Water, Acidosis, and Experimental Pyelonephritis

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ABSTRACT The effect of water restriction and ammonium chloride acidosis on the course of *Escherichia coli* pyelonephritis was determined in the nonobstructed kidney of the rat. To alter the chemical composition of the renal medulla, water intake was reduced in rats to one-half the normal daily intake. Water restriction increased the incidence of coliform pyelonephritis. Systemic acidosis, produced by giving a 300 mM solution of ammonium chloride, increased urinary osmolality to values comparable to water restriction and also predisposed to pyelonephritis. However, when rats were fed the same solution of ammonium chloride but were allowed access to tap water ad lib., urinary osmolality values were comparable to those observed in normal animals, and susceptibility to pyelonephritis was reduced or eliminated despite a degree of systemic acidosis similar to that observed in rats fed ammonium chloride solution without access to tap water. These results suggest that water diuresis may overcome the inactivation of complement produced by ammonium chloride acidosis and that renal medullary hypertonicity, produced by either water restriction or ammonium chloride acidosis, is a major determinant of this tissue's unique susceptibility to infection.

INTRODUCTION

Earlier studies concerned with the pathogenesis of pyelonephritis have demonstrated that the medulla of the kidney is much more susceptible to infection than the cortex (1–5). Various studies have suggested that the vulnerability of the renal medulla may result from its anatomical location (6), its relatively poor circulation (7, 8), its high concentration of ammonia (9), and its habitual hypertonicity (5, 10). Two factors known to predispose the renal medulla to infection are acidosis and dehydration. Acidosis is thought to result in complement inactivation in the kidney (9), and its effect has been demonstrated on experimental *Escherichia coli* pyelonephritis (11). The effect of medullary hypertonicity on infection, however, has thus far been demonstrated only on experimental staphylococcal, candida, and enterococcal pyelonephritis (5, 10).

The present experiments therefore were designed to study the effect of hydration and medullary tonicity on *Escherichia coli* pyelonephritis and to discern, if possible, the relative importance of dehydration and acidosis in predisposing to renal infection. The results of these studies describe a simple method for inducing coliform pyelonephritis in the nonobstructed kidney of the rat which provides a model for studying many aspects of experimental pyelonephritis. Coliform pyelonephritis can be produced in rats by increasing renal medullary tonicity by means of a decrease in water intake. Acidosis produced by ammonium chloride also increased renal medullary tonicity, and increased the susceptibility of the rat kidney to coliform pyelonephritis but only when water intake was also restricted. These results suggest that water diuresis may overcome the deleterious effect of ammonium chloride acidosis on complement activity and that the increase in renal medullary tonicity produced by ammonium chloride acidosis is the major factor responsible for the increased susceptibility of the medulla to infection.

METHODS

Animals. White female Sprague-Dawley CFE strain rats (Carworth Farms, New City, N. Y.), weighing 125–200 g were used. Animals were weighed on the first day, on the day of challenge, and the last day of each experiment, and were housed individually in metabolic cages which permitted the collection of urine without contamination by feces. Urine was collected under mineral oil with thymol used as a preservative. The diet consisted of Purina lab chow pellets.

Bacteria. The strain of *E. coli* (ECY 9) used in the present experiments is nontypable when tested against 17 serotypes and the details of its handling have been described previously (12). A volume of 0.5 ml of a 4-hr culture (containing $2–3 \times 10^8$ organisms per ml) was injected into the lateral tail vein of each rat. Tenfold dilutions in 0.85%

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sodium chloride solution were incubated in agar pour plates to enumerate each inoculum.

Microbiologic and histologic studies. Animals were killed 8 days after intravenous bacterial challenge. Sterile technique was maintained during all surgical procedures. Under pentobarbital anesthesia, the abdomen was cleansed with 70% alcohol and incised so that the entire urinary tract and the abdominal aorta were exposed. The animals were killed by aortic exanguination and the blood obtained was collected under oil in heparinized syringes. After urine was collected in some animals, the kidneys were removed and placed in Petri dishes for macroscopic examination and sectioning. A midline longitudinal section was made in some kidneys. Half the kidney was fixed in 10% formalin, serially sectioned, and every tenth section was mounted and stained with hematoxylin and eosin for histologic examination. The remaining half was homogenized in 4.5 ml of sterile saline. Whole kidneys were cultured after homogenization with 9.0 ml of sterile saline. These concentrations represented a 10^{-1} dilution. Subsequent tenfold dilutions were prepared in sterile saline. Agar pour plates were made from these dilutions, and colony counts were determined after incubation for 48 hr at 37°C as previously described (5). The identification of *E. coli* was confirmed by subculturing some colonies on desoxycholate, Kliger's iron, and Simmons citrate agar (13).

Urine culture. Immediately after opening the abdominal cavity, approximately 0.5 or 1.0 ml of urine was aspirated through the bladder wall by means of a sterile needle and syringe. One loopful of urine was streaked on blood agar. In some animals 10^{-1} and 10^{-3} dilutions of urine were made in nutrient broth, and pour plates were made for final bacteriologic counts. In addition, the bladder mucosa of most animals was swabbed with a sterile cotton applicator which was then streaked on blood agar base (10).

Criteria of infection. Kidneys were considered infected when they contained 5×10^4 or more colonies (12). Urine cultures were considered positive when 10 or more colonies of *E. coli* per milliliter of urine were recovered from quantitative cultures, or 10 or more colonies of *E. coli* were recovered from the bladder swab.

Chemical studies. All determinations were performed in duplicate. Sodium and potassium in serum were determined with an internal standard flame photometer, blood urea nitrogen and urine ammonia by the Conway microdiffusion method, serum chloride by amperometric titration, serum CO_2 content by the method of Van Slyke and Neill, and urine titratable acidity by a modification of the method of Peters and Van Slyke (14). Occasionally it was necessary to discard urine samples because of obvious fecal contamination which caused elevation of the urinary pH above 8.

Statistics. The mean, standard deviation, and standard error of the mean was calculated for the daily fluid intake, urine osmolality, serum bicarbonate, chloride, sodium, potassium, blood urea nitrogen, urine ammonia, and titratable acid in each group. Probability values and statistical significance were determined by the *t* test. Statistical significance of infectivity rates was obtained by determining the values of chi-square employing the Yates correction factor (15).

Experiment I. Effect of water restriction on the susceptibility of the kidney to E. coli pyelonephritis. 68 rats were given 15 ml of tap water by stomach tube daily throughout the experiment. The tube feedings were divided into two equal doses of 7.5 ml each given in the morning and afternoon. 38 animals (*group I, controls*) were allowed access to tap water ad lib. No additional tap water was given to the remaining 30 rats (*group II, water restricted*). On the third day, all animals were challenged intravenously with *E. coli.* 8 days after bacterial challenge the animals were killed, the kidneys and urine were cultured, and aortic blood was obtained for blood urea nitrogen and electrolyte determinations. During the experiment, daily intake, urine output, and urine osmolality values were measured. Urine samples were analyzed for osmolality with an Advanced osmometer (model No. 31-LAS). Furthermore, to determine the effect of water restriction on uninoculated control animals, six rats were given 7.5 ml of tap water by stomach tube in the morning and again in the afternoon each day throughout the experiment. They received no additional fluid intake nor were they challenged with *E. coli.* The animals were killed on the 10th day of the experiment and the kidneys were removed for microbiologic and histologic studies.

Experiment II. Effect of ammonium chloride on urinary osmolality. 12 rats were given tap water ad lib for 4 days. The tap water was removed on the fifth day and the animals were allowed to drink a 300 mM solution of ammonium chloride ad lib for 7 days. On the 12th day the ammonium chloride was removed and the rats were again given tap water al lib. Fluid intake, urine output, and urine osmolalities were determined daily for each animal.

Experiment III. Effect of water intake on E. coli pyelonephritis induced by ammonium chloride. 58 rats were given 10 ml of a 300 mM solution of ammonium chloride by stomach tube daily, that is, each rat received 3 mmoles of ammonium chloride per day. The tube feedings were divided into two equal doses of 5.0 ml each given in the morning and afternoon. 37 animals received no additional fluid intake (*group III, 3 mM NH_4Cl, no water*). However, the remaining 21 rats were allowed daily access to tap water ad lib. (*group IV, 3 mM NH_4Cl + water*). After 2 days of ammonium chloride administration all animals were challenged intravenously with *E. coli.* 8 days after bacterial challenge the animals were killed, the kidneys and urine were cultured, and aortic blood was obtained for urea nitrogen and electrolyte determinations. During the experiment, daily intake, urine output, and urine osmolality values were measured.

An additional 22 rats were given 15 ml of a 300 mM solution of ammonium chloride by stomach tube daily, administered in two equal doses of 7.5 ml each in the morning and afternoon, and were allowed access to tap water ad lib. (*group V, 4.5 mM NH_4Cl + water*). 16 additional rats were given 20 ml of a 300 mM solution of ammonium chloride by stomach tube, administered in three doses of 7.5, 5.0, and 7.5 ml daily, and were allowed access to tap water ad lib. (*group VI, 6 mM NH_4Cl + water*). The microbiologic and chemical determinations described earlier were obtained in some animals in both groups (V and VI) 8 days after intravenous challenge with *E. coli.*

Experiment IV. Effect of water intake on renal capacity to excrete acid. 24 rats were given 20 ml of a 300 mM solution of ammonium chloride daily by stomach tube for 8 consecutive days. 16 of these were allowed access to tap water ad lib. (*group VI, 6 mM NH_4Cl + water*). However, the remaining animals received no additional fluid intake (*group VII, 6 mM NH_4Cl, no water*). Urine was collected under oil and analyzed for NH_4 and titratable acid during the last 2 days of the experiment.

RESULTS

Effect of water restriction on the susceptibility of the kidney to E. coli pyelonephritis. The mean daily osmo-

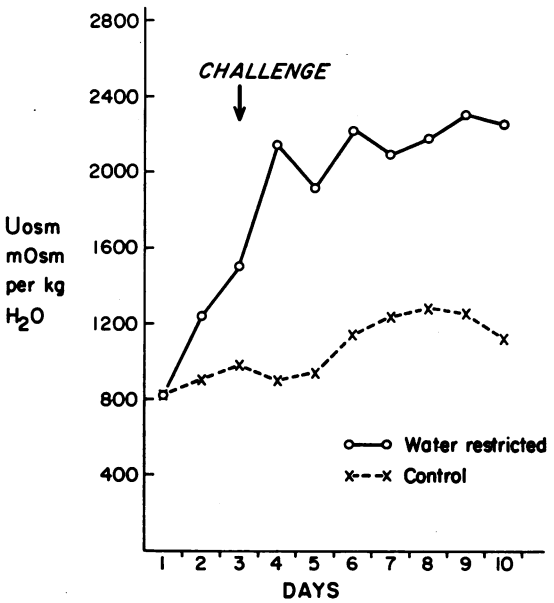

FIGURE 1 Mean daily levels of urinary osmolality (Uosm) of 38 control and 30 water-restricted rats. The arrow marked *challenge* indicates the time of intravenous coliform inoculation.

lality of the urines of 38 control (group I, tube fed 15 ml of water daily + water ad lib.) and 30 water-restricted (group II, tube fed 15 ml of water daily, no water ad lib.) rats are shown in Fig. 1. The first day's values are base line observations and closely approximate values obtained in previous studies (5, 8). These values remained relatively constant in control (group I) animals throughout the study. In contrast, the osmolality of the urine almost doubled in water-restricted rats (group II) tube fed only 15 ml of tap water daily. On the day of bacterial challenge the urinary osmolality observed in control rats averaged about 1000 mOsm/kg of water as compared with urinary osmolality values of over 2000 mOsm/kg of water in water-restricted animals. When the rats were killed 8 days after bacterial challenge, pyelonephritis was observed in 19 of 30 water-restricted (group II) animals and in only 5 of 38 control (group I) rats (Table I). Gross abscesses were seen in 35% of the infected kidneys of water-restricted rats and in only one control kidney. Urine cultures were obtained in 35 control and 27 water-restricted animals at the time the rats were killed. *E. coli* bacteriuria was observed in the five control animals with *E. coli* pyelonephritis and in one control animal without evidence of renal infection. Urine cultures were sterile in the remaining 29 control animals who had no evidence of renal infection. Similarly, coliform bacteriuria was observed in 16 of 17 water-restricted rats with *E. coli* pyelonephritis and from whom urine could be obtained for culture. Urine cultures were sterile in 9 of 10 water restricted rats studied who had no evidence of renal infection.

TABLE I

Effect of Water Restriction on E. coli Pyelonephritis

Group	Kidneys*	Rats*	Urine culture
I. Control	6/76	5/38	6/35
P	<0.005	<0.005	<0.005
II. Water restricted	29/60	19/30	17/27

* Number infected per number studied.

The water intake of control (group I) animals averaged 29 ml/day throughout the study period as compared with 15 ml/day in water-restricted rats (group II). Urinary osmolality values for all control (group I) animals throughout the study averaged 1019 mOsm/kg of water as compared with a mean of 2124 mOsm/kg of water in those animals (group II) tube fed only 15 ml

TABLE II

*Effect of Water Restriction on Chemical Composition of Blood and Urine**

Group	Water intake	Uosm	Serum CO_2	Serum Cl	Serum Na	Serum K	BUN
	ml	*mOsm/kg H_2O*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mg/100 ml*
I. Control	29	1019	21.7	99	142	5.0	20
	±5	±254	±2.5	±6.6	±2	±0.83	±2
	n = 380	n = 193	n = 25	n = 19	n = 8	n = 8	n = 7
II. Water restricted	15	2124	19.9	104	148	4.5	25
		±494	±3.4	±6.7	±8	±0.63	±4.6
	n = 300	n = 169	n = 21	n = 23	n = 15	n = 15	n = 17
P	<0.001	<0.001	NS	NS	NS	NS	NS

NS = not significant (P = >0.01).
Uosm = urinary osmolality.
* Values are mean ±SD.

of water/day. Serum sodium, potassium bicarbonate, chloride, and blood urea nitrogen concentrations in water-restricted (group II) rats were not significantly different from those observed in control (group I) animals (Table II). Although infected rats in both groups lost an average of 12% of their original body weight by the end of the experimental period, there was no weight loss at the time of bacterial challenge. Weight changes in noninfected animals ranged from a 5% loss to a 7% gain. These weight changes are not sufficient to make the rat kidney susceptible to infection with this strain of *E. coli* (11).

To determine the effect of water restriction on uninoculated controls, six rats were given 15 ml of water by stomach tube daily. They received no water ad lib. nor were they challenged with *E. coli*. When they were killed 10 days later, their kidneys were normal on gross and microscopic examination and sterile on culture.

Half of each kidney from 12 control and 12 water-restricted rats was studied histologically. The remaining half was quantitatively cultured. The results observed by quantitative culture were then compared to gross and microscopic observations for each kidney. Histologically three distinct patterns were observed. *Normal* architecture was seen in those kidneys in which gross abscesses were absent and from which less than 5×10^4 colonies of *E. coli* were recovered on culture. *Medullary abscesses* were seen with fairly large areas of inflam-

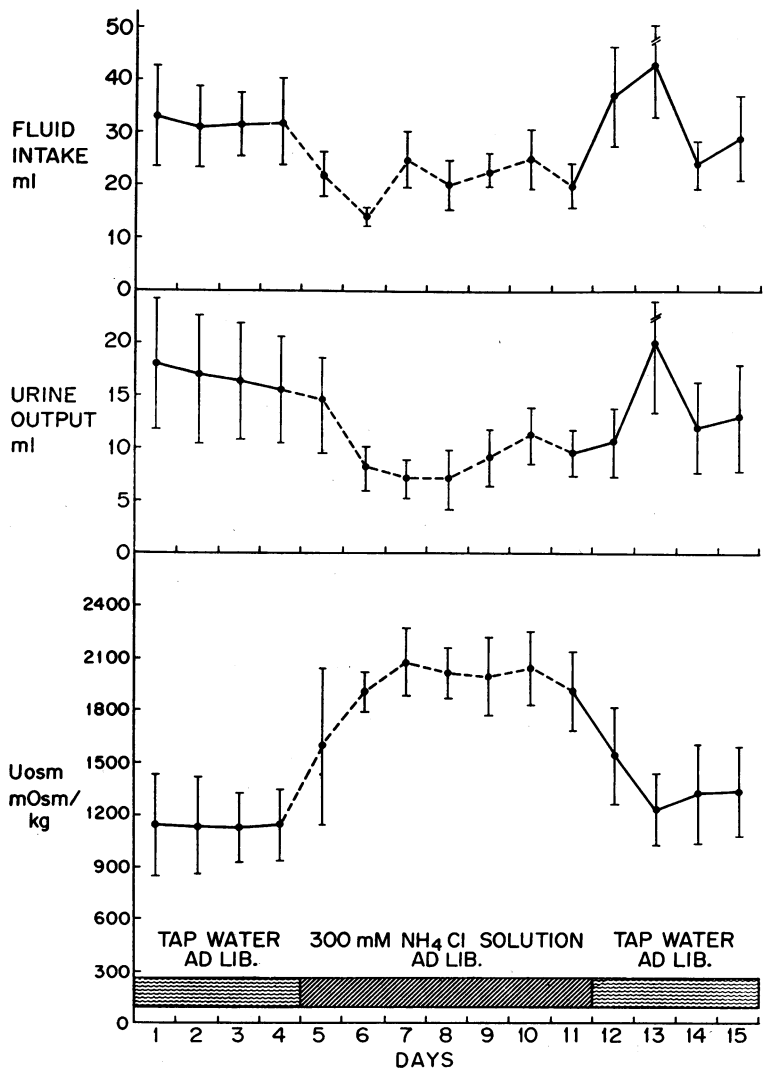

FIGURE 2 Mean daily values of fluid intake, urinary output, and urinary osmolality (Uosm) in 12 rats drinking tap water for 4 days, followed by ammonium chloride solution for 7 days, and then tap water for 4 days.

mation in the renal medulla, with the apex of the inflammatory reaction either in or pointing toward the papilla. This lesion was characterized by dense polymorphonuclear leukocytic exudates within and outside of the tubular lumen, tubular destruction, and collections of amorphous eosinophilic material in the lumen of some tubules. In some, an inflammatory exudate was seen in the mucosa of the renal pelvis. This lesion was present in those kidneys in which gross abscesses were absent but from which more than 5×10^4 colonies of *E. coli* were recovered on culture. *Wedge-shaped abscesses* were seen in those kidneys in which gross abscesses were observed and from which more than 5×10^4 colonies of *E. coli* were recovered on culture. These lesions were characterized by a wedge-shaped area of inflammation with apex in the medulla and base in the cortex. Collections of polymorphonuclear leukocytes were observed within and outside of the tubules, in the cortex, and on occasion, surrounding tubules containing colloid casts. The glomeruli were normal. Only two exceptions were noted. One kidney contained more than 5×10^4 colonies of *E. coli on* culture but was normal on histologic examination. Also, one kidney was grossly abscessed, containing more than 5×10^4 colonies of *E. coli* on culture, but had medullary abscesses without cortical involvement on microscopic examination; it seems possible that the grossly abscessed portion of the cortex was homogenized and cultured.

Effect of ammonium chloride on urinary osmolality. The mean daily values of fluid intake, urinary output, and urinary osmolality in 12 rats given tap water ad lib. for 4 days, followed by ammonium chloride (300 mmoles/liter) ad lib. for 7 days, and then tap water ad lib. for 4 days, are shown in Fig. 2. Mean urinary osmolality values ranged between 1150 and 1450 mOsm/kg of water during the initial 4 days of study. Subsequently, however, mean urinary osmolality values rose above 2000 mOsm/kg of water after 2 days of ammonium chloride ingestion and remained in that range until the animals were given tap water to drink. Within 2 days after ammonium chloride was discontinued, urinary osmolality values fell to pre-ammonium chloride levels. Volume intake during ammonium chloride ingestion fell approximately 35% when the rats were drinking the ammonium chloride solution but still averaged 20 ml/day.

Effect of water intake on E. coli pyelonephritis induced by ammonium chloride. 37 rats were tube fed 10 ml of a 300 mM solution of ammonium chloride daily (group III, 3 mM NH_4Cl, no water). These animals received no additional fluid intake. Another group of 21 rats were also tube fed 10 ml of the same ammonium chloride solution daily but were allowed access to tap water ad lib. each day (group IV, 3 mM NH_4Cl + water). The mean urinary osmolality of group III rats was 2100 mOsm/kg of water on the day of bacterial challenge as compared with a mean urinary osmolality of 1350 mOsm/kg of water in rats given ammonium chloride plus water (group IV). When the rats were killed 8 days after bacterial challenge, pyelonephritis was observed in 29 of 37 ammonium chloride (group III) animals and in only 7 of 21 rats (group IV) given ammonium chloride plus water (Table III). Gross abscesses, seen in one-third of the infected kidneys of group III rats, were absent in the kidneys of infected rats given ammonium chloride plus water. Urine cultures were obtained in 36 animals in group III and 15 animals in group IV at the time the rats were killed. Bacteriuria was observed in 27 animals with pyelonephritis which were given ammonium chloride to drink without added water (group III) and in one animal who had no evidence of pyelonephritis. One animal in the same group who had evidence of pyelonephritis had sterile urine as did seven animals in this group which had no evidence of pyelonephritis. Similarly, bacteriuria was observed in five of the rats studied which were given ammonium chloride plus water to drink (group IV) and which had evidence of renal infection. Urine cultures were sterile in 10 animals in group IV which had no evidence of renal infection.

The fluid intake (ammonium chloride + water) averaged 29 ml/day for animals in group IV as compared with 10 ml/day for animals (group III) tube fed ammonium chloride only (Table IV). Furthermore, all

TABLE III

Effect of Water Intake on E. coli Pyelonephritis Induced by Ammonium Chloride

Group	Kidneys*	*P*	Rats*	*P*	Urine culture*	*P*
III 3 mM NH_4Cl, no water	47/74		29/37		28/36	
IV 3 mM NH_4Cl, +water	10/42	<0.005	7/21	<0.005	5/15	<0.01
V 4.5 mM NH_4Cl, +water	0/10	<0.005	0/5	<0.005	NT	
VI 6.0 mM NH_4Cl, +water	3/32	<0.005	2/16	<0.005	0/11	<0.005

NT = not tested.

* Number infected per total number studied.

determinations of urinary osmolality averaged 1544 and 2501 mOsm/kg of water for animals in group IV and III respectively. Except for serum chloride, concentrations of serum sodium, potassium, bicarbonate, and blood urea nitrogen were not significantly different in the two groups of animals studied (Table IV).

Animals tube fed 3 mmoles of ammonium chloride per day lost an average of 10.2% of their original body weight by the end of the experimental period. However, there was no weight loss at the time of bacterial challenge. Furthermore, these changes in body weight are are not sufficient to make the rat kidney susceptible to infection with this strain of *E. coli* (11).

Half of each kidney from twelve rats tube fed 3 mmoles of ammonium chloride was studied histologically and compared with the remaining half which was quantitatively cultured. These observations were similar to the comparison described above. With one exception, the kidneys were normal when less than 5×10^4 colonies of *E. coli* were recovered on culture, or contained abscesses in the medulla, or medulla and cortex when more than 5×10^4 colonies of *E. coli* were recovered on culture.

During this experiment two additional groups of rats were tube fed 4.5 (group V) and 6 (group VI) mmoles of ammonium chloride daily equivalent to 1½ and 2 times the daily millimoles of ammonium chloride given to rats in groups III and IV. All animals in both groups (V and VI) were allowed access to tap water ad lib. None of five animals given 4.5 mmoles of ammonium chloride plus water (group V) each day had pyelonephritis 8 days after bacterial challenge. Similarly, renal infection was observed in only 2 of 16 rats given 6 mmoles of ammonium chloride plus water (group VI) each day and urine cultures, obtained in 11 animals in this group who also had no evidence of pyelonephritis, were sterile (Table III). Forcing fluids was therefore effective in preventing the susceptibility to pyelonephritis induced by ammonium chloride.

Daily fluid intake averaged 33 and 36 ml and urinary osmolality values averaged 1237 and 1220 mOsm/kg of water for animals in group V and VI respectively (Table IV), and were significantly different from the daily fluid intake and urinary osmolality observed in group III rats. In contrast, serum electrolytes and blood urea nitrogen values for animals in both groups (V and VI) were not significantly different from similar determinations in group III animals fed ammonium chloride with no additional water.

Effect of water intake on renal capacity to excrete acid (Table V). Total excretion of hydrogen ion as urinary titratable acid and ammonium was not significantly different in animals given 6.0 mmoles of ammonium chloride daily without additional water (group VII) as compared with rats fed 6.0 mmoles of ammonium chloride daily and allowed access to tap water ad lib. (group VI). Fluid intake averaged 36 and 20 ml and urinary osmolality 1220 and 1816 mOsm/kg

TABLE IV

*Effect of Water and Ammonium Chloride on Chemical Composition of Blood and Urine**

Group	Intake	Uosm	Serum CO_2	Serum Cl	Serum Na	Serum K	BUN
	ml	*mOsm/kg H_2O*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mg/100 ml*
III 3 mM NH_4Cl, no water	10	2501	14.15	113	147	4.9	31
		±522	±4.6	±12.8	±5	±0.7	±10.9
	$n = 370$	$n = 127$	$n = 24$	$n = 21$	$n = 22$	$n = 22$	$n = 21$
IV 3 mM NH_4Cl, +water	29	1533	13.96	100	144	4.7	27
	±3.6	±235	±3.5	±7.2	±4	±0.6	±11.6
	$n = 210$	$n = 171$	$n = 11$	$n = 16$	$n = 17$	$n = 17$	$n = 17$
P	<0.001	<0.001	NS	<0.005	NS	NS	NS
V 4.5 mM NH_4Cl, +water	33	1237	14.7	104	141	4.1	26
	±3.8	±145	±3.9	±7.9	±6	±0.4	±6
	$n = 220$	$n = 147$	$n = 20$	$n = 19$	$n = 4$	$n = 4$	$n = 5$
P	<0.001	<0.001	NS	NS	NS	NS	NS
VI 6.0 mM NH_4Cl, +water	36	1220	15.1	108	153	4.1	24
	±4.7	±207	±2.5	±14.8	±7	±0.5	±6.2
	$n = 160$	$n = 148$	$n = 13$	$n = 21$	$n = 4$	$n = 4$	$n = 5$
P	<0.001	<0.001	NS	NS	NS	NS	NS

NS = not significant ($P = >0.01$).

* Values are mean ±SD.

TABLE V

*Effect of Water Intake on Renal Capacity to Excrete Acid**

Group	Intake	Uosm	U_{TA}	$U_{NH_4^+}$	$U_{TA}+U_{NH_4^+}$
	ml	*mOsm/kg H_2O*	*mEq/24 hr*	*mEq/24 hr*	*mEq/24 hr*
VI 6.0 mM NH_4Cl, +water	36	1220	0.493	4.084	4.577
	±4.7	±207	±0.241	±0.885	±0.999
	$n = 128$	$n = 148$	$n = 32$	$n = 32$	$n = 32$
VII 6.0 mM NH_4Cl, no water	20	1816	0.405	4.278	4.569
		±371	±0.141	±1.206	±1.088
	$n = 64$	$n = 64$	$n = 10$	$n = 16$	$n = 10$
P	<0.001	<0.001	NS	NS	NS

NS = not significant ($P > 0.01$); U_{TA} = urinary titratable acidity; $U_{NH_4^+}$ = urinary ammonium.
* Values are mean ±SD.

of water daily for rats in groups VI and VII respectively. The increased volume intake did not appear to result in an increased ability of the kidney to excrete an acid load.

DISCUSSION

These studies demonstrate that *Escherichia coli* pyelonephritis can be induced in the nonobstructed, nonmanipulated normal kidney of the rat simply by increasing the osmolality of medullary tissue through a decrease in daily water intake. When water intake is decreased by one-half, urine osmolality doubles, and medullary susceptibility to infection is significantly increased. Previous data from this laboratory (5), together with much more accumulated by others (16–21), indicates that the blood and interstitial fluids of the medulla are hypertonic when the urine is concentrated, but approach isotonicity with a moderate water diuresis (10).

The present studies also demonstrate that the osmolality of medullary tissue, as reflected in the osmolality of the urine, is increased considerably by allowing animals to drink a 300 mM solution of ammonium chloride. Freedman and Beeson (11) observed that rats drinking a 1.6% (300 mM) solution of ammonium chloride ad lib. have an increased susceptibility to *E coli* pyelonephritis. The present studies support these observations. Freedman and Beeson suggested that the decreased resistance to renal infection was related to an increased renal content of ammonia resulting from a systemic acidosis induced by drinking ammonium chloride, which increased the output of ammonia in the urine and induced a rise in the activity of renal glutaminase (11). In the present study, however, renal susceptibility to infection was significantly greater in rats given ammonium chloride solution without additional water than in rats given the same amount of ammonium chloride solution plus water even though a similar degree of systemic acidosis was observed in both groups. Furthermore, the susceptibility of the rat kidney to coliform infection was also increased in the present studies simply by decreasing the intake of water and this was not accompanied by systemic acidosis.

The protection afforded the renal medulla of animals given ammonium chloride plus water cannot be attributed to an increased ability of the kidney to excrete more acid as a result of the increased volume intake, since urinary titratable acid and ammonium in these animals was not significantly different from that observed in animals given the same quantity of ammonium chloride daily without additional water. Water diuresis is also without important effect on acid excretion in man (22). It is unlikely that the increased susceptibility of the kidney to coliform infection during ammonium chloride ingestion can be attributed to structural alterations in the kidney produced by ammonium chloride since previous studies have shown that rats drinking a similar solution of ammonium chloride do not develop an abnormal urinary sediment, proteinuria, or morphological evidence of renal injury even after 3 months of ammonium chloride ingestion (11).

The present data strongly suggest that hypertonicity is responsible for the vulnerability of the renal medulla to coliform infection in those rats given either a restricted water intake or a solute load without additional water. There are a number of possible mechanisms which might explain the profound influence of water intake and urinary osmolality on the susceptibility or resistance of the renal medulla to bacterial infection. The importance of rapid polymorphonuclear leukocyte mobilization to the site of bacterial lodgment has been well established as a major determinant of the fate of invading microbial agents (23–26), and deficiencies in granulocyte mobilization have been shown to play an important role in the increased susceptibility of the renal medulla to infection (27). Furthermore, the effect of medullary osmolality on granulocyte mobilization has been pre-

viously established (8). In those studies, the granulocyte response to an inflammatory stimulus was delayed and diminished in intensity in the renal medulla of rats excreting a concentrated urine, whereas the mobilization of granulocytes was enhanced in the renal medulla of rats excreting a more dilute urine (8). Medullary osmolality may also influence susceptibility to infection by affecting phagocytosis per se. A direct correlation between the antiphagocytic properties of solutions and their osmolalities has been observed previously (28). Hypertonic saline solutions have been shown to inhibit phagocytosis in both in vitro (29) and in vivo (30) systems. Furthermore, phagocytosis of *Escherichia coli* by human leukocytes has been shown to be inhibited by hypertonic concentrations of sodium and urea as well as by highly concentrated human urine, whereas more dilute urines permitted phagocytosis to proceed at a more normal rate (28).

Medullary osmolality may also affect humoral mechanisms of tissue resistance. The bactericidal action of serum against Gram-negative rods has been shown to require complement and antibody which act to disrupt the cell wall (31, 32). Hypertonic saline has been shown to inhibit the activity of complement (33). Furthermore, Bulger (34) has observed that the bactericidal activity of healthy human serum against *Escherichia coli* was strikingly inhibited in a hypertonic environment. The osmolality of the environment used in Bulger's experiment was designed to reflect the most likely chemical environment of the human renal medulla during hydropenia and urinary concentration (34). In addition, Acquatella, Little, DeWardener, and Coleman (35) have observed the loss of bactericidal activity of normal human plasma against *Escherichia coli* and *Proteus mirabilis* when studied in concentrated human urine obtained from dehydrated subjects. In contrast, the bactericidal activity of plasma was maximal in human urines which were hypotonic or only moderately hypertonic. Both Bulger (34) and Acquatella and colleagues (35) noted a similar loss of bactericidal activity when serum or plasma respectively, was heat inactivated at 56°C for 30 min. These observations suggest that the hypertonic environment of the renal medulla could easily interfere with that tissue's defense mechanisms possibly through an inhibitory effect on the complement system.

Type-specific circulating antibody has been shown to be a significant determinant in the pathogenesis of both hematogenous and retrograde experimental pyelonephritis in the nonobstructed kidneys of rats infected with strains of Gram-negative bacilli, including *Escherichia coli* (36). However, the effectiveness of circulating antibody in renal environments of varying osmolality had not been previously studied and no attempt was made to do so during the course of the present experiments. Nevertheless, the possibility exists that antibody activity, like both phagocytosis and complement activity, might be inhibited in the medulla of the hydropenic animal.

The influence of medullary osmolality and medullary blood flow upon the susceptibility of the renal medulla to infection are difficult to separate since there is a close relationship between medullary blood flow and urine concentration (37). Medullary blood flow, estimated from dye dilution curves, is greatly increased during water diuresis (38). Blood flow is also increased during osmotic diuresis but is diminished during hydropenia or after the administration of antidiuretic hormone (38). Furthermore, a decrease in renal blood flow causes a rise in urine osmolality (39). These observations suggest that medullary blood flow, because of its effect on urine concentrations and medullary osmolality, may be the factor of major importance in determining this tissue's resistance to infection. Water restriction in the nonobstructed kidney would result in decreased blood flow to the medulla, increased urine concentration and medullary osmolality, and an increased susceptibility of this tissue to infection. In contrast, an increased fluid intake would increase medullary blood flow, decrease urine concentration and medullary osmolality, and decrease susceptibility to infection. The increase in renal medullary blood flow during water diuresis may also result in a more rapid delivery of greater numbers of phagocytes and other serum factors that contribute to natural tissue defenses, as well as provide a favorable environment in which these factors can operate.

Although nonobstructed renal parenchymal infections clearly benefit from water diuresis, the data should not be interpreted to mean that water diuresis is also effective in preventing coliform bacteriuria. Factors which enhance bacterial multiplication in the urine and lower urinary tract may be distinguished from host factors affecting susceptibility of the renal medulla to infection. Concentrated human urine appears to contain an antibacterial substance which is less active in dilute urine (40). In the rat, water diuresis has been shown to decrease the normally antibacterial effect of hypertonic urine against *E. coli* and to permit the multiplication and persistence of coliform organisms when they are introduced into the bladder urine (41). In the mouse, *E. coli* may multiply so rapidly in dilute urine that the kidney is invaded (42). It is well known that the multiplication of *E. coli* is inhibited in urines with osmolalities above 800–1400 mOsm/kg depending upon the pH of urine (43). In rats and mice normal urinary osmolality ranges between 1000 and 2000 mOsm/kg (5. 10). If the mechanism of dilution is responsible for enhanced bacterial

multiplication in the urine then, in the rat, water diuresis would decrease urinary osmolality from levels that inhibit bacterial multiplication to levels which support bacterial growth in urine. However, this is not likely to be of clinical importance in man where urinary osmolality is usually below 800 mOsm/kg (43). The observation that water diuresis promotes the persistence of coliform bacteriuria in rodents (41) is therefore entirely compatible with studies which indicate that water diuresis protects the renal medulla from infection.

The present data, combined with previous observations in this (5, 8, 10) and other laboratories (27–35) provides formidable evidence which indicates that medullary hypertonicity can promote renal parenchymal infection (5, 10); that medullary hypertonicity can be decreased by water diuresis (5, 10, 16–21); and, that by means of a water diuresis the renal medulla can either be protected from or cured of infection produced by a number of microbial agents, especially *Escherichia coli*.

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