hippurate with transport characteristics similar to PAH, it is estimated that the concentration in serum from normal humans may be as high as 0.05 mM/liter and in dialyzed uremic patients as high as 2.7 mM/liter. It is conceivable that the material in question accounts for some of the nonmetabolizable organic anion contributing to the well-known "anion-gap" in patients with severe uremia and acidosis (8, 18, 19).

It is interesting to speculate further on the significance of the secretory material considering the possibility that it is an organic acid of the hippurate class. Normal man excretes from about 0.5 (20) to 5 g (21) of hippurate daily. The potential impact of the secretion of this much material into the proximal tubule of a patient with a reduced number of functioning nephrons is considerable. Were the material to behave as a classical impermeant anion in the distal portion of the nephron, it is possible that it might contribute to the "adaptive" increase in potassium and sodium excretion that is so clearly a part of progressive renal insufficiency (22). Indeed, Selkurt and Shade (23) have shown that PAH infused into normal monkeys to achieve plasma levels commonly attained in studies of renal PAH excretion for the measurement of blood flow, and measurement of the maximal rate of PAH transport, caused a marked kaliuresis. Though not emphasized by the investigators, there was a substantial diuresis and natriuresis as well. We suggest, therefore, that the secretory material in patients with progressive renal insufficiency may contribute to the adaptive adjustments for excretion of sodium, potassium, and water. In the light of present evidence, however, this material cannot be viewed as the only factor responsible for the adaptation in the renal excretion of sodium in chronic renal failure. It has been reported that an acute change in sodium intake, all other factors being constant, results in an "appropriate" change in sodium excretion even in severe uremia (1, 2). Consequently, the secretory material must be regarded presently as having only a possible permissive role in adaptive changes in sodium excretion.

Finally, it seems reasonable to speculate as to the relation of the present observations to other aspects of nephron function. The effects of the secretory material may be important in conditions in which the flow of urine through nephrons is impeded. For example, it is well known that unilateral ureteral obstruction results in a kidney with nephrons of smaller lumen diameter and lower intratubular hydrostatic pressure, than when the obstruction is bilateral (24–26). Since the secretory factor would be excreted adequately in the case of unilateral obstruction, it is interesting to consider that the marked lumenal distension of bilateral ureteral obstruction may in part be a consequence of the accumulation of secretory material within the lumens.

ACKNOWLEDGMENTS

We wish to thank Alice Dworzack for her help in preparing the manuscript.

This work was supported by U. S. Public Health Research Grant AM 13476 and Career Development Research Award AM 22760.

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