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Role of Type 1 Pili and Effects of Phase Variation on Lower Urinary Tract Infections Produced by *Escherichia coli*

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Phase variation of type 1 pili (fimbriae) was studied during the in vivo growth of *Escherichia coli* in two animal models. In the first, a heavily piliated urinary tract isolate (strain 149) was placed in 1-cm polypropylene chambers sealed with 0.22-μm-pore-size filters. The chambers were surgically implanted intraperitoneally in mice and recovered at various times. Piliation, as determined by electron microscopy and by measuring the minimum number of bacteria needed to produce mannose-sensitive hemagglutination, gradually decreased, and by day 5, most of the organisms were nonpiliated. In the second model, piliated and nonpiliated *E. coli* phase variants were inoculated into the bladders of BALB/c mice via urinary catheters, and their fate in the lower urinary tract was studied. Viable counts of bladder homogenates revealed that piliated phase variants were significantly more effective in colonizing the bladder urothelium than were their nonpiliated counterparts. Specific antibody to type 1 pili prevented colonization by the piliated organisms. After inoculation of piliated variants, the bladder-associated bacteria gave rise to approximately 80% mannose-sensitive hemagglutination-positive colonies, and immunocytochemistry of bladder lavages revealed large numbers of type 1 piliated bacteria adhering to the bladder transitional cells. Electron microscopy confirmed the presence of piliated bacteria in association with the bladder urothelium. The urine of these mice, whose bladders were colonized with piliated bacteria, frequently showed no growth, and when bacteria were present, strain 149 yielded less than 30% hemagglutination-positive colonies. The results suggest that for some *E. coli* strains, phase variation may be a factor in determining the fate of the *E. coli* in the urinary tract and that the urine may not necessarily reflect the bacteriologic state of the bladder mucosa.

Type 1 pili are nonflagellar, filamentous appendages found on many strains of *Escherichia coli* and other gram-negative bacteria (5, 7). These structures mediate bacterial hemagglutination (HA) of various erythrocytes (RBCs), and this reaction is inhibited by mannose and certain mannose-containing oligosaccharides (6, 8, 29, 34). Type 1 pili also mediate mannose-sensitive (MS) binding to a variety of other eucaryotic cells (3, 23, 26, 27, 33, 37). The expression of type 1 pili varies from strain to strain and even between individual organisms of a given culture and is influenced by growth conditions (4–6, 8, 13, 42). Growth in static broth selects for piliated organisms (30), while repeated passage on agar often selects for nonpiliated organisms, although some strains retain their type 1 pili despite repeated subculture on agar (10). The rate at which type 1 pili are phenotypically expressed or are lost, after growth in broth or on agar, respectively, also differs between strains (10). The mechanism by which bacterial cells control pilus expression is unclear. Eisenstein (11) has provided evidence that piliation is under transcriptional control similar to flagellar phase variation in salmonella (39), and Nowicki et al. (25) have reported the occurrence of phase variation among different fimbrial types. It was recently shown that four gene products are involved in type 1 pilus production (32), and one of these, a 23-kilodalton polypeptide, is involved in pilus regulation (31).

Investigations of the role of type 1 pili in urinary tract infections (UTI) have led to conflicting results. Evidence for the importance of type 1 pili in establishing lower UTI has come from in vitro experiments (35, 43), from the fact that bacteria isolated from the urine of patients with UTI expressed MS adhesins (22), from the observation that methyl-α-D-mannopyranoside protected mice from experimental UTI (1), and from studies with animal models (14, 15, 18, 21) which showed that inoculation of piliated organisms into the bladder resulted in significantly more colonization of the urinary tract than when nonpiliated organisms were inoculated. However, conflicting results have been obtained with animal models (41), and it has also been reported that urinary pathogens isolated from patients with UTI were nonpiliated until repeated passage in broth (19, 28). These conflicts may be attributed to the occurrence of phase variation of type 1 pili in vivo. In fact, come investigators have suggested that phase variation may itself be a virulence factor (11, 12, 28). *Proteus mirabilis*, which exhibits phase variation of pili, is much more likely to cause pyelonephritis in experimental ascending infections if it is in the fimbriate phase (36). In contrast, the nonpiliated phase is more virulent when the route of inoculation is hematogenous (38), possibly because leukocytes recognize pili via mannose receptors (2, 3, 23, 37).

The experiments described here show that piliated *E. coli* cells become nonpiliated during growth in vivo in chambers implanted in the peritoneal cavities of mice. Furthermore, in an animal model of UTI, piliated organisms were found to be associated with bladder urothelium, but bacteria recovered simultaneously from the urine were frequently nonpiliated.

MATERIALS AND METHODS

Bacteria. The four strains of *E. coli* used, 149, 92, 75, and 21, were voided urinary isolates from women with cystitis. When grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), all four strains possessed type 1 pili as indicated by their ability to produce MS HA of guinea pig

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RBCs. Strains 92, 75, and 21 also produced MS HA of human erythrocytes. On blood agar (GIBCO Laboratories, Grand Island, N.Y.), only strain 21 remained MS HA positive for guinea pig (and human) RBCs after multiple passages, while the other three strains became HA negative against human and guinea pig RBCs. Transmission electron microscopy (EM) confirmed that all four strains were piliated when grown in broth and that strains 149, 92, and 75 became nonpiliated after passage on agar while strain 21 retained its type 1 pili. The degree of piliation of strain 21 grown on agar was slightly less than that of the strain grown in broth as determined by HA titer. All four strains were continuously passed both on agar and in broth throughout this study. Strain 92 produced two distinct colony types: a large flat colony with irregular borders that was nonpiliated and a small smooth round colony that was piliated. No differences in colony morphology were observed between piliated and nonpiliated cells for the other three strains.

Inoculum properties. For inoculation with piliated bacteria, *E. coli* were taken from ongoing (brain heart infusion) broth cultures which had been continuously subcultured at least six times. Overnight cultures (1 ml) were centrifuged in an Eppendorf centrifuge for 1 min, and the pellets were suspended in 0.1 ml of phosphate-buffered saline (PBS, pH = 7.3; Difco), which corresponded to a concentration of approximately 10^{10} bacteria per ml. For inoculation with nonpiliated phase variants, *E. coli* colonies which had been subcultured previously on blood agar at least six times were suspended in brain heart infusion broth to an optical density at 540 nm of 0.73. A 1-ml portion of this suspension was then centrifuged for 1 min, and the pellets were suspended in 0.1 ml of PBS, which corresponded to a concentration of approximately 10^{10} bacteria per ml. Four mice were inoculated with a spontaneous streptomycin-resistant mutant of strain 149, and bladder homogenates were plated on agar containing streptomycin to demonstrate that the organisms recovered from the urinary tracts of mice were the same as those that had been inoculated.

Animals. Female BALB/c mice (Cumberland), 6 to 8 weeks old, were housed eight to a cage and fed pellets and tap water ad libitum.

Inoculation procedure. Inoculation of *E. coli* into the mouse bladder was done as previously described (17). Briefly, 0.05 ml of the inoculum, prepared as described above, was instilled into the urinary bladder through a soft polyethylene catheter (outer diameter, 0.61 mm) adapted to a 28-gauge needle. Controls were inoculated with sterile PBS. Mice were sacrificed at various times after inoculation by cervical dislocation, and voided urine was collected aseptically by clean catch. The bladders were removed aseptically and homogenized in 1 ml of sterile PBS in a Teflon tissue grinder (Kontes Scientific Glassware/Instruments, Morton Grove, Ill.). Viable counts were performed on serial dilutions in PBS plated on blood and MacConkey agar.

Statistical analysis. Differences in bladder colonization between piliated and nonpiliated bacteria were examined by two-sample *t* tests (separate variances) after the viable-count values were transformed to a logarithmic scale (after addition of 1.0 to each untransformed value to eliminate values of zero) to stabilize the variances among the groups.

HA tests. HA of guinea pig RBCs was tested on 10 randomly selected colonies arising from cultures of urine and bladder homogenates after 18 h of growth. Colonies were picked off the agar with an inoculating loop and vigorously mixed with 25 μl of a 2% guinea pig RBC suspension on a slide. The slide was rocked back and forth by hand and examined for RBC agglutination within 1 min. Antiserum against type 1 pili of strain 149 (prepared as described below) was used to inhibit the HA reaction of HA-positive colonies arising from bladder and urine viable counts of mice that had been inoculated with strain 149. EM was also performed on both HA-positive and HA-negative colonies as described below. Titration of the minimum number of bacteria producing HA was carried out by serially diluting 25 μl of bacterial suspension in 25 μl of PBS in wells of a microtiter plate. Guinea pig RBCs (25 μl) were dropped into each well, and the plate was gently tapped for 2 min to mix each suspension. The plate was then covered and refrigerated at 4°C for at least 2 h. The HA endpoint was visually determined by observing the greatest dilution that produced HA. Viable counts of the bacterial suspension were determined, and the minimum number of bacteria causing HA was then calculated.

Bladder lavages. After cervical dislocation, the mouse voided, and the introitus was then ligated with surgical silk (5-0 Ethicon). After laparotomy, the bladder was grasped with sterile forceps while a 28-gauge needle on an insulin syringe filled with 0.15 ml of 0.1 M sodium cacodylate buffer (pH 7.3) was inserted into the bladder. The bladder was lavaged vigorously with the buffer to remove adherent bacteria and surface bladder cells. The lavage suspension was prepared for EM to observe whether or not type 1 pili were present on bladder epithelium-associated bacteria and was prepared for cytologic examination (as described below) to observe in vivo adherence properties.

Analysis of fresh urine. Bacteria were harvested from the voided urine by centrifugation and then suspended in 10 μl of 0.1 M sodium cacodylate buffer and prepared for EM. Urine samples were also prepared for cytologic examination as described below.

EM. Drops of bacterial cells suspended in 0.1 M sodium cacodylate buffer (pH 7.3) were placed on Parafilm, and 200-mesh Formvar-carbon-coated copper grids (Ernest F. Fullam, Schenectady, N.Y.) were floated on top of the bacterial suspensions for 1 min. The specimens were fixed by floating the grids on top of drops of 2% paraformaldehyde–glutaraldehyde (pH 7.3) for 30 min at 4°C and stained on top of drops of uranyl acetate for 10 s. For immunological detection of type 1 pili of strain 149, the grids were floated on top of bacterial suspensions for 1 min and then transferred to drops of reagents in the following sequence (duration of incubation is given in parentheses): (i) 1% anti-type 1 pilus antiserum in sodium cacodylate–1% bovine serum albumin (2 min); (ii) sodium cacodylate (three times for 3 min each); (iii) 50% gold-conjugated antirabbit antiserum (Janssen Pharmaceutica, Piscataway, N.J.) in sodium cacodylate (1 min); (iv) sodium cacodylate (three times for 3 min each); (v) 2% paraformaldehyde–glutaraldehyde (30 min); (vi) uranyl acetate (10 s). Between each step the edges of the grids were held against filter paper to remove residual fluid.

Cytology. Urine specimens and bladder lavages were centrifuged for 5 min at 2,000 rpm in a Shandon cytocentrifuge (Sewickley, Pa.), fixed in 95% ethanol, and stained by a modified Papanicolaou method (20). Peroxidase-antiperoxidase (PAP) staining was done by the method of Sternberger et al. (40).

Preparation of antiserum to type 1 pili. Polyclonal anti-type 1 pilus antibody was prepared in 2- to 3-kg New Zealand White rabbits. Pilus protein (1 mg), purified as previously described (24), in TES buffer was emulsified with an equal

TABLE 1. Phase variation of type 1 pili in vivo[a]

Time (days)	Viable count (bacteria/ml)	Piliation[b]				HA titer[c]
		Nonpiliated	Slight (1–9 pili/cell)	Moderate (10–30 pili/cell)	Heavy (>30 pili/cell)	
0	1.2×10^3	0	6	0	94	1.2×10^5
1	1.5×10^7	40	22	22	16	1.8×10^6
3	2.4×10^7	58	40	2	0	1.8×10^7
5	2.5×10^7	60	35	5	0	1.2×10^7
7	2.5×10^7	60	38	2	0	1.3×10^7

[a] Heavily piliated organisms of *E. coli* 149 were placed in polypropylene chambers sealed with 0.22-μm-pore-size filters and implanted into the peritoneal cavities of mice. At various times, the chambers were recovered, and the viable count, degree of piliation, and HA titer of the in vivo-grown bacteria were determined.

[b] Piliation was determined by counting the number of pili per cell on 50 cells examined by EM. The numbers show the percentage of total cells in each category.

[c] Minimum number of bacteria required to produce HA of a 0.5% guinea pig RBC suspension.

volume of Freund complete adjuvant and administered by multiple subcutaneous injections. Booster injections in Freund incomplete adjuvant were administered 2 and 4 weeks later. After week 5, the animals were bled, and the sera were filter sterilized and stored at −4°C. Antiserum against type 1 pili from strain 149 was absorbed against the nonpiliated variant of strains 149 and 75. Fab fragments were prepared by precipitating the antiserum with 50% $(NH_4)_2SO_4$ and then treating the resuspended antibody with immobilized papain (Pierce Chemical Co., Rockford, Ill.). The Fab fragments were recovered after protein A affinity chromatography (Pierce Chemical).

Chambers. Polypropylene cylinders (1 cm), sealed at each end with 0.22-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.) were prepared as previously described (9). Each chamber contained approximately 10^3 cells of strain 149 diluted in PBS. The chambers were then surgically implanted into the peritoneal cavities of mice and recovered at various times to test the HA titer and to visually quantitate piliation via EM. Organisms adherent to the chamber walls and filters were recovered by vigorously vortexing the chambers in 1 ml of PBS.

RESULTS

Effect of in vivo growth on piliation. It is well documented that environmental conditions influence the expression of type 1 pili in vitro, but little is known about phase variation in vivo. To study the effect of in vivo growth on expression of type 1 pili, the piliated variant of strain 149 was placed in polypropylene chambers sealed with 0.22-μm-pore-size filters and surgically implanted intraperitoneally in mice. The bacteria were retrieved at various times, and their degree of piliation was determined by EM and by measuring the minimum number of bacteria required to produce MS HA. The results are shown in Table 1. The initial inoculum of 10^3 bacteria, 94% of which were heavily piliated, grew to 10^8 bacteria per ml within 24 h. In this short time, EM revealed a substantial reduction in the degree of piliation, and this corresponded to the 10-fold increase observed in the minimum number of bacteria needed to cause HA (HA titer). After 1 day of growth, only 16% of the bacterial population remained heavily piliated (>30 pili per cell); 22% had become moderately piliated (10 to 30 pili per cell); another 22% had become slightly piliated (1 to 9 pili per cell); and 40% were now nonpiliated. By day 3, no heavily piliated bacteria remained, and in fact, 98% of the bacteria were nonpiliated or slightly piliated as determined by EM.

Behavior of phase variants in experimental UTI. Piliated variants of *E. coli* 149 and 92 were inoculated into mouse bladders. Dilutions of bacteria recovered from urine and from bladder homogenates at various times were plated on agar, and colonies arising after approximately 18 h of growth were tested for their ability to produce MS HA (Fig. 1). A total of 78% of the bacteria recovered from bladder homogenates of mice that had been inoculated with strain 149 and 96% of the bladder-associated bacteria of mice that had been inoculated with strain 92 gave rise to piliated MS HA colonies. EM confirmed that the HA-positive colonies consisted of piliated bacteria and the HA-negative colonies were nonpiliated. Furthermore, type 1 pili apparently mediated the HA reaction, since antiserum prepared against purified type 1 pili of strain 149 reacted specifically with the

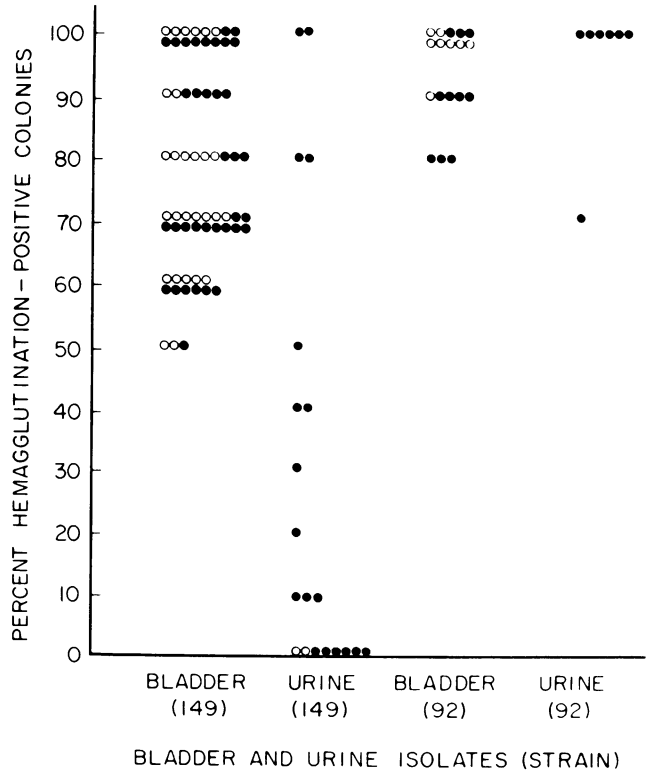

FIG. 1. Percentage of HA-positive colonies arising from bladder homogenates and urine cultures of mice inoculated with piliated phase variants of strains 149 and 92. Symbols: ●, recovered 24 h after inoculation; ○, recovered more than 24 h after inoculation. Each point represents the result of 10 individually tested colonies.

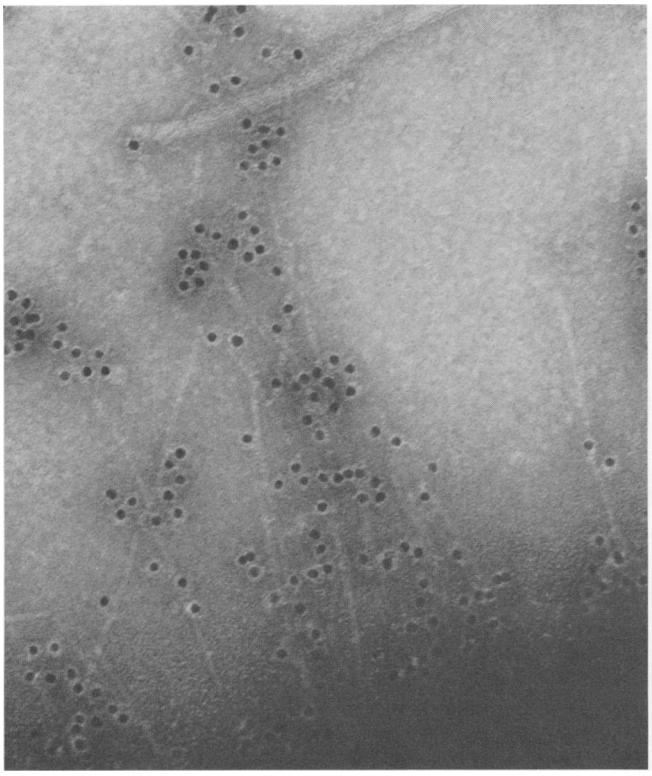

FIG. 2. Type 1 pili stained by the immunogold procedure. The cell is from a bladder homogenate grown overnight on agar (×30,200).

pili as shown by immunogold EM (Fig. 2), and the antiserum also inhibited the HA reaction of that strain.

The bacteriologic state of the urine was often unlike that of the bladder in mice which had been inoculated with piliated bacteria. Of those mice having piliated bacteria colonizing their bladder urothelium, 59% yielded sterile (<250 bacteria per ml) urine cultures even when as many as 10^4 bacteria were present on their bladder surfaces. Often, when the bladder walls were colonized with the piliated variant of strain 149, the nonpiliated variant would simultaneously be expelled in the urine. Of the bacteria recovered from the urine of mice that had been inoculated with the piliated variant of strain 149, 72% gave rise to nonpiliated and HA-negative colonies (Fig. 1). The two open circles (urine isolates of strain 149) in Fig. 1 represent an experiment in which urine cultures were obtained at 7 and 14 days postinoculation. Approximately 10^6 bacteria per ml were present at day 7 and 10^3 bacteria per ml were present at day 14, and all of the colonies tested from urine cultures arose from the nonpiliated variant as they were HA negative. In the same experiment, bacteria associated with the bladder at days 7 and 14 were piliated. Thus, after inoculation of the piliated variant of strain 149, piliated bacteria were found to be colonizing the bladder urothelium while nonpiliated phase variants were expelled in the urine.

Immunocytochemical and EM studies done on bladder lavages confirmed that the bacteria associated with the bladder urothelium possessed type 1 pili. Mice that had been inoculated with the piliated variant of strain 149 were sacrificed by cervical dislocation, which caused them to urinate. Bladder urothelial cells were then obtained by lavaging the empty bladder with sodium cacodylate buffer. Cytologic preparations of the lavage specimens demonstrated that bacteria adhered to both superficial umbrella cells and deeper, small, round transitional cells. When this preparation was stained for the presence of type 1 pili by the PAP technique, the adherent bacteria stained bright red, indicating that they were heavily piliated (Fig. 3). Free bacteria were also stained although not as intensely as the adherent bacteria; presumably the free bacteria were less heavily piliated. Control (nonpiliated) organisms were unstained. EM of these bladder lavage specimens confirmed that most of the bacteria were heavily piliated. When the bacteria were treated with specific anti-type 1 pilus antibody in the immunogold procedure, gold particles were observed to be specifically attached to the pili.

Figure 1 shows that the urine samples from mice inoculated with the piliated variant of strain 149 gave rise to primarily HA-negative colonies but on occasion yielded predominantly HA-positive colonies; urine samples from mice inoculated with the piliated variant of strain 92 always yielded predominantly HA-positive colonies. Cytologic observations on those urine samples from mice that had been inoculated with the piliated variant of strain 149 and gave rise to predominantly HA-positive colonies revealed a large number of exfoliated uroepithelial cells densely covered with adherent bacteria. The adherent organisms gave a positive reaction for the presence of type 1 pili by the PAP technique (Fig. 4), while many of the free bacteria were nonpiliated. Urine cultures from strain 149-colonized mice that produced mainly HA-negative colonies consisted mostly of free bacteria.

Role of type 1 pili in establishing bladder infections. Several experimental approaches were used to investigate the role of type 1 pili in establishing UTI. The first was to inoculate piliated and nonpiliated phase variants into the mouse bladder and at various times plate dilutions of bacteria recovered

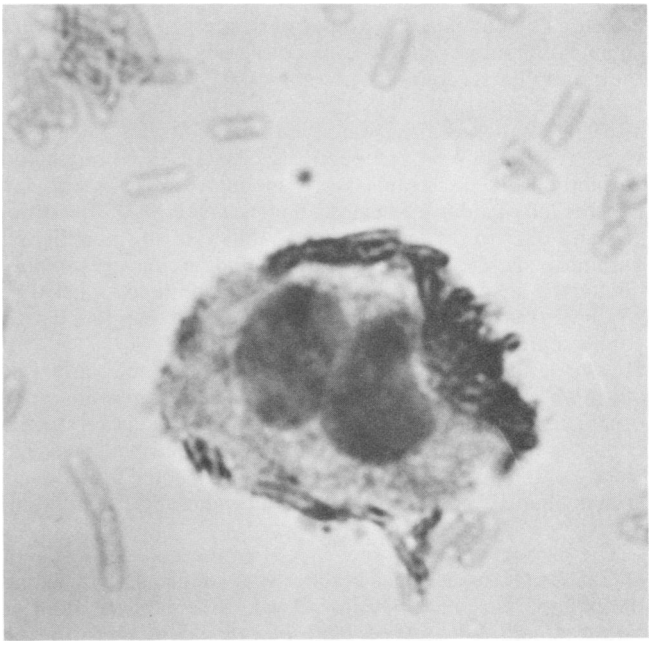

FIG. 3. Cell(s) from a bladder lavage 24 h after inoculation of the piliated variant of strain 149. All of the bacteria show a positive reaction for the presence of type 1 pili by the PAP technique; however, the adherent bacteria are stained more intensely (×3,500).

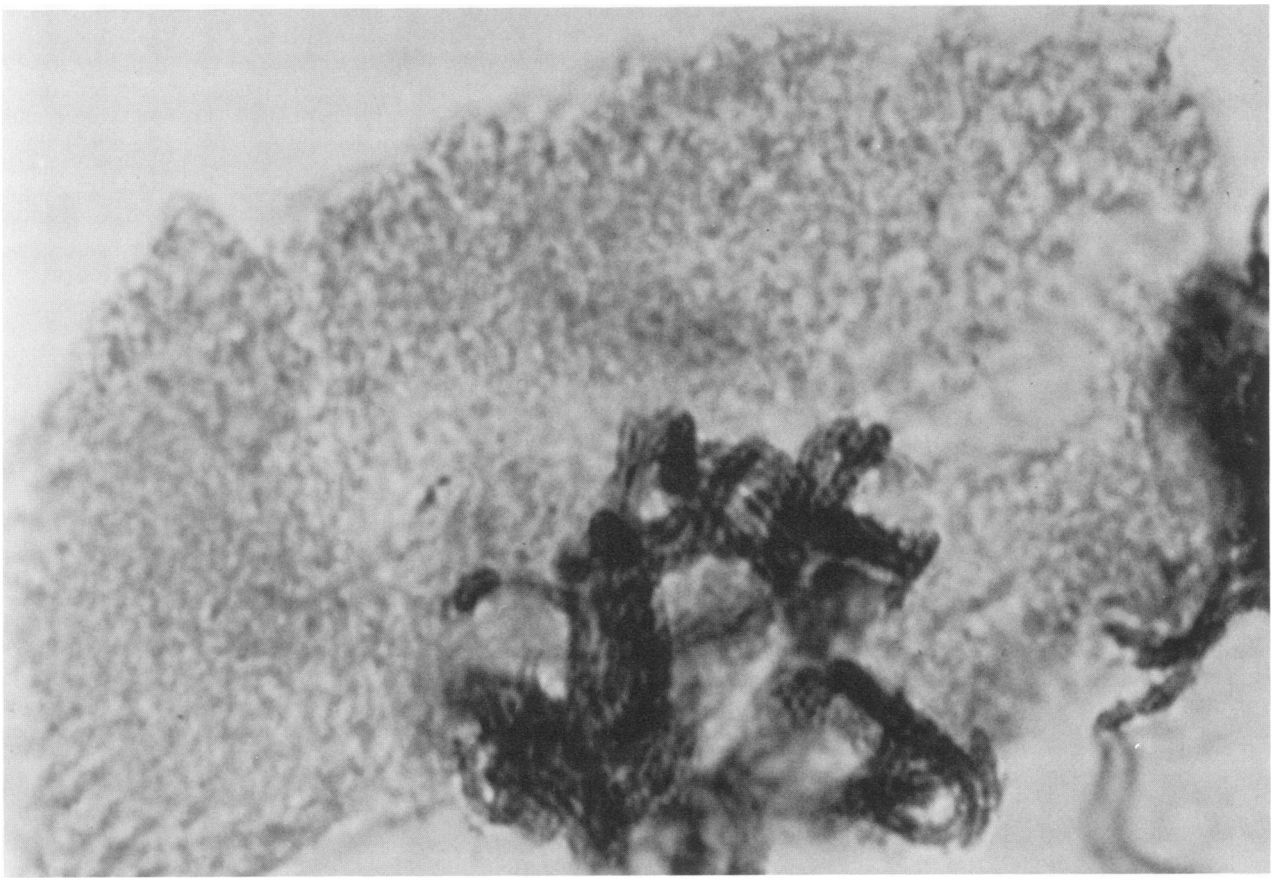

FIG. 4. Exfoliated transitional cell from a urine culture which gave rise to 80% HA-positive colonies. Adherent bacteria show a positive reaction for the presence of type 1 pili by the PAP procedure (×3,500).

from urine and bladder homogenates to determine viable counts. Piliated phase variants of strains 149 and 92 were significantly more effective in colonizing the bladder than their nonpiliated counterparts ($P < 0.01$; Fig. 5). The mean log numbers of bladder-associated bacteria at various times after inoculation of the piliated variants of strains 149 and 92 were 4.04 and 3.64, respectively. When the nonpiliated variants of these strains were inoculated, the mean log number of bladder-associated bacteria was 0.72 for strain 149 and 0.65 for strain 92. The inability of the nonpiliated variant to significantly colonize the bladder was presumably due to the absence of type 1 pili. The nonpiliated variant of strain 75 was also significantly less effective than its piliated counterpart in colonizing the mouse bladder ($P < 0.05$). However, in some instances after the nonpiliated variant of strain 75 was inoculated into the bladder, more than 10^3 organisms were recovered. This may have been due to the ability of the organisms to switch to a piliated phase in vivo since most of these bladder-associated bacteria (at levels greater than 10^3 organisms per bladder) gave rise to piliated, MS HA-positive colonies.

The importance of type 1 pili in the initial stages of colonization of the mouse bladder was confirmed by treating the piliated variant of strain 149 with specific antiserum or Fab fragments to its type 1 pili before inoculation into the mouse. In three separate experiments this treatment significantly reduced or completely inhibited bladder colonization (data not shown).

To investigate whether other factors besides type 1 pili varied between agar-grown and broth-grown organisms and affected their ability to colonize the mouse bladder, strain 21, which does not show phase variation of type 1 pili in vitro, was inoculated into the mouse bladder after growth on agar or in broth. Both inocula were equally capable of colonizing the bladder, presumably because both possessed type 1 pili (Fig. 5).

The viable counts of bladder-associated bacteria at various times after inoculation of strain 149 are shown in Fig. 6. The amount of time between inoculation and recovery of the bacteria did not seem to alter the observation that piliated variants were significantly more effective in colonizing the urothelium than their nonpiliated counterparts ($P < 0.01$). When the piliated variant was inoculated into the mouse bladder, it persisted over the 7-day period of study. Furthermore, these bacteria remained in the piliated state during this time as they primarily gave rise to MS HA-positive colonies. In contrast, when nonpiliated variants were inoculated into the mouse bladder, they were quickly eliminated from the urinary tract. The few bacteria that were recovered from the bladder continued to give rise to nonpiliated HA-negative colonies.

DISCUSSION

The occurrence of phase variation of type 1 pili of *E. coli* in vitro is well documented (4, 5, 11, 25, 42), and some investigators have speculated that this phenomenon occurs in vivo as well (12, 28). Phase variation in *E. coli* has been suggested to be a virulence factor (12, 28), and evidence for

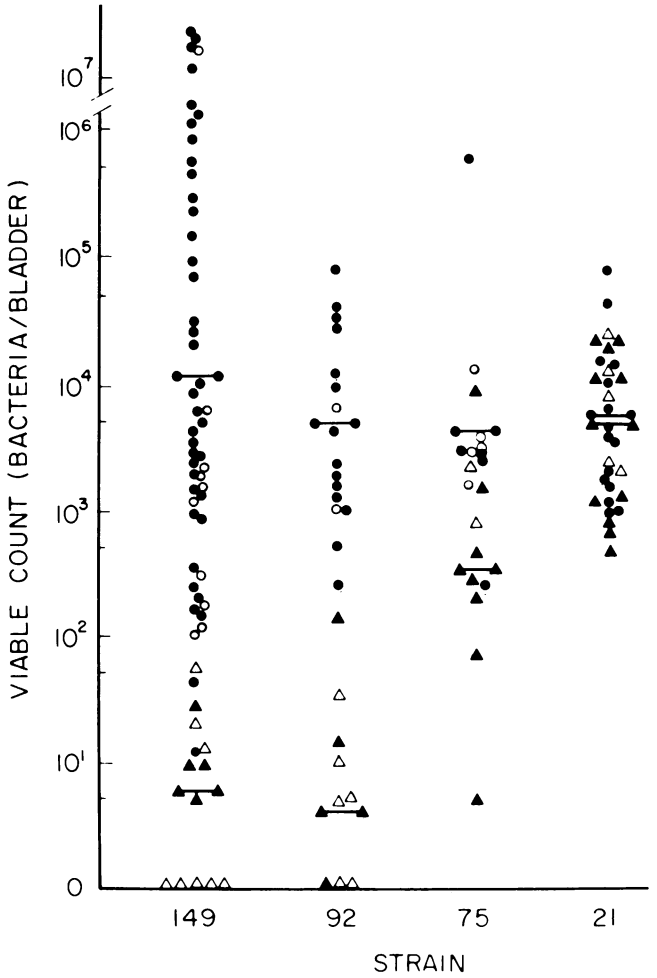

FIG. 5. Effect of piliation on ability of bacteria to colonize the bladder. Mice were inoculated with various strains of bacteria after serial growth in broth (●, ○) or on agar (▲, △). Bladders were removed, homogenized, and plated at 24 h (closed symbols) or at 48 to 72 h (open symbols) postinoculation. ●——● and ▲——▲, Antilogs of the means of the transformed data.

its occurrence in vivo has been presented by Guerina et al. (16) and with *P. mirabilis* by Silverblatt (36). The work presented in this study suggests that phase variation of type 1 pili in *E. coli* may occur in vivo and documents the fate of phase variants in the lower urinary tract.

The strains of *E. coli* used in these studies were carefully chosen from 20 UTI isolates previously examined for phase variation of type 1 pili in vitro (10). Many phase variants rapidly lose type 1 pili during growth on agar. The three strains described here were selected because their ability to produce MS HA of guinea pig RBCs did not change after a single overnight culture on agar. For this reason, the HA characteristics of these colonies arising from urine and bladder homogenates of infected mice are thought to reflect the state of piliation occurring in vivo.

Two animal models were used to study phase variants in vivo. In the first, growth of piliated bacteria in chambers implanted in the peritoneums of mice resulted in the loss of pili among the progeny. This loss occurred despite growth in a fluid environment in the chambers, but even the organisms recovered from the chamber walls and filters were predominantly nonpiliated. These results suggest that the heavily piliated cells in the inoculum may have undergone phase variation during growth in vivo, so that after 24 h, most of the population had become nonpiliated or slightly piliated. An alternative explanation, that a few nonpiliated cells in the original inoculum outgrew the piliated cells, cannot be ruled out, but seems less likely. The shift toward a nonpiliated population continued between days 1 and 3, long after the stationary phase of growth had been reached.

Phase variation of type 1 pili may play a role in experimental UTI in the mouse. Inoculation of piliated bacteria into the mouse bladder invariably resulted in bladder colonization; bacteria were always recovered from the bladder urothelium up to 7 days after inoculation (Fig. 5). Furthermore, the bladder-associated bacteria were predominantly piliated as detected by HA characteristics (Fig. 1), by EM, and by the demonstration that bacteria adherent to bladder transitional cells gave a positive reaction for the presence of type 1 pili in the PAP procedure. On the other hand, nonpiliated organisms appear to be more frequently eliminated in the urine. For example, 72% of the bacteria in the urine of mice inoculated with the piliated variant of strain 149 were in the nonpiliated state while the bladder-associated bacteria continuously expressed type 1 pili.

Cytologic examination of urine samples that gave rise to primarily HA-positive colonies showed large numbers of bacteria adherent to exfoliated transitional cells. Immunocytochemical staining with specific antibody revealed that the adherent bacteria possessed type 1 pili. Thus, the presence of piliated organisms in the urine may often be due to their being carried along with exfoliated bladder transitional cells.

Several additional experiments provided further support

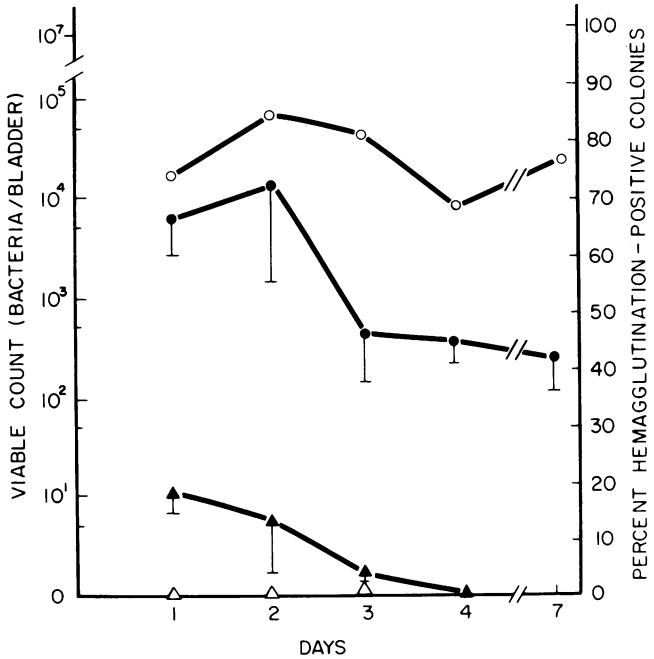

FIG. 6. Effect of piliation on persistence of bacteria in the bladder. Mice were inoculated with piliated (●, ○) or nonpiliated (▲, △) bacteria of strain 149, and at various times the bladders were removed, homogenized, and plated. After overnight growth, the viable counts (closed symbols) and HA characteristics (open symbols) were determined. Each point represents the mean of four to six experiments.

for the idea that type 1 pili are important in the initial colonization of the bladder mucosa. Nonpiliated variants were significantly less effective in colonizing the urothelium after inoculation into mouse bladders, presumably due to their inability to adhere to the bladder mucosa. Other investigators have reported similar results (14, 15). Support for the idea that the type 1 pili are responsible for early colonization events came from the observation that when piliated organisms were treated with specific anti-type 1 pilus antiserum or Fab fragments they behaved like their nonpiliated counterparts. In some instances, inoculation with the nonpiliated variant of strain 75 led to colonization of the bladder mucosa (Fig. 5). This appeared to be mediated by the piliated variant, present either as a minor proportion of the original inoculum or arising during in vivo growth in the bladder, because the majority of colonies arising from bladder-associated bacteria were HA positive when the degree of bladder infection was greater than 10^3 organisms.

Our results suggest that organisms in urine do not necessarily mirror the organisms colonizing the bladder mucosa: nonpiliated bacteria may exist in the urine while the piliated phase variants are simultaneously colonizing the urothelium. This may account for the results of other investigators (19, 28) who found that fresh urinary isolates were (i) unable to mediate MS HA, (ii) unable to adhere to epithelial cells in vitro, and (iii) nonfimbriate as determined by EM. The work presented here might predict that nonpiliated organisms would be recovered from the urine but that these organisms would not necessarily reflect the state of piliation of bladder-associated bacteria. Furthermore, the absence of organisms in the urine may not reflect the bacteriologic state of the bladder mucosa. We found that 59% of mice inoculated with the piliated variant of strain 149 yielded no growth on urine cultures, yet all of these mice were shown to have colonized bladders. All of the urine samples examined cytologically also revealed a state of pyuria whether or not bacteria were present. Therefore, if this model can be extrapolated to the human situation, patients with pyuria and dysuria may have unrecognized UTI.

ACKNOWLEDGMENT

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