Urinary Concentrating Ability in Early Experimental Pyelonephritis

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ABSTRACT The effect of early bilateral pyelonephritis on urinary concentrating ability was studied in rats injected intravenously with enterococci or *Staphylococcus aureus* and in rats inoculated with *Escherichia coli* into the medullae of both kidneys. The mean maximum urinary osmolality of normal rats was 2352 mOsm/kg of water. Inoculation of *E. coli* caused reversible pyelonephritis with sterilization of the kidneys within 12 wk. By 1 day after injection the mean maximum urinary osmolality had decreased to about 1100 mOsm, remained at this level for 3 wk, and then rose to normal by 12 wk. After injection of enterococci and staphylococci, the mean maximum urine osmolality decreased over 3–4 days to about 1000 and 800 mOsm respectively. In the enterococcal infection (which is chronic) the maximum urine osmolality remained about 1200 mOsm for at least 12 wk whereas in the staphylococcal infection (which is reversible) the osmolality gradually rose.

Antimicrobial therapy of *E. coli* renal infection with colistimethate sodium and *S. aureus* infection with ampicillin rapidly reduced bacterial titers in the kidneys with an associated rise in maximum urinary osmolality. Therapy of enterococcal renal infection with ampicillin produced less impressive decreases in bacterial titers in the kidneys and little or no improvement in urinary concentrating ability. With antimicrobial therapy or with the self-limited infections, the rate of increase in concentrating ability was directly correlated with the rate of decrease of bacterial titers. However, there was poor correlation between histological findings in the kidneys and urinary concentrating ability.

These studies demonstrate that early experimental pyelonephritis is associated with a concentrating defect that can be rapidly reversed and therefore is not related to permanent renal damage.

This work was reported in part in abstract form; Kaye, D., and H. Rocha. 1969. Urine osmolality in experimental pyelonephritis. *J. Clin. Invest.* **48**: 43a.

Received for publication 20 January 1970 and in revised form 27 March 1970.

INTRODUCTION

A defect in the urinary concentrating mechanism may be the earliest functional abnormality in pyelonephritis in humans (1, 2). Two studies (3, 4) have clearly demonstrated a urinary concentrating defect early in experimental pyelonephritis produced by intravenous inoculation of gram positive cocci (staphylococci and enterococci), but there is little information available on concentrating ability in early experimental pyelonephritis caused by gram negative bacilli. Furthermore, there are no studies on the rapidity of onset of the defect or reversibility with antimicrobial therapy and there is little information available on the correlations between the concentrating defect and the number of organisms in the kidney, the glomerular filtration rate, and the histological changes in the kidney.

The present study was undertaken to investigate urinary concentrating ability in early experimental pyelonephritis caused by both gram positive cocci and gram negative bacilli and to elucidate the mechanism of the defect by correlating the numbers of organisms in the kidneys, the results of creatinine clearance determinations, and histology of the kidneys with urinary concentrating ability. The effect of antibiotic therapy of pyelonephritis was also studied to evaluate rate of reversibility of the concentrating defect.

METHODS

Animals. White male Sprague-Dawley rats (Blue Spruce Farms, Altamont, N. Y.) weighing between 150 and 250 g were used for all experiments. The animals were given food (Rockland Rat and Mouse Food, Bound Brook, N. J.) and water ad lib. except as outlined below. The food was free of antimicrobial drugs.

Bacteria. One strain each of *Escherichia coli, Staphylo-*

TABLE I

*Effect of Vasopressin and/or 24 hr of Food Deprivation on Urine Osmolality (mOsm/kg of Water) in Rats Deprived of Water for 24 hr**

Infecting organism and days after infection	Fed during 24 hr before sacrifice	Deprived of food during 24 hr before sacrifice	
	No vasopressin	No vasopressin	Vasopressin
No infection (normals)	2418 ±357	2458 ±273	2400 ±198
Enterococcus 3 wk after infection	1334 ±351	1310 ±293	1257 ±366
S. Aureus 3 days after infection	1109 ±428	1241 ±485	1084 ±387
E. coli 2 wk after infection	1347 ±363	1460 ±225	1403 ±143

There were 6–17 rats in each group.
* Means ±SD.

coccus aureus, and enterococcus was used for all experiments. The *E. coli* (Yale strain) (5) was inhibited by 6.3 μg/ml of colistimethate sodium; the *S. aureus* (502A strain) (6) was inhibited by 0.4 μg/ml of ampicillin; and the strain of enterococcus was inhibited by 3.13 μg/ml of ampicillin.

Stock cultures were maintained by storing aliquots of an 18 hr culture in trypticase soy broth (Baltimore Biological Laboratory, Baltimore, Md.) at −20°C. Inocula for each experiment were prepared by subculturing an aliquot of the stock culture in trypticase soy broth and incubating at 37°C for 18 hr.

Bacterial enumeration. The numbers of bacteria in broth or in blood were determined by plating 0.1 ml of each specimen and making serial 100-fold dilutions in saline solution and plating 1- and 0.1-ml aliquots of each dilution in trypticase soy agar pour plates.

Numbers of bacteria in tissue were determined in the same manner after homogenizing the tissue in trypticase soy broth using Teflon tissue grinders (Tri-R Instruments, Jamaica, N. Y.). The total number of viable bacteria in a tissue or fluid was calculated from colony counts after incubation of the plates for 24 hr at 37°C. In each experiment, representative colonies were identified to assure identity with the microorganisms inoculated.

Experiments. Enterococci and *S. aureus* were inoculated intravenously in a tail vein in 1 ml of trypticase soy broth, 10^9–2×10^9 for enterococci and 3×10^8–6×10^8 for *S. aureus.* Controls were injected intravenously with 1 ml trypticase soy broth containing 10^9–2×10^9 heat-killed enterococci or 3×10^8–6×10^8 heat-killed *S. aureus* (enterococci were killed by boiling for 5 min; *S. aureus* were killed by heating at 60°C for 60 min).

E. coli was injected directly into both kidneys. Animals were anesthetized with intraperitoneal injection of pentobarbital sodium, a midline abdominal incision made, and 0.1 ml trypticase soy broth containing 5×10^6–10^7 *E. coli* was injected into the upper and lower pole of each kidney so as to deposit the inocula in the medullary portion of the kidney. Controls were injected in the same fashion at each of the four sites with 0.1 ml trypticase soy broth containing 5×10^6–10^7 heat-killed *E. coli* (killed at 60°C for 60 min). After inoculation, the abdominal incisions were closed.

Animals were weighed daily and periodically studied for

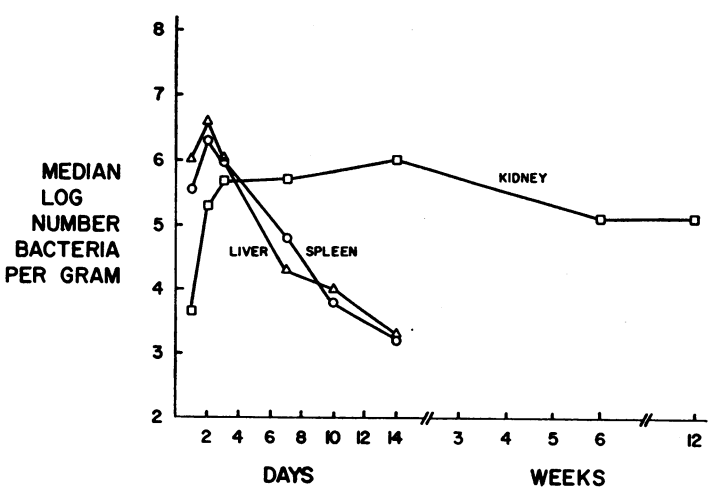

FIGURE 1 Median log number of bacteria per gram of kidney, liver, and spleen after intravenous injection of enterococci. There were five or six rats in each group at 1–14 days and 33 and 10 rats at 6 and 12 wk respectively.

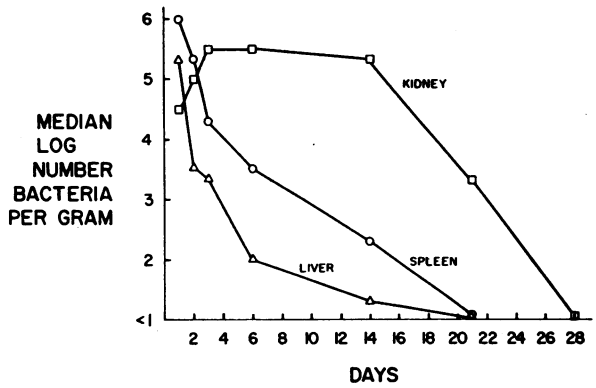

FIGURE 2 Median log number of bacteria per gram of kidney, liver, and spleen after intravenous injection of *Staphylococcus aureus*. There were five rats in each group.

determination of maximum urinary concentrating ability and numbers of bacteria in various tissues. Before study, the rats were dehydrated by removal of all fluids for 24 hr. In some experiments in addition to water deprivation, food was also removed for 24 hr before study; and in other experiments in addition to water and food deprivation, 100 mU of vasopressin were injected intraperitoneally 2 hr before study. As shown in Table I, food deprivation alone or food deprivation with administration of vasopressin did not significantly increase maximum concentrating ability as compared with water deprivation alone ($P > 0.05$ for all comparisons).

To determine urine osmolality, rats were anesthetized by intraperitoneal injection of pentobarbital sodium. The urinary bladder was exposed through a midline abdominal incision, urine was aspirated through a needle and syringe, and the osmolality was determined in an osmometer (Fiske Osmometer, model G-12, Fiske Associates, Inc., Uxbridge, Mass.) requiring only 0.2 ml of urine for each determination. Blood was aspirated directly from the heart after opening the chest, and the rat was killed by exsanguination into the pleural cavities after removal of the lungs. The kidneys, liver, and spleen were each removed separately with sterile instruments. The lungs, spleen, kidneys, and a portion of liver were each weighed and the numbers of organisms determined. One half of one kidney from each rat was fixed in formalin, and sectioned and stained with hematoxylin and eosin.

In some of the experiments control groups were pair fed with infected animals to maintain equal weights in the two groups. In other experiments infected rats were treated by intramuscular injection of ampicillin or colistimethate sodium. The dose of ampicillin was 40 mg every 12 hr and the dose of colistimethate sodium was 2 mg every 12 hr in 0.2 ml of water.

Serum creatinine and creatinine clearances. Creatinine clearances were measured in normal rats and at various time periods after initiation of infection with *E. coli*, *S. aureus*, and enterococcus using a 24 hr urine collection. Urine was collected into refrigerated bottles from individual rats in metabolic cages, the sides of which had been siliconized. At the end of the 24 hr collection, blood was aspirated from the heart and the serum separated. Creatinine determinations in serum and urine were performed by the method of Bonsnes and Taussky (7).

RESULTS

Tissue populations of bacteria and urinary concentrating ability. 24 hr after intravenous injection of *S. aureus* or enterococci (the earliest time of study) there was bacteremia with low numbers of organisms (up to 150–ml of blood) in some of the rats and this persisted for up to 2 days with *S. aureus* and up to 7 days with enterococci. As shown in Figs. 1 and 2, the liver and spleen contained more bacteria than the kidneys for up to 3 days after injection after which the titers in liver and spleen decreased rapidly. The lungs always contained fewer organisms than liver or spleen. The kidneys contained relatively few organisms 24 hr after challenge but by 48–72 hr, multiplication to titers over 10^6 per kidney had occurred. The numbers of organism in the kidneys remained high (over 10^5 per kidney) for as long as 12 wk in rats infected with enterococci whereas titers dropped in rats infected with staphylococci and most

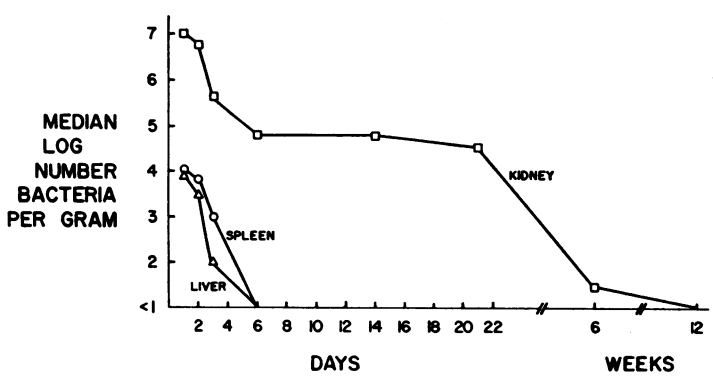

FIGURE 3 Median log number of bacteria per gram of kidney, liver, and spleen after intramedullary inoculation of *Escherichia coli*. There were five rats in each group at 1–6 days and 11–28 rats in each group at 2–12 wk.

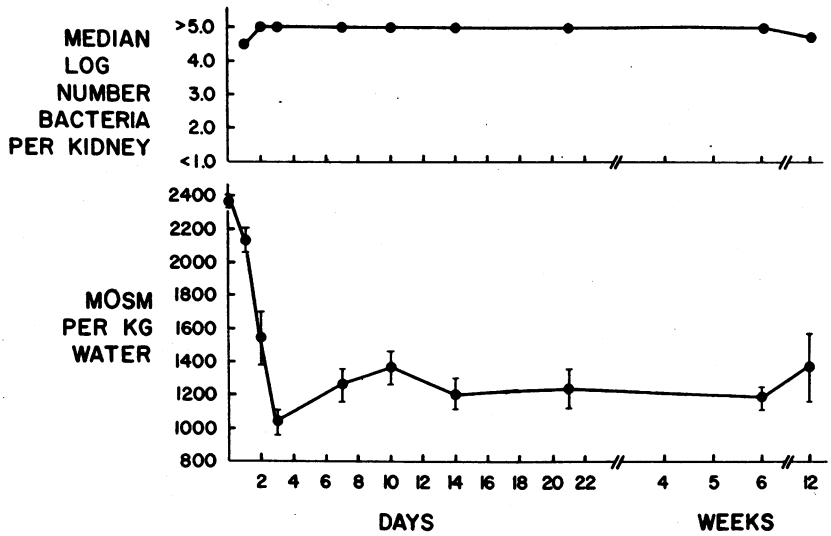

FIGURE 4 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intravenous injection of enterococci. There were 10–33 rats in each group.

kidneys were sterile by 4 wk. The mortality rate was about 10% after inoculation of staphylococci and about 5% after injection of enterococci.

After intramedullary inoculation of *E. coli,* bacteremia was not detected, even at the earliest time of study, 24 hr after inoculation. As shown in Fig. 3, the kidneys contained more bacteria than the liver or spleen at all time periods studied. Median renal titers of *E. coli* remained over 10^4 for at least 3 wk after which the numbers of organisms decreased. The mortality rate was about 10%.

Figs. 4–6 demonstrate the mean maximum urinary osmolalities and the median log numbers of bacteria in the kidneys of the same groups of rats. The mean maximum osmolality of the urine was 2352 mOsm/kg of water in 135 normal rats.

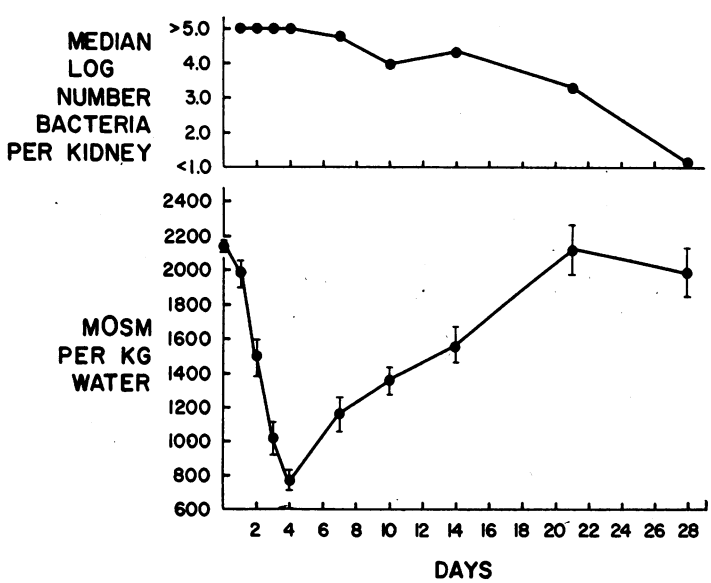

FIGURE 5 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intravenous injection of *Staphylococcus aureus.* There were 9–26 rats in each group.

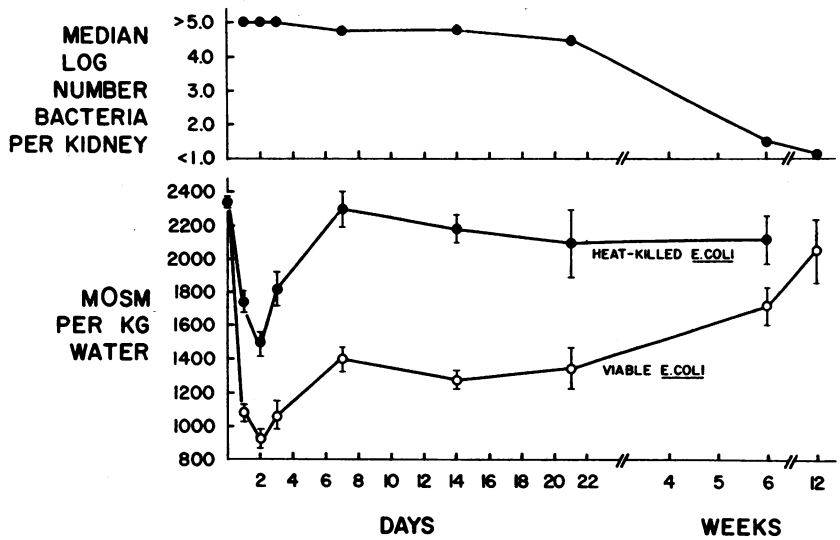

FIGURE 6 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intramedullary inoculation of *Escherichia coli*. There were 9–28 rats in each group.

After intravenous injection of enterococci (Fig. 4) or staphylococci (Fig. 5), there was a gradual decrease in mean urinary osmolality which reached minimal mean values of 772 mOsm/kg of water after injection of staphylococci and 1015 mOsm/kg of water after injection of enterococci at 4 and 3 days, respectively. The urinary osmolality of rats infected with enterococci remained low for the duration of the experiment (12 wk). In contrast the urinary osmolality began to rise after 4 days following inoculation of staphylococci and by 21 days the mean urinary osmolality was back to normal. The maximum urine osmolalities in rats infected with enterococci were significantly lower than normal ($P < 0.01$) at all times shown in Fig. 4. The maximum urine osmolalities in rats infected with staphylococci were significantly lower than normal ($P < 0.01$) at all times shown in

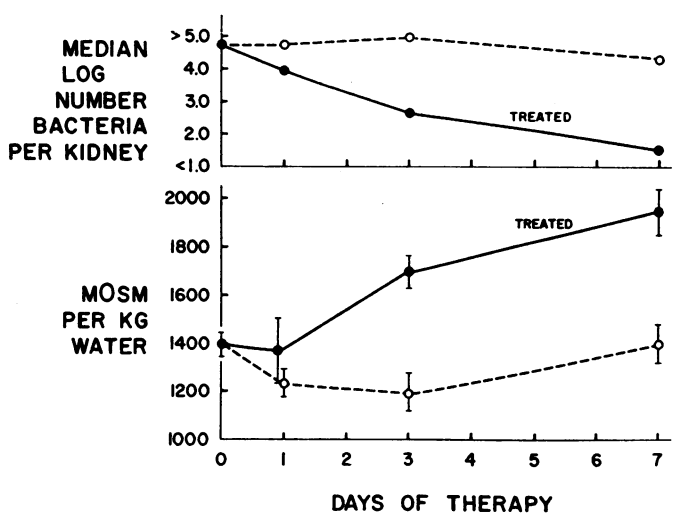

FIGURE 7 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intramedullary inoculation of *Escherichia coli*. The solid lines represent the rats that received colistimethate sodium and the broken lines represent rats that were injected with water; therapy was started 7 days after infection with *Escherichia coli*. There were 8–15 rats in each group.

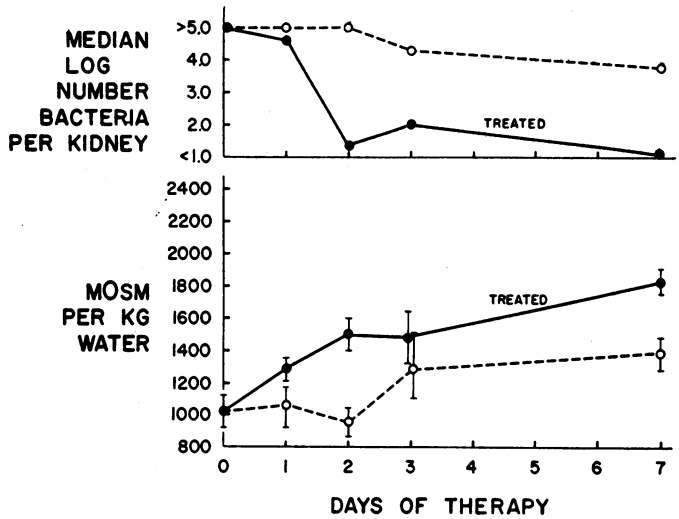

FIGURE 8 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intravenous injection of *Staphylococcus aureus*. The solid lines represent the rats that received ampicillin and the broken lines represent rats that were injected with water; therapy was started 3 days after infection with *Staphylococcus aureus*. There were 9–22 rats in each group.

Fig. 5 except at 21 and 28 days ($P > 0.05$ at these times). Intravenous injection of heat-killed bacteria as a control did not affect urinary concentrating ability.

As shown in Fig. 6, 24 hr after intramedullary inoculation of viable *E. coli* the mean maximum urine osmolality was 1087 mOsm as compared with 1737 mOsm for controls receiving heat-killed *E. coli* ($P < 0.01$). The controls regained normal concentrating ability by 1 wk ($P > 0.05$ as compared with normals). However the mean urinary concentrating ability remained significantly depressed for 3 wk in rats injected with viable *E. coli* as compared with controls ($P < 0.01$ at all times

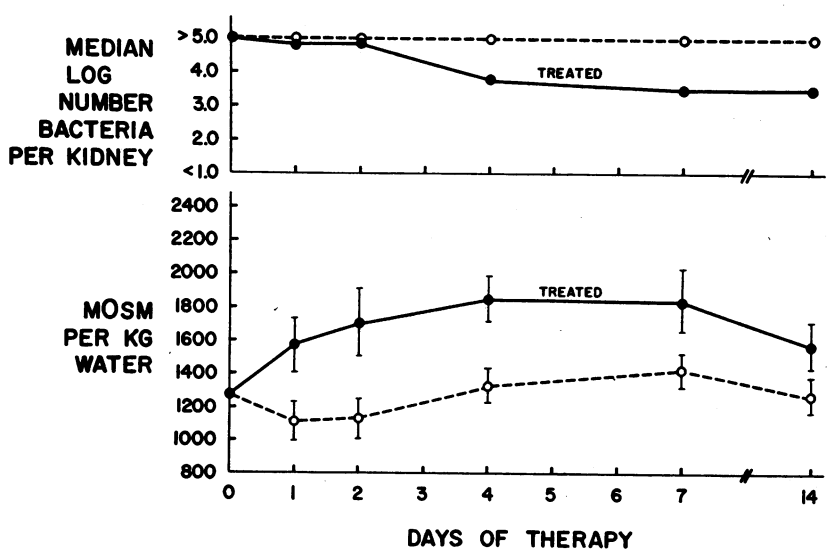

FIGURE 9 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intravenous injection of enterococci. The solid lines represent the rats that received ampicillin and the broken lines represent rats that were injected with water; therapy was started 7 days after infection with enterococci. There were 7–15 rats in each group.

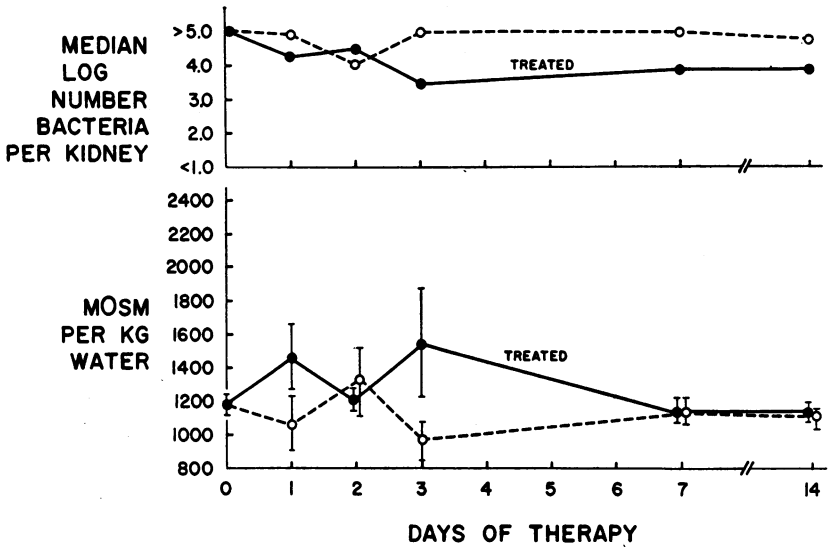

FIGURE 10 Mean maximum urinary osmolality (mOsm/kg of water) ±1 SEM and median log number of bacteria per kidney after intravenous injection of enterococci. The solid lines represent the rats that received ampicillin and the broken lines represent rats that were injected with water; therapy was started 6 wk after infection with enterococci. There were 7–13 rats in each group.

before and including 3 wk). After 3 wk, the concentrating ability in rats injected with viable *E. coli* began to rise and was not significantly different from normal by 12 wk ($P > 0.05$). Intramedullary inoculation of isotonic saline solution reduced the urinary osmolality in exactly the same manner as heat-killed *E. coli*.

In general, as shown in Figs. 4–6, the mean maximum urinary osmolality varied inversely with the numbers of bacteria in the kidneys.

In other experiments the effect of pair-feeding controls with infected animals to maintain equivalent weight change was studied. The controls for rats infected with staphylococci or enterococci received trypticase soy broth intravenously and were then pair fed with the infected animals. Mean maximum urine concentrations in these rats were essentially normal (2100 mOsm/kg of water or greater at all times). Controls for rats infected with *E. coli* were inoculated with heat-killed *E. coli* into the renal medullae and were then pair fed with the infected animals. The mean maximum urinary osmolalities of the pair-fed controls did not differ significantly ($P > 0.05$) from the mean maximum urinary osmolalities of the controls injected with heat-killed *E. coli* but not pair fed.

The effect of antibiotic therapy on maximum urinary osmolality of infected rats was studied. Figs. 7–10 show the results of these experiments. As shown in Fig. 7, treatment of rats with *E. coli* renal infection of 1 wk duration with colistimethate sodium resulted in reversal of the concentrating defect as compared with infected rats receiving injections of equal volumes of distilled water as a control ($P < 0.01$ at 3 and 7 days). Similarly as shown in Fig. 8, therapy of rats with *S. aureus* renal infection of 3-days duration with ampicillin partially reversed the defect in urinary concentrating ability as compared to untreated rats ($P < 0.01$ at 2 and 7 days but not at 3 days).

Results of therapy of enterococcal infection with ampicillin were variable. As shown in Fig. 9, initiation of treatment 1 wk after inoculation of enterococci resulted in at least partial reversal of the concentrating defect as compared with untreated controls ($P < 0.05$ at 2 days; $P < 0.01$ at 4 days; and $P > 0.05$ at all other times).

TABLE II
Serum Creatinine Concentrations

Infecting organism	Days after infection	No. of animals	Mean creatinine	±SD
			mg/100 ml	
No infection (normals)		20	0.47	0.13
Enterococcus	1–3	11	0.56	0.23
Enterococcus	7–14	5	0.56	0.08
Enterococcus	21–42	6	0.57	0.26
S. aureus	1–3	10	0.53	0.12
S. aureus	7–14	6	0.69*	0.06
S. aureus	21–42	3	0.53	0.24
E. coli	1–3	11	0.47	0.11
E. coli	7–14	5	0.37‡	0.06
E. coli	21–42	5	0.51	0.10

* Significantly higher than normal ($P < 0.01$).
‡ Significantly lower than normal ($P < 0.05$).

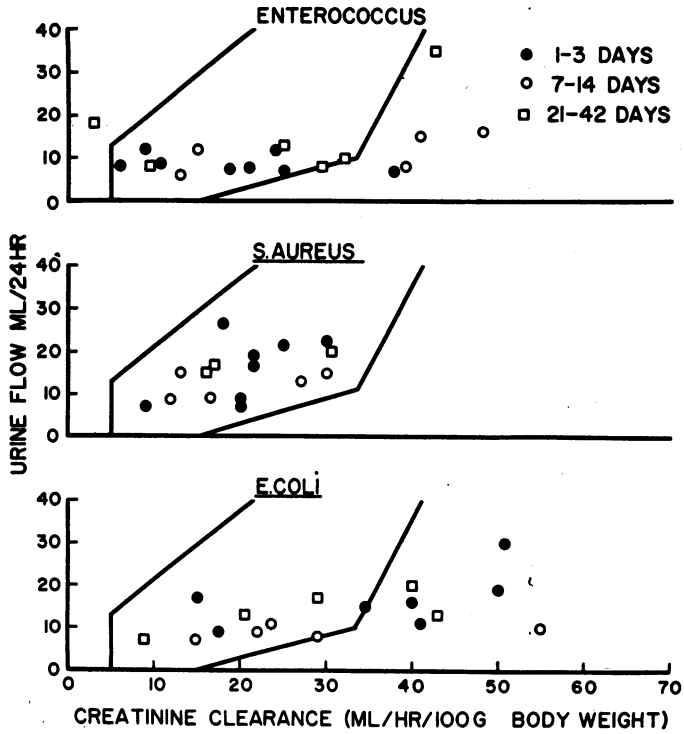

FIGURE 11 Creatinine clearances at various time periods after infection with enterococci, *Staphylococcus aureus,* and *Escherichia coli.* Each symbol represents one rat. The ranges for 20 uninfected rats fall between the lines.

However, if initiation of treatment was delayed for 6 wk after inoculation of enterococci, as shown in Fig. 10, there was no increase in maximum urinary concentrating ability as compared with untreated controls ($P > 0.05$ at all times). Treatment of enterococcal infection with colistimethate sodium for 1 wk had no effect on renal titers of enterococci or on urinary concentrating ability.

In general as shown in Figs. 7–10, improvement in concentrating ability was correlated with a decrease in the numbers of bacteria in the kidneys. Therapy resulted in sterilization of 40% of the kidneys in rats infected with *E. coli* and 100% of the kidneys in rats infected with *S. aureus,* and in concomitant reversal of the urinary concentrating defect. In contrast, the decrease in numbers of bacteria in the kidney was much less impressive with enterococcal infection and the increase in concentrating ability was much less striking or did not occur at all.

Serum creatinine and creatinine clearance. Determinations were made of serum creatinine values in rats at various time intervals after infection with *S. aureus,* enterococcus, and *E. coli.* As shown in Table II, the mean serum creatinine values for 20 normal rats was 0.47 mg/100 ml. Mean serum creatinines were significantly higher than normal ($P < 0.01$) in rats 7–14 days after infection with *S. aureus* and significantly lower than normal ($P < 0.05$) in rats 7–14 days after infection with *E. coli.* None of the other groups of rats shown in Table II differed significantly from normal ($P > 0.05$) in mean serum creatinine values. The changes in creatinine values had no regular relationship to either maximum osmolality of the urine or to numbers of organisms in the kidneys. The mean serum creatinine was above normal in staphylococcal infection and below normal in *E. coli* infection at times when there were high renal titers of bacteria and markedly decreased urinary concentrating ability.

Creatinine clearances were calculated in most of these rats using 24-hr urine outputs. Values were also determined for normal rats with different urine flows produced by dehydration, by allowing water ad lib. or by offering 5% glucose in water which increases water consumption (8). Fig. 11 shows the normal ranges of creatinine clearances and the creatinine clearance values at various time periods after infection with enterococcus, *S. aureus,* and *E. coli.* The creatinine clearances were within the normal range or higher than normal after each type of infection. The clearances tended to be higher in rats infected with *E. coli* than in rats in-

fected with enterococci or *S. aureus* and higher in rats infected with enterococci than in rats infected with *S. aureus*.

Histological studies. Hyperemia in both the cortex and medulla were common findings after infection with *E. coli, S. aureus,* or enterococci. Histological changes in the kidneys were most striking after intramedullary injection of viable *E. coli.*

24 hr after injection of viable *E. coli,* there was a triangular-shaped area of marked polymorphonuclear leukocytic infiltration surrounding the needle tract with the apex in the medulla and the base in the cortex at the renal capsule. The needle tract was also visible in kidneys of control rats injected with heat-killed *E. coli* and was sometimes hemorrhagic; however there was no leukocytic reaction. By 48 hr, both control and infected kidneys contained dilated tubules in the medulla some of which were filled with an amorphous eosinophilic material; these changes were most marked in the infected kidneys. In addition some of the dilated tubules in infected kidneys contained clumps of polymorphonuclear leukocytes. By 1 wk fibrosis which was most marked in the medulla was noted along the needle tracts of both infected and control kidneys, and the cellular infiltrate in the infected kidneys was mainly round cells rather than polymorphonuclear leukocytes. By 2 wk the area of fibrosis had increased in size and there was retraction of the capsule over the cortex. The inflammatory reaction in infected kidneys was found mainly in the cortex and consisted of round cells; however polymorphonuclear leukocytes were found in dilated tubules in the medulla. The inflammatory reaction subsided progressively between the 2nd and 12th wk. Therapy of *E. coli* renal infection with colistimethate sodium starting on the 7th day of infection did not alter the rate of resolution of the histological changes.

24 hr after injection of *S. aureus* there were usually no histological changes other than hyperemia; however occasionally a rat had an abscess in the medulla. By 48 hr after injection one-third of the kidneys contained medullary abscesses (masses of polymorphonuclear leukocytes) and by 72 hr essentially all had medullary abscesses. The area of polymorphonuclear leukocytic infiltration was usually restricted to the medulla (often the papillary part) but occasionally extended into the cortex. Dilated tubules were frequently observed in the medulla and often contained polymorphonuclear leukocytes and/or an amorphous eosinophilic material. By 4 days after injection, half of the medullary abscesses extended into the cortex in a triangular shape with the apex in the medulla. By 1 wk most kidneys had extensive triangular areas of inflammation and these contained mainly round cells. Subsequently, areas of inflammation began to subside and by 4 wk the kidneys of most rats looked normal except for thinning of the cortex in areas of some of the kidneys. Therapy of *S. aureus* renal infection with ampicillin starting on the 3rd day of infection resulted in rapid disappearance of inflammatory changes and normal histology by the 7th day of treatment.

24 hr after inoculation of enterococci there were usually no histological changes other than hyperemia. By 48 hr small collections of polymorphonuclear leukocytes were seen in about half of the renal medullae. By the 4th–7th days after inoculation of enterococci, abscesses were noted in both the medulla and cortex of about half of the kidneys. Occasionally the area of polymorphonuclear leukocytic infiltration extended in a triangular area from the medulla to involve the cortex. Thereafter up to 12 wk about half of the kidneys looked essentially normal except for hyperemia and tubular dilatation in the medulla. Most of the remainder of the kidneys had small areas of round cell infiltration (usually in the cortex but occasionally in the medulla) and clumps of polymorphonuclear leukocytes in dilated tubules in the medulla. Depression of the renal capsule over an area of round cell infiltration was a common finding. Occasionally abscesses were seen. Therapy of enterococcal renal infection with ampicillin produced absolutely no change in histology.

In untreated rats infected with *E. coli,* the extent of inflammatory changes on histological examination correlated well with the number of bacteria in the kidneys and the degree of impairment of urinary concentrating ability. In untreated rats infected with enterococci and to a lesser degree *S. aureus,* the extent of histological changes correlated poorly with the number of bacteria in the kidneys and with the concentrating defect. In these rats high renal titers of bacteria and a severe concentrating defect were often found in the presence of relatively normal histology.

Therapy of *E. coli* infection did not alter histological changes despite a rapid decrease in numbers of bacteria in the kidneys and a rapid increase in urinary concentrating ability. Therapy of enterococcal infection 1 wk after inoculation of enterococci did not alter histology but did decrease renal populations of bacteria and increased concentrating ability. Therapy of *S. aureus* infection did seem to improve histological changes in association with a decrease in renal titers of bacteria and an increase in concentrating ability.

DISCUSSION

The present studies have demonstrated that there is a severe urinary concentrating defect which occurs very early in the course of experimental pyelonephritis produced either by intravenous inoculation of gram positive cocci (*S. aureus* and enterococci) or by intramedullary inoculation of gram negative bacilli (*E.*

coli). The use of these three different models of infection not only allows comparison of results of infection produced by different methods of inoculation and by different types of bacteria (both gram negative bacilli and gram positive cocci), but also allows comparison of self-limited infections and infections that are not self limited. The use of antibiotic therapy adds the dimension of rapid reversibility of infection. With the three different experimental models (with and without antibiotic therapy), it has been possible to demonstrate a striking correlation between numbers of bacteria in the kidney and the degree of impairment of urinary concentrating ability.

In previous studies Gonick, Goldberg, Rubini, and Guze (4) demonstrated a urinary concentrating defect 1 wk after intravenous injection of enterococci; and Beck, Freedman, Levitin, Ferris, and Epstein (3) found a concentrating defect 3 wk after intravenous injection of *S. aureus*. These were the earliest times of study. The present investigation has demonstrated the presence of a concentrating defect as early as 24 hr after intravenous injection of enterococci or *S. aureus*. In addition infection caused by intramedullary inoculation of *E. coli* resulted in a prompt and striking decrease in urinary concentrating ability which was much greater than in controls injected with killed *E. coli* into the renal medulla.

The infection produced by the strain of enterococcus used in this study was comparable to that described by Guze (9). However the renal infection produced by the 502A strain of *S. aureus* in the present study was spontaneously reversible at a much earlier time than that caused by the strain of *S. aureus* used by Beck et al. (3).

Bricker, Dewey, Lubowitz, Stokes, and Kirkensgaad (10), Bricker, Morrin, and Kime (11), and Bricker, Kime, and Morrin (12) in advancing the "intact nephron" hypothesis gave evidence indicating that only intact nephrons contribute to renal function and that partially disabled nephrons disappear from the population of functioning units. They postulated that loss of urinary concentrating (and diluting) ability in renal disease including pyelonephritis is not related to destruction of specific functional sites in the tubule but that the concentrating (and diluting) processes in the persisting nephrons remain essentially normal. They suggested that the loss of concentrating ability is related to an osmotic diuresis resulting from an increased glomerular filtration rate per remaining functioning nephron. This explanation for loss of concentrating ability presupposes the destruction of a sufficient number of nephrons to significantly increase the glomerular filtration rate in the remaining nephrons. However, Bank and Aynedjian (13, 14) found that the urinary concentrating defect in rats with pyelonephritis was much more severe than in rats with equivalent surgical reduction of renal mass. They postulated that the more severe defect in pyelonephritis seemed to be related to a disturbance in medullary function, perhaps caused by disruption of the medullary architecture.

The results of the present study do not support the hypotheses of Bricker et al. or of Bank and Aynedjian concerning the mechanism of the concentrating defect in early experimental pyelonephritis. The rapid reversibility of the defect in *E. coli* and *S. aureus* renal infection (especially with antibiotic therapy) in the present study suggests a much more transient defect than profound destruction of nephrons (as required by the hypothesis of Bricker et al.) or disruption of medullary architecture (as suggested by Bank and Aynedjian). However either of these hypotheses could explain the concentrating defect in severe chronic pyelonephritis similar to the experimental models studied by these investigators (i.e., large decreases in glomerular filtration rate).

Although the reason for the urinary concentrating defect in early experimental pyelonephritis is not known, the two most likely explanations seem to be the following: (*a*) changes in permeability in the collecting tubules to water or solute, and/or (*b*) decreased osmolality in the interstitium of the medulla. Gonick et al. (4) measured the concentrations of sodium and urea in the renal medullae of rats with pyelonephritis produced by enterococci and found normal sodium concentrations but decreased urea concentrations. While the decrease in medullary osmolality resulting from the decrease in urea concentration would probably contribute to impaired concentrating ability, other factors such as decreased permeability of the collecting tubules to water may also play a role. The mechanisms by which decreases in medullary osmolality or changes in permeability occur in early experimental pyelonephritis are unknown. The striking correlation between numbers of bacteria in the kidneys and the degree of impairment in concentrating ability in the present study suggests that the defect may be related at least in part to the presence of actively metabolizing bacteria. The interaction could be one of products of bacterial metabolism or of inflammation changing the permeability of the tubular membrane or increasing blood flow in the medulla (and thereby decreasing medullary interstitial tonicity). Alternatively the products of metabolism or inflammation could inhibit enzymes necessary for solute transport or for membrane permeability. Some evidence against the importance of the inflammatory reaction per se is the fact that no difference in inflammation was apparent between kidneys of untreated and treated rats with *E. coli* infection and that there was a poor correlation

between histological changes and concentrating ability in rats with *S. aureus* or enterococcal renal infection.

ACKNOWLEDGMENTS

We are grateful to Miss Judith Gips for technical assistance.

This investigation was supported by Grants AI 07581 and AI 09489 from the U. S. Public Health Service.

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