Bacteremia in the Pathogenesis of Retrograde E coli Pyelonephritis in the Rat

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Female rats deprived of water overnight, and then given 1.0 ml of *E coli* 0111:B4 via the urethra, developed pyelonephritis. A nearly absolute association was found between the occurrence of bacteremia after the transurethral infusion and the development of pyelonephritis. An identical lesion was produced by a combination of forniceal damage and intravenous injection of *E coli*. The kidney damaged by reflux was shown to be more susceptible to hematogenous pyelonephritis than the obstructed kidney and the distribution of the infection was due to localization of bacteria in the damaged fornix but not to the route of infection. The induction of retrograde *E coli* pyelonephritis in the rat required a tear in the pelvic epithelium creating pyelovenous communications, and the resultant bacteremia produced pyelonephritis. The incidence of ureteral reflux and the volume of inoculum that refluxed to the renal pelvis was shown radiologically to be a function of bladder distensibility, which is reduced by withholding water for a few hours. In this system, retrograde *E coli* pyelonephritis developed from a combination of two factors: (1) reflux-induced damage to the renal pelvis so that *E coli* are introduced into the kidney and (2) hematogenous infection of the damaged kidney. (Amer J Path 64:443–456, 1971)

THE TWO MOST POPULAR THEORIES of the pathogenesis of *E coli* pyelonephritis are the "hematogenous" and the "ascending" theories.[1] The hematogenous theory postulates that bacteremia initiates renal infection and bacteriuria is a secondary phenomenon. The ascending theory assumes that *E coli* organisms in the bladder ascend via the ureters, penetrate the renal pelvis and infect the medulla. Attempts have been made to demonstrate in experimental models that either pathway is feasible. It is difficult, however, to create *E coli* pyelonephritis by any route in animals unless the kidneys are traumatized or the ureters obstructed. Braude, Shapiro and Siemienski[2] found that external renal massage made unobstructed rat kidneys susceptible to infection after the intravenous injection of *E coli* and other bacteria. Anderson and Jackson[3] first caused ascending pyelo-

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nephritis in rats by infusing *E coli* through their urethras. Most rats developed pyelitis but a few had a parenchymal infection as well. Multiple urethral infusions of *E coli,* repeated several times a week, produced pyelonephritis in over 80% of rats.[4] This was the first demonstration that *E coli* placed in a rat's bladder could consistently infect the unobstructed kidney. Subsequently, Heptinstall[5] reported that rats developed acute pyelonephritis after *E coli* was infused only once through the urethra in a large volume of fluid (1.5 ml) and the bladder massaged. These studies appeared to demonstrate that *E coli* could indeed cause experiemental pyelonephritis.

In the work described in this report, we reexamined ascending pyelonephritis in rats due to *E coli* and found this infection to be related to the occurrence of bacteremia. We found that a simplification of Heptinstall's technic produced a histologic pattern of pyelonephritis hitherto regarded as ascending in origin. Careful monitoring of the blood cultures, however, showed that this lesion developed in the wake of bacteremia and could be duplicated by intravenously injecting *E coli* into rats that had been prepared with a urethral infusion of sterile broth. Trauma to the renal pelvis appeared to be responsible for the bacteremia and necessary for the initiation of an ascending infection.

Methods

Female Sprague-Dawley rats, weighing 160–200 g each, were fed laboratory chow without antibiotics and deprived of water overnight before they were infected. Animals were secured supine to a small-animal board (Fisher Bros). Under ether anesthesia, the external urethra was rinsed with tincture of iodine and then the polished hub of a No. 23 needle inserted and kept secure with a loop of thread. The inoculum was infused from a tuberculin syringe with application of minimal pressure (1 ml was delivered over 90–120 seconds). Three minutes after completion of the urethral infusions, 0.2 ml of cardiac blood was obtained. Serial dilutions of 0.1 ml of blood were made in trypticase soy broth for pour plates. If the undiluted blood was sterile, the result was recorded as <10/ml.

E coli 0111:B4 was originally obtained from E. C. Heath. On the day of the experiment, 0.1 ml of a fresh overnight culture was inoculated into 50 ml of trypticase soy broth and placed on a shaker at 37 C. At 3 hours, the inocula were drawn into syringes and refrigerated until used. The bacterial count of the undiluted culture was between 10^8 and 10^9 bacteria/ml. More dilute suspensions of 3-hour cultures were made in trypticase soy broth for certain experiments.

Rats were killed by ether anesthesia after clamping the external urethra. The urinary tract was exposed and examined and then the kidneys were removed aseptically. Bladder urine was aspirated or the bladder was rinsed with trypticase soy broth. The kidneys were stripped of their capsules and sectioned through a lesion; one part used for H&E sections and the other ground with a Teflon grinder in 1 ml of sterile broth. Kidneys and urine were cultured on eosin–methylene blue agar and on trypticase soy agar (Difco) containing 5% rabbit blood + 0.001%

azide. The amount of growth was graded from 0 to 4+ by criteria previously reported.[6] The identity of *E coli* 0:111 was confined by slide agglutination with antisera (Difco).

Bacteria were found in the bladder urine aspirated after clamping the external urethra in 25% of unmanipulated female rats, despite sterile kidneys. These bladder organisms were enterococci, aerococci, Proteus sp and *E coli*. They were also cultured from kidneys of 10–15% of control animals given sterile broth via the urethra and, in a smaller percentage, in mixed culture with *E coli* 0:111 after that organism had been infused into experimental animals via the urethra. If these urethral contaminants were recovered from kidneys, the animal was excluded from consideration as noted in each table. Occasional animals with persistent urethral obstruction, distended bladders and bilateral hydroureter due to the trauma of the infusion were also excluded from tabulation.

Results

E coli Suspension, 1 ml, via Urethra

Table 1 shows the frequency of immediate bacteremia and its relationship to the development of acute pyelonephritis. In this table and in subsequent analysis, rats have been divided into three groups according to the degree of bacteremia that developed. The rats in these experiments received between 5×10^6 and 5×10^9 bacteria in a volume of 1 ml. There was no difference in the incidence of bacteremia with the different inoculums, though all the intermediate level bacteremias (10^1–10^3/ml) occurred when 5×10^6 bacteria were used. Heavy bacteremia ($>10^3$/ml) occurred in 55 of 68 rats and 54 of these developed bilateral acute pyelonephritis when examined 1–7 days later. In contrast, none of the 8 animals with sterile blood cultures (<10 *E coli*/ml) developed pyelonephritis. In the 5 rats with an intermediate degree of bacteremia, acute pyelonephritis was found in half the kidneys.

Table 1—Results of Urethral Infusion of 1.0 ml Volume of **E coli** 0111:B4 in Relation to Bacteremia

E coli/ml blood	No. of animals	No. with pyelonephritis	Proportion of kidneys with pyelonephritis	Proportion of kidneys with (+) cultures
$>10^3$	55*	55 (100%)	109/110 (99%)	99/110 (90%)
10^1–10^3	5	3 (60%)	4/10 (40%)	6/10 (60%)
$<10^1$	8†	0 (0%)	0/16 (0%)	7/16 (44%)
Total	68	58 (85%)	113/136 (83%)	112/136 (82%)

* Excludes 2 animals with bilateral pyelonephritis with enterococcus cultured from kidneys and 2 animals with bilateral pyelonephritis and hydronephrosis.

† Excludes 2 animals with enterococcus cultured from kidneys without pyelonephritis.

There was at least a 1+ growth of *E coli* from 99 of 110 pyelonephritic kidneys, as shown in Table 1. Eleven kidneys were sterile; this was in part due to the focal nature of the infection and difficulty in dividing a small lesion and in part to the rapid clearance of *E coli* from infected kidneys during the first week after infection (see below). Seven kidneys from animals with sterile blood cultures were infected. The parenchyma of these kidneys was normal but there was infiltration of polymorphonuclear leukocytes (PMNs) in the submucosa and mucosa of the renal pelvis and squamous metaplasia of the pelvis.

Seventy-four rats were infected with 10^8–10^9 *E coli* 0111:B4 suspended in 1.0 ml and sacrificed at intervals up to 3 months to determine the natural history of this infection. Characteristic gross lesions were already present at 24 hours on the flat surfaces of the kidneys in a semicircular area midway between the hilum and the greater curvature at the level of the fornix. These lesions were hyperemic without distinct abscesses on the capular surface. Abscesses were present from day 2–7 and then evolved into a pigmented atrophic scars in the area where the acute lesions had been present.

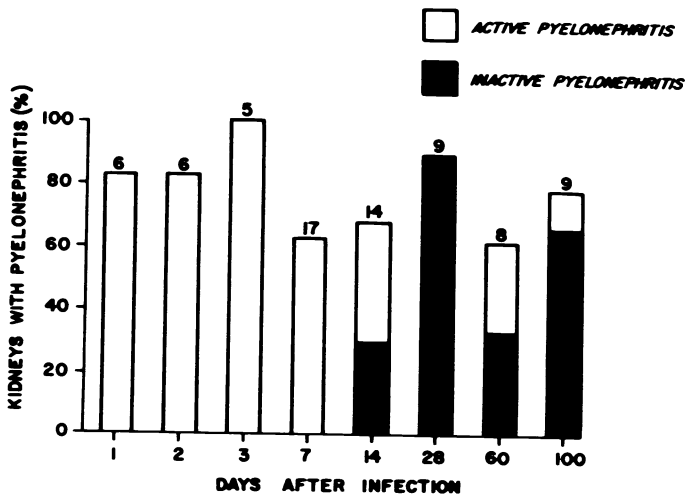
On microscopic examination at 24 hours, there were masses of bacteria, usually within tubules and collecting ducts, extending in a wedge from the fornix to the cortex. PMNs were seen around the periphery of the bacterial masses at 24 hours but thereafter came to be intimately mixed with the bacteria. This process continued until day 3, when it was impossible to distinguish bacteria on H&E section except within the renal papilla where mixed bacteria-cellular casts were sometimes noted. The lesion underwent rapid typical evolution of acute pyelonephritis from abscess formation to infiltration with fibroblasts and round cells (already present by day 3) and progressive destruction of tubules. Slight periglomerular fibrosis was apparent by day 7 but there was no thyroidization of tubules. Dilatation of tubules was found in infected and control rats given only sterile broth via the urethra. Microscopic areas of acute inflammation were still found as late as 100 days after infection, in kidneys with positive cultures. Pyelitis was present in all kidneys with pyelonephritis. Papillitis was a rare lesion, however, and generally took the form of interstitial inflammatory cells in the company of bacterial casts in collecting ducts that drained proximal areas of infection.

The trauma from the urethral injection was evident on microscopic examination of controls given sterile broth. In the fornix, there was disruption of the pelvic epithelium and hemorrhage for the first 48 hours. After that, the pelvic epithelium appeared to be intact but the

fornix was disrupted and the tubules passing through were replaced by fibroblasts. There was intrarenal hydronephrosis as indicated by dilated tubules in the medulla in the area proximal to the fornix. This is the area that most often showed pyelonephritis in infected animals.

The percentage of kidneys with active pyelonephritis (*ie*, interstitial infiltration with PMNs and a positive culture for *E coli* 0:111) at varying times is illustrated in Text-fig 1. The percentage of active infections did not decrease until after the first week although there were already fibroblasts and mononuclear cells present by day 3. There was a rapid fall in the number of bacteria recovered from the kidneys from day 1 when the mean score was 3.2 to a mean of 1.7 on day 3 and only 1.1 at the end of 1 week. This occurred despite the continued presence of gross abscess formation. At 100 days, 2 of 18 kidneys showed areas of active pyelonephritis within otherwise inactive, contracted scars. These were the only kidneys still infected with *E coli* 0:111.

The kidneys were identified as the source of the initial bacteremia by the following experiments. The right ureter was ligated and divided and the left renal vein cannulated with 0.047-inch outer-diameter silicone elastic tubing (Vivosil, Bard Parker) and obstructed distal to the cannula. Rats were given 50 units of heparin intravenously and blood was collected from the renal vein through the tubing into sterile tubes for 30-second periods. After a control bleeding from the renal vein, a 1.0 ml. suspension of *E coli* 0:111 was infused



TEXT-FIG 1—Incidence and activity of pyelonephritis after urethral infusion of 1.0 ml. *E coli* 0111:B4. Number of animals in each group is indicated at the top of each column.

into the urethra. Renal vein blood was collected for 30-second periods and, after 3 minutes, blood was obtained from the heart. Pour plates were made with each specimen of blood collected. Control samples from the renal vein were sterile but renal vein blood became positive during or within 30 seconds after the urethral infusion ended. Cardiac blood culture was also positive, however, indicating that bacteria entered the circulation by a route other than the renal vein. The left kidney is rich in collateral veins and so the experiment was repeated after ligating both ureters. No bacteremia was found in any of 4 animals. This excluded the bladder as the source of bacteremia and indicates that bacteremia originated from the "reflux" kidney. The exact spot where the bacteria gain entrance to the circulation is unknown but the rapidity with which bacteremia appears and the histologic evidence of pelvic hemorrhage suggest that the subepithelial blood vessels in the forvix of the renal pelvis are the entrance site.

E coli 0:111, 0.5 ml, via Urethra

The preceding experiments suggested either that bacteremia was necessary to initiate pyelonephritis in this retrograde model or that the injury from reflux necessary to initiate pyelonephritis also produced bacteremia. Varying the number of bacteria in the infusate from 10^6 to 10^9 bacteria or the volume between 1.0 and 0.7 ml did not change the incidence of pyelonephritis and volumes less than 0.4 ml produced neither bacteremia nor pyelonephritis. However, when 0.5 ml was used, there was some change in the relationship of bacteremia to pyelonephritis (Table 2). Only 30 of 55 animals developed bacteremia of $>10^3$/ml. As in the 1.0 ml group, however, over 90% of these animals developed pyelonephritis. In contrast to the previous experiment with 1.0 ml, 5 of 20 animals without demonstrable bac-

Table 2—Results of Urethral Infusion of 0.5 ml Volume of **E coli** 0111:B4 in Relation to Bacteremia

E coli/ ml blood	No. of animals	No. with pyelonephritis	Proportion of kidneys with pyelonephritis	Proportion of kidneys with (+) cultures
$>10^3$	30	28 (93%)	50/60 (83%)	56/60 (93%)
10^1–10^3	5	2 (40%)	3/10 (30%)	3/10 (30%)
$<10^1$	20*	5 (25%)	6/40 (15%)	10/40 (25%)
Total	55	35 (64%)	59/110 (54%)	69/110 (62.5%)

* Excludes 4 animals with bilateral pyelonephritis with proteus and 1 animal without pyelonephritis and enterococcus isolated from kidneys.

Table 3—Incidence of Pyelonephritis after Sterile Broth Was Infused into the Bladder and E coli 0111:B4 Injected Intravenously

No. of bacteria injected intravenously	Sterile broth via urethra	Gross or microscopic pyelonephritis/total kidneys
10^3	Yes	0/16 (0%)
10^4	Yes	2/22 (9.0%)
10^5	Yes	8/18 (44%)
10^6	Yes	21/30 (70%)
10^7	Yes	21/35 (60%)
10^8	Yes	10/14 (71%)
10^7 or 10^8	No	0/60 (0%)

teremia did develop small pyelonephritic lesions in 6 of 40 kidneys (15%) in a distribution similar to that in the bacteremia animals. The overall incidence of pyelonephritis was significantly lower in the 0.5 ml group than in the 1.0 ml group (χ^2, $P < 0.01$) due to the smaller percentage of animals with bacteremia.

Intravenous E coli and 1.0 ml Sterile Broth via Urethra

The strong positive correlation between bacteremia and pyelonephritis suggested that they were casually related. To determine whether bacteremia *per se* could initiate an infection, female rats were prepared as described above but were given 1.0 ml sterile broth into the urethra rather than bacteria. After 3 minutes, *E coli* 0:111 was injected intravenously into the tail vein. From Table 3, it can be seen that the 50% effective dose for initiating pyelonephritis was between 10^5 and 10^6 bacteria and the infection was produced regularly by 10^7 or more bacteria. The pathology of the infection was identical to that produced by retrograde infusion of *E coli*, including fornicitis and pyelitis. Pyelitis did not occur without concomitant pyelonephritis in this group. The last group in Table 3 emphasizes the importance of the reflux in increasing the susceptibility of the kidney to pyelonephritis. Thirty rats were injected intravenously with 10^7–10^8 *E coli* 0:111 but they were not given a preliminary urethral infusion. None developed pyelonephritis and all kidneys were sterile at 3 days.*

* All kidneys from the 75 rats in this experiment were cultured and the identity of *E coli* 0:111 was confirmed by agglutination tests with specific antisera. Eleven kidneys yielded either enterococcus (5), Aerococcus (3), or Proteus sp (3), which were felt to be contaminants introduced from the urethra. These 11 kidneys were excluded from Table 3.

Comparison with Obstructed Kidney

The susceptibility of the reflux model to hematogenous infection was compared to that of rats with a totally obstructed kidney. The left ureter was ligated and divided under ether anesthesia. Twenty-fours later, the animal was dehydrated overnight and then given 1.0 ml of sterile broth via the urethra (to produce reflux damage on the unobstructed right side). *E coli* 0:111 was injected in the tail vein. Table 4 shows that the reflux kidney was significantly more susceptible to 10^6 *E coli* ($P < 0.05$) than the obstructed side. At a dose of 10^8 bacteria, there was no significant difference between the obstructed and the reflux kidney.

Effects of Water Deprivation

Because the high incidence of bacteremia in these rats occurred after water deprivation, we studied its effect on the development of bacteremia. Table 5 shows the distribution of blood cultures and the incidence of pyelonephritis after 1.0 ml of *E coli* 0:111 via the urethra in hydrated and water-deprived rats. Blood cultures were positive in all water-deprived rats but in only 7 of 20 hydrated rats. All animals in both groups with bacteremia of $>10^3$/ml developed pyelonephritis. In addition, at the lower level of bacteremia (10^1–10^3), the water-deprived animals developed pyelonephritis and the other group did not. These two effects account for the significantly higher incidence of pyelonephritis in the water-deprived group.

Cystograms were performed on rats given no water overnight and on a group given water *ad libitum* in order to determine the effects of water deprivation on reflux. Rats given no water overnight were found to have nondistensible bladders so that ureteral reflux was observed after 0.2 ml had been infused. The renal pelvis was distended after 0.5 ml had been infused and rupture of the pelvis with retrograde filling of the renal vein was noted after 1.0 ml (Fig 1). Over half the infused volume appeared to be present in the ureters and renal pelvis. It was found that if the rats were given water to drink *ad li-*

Table 4—Hematogenous Pyelonephritis Due to **E coli** 0111:B4 in the "Reflux" Kidney and the Obstructed Kidney

E coli injected	Reflux kidney*	Obstructed kidney
10^6	10/20	2/20
10^8	13/22	9/22

* Number of kidneys with pyelonephritis/total kidneys.

Table 5—Effect of Water Deprivation on Bacteremia and Pyelonephritis after Urethral Infusion of **E coli** 0111:B4

Water withheld	E coli/ml of blood*			
	$>10^3$	10^1–10^3	$<10^1$	Total
Yes	18/18*	5/6	0/0	23/24
No	14/14	0/10	0/16	14/40

* Findings are the number of kidneys with pyelonephritis/total kidneys.

bitum the bladder distended during the cystogram and only minimal reflux was evident after 0.5 ml was infused. Reflux did occur at 1.0 ml but it was sometimes unilateral and only slightly distended the renal pelvis. Later in the experiment, under the stress of activity, the hydrated animals stopped drinking water, their bladders became noncompliant and reflux occurred equally in the water-deprived rats and the supposedly hydrated rats.

Discussion

This series of experiments reopens the question of whether ascending pyelonephritis due to an *E coli* infection can be created in rats with intact urinary tracts. The conditions of our experiment resulted in a high incidence of bacteremia and subsequent pyelonephritis. Furthermore, relatively low-grade bacteremia (produced by intravenously injecting 10^6 bacteria) caused pyelonephritis in female rats prepared by infusion of sterile broth into the urethra. The lesion produced by this maneuver duplicated the ascending lesion exactly; it involved the flat surface of the kidneys, radiated from the fornix and spared the poles and greater curvature (the usual sites of hematogenous pyelonephritis). Examination of the kidneys revealed that local trauma to the fornix produced by reflux was responsible for the distinctive distribution of the infection whether the bacteria entered the kidney retrograde from the renal pelvis or were injected intravenously.

There was an invariable association between bacteremia and the initiation of pyelonephritis if rats were deprived of water overnight and then given a volume of bacteria that exceeded the usual bladder capacity (*ie*, more than 0.5 ml). After finding that bacteremia originated in the kidney, we performed cystograms to determine the influence of overnight water deprivation on reflux. In rats given no water the night before, the bladders were contracted and nondistensible. Ureteral reflux occurred after only 0.2 ml had been infused. The complete 1.0 ml inoculum distended both renal pelves and ruptured into the renal veins. The bladders of hydrated animals, on the other hand,

distended to accommodate the infusion and only minimal reflux occurred after 0.5 ml had been given. Even after the infusion of 1.0 ml, reflux was not invariably evident in the cystograms and breaks in the renal pelvis were never seen.

It appears then that withholding water overnight may have been responsible for the higher incidence of pyelonephritis and bacteremia in our experiments than in experiments previously reported by Anderson and Jackson[3] and Heptinstall.[5] Heptinstall produced ascending pyelonephritis in almost all animals with ureteral infusion of 1.5 ml, followed by squeezing the distended bladder 20 times. The large volume and the bladder squeezing were both necessary to deliver enough bacteria to the kidney to initiate an infection. Heptinstall did find that these maneuvers resulted in heavy bacteremia in 5 of 12 of his animals and it is almost certain that he succeeded in forcing bacteria into the renal parenchyma through tears in the pelvis. He noted that evidence of forniceal hemorrhage was present in those animals that developed the most severe infections and emphasized the importance of this trauma in initiating ascending pyelonephritis. However, he felt that bacteremia played no role for two reasons: (1) the distribution of the lesions did not resemble the pattern seen with hematogenous infection of an obstructed kidney; (2) the level of bacteremia was insufficient to initiate pyelonephritis in obstructed kidneys. We have shown, however, that the distribution of the infection is the result of reflux damage to the pelvis rather than the route by which the bacteria reach the kidney; and that pyelonephritis can be produced by intravenously injecting only 10^6 bacteria because the reflux kidney is more susceptible to infection than the obstructed kidney. It is likely therefore, that bacteremia played an important role in Heptinstall's experiments, as it did in ours.

Smaller volumes infused into the bladder would be expected to cause less trauma to the renal fornices, less bacteremia and a lower incidence of pyelonephtitis. As anticipated, 20 of 55 rats given a 0.5 ml suspension of *E coli* did not develop bacteremia and only 6 of 40 kidneys showed evidence of pyelonephritis. Four kidneys showed only pyelitis. The first successful model of retrograde infection in rats was produced by injecting 0.6 ml of bacterial suspension via the urethra into hydrated rats.[3] There was less bacteremia than we found (2 of 24 versus 33 of 55) and most of the animals infected with *E coli* in that experiment developed isolated pyelitis. In later experiments, *E coli* was infused several times a week for weeks and produced pyelonephtitis.[4] Progression of pyelitis to parenchymal infection

as a result of repeated challenges was postulated. An alternative explanation for the effect of repeated infusions is that each retrograde infusion is associated with a certain probability of forniceal rupture and bacteremia, and a single such event is responsible for establishing pyelonephritis. On the basis of our cystograms, it can be anticipated that reflux damage to the renal pelvis and subsequent bacteremia would develop in some animals subjected to repeated challenges, especially if hydration was not complete between infusions or if the rat bladder was rendered nondistensible either by cystitis or several hours of water deprivation. *E coli* pyelitis may evolve into pyelonephritis but direct evidence for this is lacking.

Multiple retrograde infusions of bacteria have been used to study defense mechanisms against pyelonephritis.[7] It was found that increased clearance by the reticuloendothelial system (RES) produced by injection of endotoxin or active immunization with killed bacteria protected the rats from ascending pyelonephritis whereas RES blockade had an opposite effect. These observations are difficult to explain if one assumes that all the events leading to infection take place within the ureter and renal medulla. If, however, bacteremia plays an important role in initiating such infections, these alterations in RES function and immunity would effect clearance of bacteria and so prevent or promote renal infection.

Bacteremia and the disruption of the renal pelvis are important for initiating ascending pyelonephritis in rats with *E coli* but are apparently not necessary for other bacteria. When Klebsiella[3] and Proteus[8] are introduced into the bladder, they most often cause pyelonephritis rather than isolated pyelitis. Cotran *et al* carefully excluded bacteremia in their experiments with Proteus[8] and *E coli*.[9] They were able to document penetration of the renal pelvis by Proteus but not by *E coli*, using fluorescent antibody to the lipopolysaccharide.

On the basis of our observations, it seems that the pathogenesis of ascending pyelonephritis in rats due to *E coli* involves both direct entry of bacteria into the parenchyma through the damaged fornix and the recirculation of bacteria through pyelovenous communications back to the damaged kidney. (This phenomenon of indirect infection via the hematogenous route was predicted by Dominguez and Adams[10] from their work in dogs with radioactive Diodrast infused into the ureter). We cannot yet determine which mode of infection is more important, but both routes no doubt contribute in animals with bacteremia.

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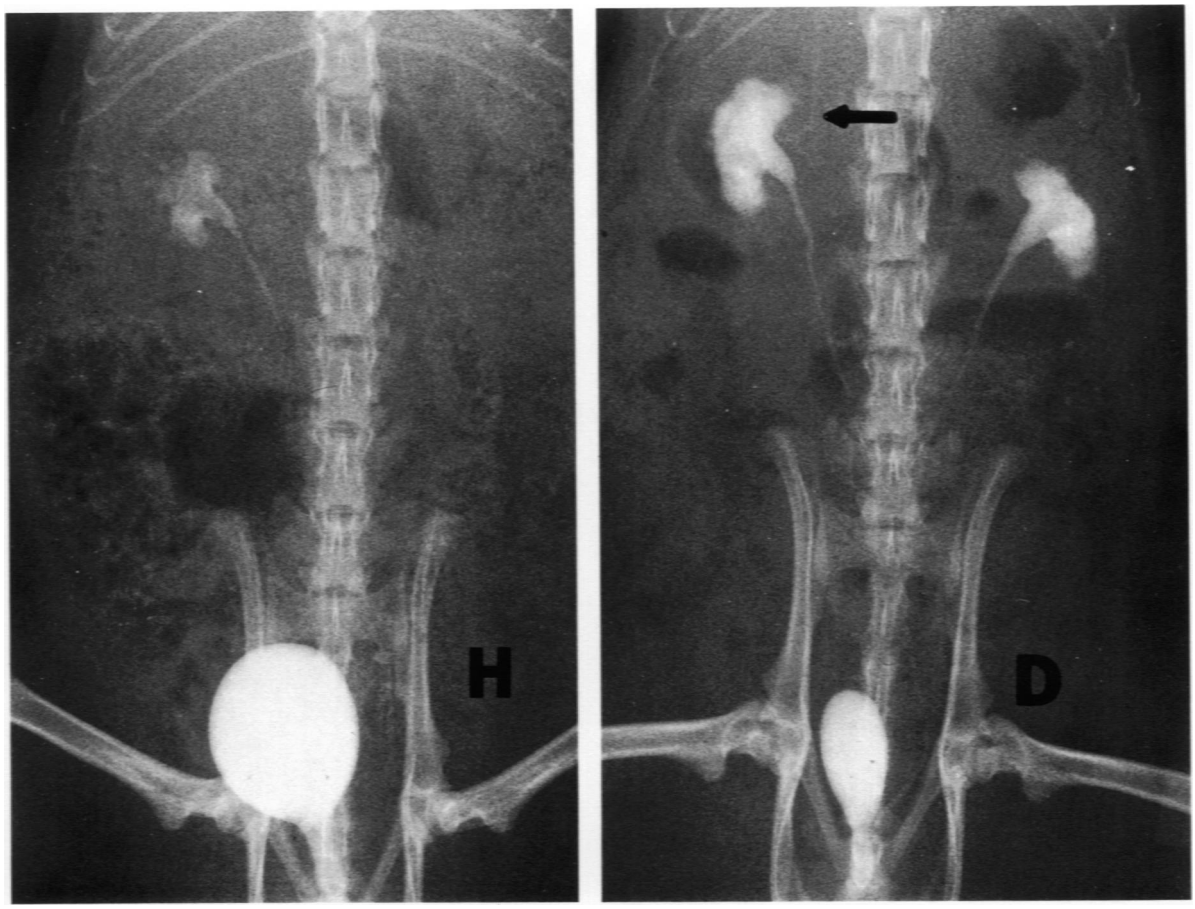

Fig 1—Cystograms on hydrated (*H*) and water-deprived (*D*) rats. The hydrated animal has a large bladder and minimal reflux on the right side. The water-deprived animal has a small contracted bladder and large volume of contrast material in the renal pelvis. Contrast material has refluxed into the right renal vein and inferior vena cava (*arrow*).

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