

## Urethral Obstruction of 6 Hours or Less Causes Bacteriuria, Bacteremia, and Pyelonephritis in Mice Challenged with "Nonuropathogenic" *Escherichia coli*

DAVID E. JOHNSON,<sup>1,2\*</sup> ROBERT G. RUSSELL,<sup>2,3,4</sup> C. VIRGINIA LOCKATELL,<sup>1</sup>  
JOSEPH C. ZULTY,<sup>4</sup> AND JOHN W. WARREN<sup>1</sup>

*The Division of Infectious Diseases, Department of Medicine,<sup>1</sup> Program of Comparative Medicine,<sup>3</sup> and Department of Pathology,<sup>4</sup> University of Maryland School of Medicine, and The Department of Veterans Affairs Medical Center,<sup>2</sup> Baltimore, Maryland 21201*

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Urethral obstruction may be caused by prostatic hypertrophy, urethral stricture, or encrustation of a urethral-catheter lumen. Bacteriuria often complicates these obstructions. The sequelae include fever, acute pyelonephritis, chronic renal inflammation, and death. We hypothesized that even brief obstruction of the urinary tract containing a nonvirulent bacterium would result in these complications. Mice challenged transurethrally with *Escherichia coli* FN414, which is rapidly eliminated from normal mice without causing bacteriuria, bacteremia, or renal pathology, were subjected to reversible urethral obstruction by coating the urethral meatus with collodion for 1, 3, or 6 h. The majority of mice obstructed for 1 h demonstrated parenchymal renal inflammation 48 h later. At the end of 3 h of obstruction, 9 of 10 mice were bacteremic; some bacteremias were present at 48 h after removal of the obstruction. At that time, more severe renal inflammation was seen in these mice. As little as 6 h of obstruction resulted not only in the acute changes described above but also in chronic renal inflammation and fibrosis in the majority of animals sacrificed 3 and 6 weeks later. Additional studies demonstrated that urethral obstruction enhanced the uropathogenicity of another nonpathogenic *E. coli* strain (K-12 strain HB101) and caused more severe renal lesions in mice challenged with *E. coli* CFT073, isolated from a patient with symptoms of pyelonephritis. These findings demonstrate that brief urethral obstruction may (i) induce organisms which are cleared rapidly from the normal urinary tract to cause bacteriuria, bacteremia, and pyelonephritis and (ii) intensify the renal lesions caused by a uropathogen.

In the presence of bacteriuria, obstruction of urine outflow is recognized clinically as a prelude to symptomatic urinary tract infection (4). Common causes of obstruction of urine outflow include prostatic hypertrophy, urethral stricture, and encrustations within the lumen of an indwelling urethral catheter. Although gradual in onset, complete obstruction of urine outflow is often short-lived because increasing discomfort prompts the patient to seek medical care. Complete obstruction may indeed be brief in patients with obstructed catheters, because lack of urine output is observable whenever urine collection bags are emptied, usually during each nursing shift. Nevertheless, if the patient is bacteriuric (even with relatively avirulent organisms, as is often the case in patients with long-term catheters in place), fever (21), bacteremia (4), and acute pyelonephritis (22) may develop. Nishi and Tsuchiya (16) demonstrated that urethral obstruction enhanced the uropathogenicity of *Pseudomonas aeruginosa* by using a strain which was uropathogenic in normal mice. We hypothesized that a nonpathogenic organism could also cause bacteriuria, bacteremia, and pyelonephritis following brief urethral obstruction. To test this hypothesis, we chose as the challenge organism an *Escherichia coli* strain which has been used as a nonpathogenic control by several investigators. This strain does not express known uropathogenic virulence factors and is eliminated rapidly from the urinary tracts of normal mice (5). We further assessed the effect of brief urethral obstruction on the uropathogenicity of

*E. coli* HB101, a K-12 strain, and *E. coli* CFT073, which is uropathogenic in normal mice.

### MATERIALS AND METHODS

**Bacteria.** *E. coli* FN414, serotype O19,O23 (K nontypeable) (kindly provided by Richard Hull, Baylor College of Medicine), is an organism isolated from the stool of a healthy child. This organism is susceptible to human and mouse sera, does not elaborate hemolysin, does not adhere to human or mouse uroepithelial cells, and does not express type 1 or P fimbriae (5). *E. coli* HB101 is a K-12 strain that has been used as a recipient in genetic-transfer experiments designed to identify bacterial-pathogenicity factors (2). It does not express type 1 or P fimbriae and is nonuropathogenic in normal mice. *E. coli* CFT073 is a urinary tract isolate from a patient with clinical symptoms of pyelonephritis. It expresses type 1, P, and S fimbriae and hemolysin and is uropathogenic in a dose-responsive fashion in normal CBA mice (13). For storage, these organisms were lyophilized onto sterile inert silica boiling stones (Cargille Scientific, Inc., Cedar Grove, N.J.) suspended in Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) plus 12% sucrose and were stored at 4°C. Inocula for mouse challenge studies were prepared by culturing a stone in 2 ml of TSB at 37°C for 6 h, subculturing onto Trypticase soy agar (TSA) slants, and incubating overnight at 37°C. Confluent growth was washed from the agar slants with sterile phosphate-buffered saline (PBS; pH 7.2). Bacterial suspensions were standardized to  $4 \times 10^{10}$  CFU/ml, which was confirmed by quantitative culture on TSA. Lower

\* Corresponding author.

concentrations of organisms required for some experiments were prepared as dilutions from the standardized suspensions and were confirmed by quantitative culture on TSA.

**Animals.** Female CBA/J mice (Jackson Laboratory, Bar Harbor, Maine) (22 to 24 g) were acclimated to the animal facility for 1 week after receipt from the breeder. During acclimation and throughout the experiment, the mice had ad libitum access to water and Purina Lab Chow. Twenty-four hours prior to each urinary tract challenge experiment, a specimen of spontaneously voided urine from each mouse was collected in a sterile petri dish. Urine was cultured on TSA and Levine eosin-methylene blue agar. Mice with bacteriuria of  $\geq 8 \times 10^2$  CFU/ml (our lower limit of detection) were not used.

Mice were anesthetized with methoxyflurane (Metofane; Pittman-Moore, Washington Crossing, N.J.). Periurethral and perianal areas were swabbed with 10% povidone iodine solution and then with a sterile swab soaked with sterile PBS. A sterile 25-mm-long polyethylene catheter (inside diameter, 0.28 mm; outside diameter, 0.61 mm; Clay Adams, Parsippany, N.J.) was gently inserted into the bladder through the urethra. A 30-gauge needle attached to a tuberculin syringe containing the bacterial suspension was inserted into the catheter lumen, 0.05 ml ( $2 \times 10^9$  CFU per mouse) was infused into the bladder over 30 s, and the catheter was then removed. We previously determined by culture and use of India ink that the inoculum does not reflux into the ureters by this procedure (7). Immediately after bacterial challenge, temporary urethral obstruction for 1, 3, or 6 h in some mice was accomplished by coating the urethral meatus with collodion (USP; J. T. Baker Chemical Co., Phillipsburg, N.J.). During urethral obstruction, mice were caged individually on absorbent paper to document the integrity of the collodion seal by the absence of urination. At the specified time, the collodion film was softened with acetone and removed from the urethral meatus. The mice then were caged in groups and cared for by the normal routine for up to 6 weeks. Blood specimens, collected from the retroorbital sinus at the time at which obstruction was relieved and 48 h after relief of obstruction, were cultured on TSA. The mice were sacrificed with an overdose of methoxyflurane, the abdomens were opened aseptically, and urine samples were aspirated from the bladders. The bladders were then rinsed twice with 0.1-ml volumes of sterile PBS by using sterile tuberculin syringes and needles. Each bladder and a segment of each kidney were removed aseptically, separately weighed, and separately homogenized in glass grinders (Kontes, Inc., Vineland, N.J.) containing TSB.

**Bacteriology.** Urine and homogenates of bladder and kidney tissues were separately cultured quantitatively on eosin-methylene blue agar by the spread plate technique with 10-fold dilutions. Results were expressed as CFU per milliliter or gram of specimen. The lower limits of detection were as follows: urine,  $8 \times 10^2$  CFU/ml; bladders,  $5 \times 10^2$  CFU/g; and kidneys,  $3 \times 10^2$  CFU/g. On the basis of the observations of Hagberg et al. (5), we recorded the number of animals with  $\geq 10^3$  CFU/g of kidney tissue 48 h after removal of the urethral obstruction as an index of uropathogenicity.

Slide agglutination with polyclonal antibody prepared in rabbits was used to document that urinary tract isolates from experimentally infected mice challenged with *E. coli* CFT073 and FN414 were the challenge organisms. Cross-reactivity with numerous *E. coli* strains was obtained when undiluted antisera were used. At a dilution of 1:64 for antiserum to *E. coli* CFT073 and a dilution of 1:16 for antiserum to *E. coli*

FN414, we obtained 4+ homologous slide agglutination. No agglutination was observed when these dilutions were used with suspensions of 10 enteric strains of *E. coli* isolated from CBA mice. These antiserum dilutions were used to identify isolates from experimentally infected mice as the challenge organisms. Since antiserum to *E. coli* HB101 was not available, we could not specifically identify urinary isolates from mice challenged with that organism. However, as was the case with mice challenged with the other strains, urinary specimens from mice challenged with *E. coli* HB101 grew pure cultures of *E. coli*.

Slide agglutination of urinary isolates from experimentally infected mice with guinea pig and human type O erythrocytes (with and without mannose) was used to further identify *E. coli* CFT073 and to determine whether mouse passage induced the expression of mannose-sensitive (type 1) or resistant P fimbriae in mice infected with *E. coli* FN414 and HB101.

**Histology.** A cross section of each kidney was fixed in 10% buffered formalin, pH 7.2. Tissue sections were stained with hematoxylin and eosin and examined by light microscopy. Pyelitis was defined as uroepithelial damage and infiltration of neutrophils into the epithelium. Purulent exudate accumulated in the lumen of the pelvic cavity. The lesions were classified as mild, moderate, or severe. This was based primarily on the extent and severity of damage to the uroepithelium. Moderate or severe pyelitis was usually accompanied by more-pronounced purulent exudate in the pelvic cavity, and in severe cases, inflammatory-cell infiltration was also present in the parenchyma immediately adjacent to the pelvis. Pyelonephritis was seen in mice examined at 48 h and 1 week postchallenge and was defined as infiltration of inflammatory cells, composed of moderate to large numbers of polymorphonuclear leukocytes, into the medulla and cortex. In mice examined at 3 and 6 weeks, the pyelonephritis was characterized by infiltration of lymphocytes, plasma cells, and macrophages and by fibrosis replacing the tubules in the medulla and cortex.

**Mouse lethality.** CBA mice were challenged intravenously with  $2 \times 10^7$  CFU (0.1 ml) of *E. coli* strain FN414 or CFT073 per mouse. Mortality was recorded for up to 1 week after challenge. At 1 week after challenge, the mice were sacrificed and blood samples, homogenates of spleen, and a segment of liver tissue were cultured quantitatively.

**Statistics.** Differences between groups were compared by Student's *t* test or by Fisher's exact test.

## RESULTS

**Urinary tract challenge experiments (i) Saline controls.** All blood and urine cultures and homogenates of bladder and kidney tissues were negative from mice (10 per group) challenged transurethrally with sterile saline, obstructed for 6 h, and sacrificed up to 1 week later (Table 1). Two of the mice showed mild pyelitis upon histologic examination of the kidneys, with low numbers of neutrophils in the lumen of the pelvic cavity and mild damage to the uroepithelium (Table 2).

**(ii) Mice challenged with *E. coli* FN414. (a) Unobstructed mice.** *E. coli* FN414 was not recovered from blood and urine samples or kidney homogenates of mice ( $n = 10$ ) at 48 h after challenge (Table 1; Fig. 1). Histologic examination of the kidneys showed mild pyelitis in 2 of 10 mice (Table 2). There were low numbers of neutrophils in the lumen of the pelvic cavity. The uroepithelium was intact (Fig. 2).

**(b) One-hour obstruction.** No renal lesions were observed

TABLE 1. Bacteremia at sacrifice in CBA mice challenged transurethally with saline or *E. coli* FN414 and subjected to reversible obstruction of the urethral meatus with collodion film for various intervals

Treatment and duration of urethral obstruction (h)	No. of bacteremic mice/no. of mice examined	
	When obstruction was relieved	48 h after obstruction was relieved
Saline (controls)		
6	0/10	0/10 <sup>a</sup>
FN414 (10 <sup>9</sup> CFU)		
0 <sup>b</sup>	0/10	0/10
1	1/10	2/10
3	9/10	3/10
6	9/10	4/10

<sup>a</sup> Sacrifice 1 week after obstruction removal.

<sup>b</sup> No unobstructed mice were bacteremic at any observation period (10 mice each at 0, 1, 3, 6, and 48 h after transurethral challenge).

histologically at the time at which the 1-h obstruction was removed. At 48 h after removal of the obstruction, there was a significant increase ( $P < 0.02$ ) in the number of mice with  $\geq 10^3$  CFU/ml or g of urine or kidney homogenate, respectively (Fig. 1). Increases in the number of bladder cultures with  $\geq 10^3$  CFU/g ( $P = 0.12$ ) and an increase in the number of mice with bacteremia ( $P = 0.20$ ) were not statistically significant (Table 1; Fig. 1). Histologic examination of the kidneys showed pyelitis in 8 of 10 mice examined (Table 2). Six of the mice had mild pyelitis. The two mice with moderate pyelitis showed necrosis of individual uroepithelial cells. The uroepithelium was intact. There were moderate numbers of neutrophils in the lumen of the pelvic cavity and occasional focal infiltrations of neutrophils in the adjacent parenchyma.

TABLE 2. Renal damage in unobstructed and obstructed mice at various time intervals after challenge with saline or *E. coli* FN414, HB101, or CFT073

Treatment and duration of urethral obstruction (h)	Time of sacrifice after removal of obstruction	No. of mice examined	No. of mice with renal pathology	
			Pyelitis	Pyelonephritis
Saline (controls)				
6	1 wk	10	2	0
FN414 (10 <sup>9</sup> CFU)				
0	48 h	10	2	0
1	0 h	10	0	0
	48 h	10	8	0
	3 wks	5	0	0
	6 wks	5	0	0
3	0 h	9	0	0
	48 h	10	9	0
	3 wks	5	1	2
	6 wks	5	0	1
6	0 h	9	5	0
	48 h	10	2	8
	3 wks	5	0	4
	6 wks	5	0	5
HB101 (10 <sup>9</sup> CFU)				
0	1 wk	10	7	0
6	1 wk	10	7	3
CFT073 (10 <sup>7</sup> CFU)				
0	1 wk	10	4	0
6	1 wk	9	5	3

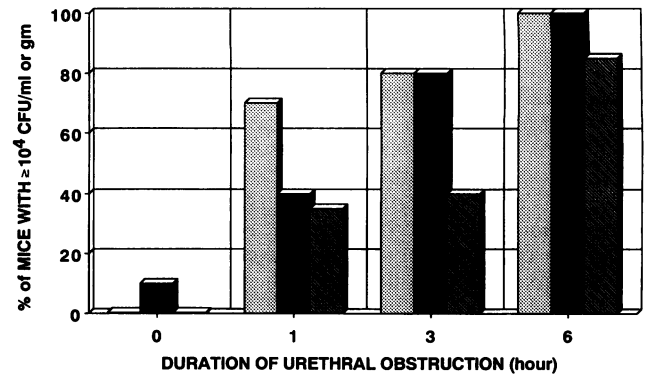


FIG. 1. Percentages of mice with urine, bladder, or kidney cultures of  $\geq 10^3$  CFU/ml or g. Mice were challenged transurethally with *E. coli* FN414 and subjected to reversible urethral obstruction for 1, 3, or 6 h. Counts are from specimens collected 48 h after removal of urethral obstruction. Dotted bars, urine; solid bars, bladder tissue; hatched bars, kidney tissue.

(c) **Three-hour obstruction.** Immediately after 3 h of obstruction, 9 of 10 mice were bacteremic ( $P < 0.001$  versus 0 h of obstruction;  $P < 0.001$  versus 1 h of obstruction [Table 1]). Five of the mice examined histologically showed dilation of the renal pelvis.

At 48 h after removal of the obstruction, 3 of 10 mice were bacteremic ( $P = 0.11$  versus 0 h of obstruction;  $P = 0.5$  versus 1 h of obstruction [Table 1]). Eight of ten mice had  $\geq 10^3$  CFU/ml or g of urine or bladder homogenate, respectively ( $P = 0.023$  versus 0 h of obstruction), and 8 of 20 kidney homogenates contained  $\geq 10^3$  CFU/g (Fig. 1). Histologic examination showed pyelitis in 9 of 10 mice (Table 2). The uroepithelial damage and inflammatory-cell infiltration were mild ( $n = 4$ ), moderate ( $n = 2$ ), or severe ( $n = 3$ ). In mice with severe pyelitis (Fig. 3), the uroepithelium showed localized areas of necrosis and disruption. There were bacterial colonies and moderate acute inflammatory-cell infiltration composed of neutrophils, with microabscesses in the adjacent subepithelial medulla and/or papilla.

At 3 weeks after removal of the obstruction, cultures of blood and kidney homogenates were negative for the challenge organism. Histologic examination showed chronic pyelonephritis in two of five mice and pyelitis in one mouse with localized inflammation in the renal papilla (Table 2).

At 6 weeks after removal of the obstruction, blood and kidney cultures were negative for the challenge organism in five of five mice. Histologic examination showed an area of healed pyelonephritis in the cortex of one of five mice (Table 2).

(d) **Six-hour obstruction.** Bacteriuria, colonization of the urinary tract, bacteremia, and renal lesions were most pronounced in mice obstructed for 6 h. Nine of ten mice were bacteremic when the obstruction was removed ( $P < 0.001$  versus 1 h of obstruction [Table 1]). Bacterial colonies invaded the parenchyma adjacent to the pelvic cavity and were located in the interstitium between tubules in five of nine mice. There was no inflammatory-cell infiltration (Fig. 4A).

At 48 h after removal of the obstruction, 10 of 10 mice had urine and bladder cultures of  $\geq 10^3$  CFU/ml or g, respectively, and 17 of 20 kidney cultures contained  $\geq 10^3$  CFU/g ( $P = 0.06$  [urine];  $P = 0.004$  [bladder];  $P = 0.002$  [kidney versus 1 h of obstruction]) (Fig. 1). Histologic examination showed pyelonephritis in 8 of 10 mice (Table 2). There was infiltra-

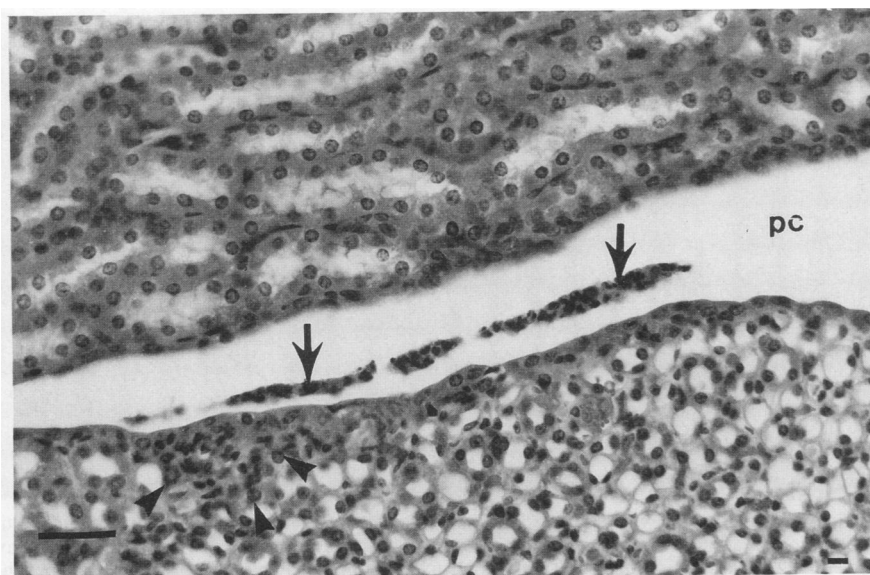


FIG. 2. Mild pyelitis in an unobstructed mouse infected with *E. coli* FN414 examined at 48 h postchallenge. Shown are low numbers of neutrophils (arrows) accumulating in the lumen of the pelvic cavity (PC) 48 h after inoculation, as well as focal infiltration of neutrophil inflammatory cells immediately subepithelial (arrowheads). The epithelium is intact. Bar, 85  $\mu$ m.

tion of moderate to large numbers of inflammatory cells, predominately neutrophils, immediately below the uroepithelium. The inflammatory-cell infiltrate extended into the medulla and cortex, replacing the tubules in the parenchyma (Fig. 4B).

At 3 and 6 weeks after removal of the obstruction, four of five and five of five mice, respectively, showed severe wedge-shaped pyelonephritis (Table 2). There was a prominent mononuclear cell inflammatory infiltrate in the cortex accompanied by marked fibrosis.

(iii) **Mice challenged with *E. coli* HB101.** Ten unobstructed mice were sacrificed 1 week after challenge with  $2 \times 10^9$  CFU of *E. coli* HB101 per mouse. The challenge organism was recovered at a concentration of  $\geq 10^3$  CFU/ml or g in 0 of 10 urine specimens, 1 of 10 bladder homogenates, and 1 of 20 kidney homogenates. Histologic examination of kidney sections showed mild pyelitis in 7 of 10 mice (Table 2).

Ten mice challenged with  $2 \times 10^9$  CFU of *E. coli* HB101 per mouse and obstructed for 6 h were sacrificed 1 week after removal of the obstruction. The challenge organism was recovered at a concentration of  $\geq 10^3$  CFU/ml or g in 4 of 10 urine specimens ( $P = 0.02$  versus unobstructed mice), 8 of 10 bladder homogenates ( $P = 0.002$ ), and 6 of 20 kidney homogenates ( $P = 0.03$ ). These mice showed mild ( $n = 1$ ), moderate ( $n = 5$ ), or severe ( $n = 1$ ) pyelitis or moderate to severe pyelonephritis ( $n = 3$ ), with extensive infiltration of macrophages, lymphocytes, and neutrophils accompanied by destruction of tubules in the medulla and cortex (Table 2; Fig. 5).

(iv) **Mice challenged with *E. coli* CFT073.** Ten unobstructed mice were sacrificed 1 week after challenge with  $2 \times 10^7$  CFU of *E. coli* CFT073 per mouse. The challenge organism was recovered at a concentration of  $\geq 10^3$  CFU/ml or g in 3 of 10 urine samples, 2 of 10 bladder samples, and 7 of 20 kidney samples. Moderate ( $n = 3$ ) to severe ( $n = 1$ ) pyelitis was observed in mice at 1 week after challenge (Table 2).

Ten mice challenged with  $2 \times 10^7$  CFU per mouse and obstructed for 6 h were sacrificed 1 week after removal of the obstruction. The challenge organism was recovered at a

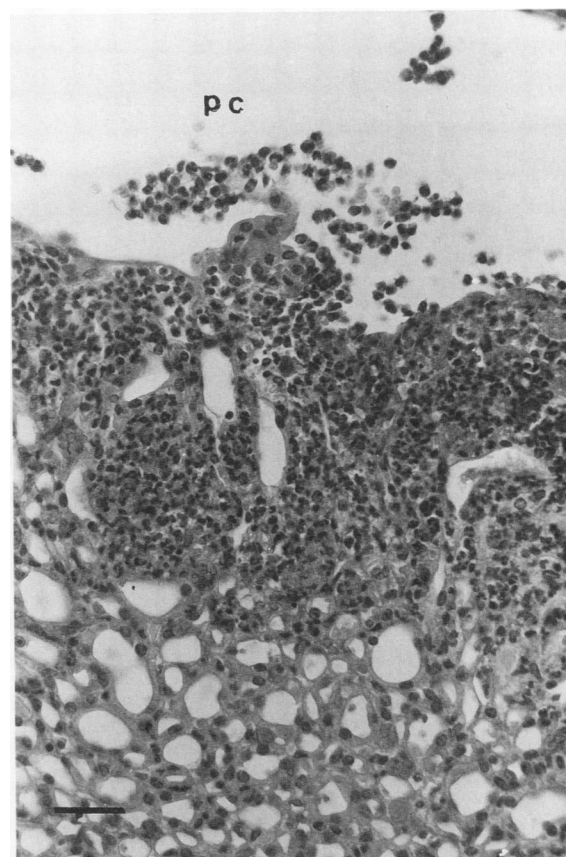


FIG. 3. Severe acute pyelitis in mice challenged with *E. coli* FN414, obstructed for 3 h, and sacrificed 48 h after removal of the obstruction. The uroepithelium lining the pelvic cavity (PC) is disrupted as a result of necrosis and loss of epithelial cells. The infiltration of moderate numbers of neutrophils extends into the parenchyma immediately adjacent to the pelvis. Bar, 36  $\mu$ m.

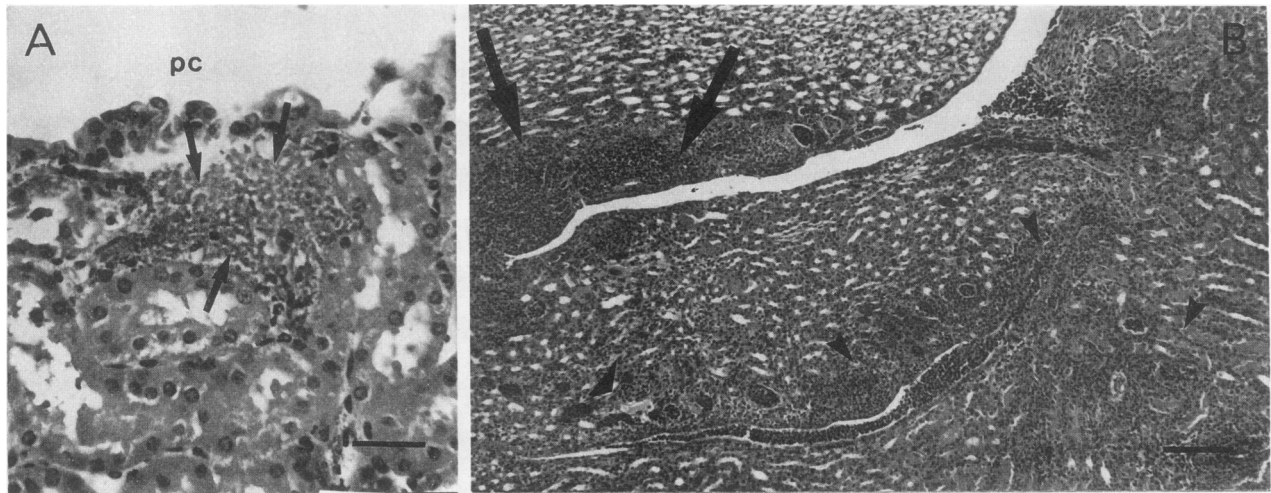


FIG. 4. Renal pathology in mice challenged with *E. coli* FN414 and obstructed for 6 h. (A) Sacrifice at the time of removal of the obstruction. Bacterial colonies are invading the renal parenchyma (arrows) immediately adjacent to the pelvic cavity (PC). There is no inflammatory-cell infiltration. Bar, 23  $\mu\text{m}$ . (B) Sacrifice 48 h after obstruction removal. Pyelonephritis extends from the pelvic fornix into the adjacent medulla (arrows). Severe inflammation replaces parenchyma deeper in the medulla, where the damage is extensive. Bar, 83  $\mu\text{m}$ .

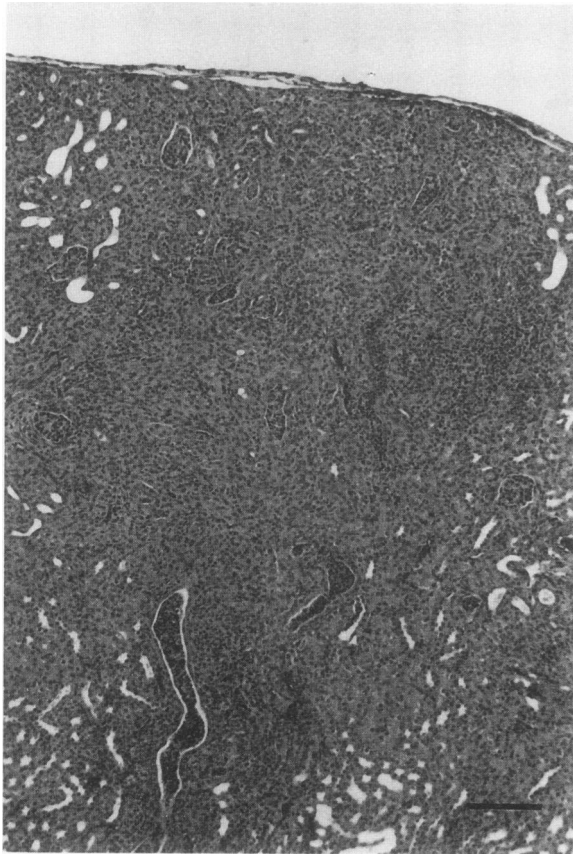


FIG. 5. Severe pyelonephritis in mouse challenged with *E. coli* HB101, obstructed for 6 h, and sacrificed 1 week after obstruction removal. There was diffuse infiltration of macrophages and neutrophils, with extensive destruction of the renal cortex. Bar, 400  $\mu\text{m}$ .

concentration of  $\geq 10^3$  CFU/ml or g in 8 of 9 urine samples ( $P = 0.0004$  versus unobstructed mice), 9 of 9 bladder samples ( $P < 0.0001$ ), and 17 of 18 kidney samples ( $P = 0.0001$ ). Moderate ( $n = 2$ ) to severe ( $n = 1$ ) pyelonephritis with microabscesses and diffuse infiltration of large numbers of macrophages and neutrophils replaced the tubules in the medulla and cortex. The remaining mice showed no renal lesions ( $n = 1$ ) or moderate ( $n = 1$ ) or severe ( $n = 4$ ) pyelitis.

**Agglutination.** Expression of type 1 and P fimbriae was detected by using guinea pig and human O erythrocytes with inocula and mouse isolates from mice challenged with *E. coli* CFT073 but not in mice challenged with FN414 or HB101. Isolates from mice challenged with *E. coli* FN414 or CFT073 were agglutinated only by homologous polyclonal antiserum. Contaminating organisms were not detected in the cultures of mouse specimens.

**Mouse lethality experiments.** No mortality was observed for 1 week after intravenous challenge with  $10^7$  CFU of *E. coli* strain FN414 or CFT073 per mouse. At 1 week after challenge with strain FN414, all blood, spleen, and liver cultures were negative for the challenge organism. For mice challenged with strain CFT073, the challenge organism was recovered at a concentration of  $\geq 10^3$  CFU/g in 1 of 10 blood specimens, 7 of 10 spleen homogenates, and 9 of 10 liver homogenates at 1 week postinfection.

## DISCUSSION

The mechanisms of urinary tract infections both experimentally induced in animals and naturally acquired in humans have been studied extensively. Expression of type 1, P, and other fimbriae, elaboration of hemolysin, aerobactin, and/or colicin, and serum resistance contribute to the uropathogenicity of *E. coli* in the normal urinary tract (19). Type 1 fimbriae bind to urinary mucus. Globoseries glycolipids on human (10) and CBA mouse (5, 6) uroepithelial cells are receptors for P fimbriae and aid in colonization by uropathogens.

Lomberg et al. (11) reported that 97% of *E. coli* urinary isolates from girls with recurrent pyelonephritis who did not have vesicoureteral reflux expressed P fimbriae. In contrast,

only 25% of *E. coli* strains isolated from girls with pyelonephritis who experienced vesicoureteral reflux expressed P fimbriae. These observations and those of Johnson et al. (8) indicate that the role of *E. coli* virulence factors in the establishment of urinary tract infection or for the development of urosepsis following that infection may be more important in the normal than in the compromised urinary tract.

Urethral obstruction caused by prostatic hypertrophy, urethral stricture, or a blocked indwelling urethral catheter may compromise the urinary tract. More than 100,000 nursing home patients in the United States at any given time have urethral catheters in place (23). Patients with long-term catheters frequently experience periodic bladder outflow obstruction (24). The great majority of these patients have a polymicrobial bacteriuria consisting of a mixture of known pathogens and organisms that are not usually recognized as uropathogens (24). The obstruction itself rather than subsequent catheter irrigation, removal, or insertion seems to be the important event leading to fever in these patients (21). This is supported by autopsy studies that indicate that obstruction is associated with the prevalence of acute pyelonephritis (22) and chronic renal inflammation (20a). Complete obstructions of urinary catheters may be quite brief; urine output is monitored at least once during each nursing shift, allowing early corrective action.

The present study tests the hypothesis that serious urinary tract infection may be caused in a briefly obstructed urinary tract by a nonpathogenic *E. coli* strain. *E. coli* FN414 is so lacking in pathogenic properties that it has been used as a negative control in previous studies of uropathogenicity (5). In normal mice, the organism was eliminated from the urinary tract in the present study within 48 h after transurethral challenge; there were no bacteriuria, bacteremia, or renal infections and no renal lesions. The absence of virulence factors was further documented by the absence of the challenge organism in spleen and liver cultures 1 week after intravenous challenge.

However, urethral obstruction for as short as 1 h caused increases in bacteriuria, bacteremia, and renal infection, with mild neutrophil infiltration in renal papillae. Some mice also developed inflammation of the uroepithelium lining the pelvic cavity. There were no permanent lesions in the kidney parenchyma. Slightly longer periods of obstruction of 3 to 6 h resulted in most mice developing bacteriuria and bacteremia and significantly increased pyelonephritis. This suggests that initial renal infection occurred along the uroepithelial lining of the pelvic cavity, with subsequent proliferation into the immediately adjacent interstitium. Subsequently, the inflammation spread into the medulla and cortex particularly from the pelvic fornix. These observations are consistent with previous studies documenting the pathogenesis of pyelonephritis in rats (1). The sequelae of this infection were loss of tubules and scarring, which were seen in five of five mice 6 weeks after the obstruction was removed.

Additional studies with two other *E. coli* strains were conducted to determine whether obstruction-induced uropathogenicity was unique to strain FN414. *E. coli* HB101 is a nonpathogenic K-12 strain which has been frequently used as a recipient and carrier of pathogenic factors in genetic-transfer experiments (2). Obstruction for 6 h after transurethral challenge resulted in an enhancement of uropathogenicity similar to that seen with strain FN414. Kidney cultures were negative for the challenge organism in all of 10 unobstructed mice but positive in 9 of 10 mice 1 week after a 6-h obstruction. We have previously reported (13) that 8 of

10 unobstructed mice challenged with  $10^9$  CFU of the human uropathogen *E. coli* CFT073 had kidney cultures of  $\geq 10^3$  CFU/g 2 days after transurethral challenge. In the present experiments, we challenged mice with  $10^7$  CFU so that an obstruction-induced increase in uropathogenicity, if present, would not be masked by a high positive rate in unobstructed mice. Obstruction significantly ( $P \leq 0.0004$ ) increased the number of mice with urine, bladder, and kidney cultures with  $\geq 10^3$  CFU/ml or g and induced more severe renal histologic changes. These results underscore those obtained with strain FN414 and further indicate that brief urinary tract obstruction may enhance the pathogenicity of a uropathogen either through enhancement of urinary tract lesions or by induction of infection with a lower initial challenge dose.

The initial result of urine obstruction may simply be to prevent elimination of the challenge organism through normal micturition, thus abrogating a very effective natural host defense mechanism by which inoculated organisms are flushed from the urinary tract before infection is established (17). The sequelae to obstruction-induced retention may include (i) bacterial proliferation and (ii) increase in bladder volume causing increased intraluminal pressure. This could result in bacterial translocation, the migration of organisms through the epithelium to regional lymph nodes (20), and/or increasing bladder pressure which may promote vesicoureteral reflux of bacteriuric urine into the pelvis and the tubular system of the kidney. In the present study, there was mild uroepithelial damage in the kidneys of 2 of 10 mice challenged with sterile saline and obstructed for 6 h. Direct bacterial damage to or invasion of pelvis or tubular epithelial cells with interstitial invasion could lead to bacteremia via direct entry into exposed adjacent blood vessels or indirectly after uptake by lymph nodes.

The fact that renal damage resulted following transurethral challenge with the nonuropathogens in obstructed mice is noteworthy. These organisms appear to lack demonstrable pathogenicity factors, and renal tissue damage may be the result of mouse leukocyte infiltration into the renal parenchyma in response to the bacterial invasion. This speculation is consistent with the observations of Brille and Glauser (3), who demonstrated that renal damage associated with bacterial infection was prevented by cyclophosphamide-induced neutropenia. The use of anti-inflammatory agents to prevent and/or treat upper urinary tract damage may be a useful adjunct to antimicrobial therapy.

Several studies have investigated the potential of bacterial fimbriae as immunogens to prevent urinary tract infections (12, 18). The challenge organism used here does not express type 1 or P fimbriae, organelles frequently implicated as *E. coli* uropathogenicity factors. However, with urethral obstruction, this nonuropathogen caused bacteremia, pyelonephritis, and fibrosis. Clearly, a type 1 or P fimbria vaccine would not have been effective in preventing urinary tract infection caused by this organism, since it does not elaborate these antigenic virulence factors.

Therefore, for patients who experience recurrent obstruction, such as those with long-term indwelling catheters, methods other than vaccination must be implemented for prevention of urinary tract infections. Development of catheters made from materials formulated to minimize adhesion of bacteria and urinary glycocalyx may decrease the incidence of obstruction. Preemptive catheter changes may preclude catheter obstruction (9, 15). Since *Proteus mirabilis* bacteriuria is associated with catheter obstruction because of crystal deposition (14), urine cultures and subsequent therapy for that organism may minimize catheter obstruc-

tion. For patients with other types of obstruction to urine flow, such as prostatic hypertrophy and urethral strictures, suitable measures to prevent or relieve obstruction will continue to be necessary.

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#### REFERENCES

- Andersen, B. R., and G. G. Jackson. 1961. Pyelitis, an important factor in the pathogenesis of retrograde pyelonephritis. *J. Exp. Med.* **114**:375-384.
- Boyer, H. W., and D. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41**:459-472.
- Brille, J., and M. P. Glauser. 1982. Protection against chronic pyelonephritis in rats by suppression of acute suppuration: effect of colchicine and neutropenia. *J. Infect. Dis.* **146**:220-226.
- Gleckman, R., N. Blagg, D. Hilbert, A. Hall, M. Crowley, A. Prichard, and W. Warren. 1982. Catheter-related urosepsis in the elderly: a prospective study of community-derived infections. *J. Am. Geriatr. Soc.* **30**:255-257.
- Hagberg, L., I. Engberg, R. Freter, J. Lam, S. Olling, and C. Svanborg Edén. 1983. Ascending, unobstructed urinary tract infection in mice caused by pyelonephritogenic *Escherichia coli* of human origin. *Infect. Immun.* **40**:273-283.
- Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg Edén. 1983. Contribution of adhesion to bacterial persistence in the mouse urinary tract. *Infect. Immun.* **40**:265-272.
- Johnson, D. E., C. V. Locketell, M. Hall-Craggs, and J. W. Warren. 1987. Uropathogenicity in rats and mice of *Providencia stuartii* from long-term catheterized patients. *J. Urol.* **138**:632-635.
- Johnson, J. R., P. L. Roberts, and W. E. Stamm. 1987. P fimbriae and other virulence factors in *Escherichia coli* urosepsis: association with patients' characteristics. *J. Infect. Dis.* **156**:225-228.
- Kunin, C. M., Q. F. Chin, and S. Chambers. 1987. Formation of encrustations on indwelling urinary catheters in the elderly: a comparison of different types of catheter materials in "blockers" and "nonblockers." *J. Urol.* **138**:899-902.
- Leffler, H., and C. Svanborg-Edén. 1981. Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. *Infect. Immun.* **34**:920-929.
- Lomberg, H., M. Hellstrom, U. Jodal, H. Leffler, K. Lincoln, and C. Svanborg-Edén. 1984. Virulence-associated traits in *Escherichia coli* causing first and recurrent episodes of urinary tract infection in children with or without vesicoureteral reflux. *J. Infect. Dis.* **150**:561-569.
- Lund, B., F. Lindberg, B. I. Marklund, and S. Normark. 1988. Tip proteins of pili associated with pyelonephritis: new candidates for vaccine development. *Vaccine* **6**:110-112.
- Mobley, H. L. T., D. M. Green, A. L. Trifillis, D. E. Johnson, G. R. Chippendale, C. V. Locketell, B. D. Jones, and J. W. Warren. 1990. Pyelonephritogenic *Escherichia coli* and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. *Infect. Immun.* **58**:1281-1289.
- Mobley, H. L. T., and J. W. Warren. 1987. Urease-positive bacteriuria and obstruction of long-term urinary catheters. *J. Clin. Microbiol.* **25**:2216-2217.
- Muncie, H. L., and J. W. Warren. 1990. Reasons for replacement of long-term urethral catheters: implications for randomized trials. *J. Urol.* **143**:507-509.
- Nishi, T., and K. Tsuchiya. 1978. Experimental urinary tract infection with *Pseudomonas aeruginosa* in mice. *Infect. Immun.* **22**:508-515.
- Norden, C. W., G. M. Green, and E. H. Kass. 1968. Antibacterial mechanisms of the urinary bladder. *J. Clin. Invest.* **47**:2689-2700.
- Pecha, B., D. Low, and P. O'Hanley. 1989. Gal-Gal pili vaccines prevent pyelonephritis by piliated *Escherichia coli* in a murine model. Single component Gal-Gal pili vaccines prevent pyelonephritis by homologous and heterologous piliated *E. coli* strains. *J. Clin. Invest.* **83**:2102-2108.
- Reid, G., and J. D. Sobel. 1987. Bacterial adherence in the pathogenesis of urinary tract infections: a review. *Rev. Infect. Dis.* **9**:470-487.
- Tacrede, C. H., and A. O. Andreumont. 1985. Bacterial translocation and gram-negative bacteremia in patients with hematological malignancies. *J. Infect. Dis.* **152**:99-103.
- Warren, J. W. Unpublished data.
- Warren, J. W., D. Damron, J. H. Tenney, J. M. Hoopes, B. DeForge, and H. L. Muncie. 1987. Fever, bacteremia, and death as complications of bacteriuria in women with long-term urethral catheters. *J. Infect. Dis.* **155**:1151-1158.
- Warren, J. W., H. L. Muncie, and M. Hall-Craggs. 1988. Acute pyelonephritis associated with bacteriuria during long-term catheterization: a prospective clinicopathological study. *J. Infect. Dis.* **158**:1341-1346.
- Warren, J. W., L. Steinberg, J. R. Hebel, and J. H. Tenney. 1989. The prevalence of urethral catheterization in Maryland nursing homes. *Arch. Intern. Med.* **149**:1535-1537.
- Warren, J. W., J. H. Tenney, J. M. Hoopes, H. L. Muncie, and W. C. Anthony. 1982. A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. *J. Infect. Dis.* **146**:719-723.