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Ascending, Unobstructed Urinary Tract Infection in Mice Caused by Pyelonephritogenic *Escherichia coli* of Human Origin

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A model for ascending unobstructed urinary tract infection was developed in mice to study the pathogenesis of urinary tract infection induced by *Escherichia coli* associated with urinary tract infection in humans. Specifically, the model was designed to monitor the initial stages of the infectious process, e.g., bacterial adhesion. Mice were selected since the specificity and intensity of bacterial attachment of pyelonephritogenic *E. coli* strains to human and mouse uroepithelial cells were similar. Female mice were infected by urethral catheterization and installation of bacteria in the urinary bladder. To maximize clearance of unattached bacteria, no obstructive manipulations were performed. After sacrifice, the persistence of bacteria in kidneys and bladder was determined by viable counts on homogenized tissues. The experimental infection was standardized by using one pyelonephritis (HU734) and one normal fecal (414) *E. coli* isolate. With both strains all of the bladders became infected, but *E. coli* 414 was eliminated more rapidly than HU734. The percentage of positive kidney cultures increased with the bacterial inoculum concentration and volume. An inoculum of 0.05 ml containing 10^{10} bacteria per ml was selected, giving the highest percentage of positive kidney cultures without detectable bacterial spread to the blood stream. The variation in the percentage of positive kidney cultures possibly depended on the degree of vesicoureteric reflux in the individual animals. Both in the kidneys and in the urinary bladders, strain HU734 yielded higher numbers of bacteria at 24 h and persisted longer than did strain 414. Several *E. coli* pyelonephritis isolates with properties associated with virulence in the human urinary tract consistently were recovered from mouse kidneys and bladders in higher numbers than *E. coli* strains of human fecal origin lacking those properties. The role of bacterial adhesion per se is the topic of the accompanying paper.

The healthy urinary tract is quite resistant to bacterial invasion (9, 26). Still, urinary tract infections (UTI) are a significant problem in humans and certain domestic animals. The low degree of susceptibility to UTI of most laboratory animals has been an obstacle in the search for useful animal models. Human UTI, on the other hand, comprises disease entities such as acute pyelonephritis with kidney parenchymal involvement, cystitis limited to the urinary bladder, and asymptomatic bacteruria. These conditions probably involve multiple bacterial virulence factors and mechanisms of host defense. Our aim was to study the initial interaction between isolates from human UTI and the tissues in the urinary tract, specifically the role of bacterial attachment. The mouse UTI model described here was developed since none of the numerous UTI animal models previously described was found to be directly applicable to this problem.

The experimental animal was selected to resemble the human in specificity of attachment, to permit study of *Escherichia coli* strains with adhesions relevant to human UTI. The majority of uropathogenic *E. coli* strains attach to human uroepithelial cells by specific binding to globoseries glycolipid receptors (14, 17; H. Lomberg, L. C Å. Hanson, B. Jacobsson, U. Jodal, H. Leffler, and C. Svanborg Edén, submitted for publication). Many strains express additional adhesins; those with "mannose"-recognizing adhesins alone attach poorly (12), but may associate with urinary slime (28) or phagocytes (21). Since globoseries glycolipid receptor-binding bacteria attached to uroepithelial cells from

mice, but not rats, and the globoseries glycolipids are the predominating nonacid glycolipid both in human (22) and mouse (1) kidney, mice were selected. This report presents the technical aspects as well as evidence that the mouse model distinguishes among strains of different uropathogenicity in the human. The role of adhesion per se and of different adhesins for bacterial persistence in the mouse urinary tract is the topic of the accompanying paper (11).

MATERIALS AND METHODS

Animals. Female CBA mice aged 6 to 8 weeks, housed five in each cage and fed pellets and tap water ad libitum, were used within a week of delivery from Anticimex (Stockholm, Sweden). The susceptibility to experimental UTI of the CBA mice was above average compared with BALB/c mice (11), C57 black mice, and C3H/HeN mice. The increase in susceptibility to experimental UTI in mouse strains with known immune defects will be the topic of a separate report (Svanborg Edén et al., manuscript in preparation). For anatomic reasons only female mice could be used.

Bacteria. *E. coli* strains HU734 and 414 were used to standardize the infection model. *E. coli* HU734 was a *lac*$^-$ nitrous acid mutant of the wild-type pyelonephritis isolate, GR12 (32; C. Svanborg Edén, R. Hull, S. Hull, S. Falkow, and H. Leffler, Nutr. Res., in press). It retained from the parent several phenotypic traits associated with virulence in the human urinary tract: serotype O75 K non typable, resistance to killing by human and mouse serum, and the ColV plasmid. However, it did not produce hemolysin. It carried adhesins binding both to GGR and to mannosides (*pap*$^+$*pil*$^+$ [11]). *E. coli* HU734 attached to human and mouse uroepithelial cells, and caused agglutination of human (mannose-resistant) and guinea pig (mannose-sensitive) erythrocytes. *E. coli* 414 was isolated from the stools of a healthy child and was selected since it lacked phenotypic traits associated with uropathogenicity: serotype O19,O23 K non typeable, sensitivity to killing by human but not mouse serum, no large plasmid, and no adhesin or hemolysin production. To test whether the mouse infection model differentiated among *E. coli* strains more or less virulent in the human urinary tract, the following strains were used. *E. coli* 4283, 1682, 3048, and O6K13 were isolated from the urine of patients with acute pyelonephritis. *E. coli* 525 and 913 were fecal isolates from healthy schoolchildren. *E. coli* Bam was kindly supplied by C. C. Brinton, Jr., Pittsburg, Pa.

The properties of the strains are summarized in Table 1. Serotype (18) and resistance to the bactericidal effect of serum (27) were tested as previously described. The ability to attach to human and mouse uroepithelial cells was assessed as described previously (31) and in the accompanying paper (11). The adhesins were classified regarding specificity for globoseries glycolipid receptors or "mannosides" by hemagglutination (31). Agglutination reversed by α-methyl mannoside (10 mg/ml) was designated mannose sensitive. Agglutination induced by coating of guinea pig erythrocytes with globotetraosylceramide was designated globotetraosylceramide sensitive.

Bacterial growth in mouse urine. To mimic in vitro conditions in the mouse urinary bladder, bacterial adhesion to mouse uroepithelial cells was tested in urine pooled from 50 CBA mice, undiluted and diluted in phosphate-buffered saline (PBS) (pH 7.2, 300 mOsm/liter). Furthermore, the ability of the different strains to multiply in mouse urine was compared with growth in broth (brain heart infusion broth; Difco, Sweden). From an overnight broth culture, 10^3 organisms were inoculated into tubes containing broth or mouse urine. The number of viable organisms and optical density were registered after different times of incubation.

Inoculum properties. For infection, the *E. coli* strains were transferred from deep agar storage cultures to lactose-bromthymol blue agar plates and grown over night in static Luria broth (19). After harvesting by centrifugation at 2,000 × *g* for 20 min, bacteria were resuspended in PBS to the appropriate concentration, as indicated by the optical density at 597 nm. The exact bacterial concentration was determined by viable counts on serial 10-fold dilutions in PBS. The adhesive and hemagglutinating properties of the inoculum strains were tested before infection.

Infection procedure. Before infection, the mouse urinary bladders were voided by gentle compression of the abdomen. A drop of urine was caught directly at the urethral orifice with a calibrated loop and spread on a lactose-bromthymol blue agar plate to test sterility. The animals were anesthetized with ether in a sealed glass jar or by intraperitoneal injection of 0.05 ml of sodium pentobarbital (50 mg/ml).

The inoculum, prepared and characterized as described below, was instilled into the urinary tract through a soft polyethylene catheter (outer diameter, 0.61 mm; Kebo Grave, Göteborg, Sweden) adapted to a 0.4- by 20-mm needle on a tuberculine syringe (ASIK, Denmark). After injection, the catheter was immediately withdrawn and no further manipulations were performed. Controls were injected with sterile PBS.

The establishment and persistence of bacteria in the mouse urinary tracts was monitored by sequential urine cultures until sacrifice. After sacrifice by cervical dislocation, kidneys and bladders were aseptically removed and homogenized in 0.5 ml of sterile PBS in a Teflon tissue grinder (A. Thomas Co., Philadelphia, Pa.), and viable counts were performed on serial dilutions in PBS plated on lactose-bromthymol blue agar plates. The number of bacteria is expressed as the number of CFU per entire tissue.

To optimize the experimental conditions separating most clearly between strains of different virulence, a mixed infection was performed with *E. coli* strains HU734 and 414. Mixed infections to correct for variation in the susceptibility to infection of individual animals have been used by a number of workers (10, 23, 30). The two strains used here were easily distinguished out of a mixture on lactose-bromthymol blue agar plates, since *E. coli* 414 fermented lactose and formed yellow colonies, whereas *E. coli* HU734 remained blue. Accordingly, the number of CFU of the respective strains in the inoculum mixture and in the homogenized tissues was determined by counting yellow and blue colonies. In this way, the relative ability of the two strains to persist in the urinary tract under different experimental conditions was compared directly for each mouse.

TABLE 1. Some virulence properties of the *E. coli* strains used

Strain	Serotype		Hemagglutination[a]		Resistance to serum killing[b]	Adhesion to uroepithelial cells		
	O	K	Human	Guinea pig		Human	Mouse	Mouse + urine[c]
HU734	O75	Knt[d]	GS	MS	R	59	37	48
4283	O6	K1	GS	MS	R	66	77	30
1682	O2	Knt	GS		R	106	40	38
3048	O4	K6, K12	GS	MS	R	97	85	61
O6K13	O6	K13		MS	R	0	73	65
414	O19, O23	Knt			S	0	0	0
525	ON	K1	MS	MS	S	23	13	8
913	O25	Knt			S	0	10	10
Bam	ON	K2	MS	MS	S	0	38	30

[a] MS, Agglutination reversed by α-methyl-D-mannoside; GS, agglutination reversed by globotetraosylceramide.

[b] R, Resistant; S, sensitive. Tested with human serum.

[c] The adhesion test was performed in mouse urine. Adhesion was measured as the mean number of bacteria attached per cell; 40 cells were counted.

[d] Knt, Kantigen not typable.

Statistical evaluation. The bacterial recovery is given as CFU per entire tissue. Due to large positive skewing of the recovery values a transformation to a logarithmic scale was undertaken and the geometric mean used for statistical analyses. Differences in ability to persist in the mouse urinary tract were evaluated for significance with the Student *t* test. The variations in infection rate, due either to difference in susceptibility or to variations in administration of the inoculum, were then evaluated by standard parametric techniques. In comparing the relative recovery of HU734 and 414 from individual animals, animals with sterile kidney cultures were excluded. In groups of mice infected with different strains, sterile kidneys were assigned the value of 1 CFU.

Morphology. *E. coli* strain HU734 was used for morphological studies. At different times after inoculation, animals were sacrificed, and the right kidney and urinary bladder were taken out. Half of the bladder and the kidney were homogenized, and bacterial cultures were performed. The other half of the bladder was used to register bacterial adhesion by scanning electron microscopy or inflammation in thick sections. Sections about 3 μ thick were made after fixation in 10% Formalin on paraffin-embedded tissues and stained with hematoxylin and eosin.

Tissue fixation for scanning electron microscopy. Bladder, ureters, and kidney tissues in their intact form were fixed overnight at 4°C in 0.1 M cacodylate buffer (pH 7.2) containing 0.5% glutaraldehyde. The next day these fixed tissues were washed three times with cacodylate buffer. They were then shipped to J. W. Costerton's laboratory in Calgary, Canada, by air freight. Upon arrival, they were immediately washed two more times with cacodylate buffer and then postfixed with 2% osmium tetroxide in cacodylate buffer for 1.5 h. The tissues were made conductive by the modified procedure of Malick and Wilson (20) for thiocarbohydrazide.

After the thiocarbohydrazide procedure, the tissues were transferred to a 10-step ethanol-water dehydration series ending in 100% ethanol. They were then transferred into a 10-step freon 113-ethanol series ending in 100% freon 113. In between each dehydration step, a 30-min interval was allowed for equilibration. From 100% freon 113 the tissues were then critical point dried by the method of Cohen et al. (7), mounted on stubs, and sputter coated with gold-palladium. A Hitachi model 450 scanning electron microscope was used at an accelerating voltage of 20 kV, and Ilford FP4 panchromatic film was used to photograph the specimens.

Vesicoureteric reflux. The degree of vesicoureteric reflux and its contribution to bacterial spread to the kidney was evaluated in two steps. (i) Bacteria suspended in India ink were injected intravesically at increasing volumes and speed. After immediate sacrifice the occurrence of ink in ureters was observed, and viable counts were performed on the homogenized kidneys. (ii) [^{14}C]mannose (ICM Pharmaceuticals, Irvine, Calif.; lot no. 76-2362) was used as a tracer. After injection of 0.05 ml, the urethra was closed with superglue. The animals were bled to detect leakage into the blood stream of labeled sugar. Kidneys and filled bladders were removed after 5 min. The tissue was dissolved in 5 ml of Protosol (New England Nuclear Corp., Boston, Mass.), and a 1-ml sample was suspended in scintillation liquid and counted in a Hewlett-Packard scintillation counter. Quench controls, consisting of homogenized tissue with increasing concentrations of labeled sugar directly added, were included.

RESULTS

Model studies. Ninety percent of the uninfected mice had sterile urine cultures; 10% harbored an O8 lactose-negative *E.coli* strain in the urethra. Cultures of homogenized bladders and kidneys from uninfected animals were sterile. In animals catheterized and injected with sterile PBS, tissues were sterile after 24 h.

The ether anesthesia lasted for about 30 s compared with about 1 h for the sodium pentobarbital. In spite of the different times the inocu-

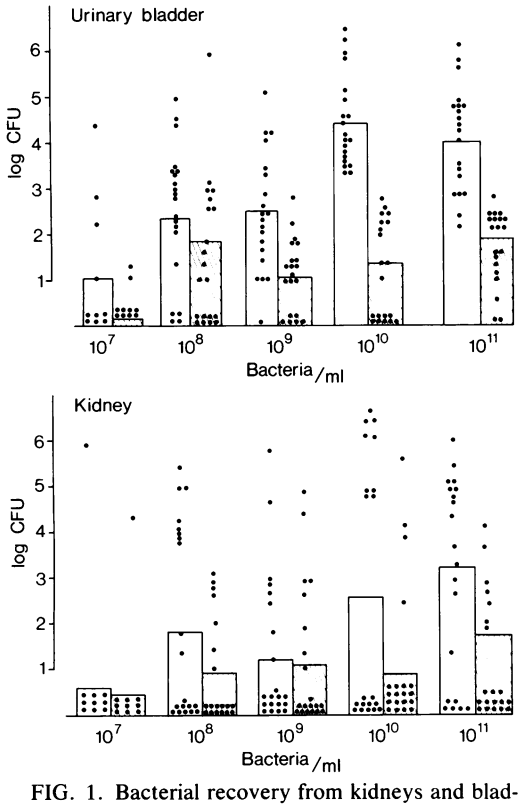


FIG. 1. Bacterial recovery from kidneys and bladders of CBA mice 24 h after inoculation with 0.05 ml of a mixture of *E. coli* HU734 (open bars) and 414 (hatched bars) at different inoculum concentrations. The histogram columns represent the mean recovery of each strain measured on a logarithmic scale. Kidneys and bladders with no detectable recovery were counted as if 1 CFU was found. The relation between the two strains is also illustrated by calculating the recovery ratio (HU734/414) within each animal. All ratios, except kidneys at 10^9 bacteria per ml, showed a significantly larger recovery of HU734 ($P < 0.01$) by the Student t test; the maximum ratio was 1,259 in the bladder and 6,310 in the kidney at 10^{10} bacteria per ml.

la were held in the urinary bladders, no significant difference in bacterial persistence after 24 h was seen (data not shown).

Inoculum properties. The recovery of *E. coli* HU734 and 414 from a mixture was evaluated for effects of increases in bacterial concentration at a fixed inoculum volume (Fig. 1) or increases in inoculum volume at a fixed bacterial content (Table 2). Increasing inoculum volumes (0.05, 0.1, and 0.2 ml) were tested with a total bacterial content of 5×10^7 bacteria. The number of animals with positive kidney cultures and the mean number of CFU recovery increased with increasing volume (Table 2).

At an inoculum volume of 0.05 ml, the mean bacterial recovery from kidneys and bladders in groups of 10 animals increased with increasing inoculum concentrations up to 10^{10} bacteria per ml and then leveled off (Fig. 1). The increase was due to an increasing number of animals with positive cultures, rather than to an increase in the recovery from each animal.

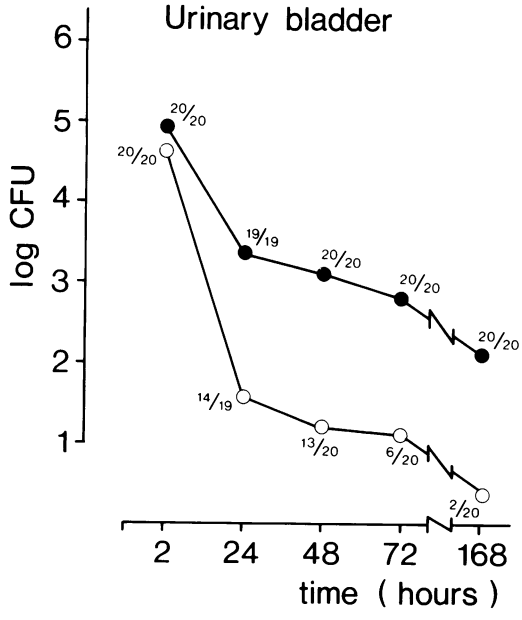
Dissemination of bacteria into the bloodstream was tested in six groups of 10 mice receiving different inoculum volumes. The animals were sacrificed within 5 min of infection, and blood cultures were obtained by cardiac puncture. At inoculum volumes of 0.05 ml, all blood cultures were sterile; at 0.1 and 0.2 ml, 1 of 20 and 8 of 20, respectively, were positive.

Infection kinetics. Groups of animals inoculated with 0.05 ml of 10^9 bacteria (a mixture of HU734 and 414) per ml were followed with urine cultures and sacrificed at 2, 24, 48, 72, and 168 h after infection. Kidneys and bladders were homogenized, and viable counts were performed. *E. coli* 414 was excreted rapidly, whereas *E. coli* HU734 persisted (Fig. 2). After 7 days (168 h) *E. coli* 414 was completely eliminated, whereas 20 of 20 animals had positive bladder cultures and 6 of 20 animals had positive kidney cultures for *E. coli* HU734. A steady decrease in bacterial numbers occurred in the bladder. In the kidneys the recovery of *E. coli* HU734 increased up until day 3. The frequency of animals with bacteruria decreased (Fig. 3), but bacteruria was found to have an inconstant relationship to positive cultures from kidneys and bladders in that 12 of 20 animals had sterile urine cultures 168 h after

TABLE 2. Influence of inoculum volume on bacterial recovery from kidneys and bladders 24 h after inoculation[a]

Inoculum vol (ml)	No. of animals with bacterial recovery/total				Geometric mean CFU			
	Bladders		Kidneys		Bladders		Kidneys	
	HU734	414	HU734	414	HU734	414	HU734	414
0.05	14/14	8/14	7/14	4/14	1,175	117	126	33
0.1	14/14	9/14	7/14	5/14	4,114	162	174	67
0.2	14/14	12/14	11/14	11/14	24,346	540	1,004	603

[a] A mixture of *E. coli* HU734 and 414 containing 5×10^7 bacteria in various inoculum volumes was used.



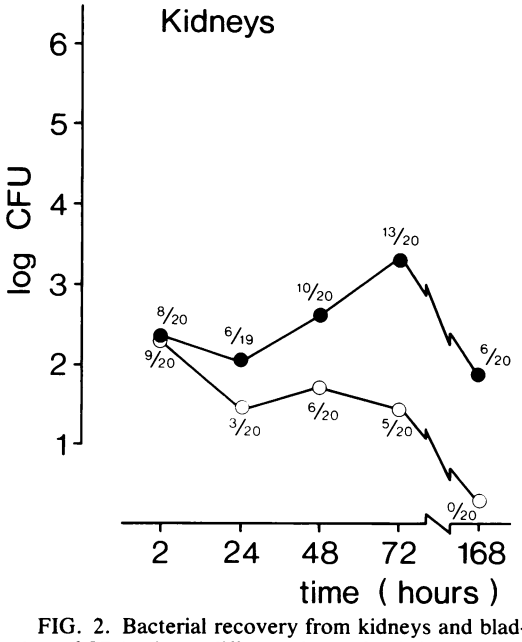


FIG. 2. Bacterial recovery from kidneys and bladders of CBA mice at different times after infection with 0.05 μl of a mixture of *E. coli* HU734 (●) and 414 (○). Kidneys and bladders with no detectable recovery were counted as if 1 CFU was found. The numbers above the circles represent numbers of infected tissues/total number of tissues. The relation between the two strains is also illustrated by calculating the recovery ratio (HU734/414) within each animal. All ratios, except in animals sacrificed after 2 h, showed significantly larger recovery of HU734 ($P < 0.01$) in the kidneys by the Student t test; the maximum was 100 in the bladder after 48 h and 632 in the kidney after 72 h.

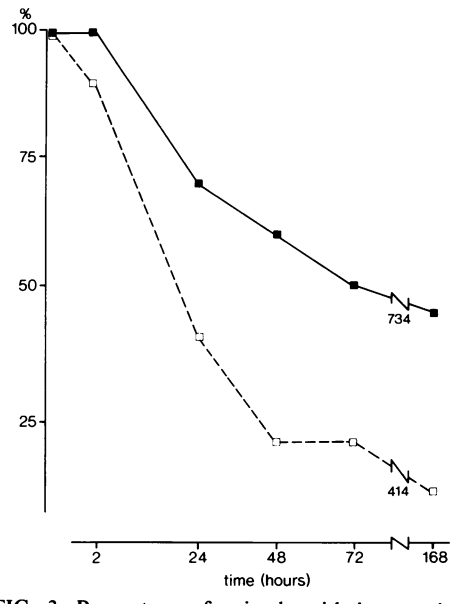


FIG. 3. Percentage of animals with bacteruria at different times after inoculation with a mixture of *E. coli* HU734 (●) and 414 (○).

infection while kidney or bladder cultures (or both) were still positive. Bacteruria was subsequently not used to monitor infection.

Morphology. Microscopy revealed signs of inflammation in kidneys and bladders (Table 3) (Fig. 4). Inflammatory cells were first detected about 4 h after bacterial inoculation. Most animals with positive kidney cultures had pyelitis with intraepithelial and subepithelial infiltration of granulocytes and accumulation of granulocytes in the renal pelvis (Fig. 4b), but without parenchymal involvement. Of the 42 animals studied, 2 had minimal pyelonephritis, i.e., focal renal tissue involvement. Even after 3 weeks, no macroscopic changes were observed in kidneys. One-third of the animals still had pyelitis. Even after the exhaustive washing and dehydration steps during tissue preparation for scanning electron microscopy, bacteria were seen adhering firmly and either in clusters or singly to the mucosal lining of bladders and kidney 24 h after infection (Fig. 5a and b).

Experimental protocol subsequently used. An inoculum of 0.05 ml containing 10^{10} bacteria per ml was chosen since it gave a high percentage of positive kidney cultures without hematogenous dissemination. To focus on the initial stages of infection, sacrifices were made after 24 h. Animals with positive urine cultures before infection were eliminated. The bacterial recovery was given as the number of CFU in each tissue.

TABLE 3. Proportion of mice with histological changes after intravesical infection with *E. coli* HU734

Time after infection	Animals with cystitis	Animals with pyelitis	Positive culture at time of sacrifice	
			Bladder	Kidney
2 h	0/6	0/6	6/6	4/6
4 h	3/6	2/6	6/6	4/6
24 h	6/6	3/6	6/6	5/6
48 h	6/6	3/6[a]	6/6	4/6
4 days	4/6	2/6	6/6	3/6
12 days	3/6	3/6[a]	6/6	3/6
21 days	1/6	2/6	6/6	2/6

[a] One animal in the group had pyelonephritic changes.

Variation in the experimental model. The reproducibility of the method as specified above was analyzed for groups of 10 mice infected with *E. coli* HU734. All urinary bladders became infected. The variation coefficient calculated from the log bacterial recovery was 34% among animals within each experiment. The variation coefficient of the mean recovery from groups of 10 mice among experiments was 13%. The percentage of kidneys with positive cultures varied. The mean number of infected animals calculated from 10 groups with 10 animals in each group was 5.9 with the standard deviation 1.97.

Vesicoureteric reflux. Attempts were made to measure vesicoureteric reflux, a factor possibly explaining the variation in kidney infection rate. The largest volume of urine collected from a single animal before anesthesia was 0.15 ml. Immediate reflux, seen as transport of india ink into the renal pelvis, occurred in 10 of 10 animals inoculated at high pressure with 0.1 to 0.25 ml. At a lower inoculum pressure, 0.25 ml could be injected without any dye ascending the urethers. At a volume of 0.05 ml, reflux occurred in 9 of 29 animals. Reflux of ink correlated with positive kidney cultures ~5 min after infection. No difference was found between sodium pentobarbital- or ether-anesthetized animals. The recovery of [^{14}C]mannose from the bladder occluded immediately after injection varied form 30 to 90% (mean, 64%) of the inoculum counts. None of the kidneys contained more than 0.01% of the radioactivity injected.

Virulence of different *E. coli* strains tested in the mouse model. Under all experimental conditions HU734 was recovered from both kidneys and bladders in significantly higher numbers than was 414. This suggested that the infection model differentiated between *E. coli* strains more or less virulent in the human urinary tract. The hypothesis was tested with other pyelonephritis or normal fecal isolates (Table 1). The pyelonephritis strains were consistently recovered from the mouse kidneys in higher numbers than the fecal or laboratory strains (Table 4). The same tendency was seen in the bladders, with two exceptions. The pyelonephritis strain 1682, recovered from the bladder in low numbers, lacked mannose-sensitive adhesins, and the fecal strain 525, recovered in high numbers, had such adhesins. To assess the influence of mouse urine on the bacterial inoculum, adhesion testing and bacterial growth were tested in mouse urine.

The adhesive properties of the strains was not significantly altered in the presence of mouse urine (Table 1). Mouse urine supported bacterial growth less well than did broth. The bacteriostatic effect of urine was equally pronounced for all strains and, thus, is not a probable reason for the difference in recovery between pyelonephritic and fecal isolates (Fig. 6).

DISCUSSION

A mouse model for ascending UTI was developed to study virulence mechanisms of urinary tract pathogens relevant to human UTI. The model was designed specifically for the study of initial events in the infection process. Hematogenous (5), intraureteral (3), intraurethral (8), and intravesical (2, 4, 6, 13, 16, 24, 25, 29, 34) infection routes have been used in experimental UTI. In analogy with human UTI, the ascending rather than hematogenous route was preferred. Colonization of the urinary tract by bacteria from the intestine has not yet been achieved experimentally. The infection of the mouse kidney was assumed to be ascending rather than hematogenous, since no bacterial spread to the circulation was detected with the protocol used. As in humans, the ascent of bacteria from bladder to kidney should be facilitated by vesicoureteric reflux. The results with labeled sugar suggested that <0.01% of the inoculum reached the kidney. Reflux, measured as ascent of india ink and bacteria into the renal pelvis, could always be provoked either by increasing volume or pressure. A variable degree of reflux might explain the variation in bacterial recoveries from kidneys of different animals, but not the differ-

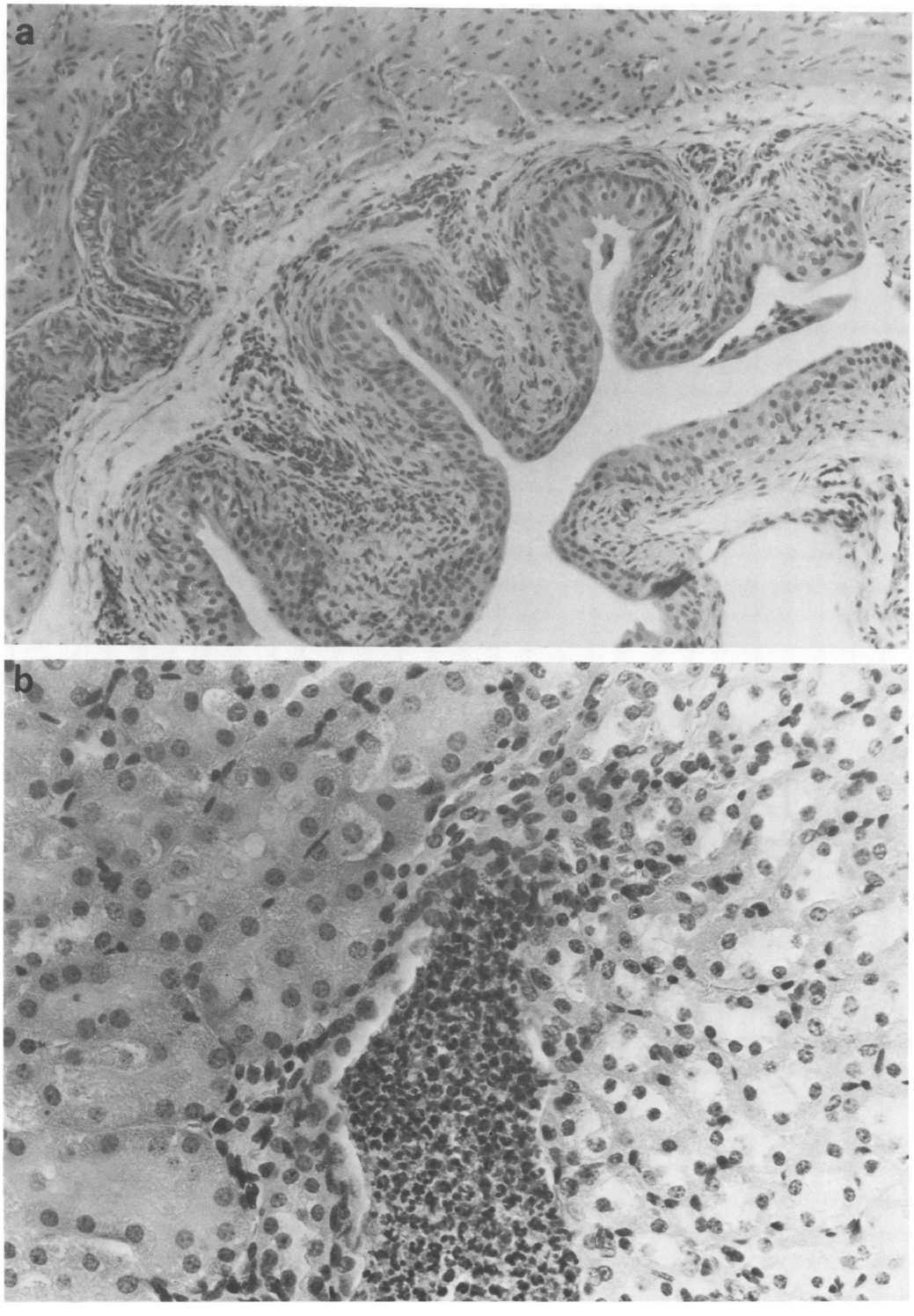


FIG. 4. Inflammatory changes in kidney and bladder. Sections were 3 μm thick, stained as described in the text. (a) Photomicrograph of an infected urinary bladder showing perivascularly situated inflammatory cells. (b) Photomicrograph of an infected renal pelvis with heavy accumulation of mainly granulocytes in the pelvis lumen.

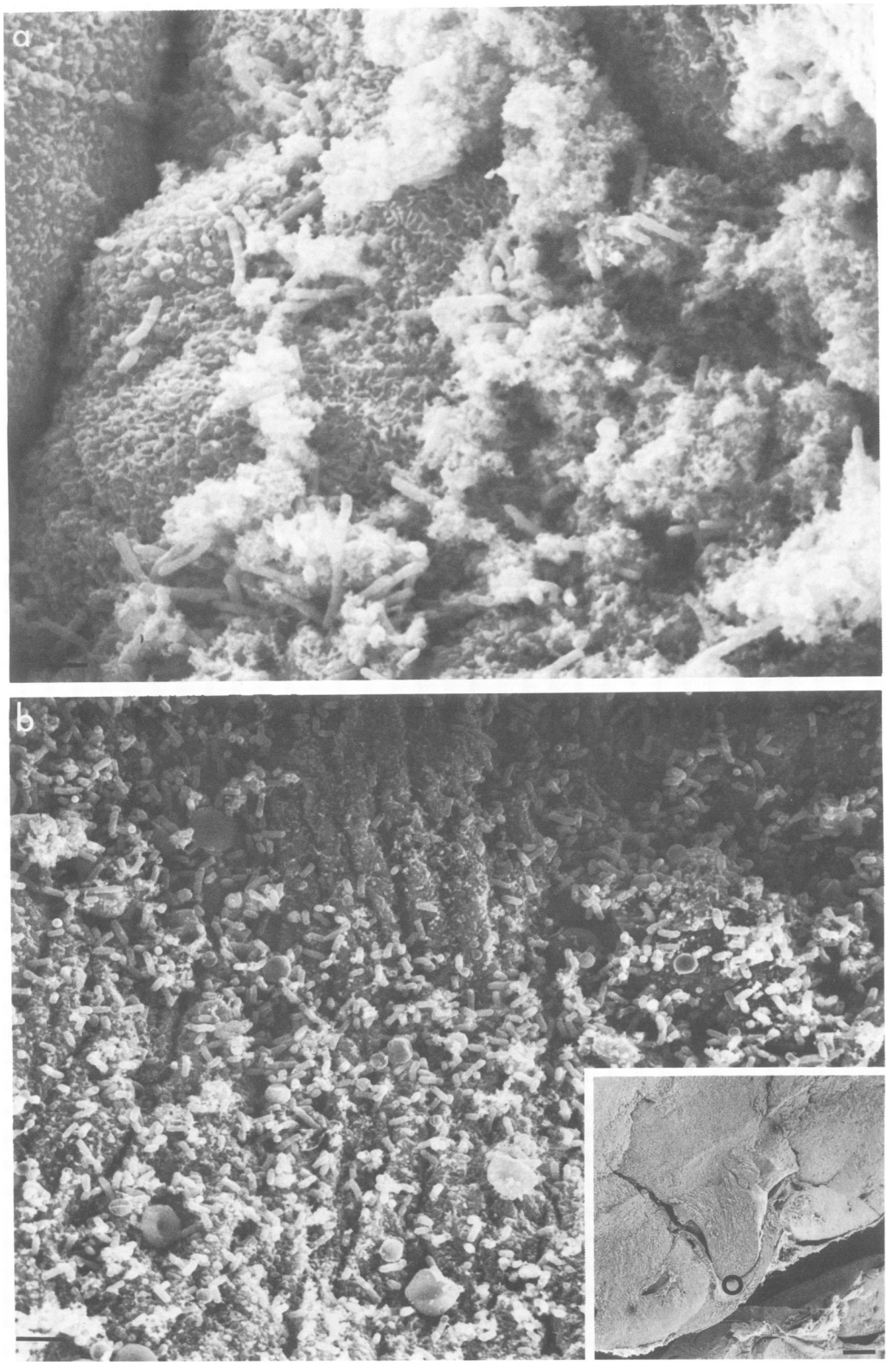


FIG. 5. Bacterial attachment in vivo, 24 h after infection. (a) Scanning electron micrograph of bladder mucosa with bacteria adhering either singly or in clusters. Bar, 5 μm. (b) Scanning electron micrograph of kidney pelvis mucosa showing heavy population of bacteria adhering all over the surface. Bar, 5 μm. This area of interest is the same as the circled area of the inset; inset bar, 500 μm.

TABLE 4. Bacterial recovery from bladders and pairs of kidneys infected with different *E. coli* strains

Strain	Origin[a]	Kidney		Bladder	
		Infectivity rate[b]	Mean CFU	Infectivity rate	Mean CFU
HU734	Py	17/30	2,630	30/30	16,595
4283	Py	17/31	2,748	27/32	2,765
1682	Py	20/31	17,732	27/32	346
3048	Py	34/47	578	45/47	2,757
O6K13	Py	26/40	1,358	38/40	17,090
414	F	8/28	33	16/28	117
525	F	5/10	68	9/10	1,467
913	F	5/10	38	9/10	149
Bam	Lab	7/10	194	3/10	33

[a] Isolated from the following: Py, patient with pyelonephritis; F, fecal flora of healthy children; or Lab, laboratory strain.

[b] Number of animals with bacterial recovery/total number of animals.

ence in persistence of bacterial strains in individual animals.

The infection endpoint studied in most previous UTI models is morphological or functional changes resulting from pyelonephritic infections. Bacterial growth after 24 h was selected as a better measure of the early bacterial establishment in the urinary tract. This endpoint assumed that bacteria associated with the mucosa would still be viable and that others would be excreted at this time. The infection provoked several signs of inflammation known from human UTI, e.g., leukocyturia and hematuria. Activation of host defense mechanisms in the tissue was indi-

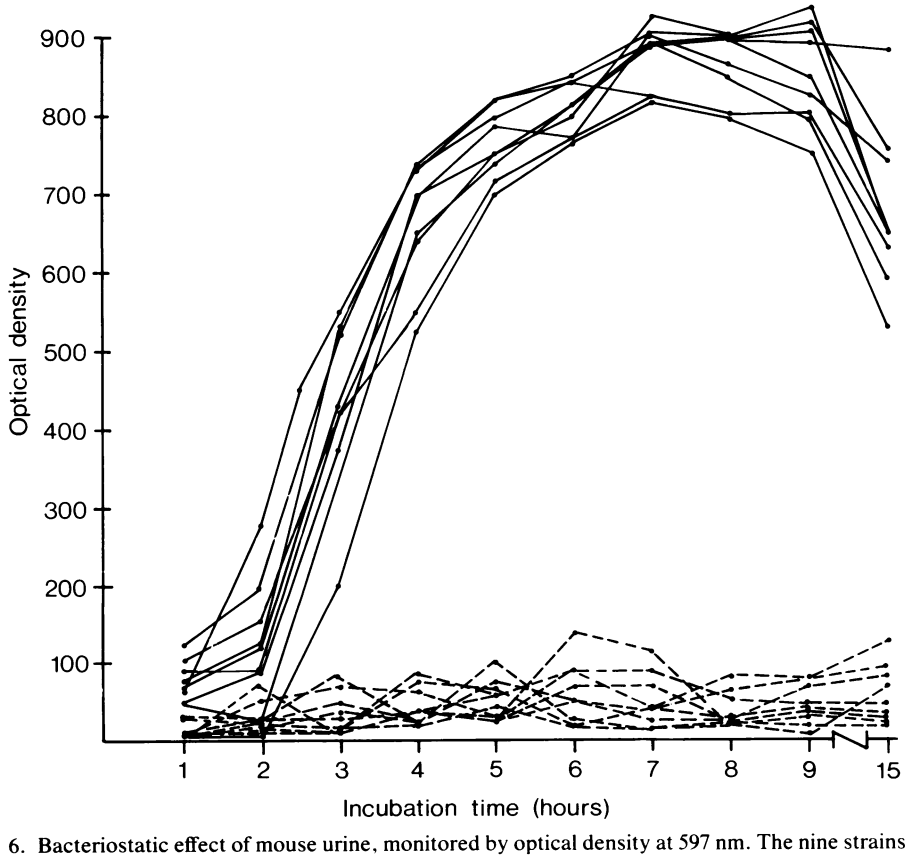


FIG. 6. Bacteriostatic effect of mouse urine, monitored by optical density at 597 nm. The nine strains in Table 4 were grown in broth (————) or urine (----).

cated by the accumulation of inflammatory cells in the kidneys and bladder tissue. Bacterial elimination may thus have resulted not only from excretion, but also from phagocytosis. Conversely, bacteria not recognized by inflammatory cells and attaching to mucosal surfaces may be the ones able to persist. These aspects of the model suggest its usefulness in the study of pathogenic events after the initial bacterial establishment.

Most animal species used for experimental UTI, like rabbits, rats, and guinea pigs, do not parallel humans in receptors for attaching bacteria and should not be used to study this aspect of UTI. In addition, many UTI infection models have depended on means to increase the susceptibility to infection-like obstructions of the ureter (6) or urethra (25, 34), kidney or bladder massage (5, 13), implantation of foreign bodies (8), or glucose-induced diuresis (15). Since defects of the urine flow which facilitate bacterial ascent into the renal pelvis in UTI patients decrease the need for bacterial adhesion for establishment of infection (Lomberg, et al., submitted for publication), obstructive manipulations were eliminated from the experimental protocol. One factor explaining the resistance of the mouse urinary tract to bacterial infection is the decreased bacterial growth rate in mouse urine. *E. coli* strains of different uropathogenicity seemed, however, equally susceptible to this factor. Our results suggest that measures to increase susceptibility are less essential if *E.coli* with the combination of virulence properties common to human pyelonephritis isolates are used at sufficiently high inoculum concentrations. The effective inoculum is in fact quickly reduced by the first voiding. It cannot be anticipated whether the bacteria detected after sacrifice are those remaining after gradual excretion or are the offspring of a few surviving and dividing bacterial cells. Results presented in an accompanying paper (11) show that bacterial attachment is quantitatively important for bacterial persistence.

During all experimental conditions tested, the pyelonephritis strain *E. coli* HU734 was recovered in higher numbers and persisted longer than the fecal strain 414. The difference in infectivity between pyelonephritis and fecal isolates was confirmed with other strains. The results suggest that the mouse infection model can differentiate between strains that are more or less virulent in the human urinary tract. The bacterial properties deciding the difference in infectivity are not clear. Strains causing human pyelonephritis often simultaneously possess certain types of lipopolysaccharide and capsular polysaccharide, are resistant to the bactericidal effect of serum, produce hemolysin, and attach to uroepithelial cells (33). A definite influence of bacterial adhesion is shown in the accompanying paper (11). The model will, however, most probably be a tool helping us to understand pathogenetic events after adhesion.

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