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Adhesion of *Escherichia coli* to Human Uroepithelial Cells In Vitro

C. SVANBORG EDEN,[1]* B. ERIKSSON,[2] AND L. Å. HANSON[1,3]

Department of Immunology, Institute of Medical Microbiology,[1] and Departments of Pediatrics,[3] Statistics, and Social Medicine,[2] University of Gothenburg, Gothenburg, Sweden

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Optimal conditions for in vitro adherence of *Escherichia coli* to uroepithelial cells, previously shown to be more efficient for strains causing acute symptomatic than that for strains causing "asymptomatic" urinary tract infections, were investigated. Uroepithelial cells from fresh morning urine of healthy individuals and *E. coli* bacteria from patients with various forms of urinary tract infection were used. Adhesion was found to vary, between individuals and epithelial cell types, with epithelial cell viability, bacterial cultivation medium and growth phase, number of bacteria added to the epithelial cells, and incubation time and temperature. Adhesion was also influenced by variations in pH and osmolarity. Optimal test conditions were obtained with post-log-phase bacterial cultures grown on nutrient broth when 10^8 bacteria were added to 10^5 epithelial cells and incubated for 60 min. Considerable variation was found between experiments done on different days, whereas the variation between duplicates was small. The method described may provide a useful tool in the study of the host-parasite relationship in urinary tract infections.

The ability to adhere to epithelial surfaces has been shown to correlate to virulence in man for enteropathogenic *Escherichia coli* in the gut (19), for gonococci in the urogenital tract (22, 29), for streptococci in the oral cavity (8), and in various animals for mycoplasma (21, 25), *Vibrio cholerae* (11, 15, 16), and *E. coli* (14, 20, 23), among others.

Infections in the urinary tract caused by *E. coli* may give symptoms of cystitis and/or pyelonephritis, but many patients have few or insignificant symptoms of their bacteriuria: "asymptomatic" bacteriuria (12a). Whether only symptomatic or also asymptomatic infections lead to tissue engagement that occasionally may progress to renal scarring is not well known.

In a preliminary report, it was suggested that the clinical expression of urinary tract infections as symptomatic or asymptomatic might be related to the ability of the bacteria to adhere to the mucous surfaces of the urinary tract (28). *E. coli* bacteria isolated from the urine of patients with acute pyelonephritis were shown to adhere in significantly larger numbers to human uroepithelial cells in vitro than did bacteria from patients with asymptomatic bacteriuria. The present report describes an in vitro model for the study of bacterial adhesion, using epithelial cells from fresh morning urine of healthy individuals and *E. coli* bacteria isolated from patients with urinary tract infection. The influence on adhesion of the type of epithelial cell, properties of the bacteria, and conditions of incubation is investigated.

MATERIALS AND METHODS

Epithelial cells. The sediment of fresh morning urine from healthy individuals was washed in phosphate-buffered saline (PBS), pH 7.1, 300 mosmol/liter, and the cells were resuspended in PBS unless otherwise stated. The number of cells per milliliter was calculated by direct-light microscopy. Uroepithelial cells from 11 apparently healthy, non-bacteriuric persons denying previous urinary tract infection were tested, but most of the experiments were done with epithelial cells from one subject, CSE.

Bacteria. Nine *E. coli* strains were selected from the 49 strains tested earlier (28) to represent varying adhesive ability from the strongest (I and II) to the weakest (V through IX). All strains had been isolated from the urine of patients with urinary tract infection. From the date of isolation until used, the strains had been kept on deep agar slab cultures.

For adhesion testing, bacteria were transferred from deep agar to Drigalski agar plates and from these to liquid growth media and were grown without shaking at 37°C for 16 h unless otherwise stated. The growth media used were brain heart infusion broth (Difco Laboratories, Detroit, Mich.), minimal salts medium (standard mineral base with lactate as a carbon source [26]), and nutrient broth according to Cowan and Steele (6) with and without glucose (0.5% and 1.5%). Bacteria were harvested by centrifugation, and the sediment was suspended in PBS. The number of bacteria per milliliter was determined by direct-light microscopy, using a Bürker chamber.

Bacterial adhesive properties were varied by heat

treatment at 56°C for 30 min or boiling for 1 h, by Formalin treatment (0.5%), and by washing by centrifugation at 250 × *g* for 10 min and resuspension in 10 ml of PBS zero, two, four, and six times before incubation with epithelial cells.

Adhesion testing. To 10^5 epithelial cells were added varying numbers of bacteria and PBS to a volume of 1 ml. The mixtures were incubated during rotation in a "Heto" rotor (Birkerød, Copenhagen, Denmark) at 20 rpm and 37°C for 60 min unless otherwise stated.

Incubation media were varied as follows. PBS was usually used as the incubation medium. The influence of pH on adhesion was tested with citrate buffer at pH 3.0, 5.2, and 6.3, Veronal buffer at pH 7.2 and 8.3, or PBS at pH 7.2. PBS to which increasing amounts of NaCl had been added, giving osmolarities of 300, 600, 900, and 1,400 mosmol/liter, was also used. Since urine contains variable components other than acids and salts, urine from 16 healthy individuals with no previously known urinary tract infection was also used as an incubation medium. The urines were free of antibody to the strains tested, as measured with the enzyme-linked immunosorbent assay (ELISA) technique (9), using a pool of O antigens representing the eight O serogroups most often found in *E. coli* causing urinary tract infections (1, 13). Incubation temperatures ranging from 4 to 56°C were tested.

After incubation, unattached bacteria were eliminated by repeated washing in PBS. Before counting, a drop of trypan blue was added to the cell suspension, to allow exclusion of the stained dead epithelial cells (10, 18). The number of bacteria adhering to each of 40 epithelial cells was determined, starting from the upper left corner of the Bürker chamber. All samples were identified in code and read by the same person.

Statistical methods. Standard statistical methods were used in the study. Location and dispersion were estimated with the arithmetic mean and standard deviation. Different sources of variation were studied, using a one-way analysis of variance (3). Relations between the means and standard deviations were investigated with simple linear regression. Due to the skewness of the distribution of the number of bacteria adhering to an epithelial cell, nonparametric tests were used for significance testing. Different samples of 40 epithelial cells obtained the same day were compared in the following way. The median number of bacteria per epithelial cell for all observations was calculated. In each sample, the number of epithelial cells with bacterial numbers above and below this median was determined. The contingency table so obtained was tested for homogeneity using an ordinary χ^2 test. When comparisons were based on experiments performed on different days, the χ^2 test was applied separately to the data from each day and pooled together (2). This χ^2 technique was used for all comparisons described below.

RESULTS

Adhering bacteria were clearly seen on the cell surface and were easy to count. When large numbers of bacteria attached to the epithelial cells, bacteria became confluent on the cell surface, and a rough estimate of the numbers of adhering bacteria had to be made. Adherence could also be demonstrated by scanning electron microscopy (Fig. 1).

Influence of bacterial growth phase on adhesion. Inocula on brain heart infusion broth of the nine *E. coli* strains were grown at 37°C, and samples taken at 30 min and 2, 5, 7, 10, 12, 14, 16, 20, and 24 h were quantitated by viable counts and used in the adhesion test. The growth rates of the nine strains were essentially the same. The adhesive ability of the four poorly adhering strains was low, regardless of growth phase. The five adhering strains adhered at all stages of growth, but maximal adhesive ability was always seen after termination of the logarithmic growth phase. For subsequent experiments, 16-h bacterial cultures were used.

Influence of bacterial culture medium on adhesion. The influence of medium on adhesion is illustrated in Table 1. Bacteria grown on brain heart infusion broth, nutrient broth, and minimal salts medium showed good adhesive ability. When bacteria were grown on glucose-rich nutrient broth, their adhesive ability decreased significantly, more with 1.5 than with 0.5% glucose ($P < 0.01$). Poorly adhering strains did not attach, regardless of cultivation medium.

Influence of pretreatment of bacteria on adhesion. Formalin or heat-killed bacteria lost the ability to adhere to uroepithelial cells. After incubation at 56°C for 30 min, the adhesive ability of *E. coli* decreased significantly. Washing of the bacteria before incubation with epithelial cells significantly impaired the adhesion of good adherers after as few as two washings (Table 2). Poor adherers remained so after washing

Influence of bacterial concentration and incubation time on adhesion. Epithelial cells (10^5) were incubated with 10^4 to 10^8 bacteria per ml of each of the nine strains tested. Samples were taken at 1, 10, 30, 60, 90, 120, 180, and 240 min. For each incubation time and bacterial concentration, the number of bacteria adhering to 40 cells was counted. Figure 2 illustrates the effect of increasing bacterial concentrations on adhesion at 60 and 180 min of incubation time. One poorly and one efficiently adhering *E. coli* strain representative of the other strains tested are shown. At a concentration of 10^4 bacteria, no adhesion was observed for any of the strains tested. Of the four poorly adhering strains, no or only a few bacteria attached to the epithelial cells even at maximal bacterial concentrations, whereas strains I through IV increased their adherence with increasing bacterial concentrations.

The number of bacteria adhering to the epi-

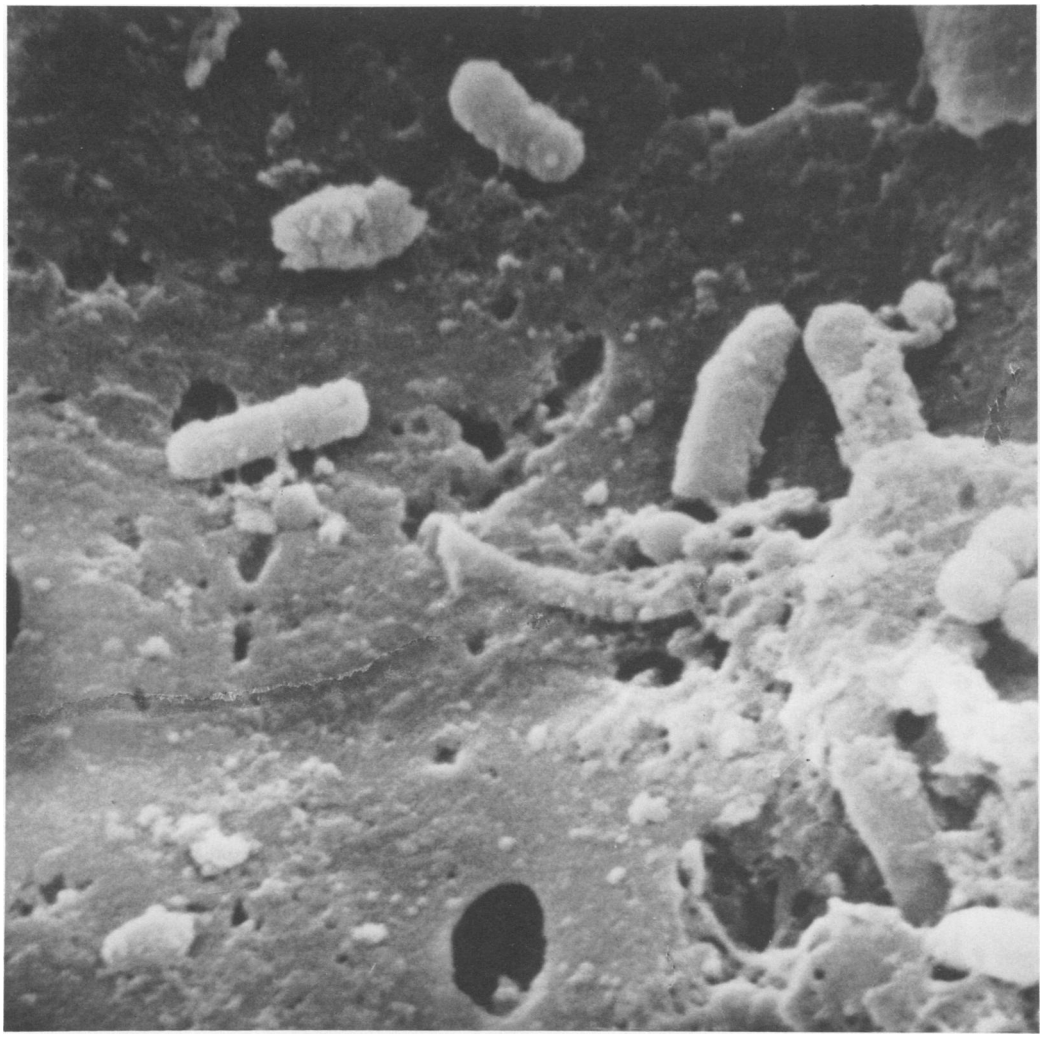

FIG. 1. *Scanning electron micrograph of E. coli on the surface of an epithelial cell from the urinary tract.* ×*11,000.*

thelial cells increased with longer incubation time. Figure 3 illustrates the effect of increasing incubation time, using as representative examples one poorly and one efficiently adhering strain. A slight, but significant, increase in adhesion could be seen in one of the poorly adhering strains at 2 h of incubation ($P < 0.05$). For all the strongly adhering strains, the most rapid increase in adhesion was observed during the first 60 min of incubation. In Fig. 4 is illustrated the change in adhesion with longer incubation time for one of the strongly adhering strains, using 10^8 bacteria per ml.

To ensure that growth during incubation did not account for the increase in adhesion observed with prolonged incubation time, control experiments were performed. First, PBS was tested for its capacity to entertain bacteria growth. Bacterial inocula were incubated for 60 and 180 min in PBS, and viable counts were performed. No increase in bacterial numbers was observed. Second, mixtures in PBS of 10^5 epithelial cells and 10^8 bacteria were incubated at 37°C, and samples for viable counts were taken at 10, 60, and 180 min. One good and one poor adherer were tested. No significant increase in bacterial numbers was observed.

Multiplication of bacteria, once attached to the epithelial cell surface, was analyzed in small chambers prepared with a microscope slide and cover slip closed at the edges with silicone grease. The chambers were filled with mixtures of 10^8 bacteria and 10^5 epithelial cells per ml, previously incubated for 10 min and washed free

TABLE 1. *Influence of culture medium on adhesion of bacteria with good adhesive ability*

Bacteria/cell	Frequency distribution[a]			
	Brain-heart infusion broth	Nutrient broth	Standard mineral base	Nutrient broth + 1.5% glucose
0–9	2	7	11	33
10–19	1	1	4	4
20–29	2	1	3	2
30–39	3	0	3	0
40–49	0	1	2	1
50–99	15	16	14	0
100–149	15	11	2	0
150–199	2	3	1	0
Mean	87	77	44	5
SEM[b]	7	8	4	1

[a] 40 epithelial cells investigated for each incubation medium.
[b] SEM, Standard error of the mean.

TABLE 2. *Effect on adhesion of washing bacteria before incubation with epithelial cells, using a good adherer*

Bacteria/cell	Frequency distribution[a]			
	0 washings	2 washings	4 washings	6 washings
0–9	12	30	32	40
10–19	3	2	3	0
20–29	0	2	2	0
30–39	0	2	1	0
40–49	1	0	1	0
50–99	15	4	1	0
100–149	9	0	0	0
Mean	58	10	6	0
SEM[b]	8	4	3	0

[a] 40 epithelial cells studied after each washing.
[b] SEM, Standard error of the mean.

of unattached bacteria. One group of five epithelial cells was selected, and the number of attached bacteria was counted after 0, 30, 60, and 180 min of incubation at 37°C. For the five nonadhering strains, no bacterial multiplication was observed on the cell surface. In contrast, the four adhering strains could be seen to multiply on the epithelial cells with an increase of less than 25% in 60 min.

To investigate what effect such bacterial growth might have on the number of adhering bacteria registered after 60 min of incubation, the following experiments were done. Continuously incubated samples of 10^5 epithelial cells to which had been added 10^8 *E. coli* of each of the nine strains tested were compared with duplicate samples for which incubation had been interrupted after 10 min and the cell suspension had been washed free of unattached bacteria and reincubated at 37°C after readjustment to a volume of 1 ml with PBS. Adhering bacteria were counted in the interrupted sample after 10 min and in both samples after 60 min of incubation. A much greater increase in the number of adhering bacteria from 10 to 60 min of incubation was seen for the continuously incubated sample compared with the washed one (Table 3). Thus, the increase in the number of adhering bacteria was mainly due to continuous adhesion of new bacteria and not to growth of those already adhering.

From the information presented above, we decided to use 10^5 epithelial cells and 10^8 bacteria per ml and to incubate the mixture for 60 min, which was done in subsequent experiments.

Influence on adhesion of variations in epithelial cells. In all uroepithelial cell preparations, both squamous and transitional epithelial cells were found. Columnar epithelial cells were rare. None of the *E. coli* strains tested adhered selectively to either of the cell types.

To find out if separation of the uroepithelial cell types was necessary, the influence on variation of different ratios of squamous and transitional cells was investigated. The epithelial cells were classified as squamous or transitional on morphological bases. Eleven samples, each containing 120 cells, were analyzed. In Table 4 are shown the weighted standard deviations for *E. coli* strain I. The mean ratio of squamous to transitional epithelial cells was 0.18 ± 0.08. The two cell types differed in mean adherence. The contribution to the total variation of differences in ratio and in mean adherence was estimated as: 2,430 − (0.18 × 1−117 + 0.82 × 2,625) = 76 (3%). The remaining 97% of the total variation

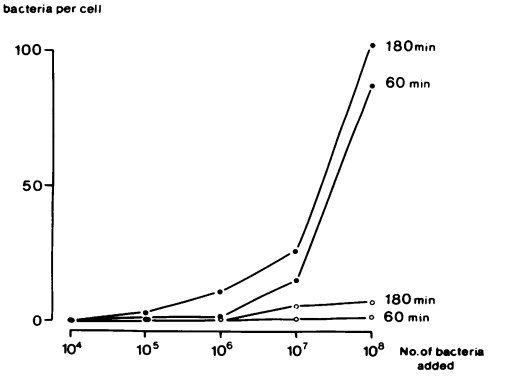

FIG. 2. *Influence on adhesion to human uroepithelial cells of bacterial concentration at 60 and 180 min of incubation time. One poorly* (○) *and one efficiently* (●) *adhering strain are presented. Each dot indicates the mean of bacteria counted on 40 cells.*

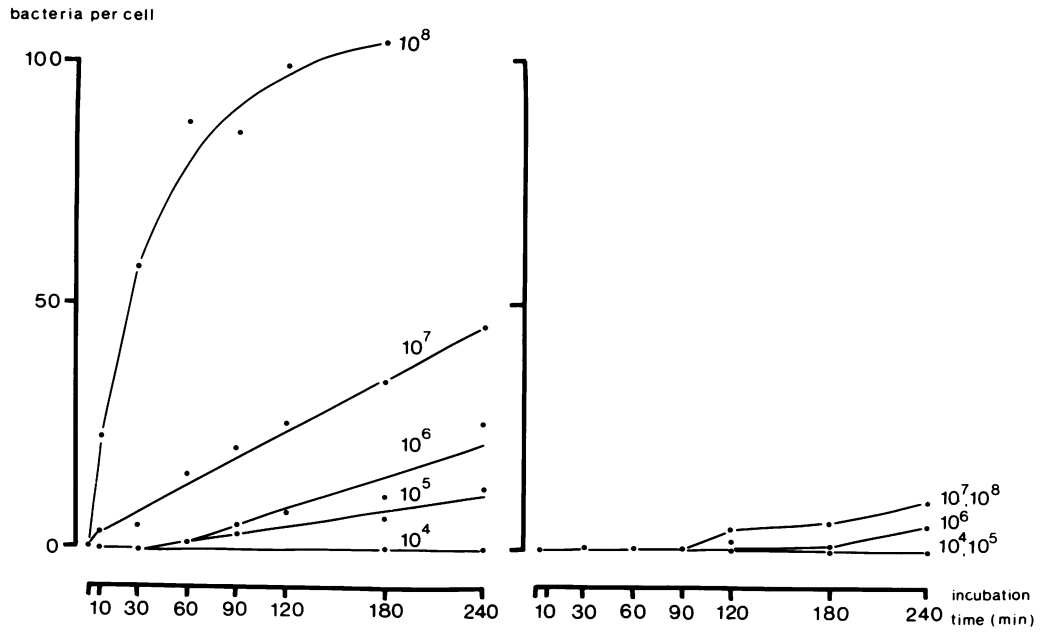

FIG. 3. *Influence on bacterial adhesion of incubation time at increasing bacterial concentrations. One strain with good and one with poor adhesive ability are presented separately. Each dot indicates the mean of bacteria attached to 40 epithelial cells.*

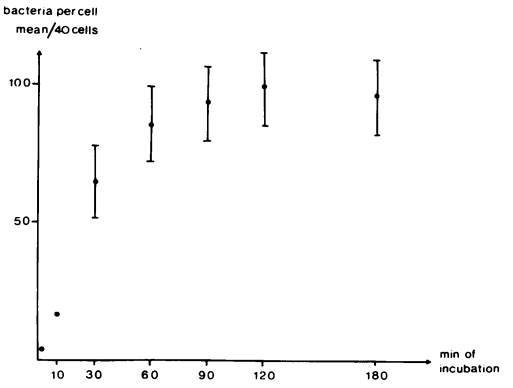

FIG. 4. *Influence of incubation time on the adhesion of E. coli I at a concentration of 10^8 bacteria. Each dot is based on data from six experiments in each of which bacteria attached to 40 epithelial cells were counted. The standard deviations were weighted together for the values at 30 min and on, since differences were small. At 1 and 10 min of incubation, the standard deviations varied extensively with the means and have been left out of the figures.*

was caused by the variation in adherence to individual epithelial cells of both types. Similar estimates were obtained for the other strains tested. Thus, no further attempts were made to study adhesion to either cell type selectively.

Uroepithelial cells from each of 10 healthy, non-bacteriuric individuals were mixed with 10^8 *E. coli* I and compared with uroepithelial cells from one subject, CSE (Table 5). The number of adhering bacteria of the other subjects ranged from 84 to 124% of that of CSE.

Adherence to trypan blue-stained cells was significantly lower than that to unstained cells ($P < 0.001$), but exceptional stained cells had the same number of bacteria attached as did unstained epithelial cells. Regardless of the adhesive ability of the *E. coli* bacteria tested, all samples contained some epithelial cells free of attached bacteria. Epithelial cells that had been kept at 56°C for 30 min retained the ability to have bacteria attached to their surface, but, with few exceptions, they were stained by trypan blue.

Incubation conditions. At each of four different pH values or four different osmolarities, the bacteria on 40 epithelial cells were counted. At pH 3, hardly any discrete cells were seen, and remnants of cells did not have bacteria attached to their surfaces. Maximal adhesion was seen at about pH 6. A decrease in adhesion was observed with increasing osmolarity from 300 to 1,400 mosmol/liter. When incubation was performed in urine from healthy individuals without detectable antibodies to the O antigen of the bacterium tested, the adhering number of bacteria was not significantly different from the adhesion obtained with PBS as the incubation medium.

TABLE 3. *Control of growth during incubation*[a]

E. coli strain no.	Bacteria/cell (mean ± standard error)		
	10 min	60 min	
		Continuously incubated	Washed
I	18 ± 3	85 ± 6	29 ± 4
II	32 ± 4	75 ± 7	36 ± 6
III	3 ± 0.6	14 ± 3	6 ± 2
IV	1 ± 0.5	1 ± 0.5	0
V-IX	0	0	0

[a] Continuously incubated samples were compared with samples with incubation interrupted after 10 min, the epithelial cell suspensions washed free of unattached bacteria, and incubated at 37°C.

TABLE 4. *Analyses of variation in adherence of E. coli I to squamous and transitional epithelial cells*[a]

Cell type	Bacteria/cell (weighted SD)	Bacteria/cell (range of individual SDs)
Squamous	51.2	41.2–60.7
Transitional	33.4	18.3–43.3
All	49.3	40.6–57.9

[a] Figures were obtained from 11 experiments, each containing 120 cells.

At temperatures between 35 and 41°C, no significant differences in adhesion were seen. At lower temperatures, slow adhesion took place, and at temperatures of >45°C, hardly any bacterial attachment could be registered.

Conditions subsequently used for adhesion testing. Suspensions in PBS of $10^5 \pm 0.11$ epithelial cells per ml and $10^8 \pm 0.14$ *E. coli* bacteria per ml (mean ± standard error) were mixed. The samples were incubated during rotation at 37°C for 60 min. Unattached bacteria were then eliminated by repeated washing in PBS, and a drop of trypan blue was added. Bacteria attached to each of 40 unstained epithelial cells were calculated by direct-light microscopy, using a Bürker chamber.

Variation in adherence within and between preparations of bacteria and epithelial cells. On each of 8 consecutive days, duplicate preparations of epithelial cells from one individual and the nine *E. coli* strains were made, using the conditions described above. The number of bacteria attached to each of 40 epithelial cells was determined in each sample. The standard deviation in adherence to individual cells within a preparation was linearly related to the mean adherence of the preparation and is given as an equation of regression (Table 6). To estimate the variation between preparations within a day and between days, a simple analysis of variance was performed. The standard deviation between preparations within a day was similar to the standard deviation within a preparation, whereas the standard deviation between days was larger.

The experimental error of interest, i.e., that between days, was calculated for the strains with good adhesion, as described here for strain I. The standard deviation for a mean of n epithelial cells was $\sqrt{16.3 + 49.6^2/n}$, using the estimates of Table 6 and ignoring the variation within a day. The experimental errors for various n, assuming a mean adherence of 90 bacteria per cell for *E. coli*, is illustrated in Table 7. Counting 40 cells gives an experimental error of 20%. An increase in the number of cells counted only

TABLE 5. *Mean number of E. coli I bacteria adhering per uroepithelial cell from 11 healthy individuals*

Subject	Mean bacteria/cell (n = 40 cells)
CSE (test person)	90
DB	76
ID	112
HL	86
MH	77
GÅ	73
KW	108
CS	100
IM	88
SE	108
AJ	81

TABLE 6. *Variations within a preparation of bacteria and epithelial cells, between duplicates and between preparations made on 8 different days*[a]

Strain	Adherence (bacteria/cell)			
	Mean	Variations		
		Within mixtures (df = 639)	Between mixtures (df = 8) within a day	Between days (df = 7)
I	90	40.8 ± 0.1 $\bar{x}$ (49.6) r = 0.23	1.9	16.3
II	109	16.8 ± 0.3 $\bar{x}$ (49.5) r = 0.57	0	15.2
III	40	9.6 ± 0.6 $\bar{x}$ (33.4) r = 0.96	3.3	19.4
IV	30	7.2 ± 0.6 $\bar{x}$ (25.3) r = 0.93	0	17.8

[a] Means ($\bar{x}$) and standard deviations were estimated for each strain and each source of variation. Standard deviations are given as an equation of regression on the mean. r indicates the coefficient of correlation between the mean and standard deviation. To estimate the variation between preparations within a day and between days the variance within a preparation should be subtracted. For this, the estimated standard deviations (parentheses) were used, i.e., the value of the regression equation for the mean of all samples.

For the strains with poor adhesive ability (V through IX), measurements like mean and standard deviation are inappropriate.

yields minor changes in the experimental error, the limit being 18% when the number of cells counted tends to infinity. For the strains with poor or intermediate adhesive ability, the experimental error based on mean and standard deviation was inappropriate; therefore, no quantitative statements were made.

DISCUSSION

E. coli bacteria causing symptomatic urinary tract infection adhere in larger numbers to epithelial cells from the urinary tract than do *E. coli* isolated from urine of patients with asymptomatic bacteriuria (30). Bacterial adhesion to epithelial cells may thus be of importance in the pathogenesis and course of urinary tract infections. The method presented in this paper provides a simple tool for the further study of bacterial adhesiveness in relation to urinary tract infection.

Epithelial cells are a normal component of human urine. Quantitatively dominating are transitional epithelial cells from the ureters and bladder and squamous epithelial cells from the trigonum area, the urethra, and, in women, the external urogenital tract. The ratio of the different cell types is known to vary between days and individuals (17, 27). These variations, however, account only for about 3% of the total variation in bacterial adhesion to the epithelial cells; therefore; no further attempts have been made to separate the cell types.

It is uncertain to what extent the epithelial cells discharged into the urine can be considered representative of the intact epithelial surfaces of the urinary tract. Trypan blue as an indicator of cell vitality is not without disadvantages. Cells take up dye in late stage 3 of cell death, after loss of deoxyribonucleic acid from the cell nucleus and cessation of respiration (18). The population of unstained cells may thus contain both viable cells and cells in early stages of cell death. The epithelial cells seem much less sensitive to various treatments than do the *E. coli* bacteria, however, and can function as recipients for adhering bacteria both after repeated washings and after heating at 56°C for 30 min. Further studies on possible metabolic activity of the discharged cells may help to explain the great variation in receptivity for adhering bacteria between individual epithelial cells.

There are several indications that pili are present on the attaching bacteria. The adhesive ability of the *E. coli* strains tested was expressed when bacteria were grown in minimal salts medium, nutrient broth, and brain heart infusion broth, but not when extra glucose was added to those media. The structure responsible for adhesion was easily washed away and was inactivated by heating for 30 min at 56°C or by Formalin treatment. Pili on *E. coli*, as described by Brinton, are almost pure polypeptides that depolymerize on heating and are easily washed off the bacterial cell surfaces (5). For *Shigella*, pili are not formed when bacteria are grown on glucose-rich media (7). This might be due to increased acidity, which is known to depolymerize pili (5). The growth conditions in the urinary bladder, with limited oxygen supply, should favor growth of piliated *E. coli* (5, 7). Presence of pili on *E. coli* bacteria also relates to their ability to attach to human uroepithelial cells (C. Svanborg Edén, H. A. Hansson, and L. Å. Hanson, manuscript in preparation), as has been suggested for *Proteus* (24). The adhesiveness of the *E. coli* strains tested is not due to acquisition of blood group-reactive substances from the cultivation medium, as suggested by R. J. Gibbons and J. U. Qureshi (manuscript in preparation), since growth on minimal salts medium did not significantly decrease adhesion.

TABLE 7. *Standard deviations (SD) and experimental error for E. coli I for a mean value of 90 bacteria per epithelial cell, for increasing numbers of epithelial cells (n)*[a]

n	SD	%
1	52.2	58
10	22.6	25
20	19.7	22
40	18.1	20
80	17.2	19
120	16.7	19
*[b]	16.3	18

[a] The figures were based on pooled data from 11 samples, each containing 120 cells.
[b] *, Limit where *n* tends to infinity.

A few connections may be found between the presented in vitro model and conditions known to exist in patients with urinary tract infections. In the urinary bladder, bacterial growth is thought to be continuously in log phase (4), since medium (urine) is continuously supplied and since part of the old culture is voided. In patients with residual urine or reflux, but also during incubation over night, it might, however, be possible to obtain post-log-phase cultures. Bacteria at both stages of growth obviously possess adhesive ability although post-log-phase cultures adhered better than did those in log phase. The bacterial concentration, 10^8 bacteria per ml, that is used in the in vitro test is high but not unbiological, since the number of bacteria per milliliter isolated from patients with significant bacteriuria ranges from 10^5 to 10^9 (4).

The normal time between voidings is about 4 h. After 4 h of incubation in vitro, the poorly adhering strains still adhered in small numbers, whereas the efficiently adhering strains showed the largest increase of attachment during the

first 60 to 120 min of incubation. Thus, even in a patient with frequency as in cystitis, voiding every hour, the time between voidings may be sufficient for bacteria to become attached to the urinary tract epithelium. Once attached, bacteria may penetrate, activate the defense mechanisms of the host, or multiply on the cell surface until the epithelial cell is discharged and new adhesion must take place. The normally slow turnover of bladder epithelium is likely to be faster during infection (12). Epithelial cells in the urine sediment of infected patients are, thus, likely to have bacteria attached to the surface. The sediments of patients with urinary tract infection are presently being studied from this aspect. Furthermore, uroepithelial cells from a larger group of normal individuals is being studied and compared with cells from patients with ongoing or recurrent urinary tract infection.

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LITERATURE CITED

1. **Andersen, H. J.** 1968. Clinical studies on the antibody response to *E. coli* O-antigens in infants and children with urinary tract infection, using a passive haemagglutination technique. Acta Paediatr. Scand. Suppl. **180**:8-28.
2. **Armitage, P.** 1973. Statistical methods in medical research, p. 372-373. Blackwell Scientific Publications, Oxford.
3. **Armitage, P.** 1973. Statistical methods in medical research, p. 189-207. Blackwell Scientific Publications, Oxford.
4. **Asscher, A. W., M. Sussman, and Weiser. R.** 1968. Bacterial growth in human urine, p. 3-13. *In* F. O'Grady and W. Brumfitt (ed.), Urinary tract infection. Proceedings of the First National Symposium, Oxford, London.
5. **Brinton, C. C.** 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans. N.Y. Acad. Sci. **27**:1003-1054.
6. **Cowan, S. T., and K. J. Steel.** 1966. Manual for the identification of medical bacteria, p. 99. University Press, Cambridge.
7. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. **74**:397-411.
8. **Ellen, R. P., and R. J. Gibbons.** 1974. Parameters affecting the adherence and tissue tropisms of *Streptococcus pyogenes*. Infect. Immun. **9**:85-91.
9. **Engvall, E., and P. Perlmann.** 1972. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J. Immunol. **109**:129-135.
10. **Evans, H. M., and W. Schuleman.** 1914. The action of vital stains belonging to the benzidine group. Science **39**:443-454.
11. **Freter, R., and G. W. Jones.** 1976. Adhesive properties of *Vibrio cholerae*: nature of the interaction with intact mucosal surfaces. Infect. Immun. **14**:246-256.
12. **Gibbons, R. J. and van Houte, F.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19-44.
12a. **Hanson, L. Å, S. Ahlstedt, A. Fasth, U. Jodal, B. Kaijser, P. Larsson, U. Lindberg, S. Olling, A. Sohl Åkerlund, and C. Svanborg Edén.** 1977. *E. coli* antigens, human immune response and the pathogenesis of urinary tract infections. J. Infect. Dis. **136**:S144-149.
13. **Hanson, L. Å., J. Winberg, H. J. Andersen, J. Holmgren, U. Jodal, and B. Kaijser.** 1971. Significance of serum and urine antibodies in urinary tract infections in childhood, p. 117-132. *In* P. Kinkaid-Smith and K. F. Fairley (ed.), Renal infection and renal scarring. Mercedes, Melbourne.
14. **Hohmann, A. and M. R. Wilson.** 1975. Adherence of enteropathogenic *Escherichia coli* to intestinal epithelium in vivo. Infect. Immun. **12**:866-880.
15. **Jones, G. W., G. D. Abrams, and R. Freter** 1976. Adhesive properties of *Vibrio cholerae*: adhesion to isolated rabbit brush border membranes and hemagglutinating activity. Infect. Immun **14**:232-239.
16. **Jones, G. W., and R. Freter.** 1976. Adhesive properties of *Vibrio cholerae*: nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. Infect. Immun. **14**:240-245.
17. **Kern, W. H.** 1970. Epithelial cells in urine sediments. Am. J. Clin. Pathol. **56**:67-72.
18. **King, D. W., S. R. Paulson, N. L. Puckett, and A. T. Krebs.** 1959. Cell death. IV. The effect of injury on the entrance of vital dye in Ehrlich tumour cells. Am. J. Pathol. **35**:1067-1079.
19. **McNeish, A. S., P. Turner, J. Fleming, and N. Evans.** 1975. Mucosal adherence of human enteropathogenic *E. coli*. Lancet **ii**:946-948.
20. **Nagy, B., H. W. Moon, and R. E. Isaacson.** 1976. Colonization of porcine small intestine by *Escherichia coli*: ileal colonization and adhesion by pig enteropathogens that lack K88 antigen and by some acapsular mutants. Infect. Immun. **13**:1214-1220.
21. **Powell, D. A., P. C. Hu, M. Wilson, A. M. Collier, and J. B. Baseman.** 1976. Attachment of *Mycoplasma pneumoniae* to respiratory epithelium. Infect. Immun. **13**:959-966.
22. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255-263.
23. **Sellwood, R., R. J. Gibbons, G. W. Jones, and J. M. Rutter.** 1975. Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. J. Med. Microbiol. **8**:405-411.
24. **Silverblatt, F. J.** 1974 Host-parasite interaction in the rat renal pelvis. A possible role for pili in the pathogenesis of pyelonephritis. J. Exp. Med. **140**:1696-1711.
25. **Sobeslavsky, O., B. Prescott, and Chanock, R. M.** 1968. Adsorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. J. Bacteriol. **96**:695-705.
26. **Stainer, R. Y., N. J. Palleroni, and M. Doudoroff.** 1966. The aerobic pseudomonads: a taxonomic study. J. Med. Microbiol. **43**:159-271.
27. **Ström, J.** 1973. Cytology of the urine in healthy persons and cytological reactions in acute infections. Scand. J. Infect. Dis. **5**:209-228.
28. **Svanborg Edén, C., L. Å Hanson, U. Jodal, U. Lindberg, and A. Sohl Åkerlund.** 1976. Variable adherence to normal human urinary tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary-tract infection. Lancet **ii**:490-492.
29. **Ward, M. E., and P. J. Watt.** 1975. Studies on the cell biology of gonorrheae, p. 229-242. *In* D. Danielsson, L. Juhlin, and P.-A. Mårdh (ed.) Genital infections and their complications. Almqvist and Wiksell Int., Stockholm.