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In Vitro Adherence of Type 1-Fimbriated Uropathogenic *Escherichia coli* to Human Ureteral Mucosa

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Type 1-fimbriated *Escherichia coli* isolated from patients with urinary tract infections adhered in vitro to the epithelial cell surface of an excised human ureter. The bacteria also adhered to a mucous coating and to Formalin-fixed human ureteral mucosa. D-Mannose strongly inhibited such adherence. The bacteria in their nonfimbriated phase lacked the ability to adhere. We concluded that type 1 fimbriae play a role, at least in part, in upper urinary tract infections in humans.

Escherichia coli is an important agent in urinary tract infections. Uropathogenic *E. coli* (UPEC) colonizes the perineum, crosses the urethra to the bladder, and occasionally ascends to the kidney (1, 6, 8). Adherence of UPEC to urinary tract mucosa is mediated mainly by bacterial surface fimbriae such as type 1 or P (17). P fimbriae, which show mannose-resistant hemagglutination with human (P-antigen-positive) erythrocytes, are generally found in *E. coli* isolated from patients with upper urinary tract infections (12). Type 1 fimbriae, which show mannose-sensitive hemagglutination with guinea pig erythrocytes, are generally found in *E. coli* isolated from patients with both upper and lower urinary tract infections (7). In a mouse model, it has been shown that type 1 fimbriae mediate adherence in the early events of urinary tract infections (9, 16). Adherence of type 1-fimbriated *E. coli* to human urinary tract exfoliated cells has also been demonstrated in vitro (15). Moreover, 96% of P-fimbriated *E. coli* isolates from patients with pyelonephritis have been found to produce type 1 fimbriae as well (2, 7, 18), suggesting that type 1 fimbriae may also play a role in upper urinary tract infections. In this study we have shown that type 1-fimbriated *E. coli* can adhere to untreated (native) human ureteral mucosa and human ureteral mucosa which has been treated with Formalin.

The *E. coli* strain (E16) used in this study was a voided urinary tract isolate from a patient (55-year-old female) with acute uncomplicated cystitis. Fimbriated E16 cells were obtained by culturing cells in nutrient broth (Eiken, Tokyo, Japan) for 48 h at 37°C without agitation, as reported for type 1-fimbriated *E. coli* (3, 13). A nonfimbriated phase variant of E16 was obtained by culturing E16 cells repeatedly on colonization factor antigen agar (5) containing 1% Casamino Acids (Difco Laboratories, Detroit, Mich.), 0.15% yeast extract (Difco), 0.005% $MgSO_4$, 0.0005% $MnCl_2$, and 2% agar (pH 7.4) at room temperature (ca. 22°C).

For the hemagglutination (HA) assay, bacterial cells were suspended at 300 Klett units (measured in a Klett-Summerson colorimeter with a red filter) in phosphate-buffered saline (pH 7.4). Twofold serial dilutions of this suspension were made, and aliquots were tested at room temperature by using the 24-well plate method (20) with 3% guinea pig or human erythrocytes (group A, p^1 antigen positive). The HA endpoint was determined by using a microscope; the HA titer represented the greatest dilution which produced positive results. Erythrocytes containing 0.5% (wt/vol) sugar (D-mannose or L-fucose) were also used to examine the effect of sugar on the HA reaction.

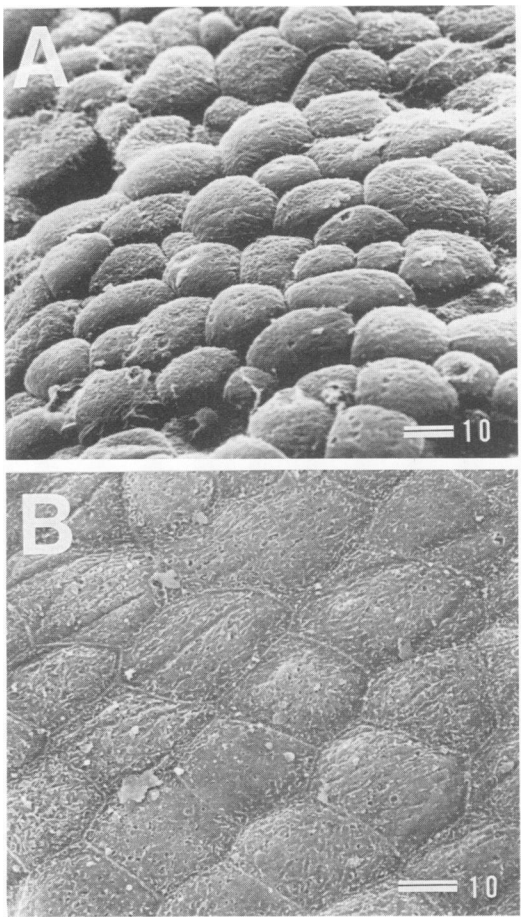

FIG. 1. Scanning electron micrograph of the control human ureteral epithelium. (A) Native. (B) Treated with 10% Formalin. Numbers represent the length of the bars (in micrometers).

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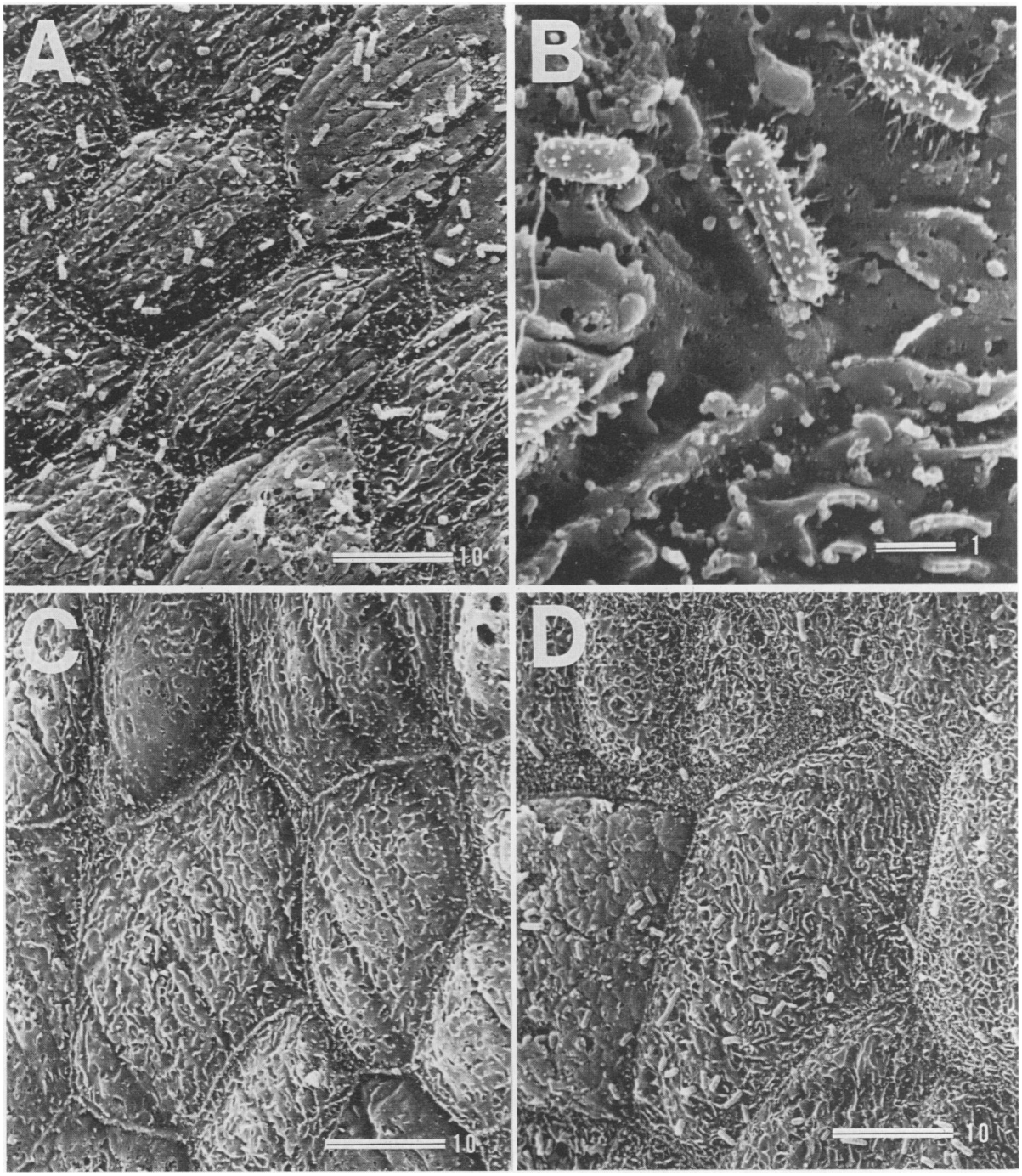

FIG. 2. Adherence of *E. coli* E16 to the surface of human ureteral epithelium. Sugars were added as follows: A and B, none; C, 0.5% (wt/vol) D-mannose; D, 0.5% (wt/vol) L-fucose. Numbers represent the length of the bars (in micrometers).

TABLE 1. Effect of sugars on the adherence of *E. coli* E16 to the epithelial cell surface of Formalin-treated human ureter

E16	Adherence index[a] with the following addition:		
	None	0.5% (wt/vol) D-mannose	0.5% (wt/vol) L-fucose
Fimbriated[b]	19.9 ± 7.5 (>1:128, 1:64)	1.2 ± 1.2 (<1:1, <1:1)	17.6 ± 9.1 (>1:128, 1:64)
Nonfimbriated[c] (off-phase variant)	0.6 ± 0.9 (<1:1, <1:1)	ND[d] (<1:1, <1:1)	ND (<1:1, <1:1)

[a] Means ± standard deviations for 30 determinations. The first titer in parentheses is the HA titer estimated with 3% guinea pig erythrocytes by the 24-well plate method. The second titer in parentheses is the HA titer estimated with 3% human erythrocytes (group A, p^1 antigen positive) by the 24-well plate method.
[b] Cultured in nutrient broth for 48 h at 37°C.
[c] Grown on colonization factor antigen agar at room temperature (see text).
[d] ND, Not done.

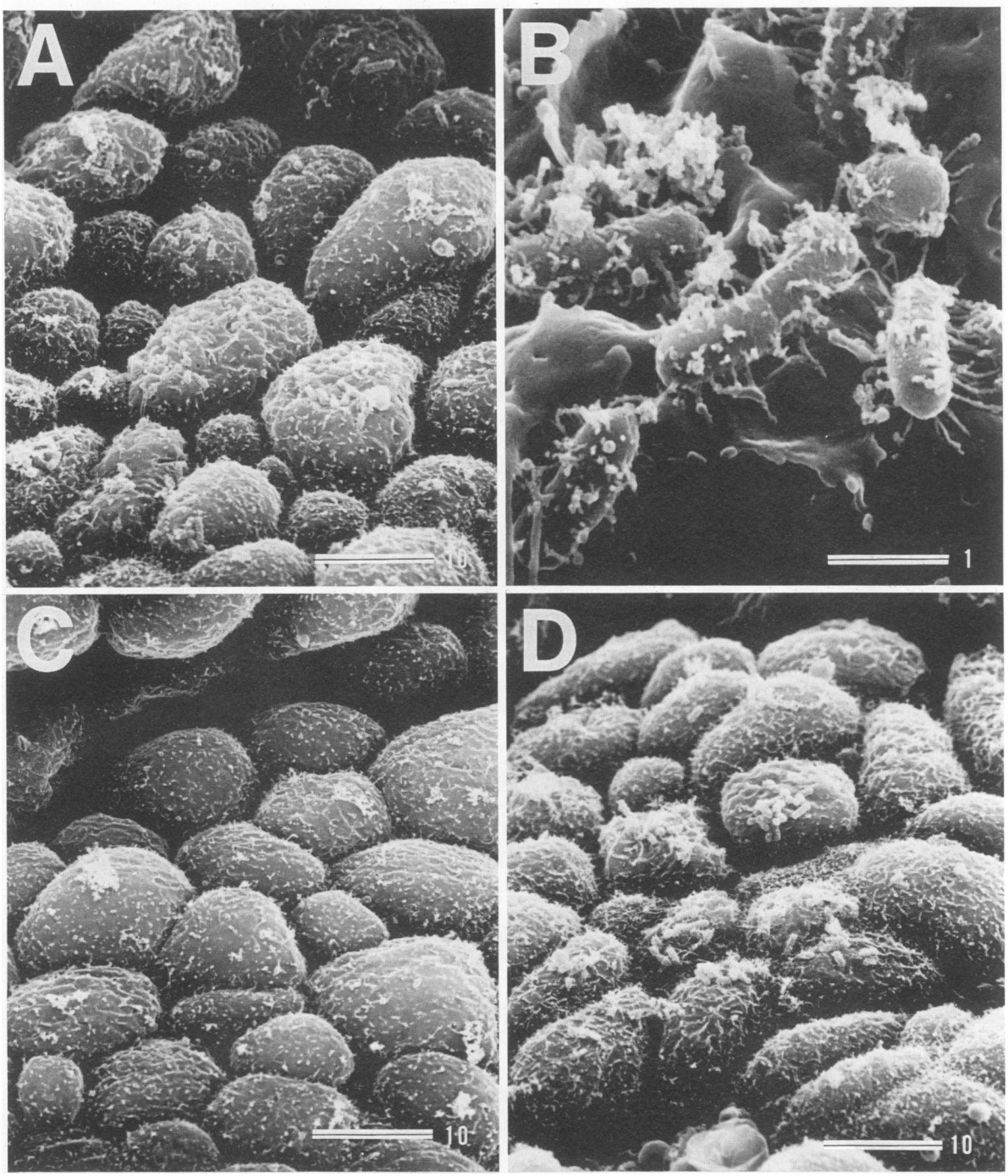

FIG. 3. Adherence of *E. coli* E16 to the surface of Formalin-fixed human ureteral epithelium. Sugars were added as follows: A and B, none; C, 0.5% (wt/vol) D-mannose; D, 0.5% (wt/vol) L-fucose. Numbers represent the length of the bars (in micrometers). Microridges and cellular junctions are well identified in the ureteral surface.

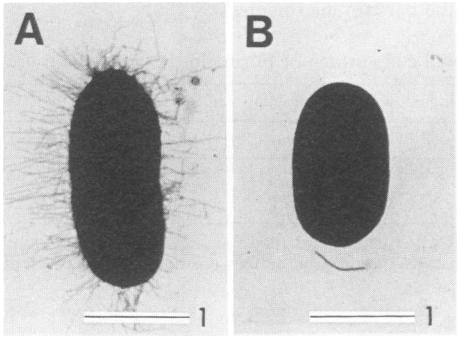

FIG. 4. Transmission electron micrograph of *E. coli* E16 in its fimbriated (A) and nonfimbriated (B) phases. Bacterial cells adhering to collodion grid screens were negatively stained with uranyl acetate as previously described (19). Numbers represent the length of the bars (in micrometers).

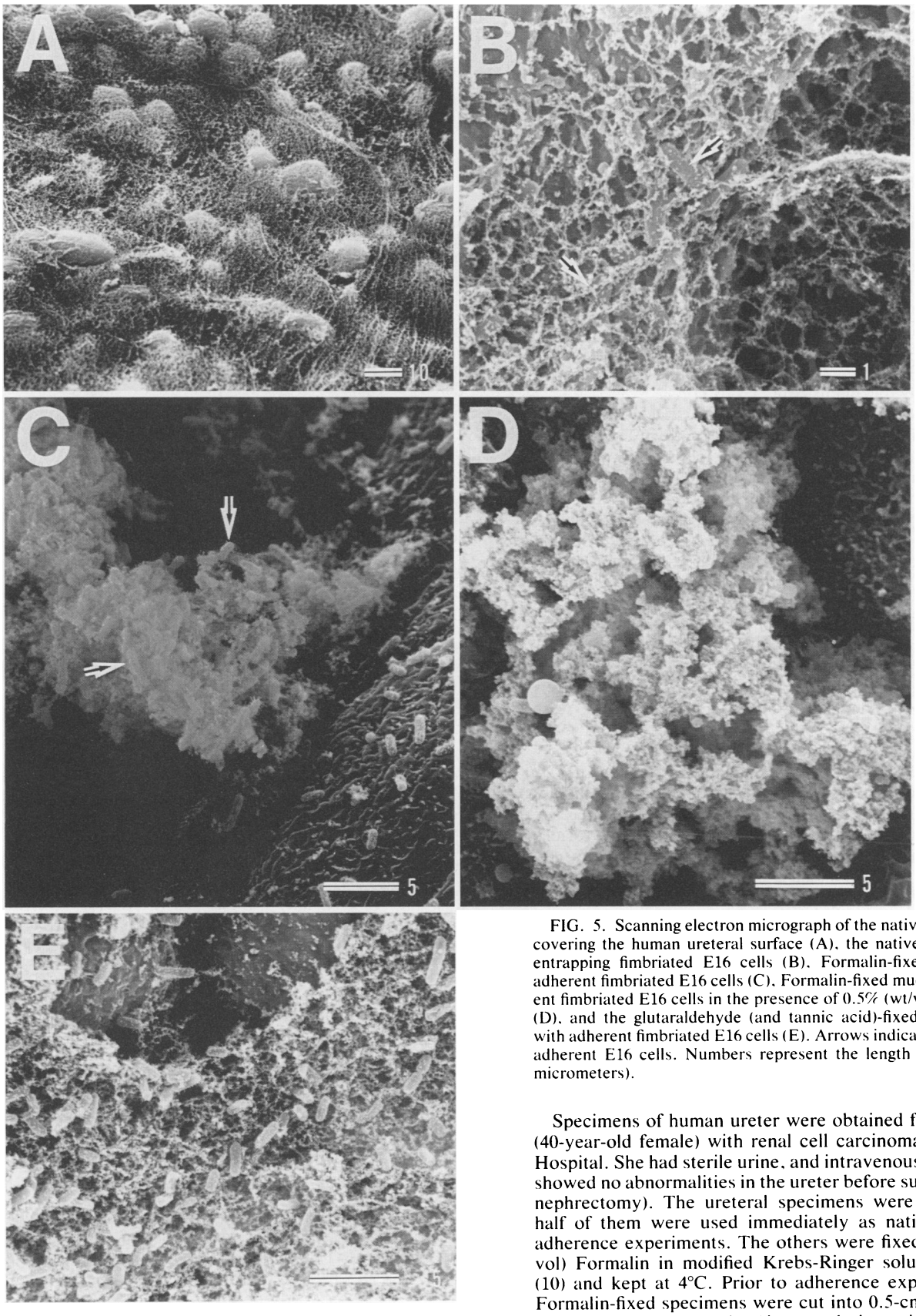

FIG. 5. Scanning electron micrograph of the native mucous layer covering the human ureteral surface (A), the native mucous layer entrapping fimbriated E16 cells (B), Formalin-fixed mucus with adherent fimbriated E16 cells (C), Formalin-fixed mucus with adherent fimbriated E16 cells in the presence of 0.5% (wt/vol) D-mannose (D), and the glutaraldehyde (and tannic acid)-fixed mucous layer with adherent fimbriated E16 cells (E). Arrows indicate entrapped or adherent E16 cells. Numbers represent the length of the bars (in micrometers).

Specimens of human ureter were obtained from a patient (40-year-old female) with renal cell carcinoma at Juntendo Hospital. She had sterile urine, and intravenous pyelography showed no abnormalities in the ureter before surgery (radical nephrectomy). The ureteral specimens were opened, and half of them were used immediately as native ureter for adherence experiments. The others were fixed in 10% (vol/vol) Formalin in modified Krebs-Ringer solution (pH 7.4) (10) and kept at 4°C. Prior to adherence experiments, the Formalin-fixed specimens were cut into 0.5-cm squares and washed with cold Krebs-Ringer solution with two buffer changes (500 ml each) for 3 h. In some experiments, the

ureteral specimens were fixed in Krebs-Ringer solution containing 2.5% (vol/vol) glutaraldehyde and 2% (wt/vol) tannic acid and, after being washed as described above, used for adherence experiments.

For adherence experiments, the bacterial cells were suspended in phosphate-buffered saline at 800 Klett units. Ureteral specimens prepared as described above were immersed in 1.5 ml of bacterial suspension and incubated for 10 min at 28°C (20, 21). The washed specimens were fixed with glutaraldehyde and subsequently with osmium tetroxide. They were examined by using a scanning electron microscope (19).

Human ureteral epithelial cells had a cobblestonelike appearance (Fig. 1). E16 cells in their fimbriated phase (grown in nutrient broth for 48 h at 37°C) adhered well to the native ureteral surface (Fig. 2A and B). D-Mannose strongly inhibited this adherence (Fig. 2C), while L-fucose had no significant effect (Fig. 2D). Similar results were noted for Formalin-fixed specimens (Fig. 3A to D).

Quantitative estimates of binding of E16 cells in their fimbriated (Fig. 4A) and nonfimbriated (grown on colonization factor antigen agar at room temperature) (Fig. 4B) phases were made (Table 1). For these estimates, 30 electron microscope observations obtained at a magnification of 4,000× (23 by 28 μm) were randomly chosen and photographed. The average number of bacteria per electron microscope field (photograph) defined the adherence index. The data in Table 1 show that the adherence of E16 cells is mediated primarily by type 1 fimbriae. Additionally, the failure of strain E16 to produce P fimbriae was confirmed by using a dry spot latex agglutination test (Orion Diagnostica, Espoo, Finland).

Human ureteral epithelium was covered with a mucous layer (Fig. 5A). E16 cells in their fimbriated phase were entrapped in this native mucous layer (Fig. 5B). Type 1 fimbria-mediated adherence to the mucous layer was clearly noted in the Formalin-fixed specimens (Fig. 5C and D). A Formalin-fixed mucous layer was slightly more rigid than a native mucous layer, and this rigidity enabled an adherence study of the surface of a mucous layer. Fimbriated E16 cells also adhered to the mucous layer of glutaraldehyde (and tannic acid)-fixed specimens (Fig. 5E). Glutaraldehyde (and tannic acid) fixation caused a meshlike appearance of the mucous layer, much like that of the mucous layer of a native specimen (compare Fig. 5A and E). In contrast to their fimbriated counterparts, E16 cells in their nonfimbriated phase showed no detectable adherence to the mucous layer (data not shown).

Our study clearly showed that type 1-fimbriated *E. coli* can adhere to human ureteral epithelial cells and the mucous layer which covers the epithelium. P fimbriae of UPEC have been characterized as the virulent factor causing pyelonephritis; P fimbriae recognize Gal-Gal receptors on urinary tract epithelial cells (12). Type 1 fimbriae, on the other hand, are believed to play no role in pyelonephritis (2, 18). Indeed, more than 90% of *E. coli* isolates from patients with acute febrile pyelonephritis are P fimbriated, and *E. coli* possessing type 1 fimbriae alone is rarely isolated (2, 11). However, because most P-fimbriated UPEC strains have the genetic potential to express type 1 fimbriae (4, 7), it is possible that UPEC possessing P fimbriae can produce type 1 fimbriae as well in vivo. It has also been reported that antibodies to type 1 fimbriae are found in patients with pyelonephritis (14). Our data strongly indicate that type 1 fimbriae facilitate bacterial adherence to ureteral mucosa and that type 1 fimbriae, together with P fimbriae, play an important role in pyelonephritis in humans.

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