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In Vivo Adherence of *Pseudomonas aeruginosa* to Rat Bladder Epithelium (41586)

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Abstract. To date, the study of bacterial adherence to uroepithelial cells has utilized considerable variation in methodology, resulting in highly divergent conclusions. In an attempt to standardize methodology, a modified method is described to more accurately measure the *in vivo* bacterial adherence to rat bladder uroepithelium utilizing *Pseudomonas* species labeled with 2-[^{3}H]adenine. Two isolates of *P. aeruginosa* were selected for more intensive study; one showed consistently good adherence; the second strain always adhered poorly, thus providing a negative control. Maximum adherence was detected after 60 min of incubation. Neither of the two *Pseudomonas* isolates when examined by transmission electron microscopy showed evidence of pili formation. The morphological features of *Pseudomonas* adherence to bladder mucosa as studied by scanning electron microscopy are described.

The defense mechanisms of the urinary bladder have assumed primary importance in understanding the pathogenesis of lower and upper urinary tract infections. The natural resistance of the bladder to colonization by pathogenic bacteria has been emphasized (1, 2, 3). In this regard, bacterial adherence to the bladder uroepithelium has been investigated to identify virulence factors among the uropathogens as well as explore bladder defense (4, 5).

Different models have been used *in vivo* including dog (6), guinea pigs (2), rabbit (7), mouse (8), and rat (9–14). Bacteria have been introduced into the bladder transurethrally or via cystostomy. Some *in vivo* techniques require preliminary treatment of bladder epithelium with acid solutions of varying concentrations (3, 7, 15, 16). Differences also exist in duration of exposure of bladder to bacteria, pH of admixture, and particularly regarding the method of measurement of adherence (3, 7). Variable attention was given to size, weight, and viability of the experimental bladder. On the other hand, the use of exfoliated human and animal uroepithelial cells *in vitro* has been criticized because of additional variables, particularly viability of cells and the heterogenous population of the low-yield cells so studied (17–21). Furthermore, variation in selection of bacteria and above all, the *in vitro* preparation of microorganisms has a profound effect on adherence (10, 18, 20, 21). Accordingly, it is no surprise that strongly divergent conclusions have resulted (5, 11, 17, 22).

In studying the *in vivo* rabbit model, Parsons *et al.* (16) have stressed the marked day-to-day variation in adherence which precludes comparison of experiments performed on different days. Adherence values measured *in vivo* and *in vitro* are frequently expressed with relatively large standard errors and wide ranges (7, 15, 16).

In an attempt to obtain a more reliable *in vivo* model of adherence, we have modified the rabbit model as described by Parsons *et al.* (3). Radiolabeled *Pseudomonas aeruginosa* in rats was utilized in a methodological study directed at identifying sources of error in estimating *in vivo* adherence.

Materials and Methods. *Bacterial strains.* Two strains of *P. aeruginosa* taken from the laboratory collection were used in the present study. The usual method for storing *Pseudomonas* strains was as follows: after first isolation from clinical material on McConkey agar (Difco Laboratories, Detroit, Mich.) and characterization according to standard cultural, morphological, biochemical, and serological techniques, two to three isolated colonies were picked and inoculated on King agar (23). Overnight incubation at 37°C was followed by preparation of stock cultures in brain–heart infusion (Difco), which were distributed in small aliquots and stored at −70°C.

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On use, one stock tube was thawed, inoculated on King agar, incubated overnight at 37°C, and incubated another 24 hr at room temperature for full development of eventual slime and/or pigment. The two *Pseudomonas* strains used were: strain TM515 isolated from a wound swab and which belonged to serotype 11, and strain TM562 isolated from urine and identified as serotype 10. Both were pigment producers, but only strain TM515 was a slime producer.

Preparation of bacteria for tests. Three to four isolated colonies from the King agar culture were inoculated in 10 ml Mueller–Hinton broth (Difco) containing 2 μCi/ml of 2-[^{3}H]adenine (Radiochemical Center Amersham, England) and incubated overnight at 37°C under stationary conditions. Next day the bacteria were harvested by centrifugation at 3000 rpm for 15 min and washed three times with 20-ml portions of sterile phosphate-buffered saline (PBS; pH 7.3). Preliminary tests had shown that under these conditions, the last supernatant did not contain any significant amount of radioactivity. The bacterial sediment was resuspended in 1.0 ml of PBS and was homogenized by aspiration and extrusion through a 26-G needle. The suspension of bacteria was finally adjusted to an absorbance of 0.58 and 620 nm in a Coleman Junior II A linear spectrophotometer, Model 6/20A (Coleman Instr., Playwood, Ill.). Previous calibrations, confirmed by colony counts expressed as colony forming units (CFU), showed that this absorbance corresponds to approximately 3.2×10^8 CFU/ml for strain TM515 and to 8.4×10^8 CFU/ml for strain TM562. The number of colony forming units per milliliter was determined by serial 10-fold dilution and plating 10-μl aliquots of the last three dilutions on McConkey agar. This measurement was repeated in each experiment. On each such occasion, the radioactivity (counts per minute, cpm) of a 0.5-ml aliquot of the final bacterial suspension was determined, after being treated exactly as were the rat bladders. An index of the labeling was calculated, which averaged 5.1 $\times 10^3$ CFU/cpm for strain TM515 and 17.2 $\times 10^3$ CFU/cpm for strain TM562.

Adherence assay. Sprague–Dawley female rats, weighing between 200 and 300 g were used throughout the experiments. The animals were anesthesized with pentobarbital sodium (Abbott), 4 mg/100 g animal weight, injected intraperitoneally. The method of Parsons *et al.* (15) was adapted for use with our much smaller animals. A pediatric feeding tube, 3½ Fr. (Sherwood, St. Louis, Mo.) was inserted into the rat's urethra and secured with a 4-O silk purse-string suture around the external genitalia. The abdomen was opened, the bladder exposed and emptied of its contents under direct vision. Except where otherwise mentioned, the following procedure was basically followed:

(i) Acid-treated bladders. After finally verifying that the urethral catheter was in place, the bladder was flushed with 0.7 ml of a 0.1 *N* hydrochloric acid solution for 1 min, then emptied and washed with 1.0 ml of 0.5 *M* dipotassium phosphate (K_2HPO_4), pH 9.4, followed by three washings with 1-ml portions of 0.1 *M* monosodium phosphate (NaH_2PO_4), pH 5.5. The bacterial suspension, 0.5 ml, was then injected through the catheter which was clamped and left at room temperature for 1 hr. After this time the animal was sacrificed, the bladder contents emptied via the catheter, and the bladder excised.

(ii) Non-acid-treated bladders. These were prepared exactly as above, by omitting the 0.1 *N* HCl-treatment step.

In all animals the opened bladder was serially rinsed in seven beakers containing normal saline, to remove nonadherent bacteria. It was then introduced into a glass scintillation vial (Packard Instr. Co. Inc., Downers Grove, Ill.), dried to constant weight at 60°C, weighed, and disintegrated in 1.0 ml of a 1.5 *N* sodium hydroxide solution for 3 hr, at 60°C. Decolorization was achieved by adding 0.1 ml of 60% perchloric acid (Fluka AG Buchs SG) and 0.2 ml of 30% hydrogen peroxide (Merck) and leaving the solution at room temperature for 2 hr. Three drops of a 15% ascorbic acid solution were added and the solution was neutralized with 2.5 *N* HCl. After adding 10 ml of Brady's scintillation liquid, radioactivity was recorded in a Packard Tricarb Scintillation Spectrometer Model 3990.

Background counts were performed on bladders removed from the animals with no acid or bacterial treatment at all, but prepared for radioactivity recording exactly as the

treated bladders, and subtracted from each assay.

The pH values of the bladders' contents after incubation with bacteria, with or without acid treatment, were recorded and found not to exceed the range of 6.5–7.0.

The bladders of animals dying during the experiment, as well as those showing signs of gross mucosal damage, i.e., macroscopic ulceration or hemorrhage, were excluded from the results. This was done after preliminary experiments showed that lower than normal counts were obtained in the former case, while exaggerated high results were recorded in damaged bladders, probably due to trapping of bacteria in crypts (24).

Quantitation of adherence. Rat bladders varied in weight and surface area, hence all measurements of radioactivity were corrected for the weight of the bladder and expressed as cpm/mg rat bladder (dry weight). In addition, since quantitative differences in adherence measured on different days could in part be explained by variations in labeling of test bacterial strains, i.e., uptake of isotope by bacteria, a correction factor was introduced in which the cpm-reading of the bladder was divided by the cpm-reading of an aliquot of bacterial test suspension, identical to the specimen introduced into the rat bladders. The calculation was as follows:

$$\frac{\text{cpm/mg} - \text{background activity of bladder}}{\text{cpm/0.5 ml bacterial suspension} \times 10^4}$$

$$= \text{corrected cpm} \times 10^{-4} \text{ (ccpm} \times 10^{-4})$$

Scanning electron microscopy. After fixation in glutaraldehyde, the specimens were washed twice with PBS and fixed in 5% osmium tetroxide in PBS for 15 min. The specimens were dehydrated in a graded ethanol series (50, 70, 90, and 100%) for 5 min each. Thereafter, they were rinsed twice in amyl acetate and then were subjected to critical-point drying for 30 min, coated with gold in a vacuum evaporator, and examined with a scanning electron microscope (Jeol, Ltd., Medford, Mass., JSM model 504).

Statistical methods. All data were subjected to statistical analysis using Student's *t* test. Differences in mean bacterial adherence are reported as significant when $P < 0.05$ and as not significant when $P > 0.05$.

Results. Initial experiments utilizing six different isolates of *P. aeruginosa* revealed somewhat variable and generally low adherence to untreated rat bladder epithelium, except for one isolate (TM562) which failed to adhere at all. Accordingly, all subsequent experiments were performed upon acid-treated bladders.

Effect of acid treatment on bacterial adherence. The effect of different concentrations of hydrochloric acid used in pretreatment of rat bladder *in vivo* prior to the addition of bacteria is seen in Fig. 1. The mean adherence after 60 min (ccpm $\times 10^{-4} \pm$ SEM) of strain TM515, upon treatment for 1 min with 0.05, 0.1, 0.2, and 0.5 *N* hydrochloric acid was 3.2 ± 0.8, 17.4 ± 3.4, 17.4 ± 6.4, and 9.6 ± 3.2, respectively.

Accordingly, in all subsequent experiments, the lowest acid concentration which resulted in good adherence, i.e., 0.1 *N* HCl, was used. Multiple histological sections of 0.1 *N* HCl acid-treated bladders were stained with hematoxylin and eosin, as well as PAS. After acid treatment the appearance of the bladder epithelium was normal, except that PAS-positive surface mucine was not detected. Similarly, there were no structural differences between the scanning electron micrographs of coded specimens of acid-treated and untreated bladders.

Effect of incubation time on bacterial adherence. A progressive increase in adherence

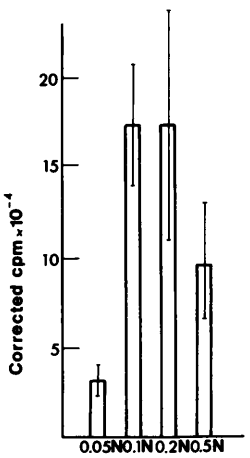

FIG. 1. Effect of hydrochloric acid concentration used in pretreatment of rat bladder on adherence of *P. aeruginosa* strain TM 515.

TABLE I. EFFECT OF INCUBATION TIME OF *P. aeruginosa* STRAIN TM 515 IN RAT BLADDER, *IN VIVO*[a]

Time (min)	Mean adherence[b] (ccpm × 10^{-4}/mg bladder ± SEM)	N^b
15	4.5 ± 2.2	5
30	12.9 ± 5.2	5
60	17.4 ± 3.4	17
120	4.8 ± 0.4	5

[a] Bladders were pretreated with 0.1 *N* hydrochloric acid for 60 sec.
[b] Number of animals tested.

to acid-treated bladders was detected, reaching a maximum at 60 min (Table I). The mean adherence (ccpm × 10^{-4} ± SEM) at 15, 30, 60, and 120 min, was 4.5 ± 2.2, 12.9 ± 5.2, 17.4 ± 3.4, and 4.8 ± 0.4, respectively. In all subsequent experiments, a 60-min incubation period was used.

Comparison of adherence of two P. aeruginosa isolates. The results of multiple experiments comparing two clinical isolates of *P. aeruginosa* are shown in Fig. 2.

In the absence of acid treatment, strain TM515 showed consistently poor adherence, with a mean of $1.4 \times 10^{-4} \pm 0.6$ ccpm/mg bladder in 11 experiments. After 0.1 *N* HCl treatment, however, a marked increase in adherence was observed with a mean of 17.4 $\times 10^{-4} \pm 3.4$ ccpm/mg bladder ($P < 0.0005$). In only 3 out of 17 consecutive experimental subjects was low adherence observed. Evaluation of duplicate samples studied on the same day revealed minor differences only, within the same experimental conditions (Table II).

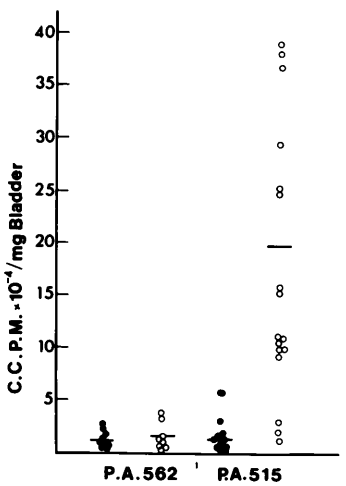

FIG. 2. Comparison of *in vivo* adherence of *P. aeruginosa* strains TM 562 and TM 515 to rat bladder epithelium. (●) Untreated and (○) pretreated with 0.1 *N* hydrochloric acid.

Strain TM562 showed similar low adherence after incubation with untreated bladder, the mean value being $0.91 \times 10^{-4} \pm 0.3$ ccpm/mg bladder. No significant increase in adherence was seen even after acid treatment, when the mean value obtained was $1.3 \times 10^{-4} + 0.5$ ccpm/mg bladder ($P > 0.025$).

In additional experiments, the adherence of 10 clinical isolates of *P. aeruginosa* to acid-treated bladders was determined and correlated with slime production. As shown in Table III no correlation was found between *in vitro* slime production and *in vivo* adherence ($P > 0.05$).

Effect of death of rat on bacterial adherence. During the course of the experimental study, 10 rats died while the bacterial test suspension was incubated within the bladder. In all 10 test animals, extremely low adherence was measured, i.e., $3.2 \times 10^{-4} + 1.5$ ccpm/mg bladder. Thus all data derived when the animals died were excluded.

With obvious hemorrhage or macroscopic erosion of the bladder, extremely high counts were always obtained even for the poorly adherent strain TM562. Accordingly, when hemorrhage or erosion was detected, results were likewise excluded.

TABLE II. COMPARISON OF *IN VIVO* ADHERENCE OF *P. aeruginosa* STRAINS TM 562 AND TM 515 TO BLADDER EPITHELIUM OF DUPLICATE RATS PERFORMED ON FOUR SUCCESSIVE DAYS. EVALUATION OF DAY-TO-DAY ADHERENCE

	Bacterial adherence (ccpm × 10^{-4}/mg bladder)[a]			
Day	TM 562		TM 515	
1st	1.3	2.6	29	41.2
2nd	0.2	0.4	13	12.2
3rd	0.7	0.6	20	26
4th	0.4	0.5	15.9	9.7

[a] Corrected counts per minute × 10^{-4}/mg rat bladder, after 60 min incubation. Rat bladder had been pretreated with 0.1 *N* hydrochloric acid for 60 sec.

TABLE III. COMPARISON OF SLIME PRODUCTION AND *IN VIVO* ADHERENCE OF 10 CLINICAL ISOLATES OF *P. aeruginosa* TO BLADDER EPITHELIUM OF RATS

Strain No.	Clinical source	Adherence (ccpm × 10^{-4}/ mg bladder)[a]	Slime production
367	Urine	4.9 ± 2.3	///
3452	Blood	11.4 ± 4.8	///
494	Urine	7.4 ± 2.9	///
207	Sputum[b]	2.1 ± 0.7	///
119	Urine	12.9 ± 5.1	///
475	Water	6.0 ± 2.4	—
1133	Wound	10.4 ± 4.0	—
1698	Pacemaker fluid	13.2 ± 4.9	—
564	Urine	9.3 ± 3.6	—
352	Urine	4.7 ± 2.1	—

[a] All *P. aeruginosa* isolates were studied at least twice, the results shown represent the mean of two experiments. In each experiment, duplicate rats were used for each organism.

[b] Patient with cystic fibrosis.

Scanning electron microscopy. In terms of surface morphology of rat bladder mucosa, no differences were observed between acid- and non-acid-treated bladders (Fig. 3a); the observer was unable to differentiate between the blindly coded specimens. No mucosal ulceration or bacteria were seen in acid-treated bladders before incubation with *Pseudomonas*. In the experimental animals treated with acid and *Pseudomonas* the organisms could be seen widely distributed and markedly adherent to the stretched uroepithelium (Fig. 3b). At higher magnification, numerous *Pseudomonas* bacteria were seen attached to epithelium cells by means of fibrin-like strands, possibly further anchoring the bacteria to the epithelium (Fig. 3c). No evidence of trapping of bacteria in the epithelial folds or crypts was observed.

Transmission electron microscopic examination of both strains used in the above studies, utilizing uranyl acetate and lead citrate staining in order to demonstrate the eventual presence of pili, failed to show pili in either strain, even when using unwashed test bacteria.

Discussion. In planning the model for studying *P. aeruginosa* uroepithelial adherence, one was faced with a multitude of methods and often divergent conclusions (3, 8, 10, 12, 18). It appears that isotopic labeling of bacteria is a superior method of quantitating adherence than methods dependent on direct light microscropy counting. The latter method is tedious and introduces observer error and bias, while measuring adherence to a very few selected cells only (5). The use of exfoliated cells collected in the urine has been criticized because of the diverse origin of the cells and in particular because of concern regarding cell viability. Thus, exfoliated epithelial cells represent a select, older population of possibly altered cells. Svanborg Eden *et al.* (17) have emphasized the importance of viability on bacterial adherence. In keeping with this observation, in our study, when the rats died during the experiment, adherence declined considerably.

In this study, the *in vivo* rat model was selected based upon the methodology of Parsons *et al.* (15) in rabbits. Similar results were obtained in terms of kinetics of adherence and the effect of acid treatment in enhancing adherence. The cystostomy was replaced by a urethral catheter in order to avoid trauma to the bladder mucosa. Parsons *et al.* stressed the marked day-to-day variation in adherence, precluding comparison of studies performed on different days (15). To decrease variability of results among rats, their data was expressed as the ratio of bacterial adherence to acid- and non-acid-treated rabbit bladders. In the present study, adherence was expressed only as corrected counts per minute, since conversions of measurements to colony forming units (CFU) introduces additional variables. It was observed that day-to-day variability of adherence was minimal.

The finding of a *Pseudomonas* strain that failed to adhere, even after acid treatment, provides a suitable negative control against which adherent strains can be compared. In particular, this strain is useful in detecting false-positive readings due to inadequate washing, nonspecific trapping, or association of bacteria with damaged or altered mucosa. Moreover, the fact that significant quantitative differences in adherence are revealed after acid treatment—i.e., removal of bladder mucin—suggests that whatever the means by which acid enhances adherence, a selective bacterial uroepithelial cell adherence interaction occurs. This is distinguished from the nonspecific bacterial mucosal association that occurs with all strains after trauma.

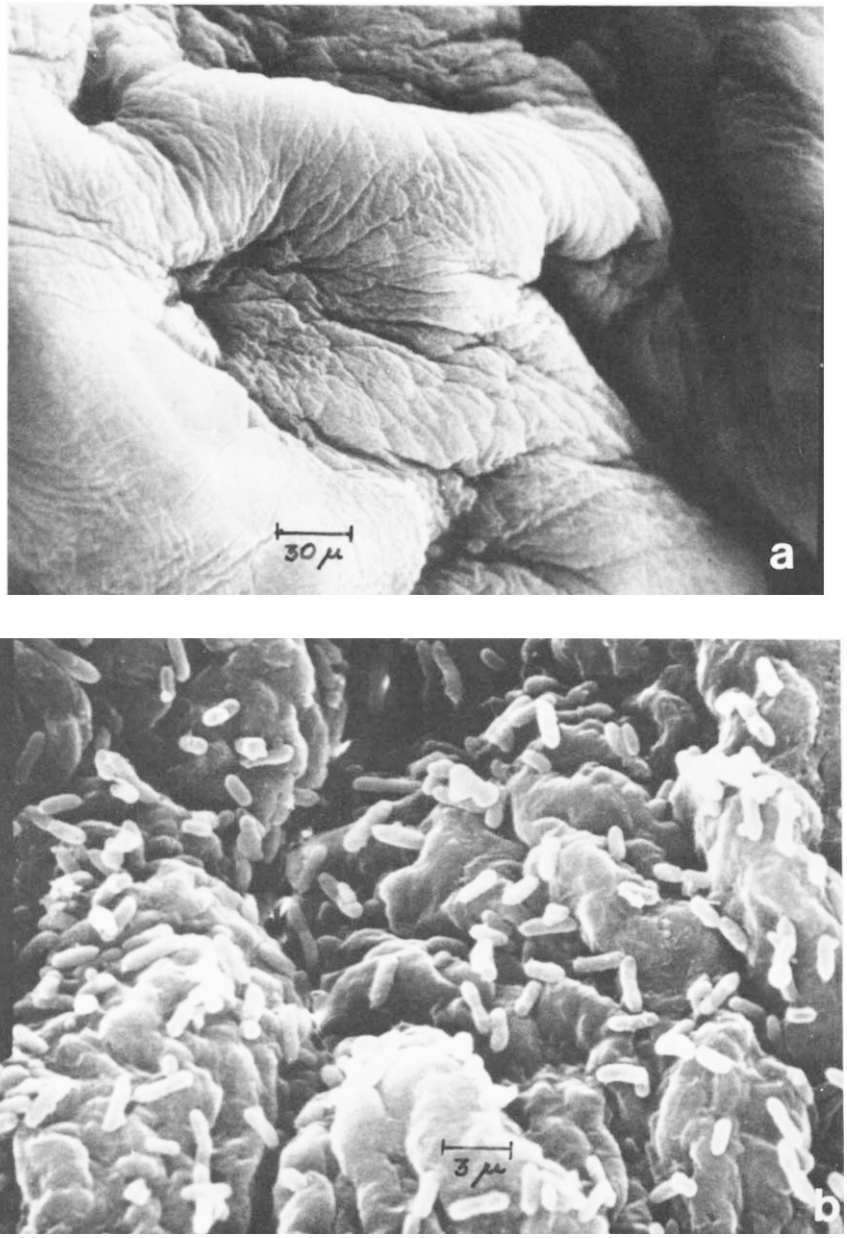

FIG. 3. Scanning electron micrographs of stretched but nondistended rat bladders. (a) 0.1 *N* hydrochloric acid-treated bladder showing multiple mucosal folds and normal appearing surface morphology. (b) *P. aeruginosa* diffusely adherent to uroepithelial cells after 1 hr incubation with acid-treated bladder. (c) *P. aeruginosa* associated with bladder are entrapped in, or anchored by fibrin-like strands to the bladder surface.

To date, little is known concerning *Pseudomonas* adherence to epithelial surfaces. Studies by Ramphal *et al.* (25), Ramirez-Ronda (26), and Woods *et al.* (27) all concluded that *Pseudomonas* adherence *in vivo* and *in vitro* occurs virtually only after the host cells have in some way been damaged. Ramphal *et al.* suggested that *Pseudomonas* ad-

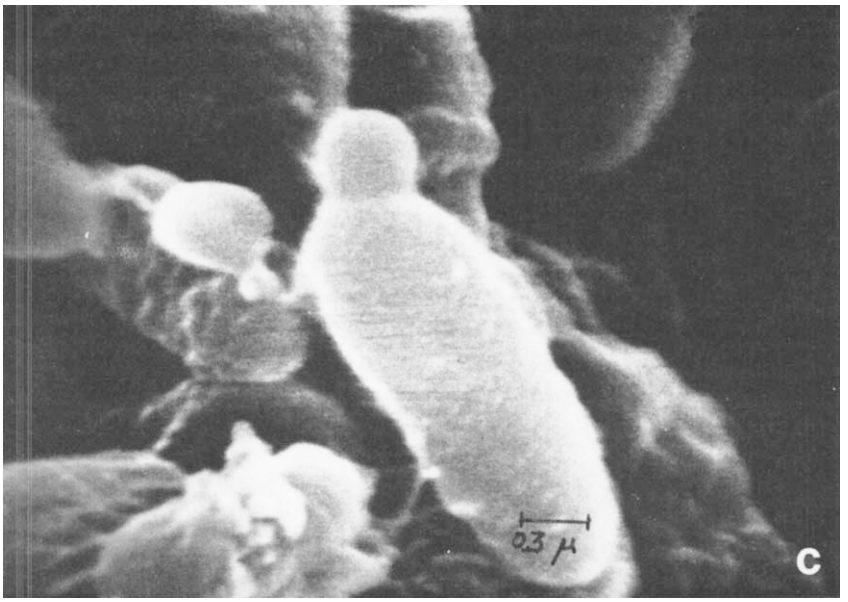

FIG. 3—*Continued.*

herence may lack the tissue specificity shown by many other pathogens but adheres to tissues of different origin after various kinds of injury, i.e., "opportunist" adherence (25). The present findings would particularly support this concept.

The role of slime formation by mucoid strains of *P. aeruginosa* in adherence has not been defined, although results from this laboratory failed to demonstrate any effect on *in vitro* adherence to exfoliated epithelial cells. Mucoid strains of *P. aeruginosa* are well known as urinary pathogens, especially in chronic urinary tract infections (28). In the present study, excellent adherence by the slime producer was observed.

Several authors have demonstrated the importance of bacterial pili in adherence to uroepithelial cells of *Escherichia coli* (22, 19), *Klebsiella pneumoniae* (10), and *Proteus mirabilis* (21). Recently, Woods *et al.* (29) described evidence of pili-dependent adherence of *P. aeruginosa* to exfoliated buccal epithelial cells. It is noteworthy that in the latter study, the *Pseudomonas* adherence was dependent on pretreatment of epithelial cells with enzymes. The *pseudomonas* isolates selected in the present study and which showed consistently good adherence were, on the other hand, nonpiliated. This fact suggests that a pili-independent attachment mechanism may also exist.

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1. Mooney JK, Mooney JS, Hinman F. The antibacterial effect of the bladder surface: An electron microscopic study. J Urol **115**:381–386, 1976.
2. Norden CW, Green GM, Kass EH. Antibacterial mechanisms of the urinary bladder. J Clin Invest **47**:2689–2700, 1976.
3. Parsons CL, Shrom SH, Hanno PM, Mulholland SG. Bladder surface mucin, examination of possible mechanisms for its antibacterial effect. Invest Urol **16**:196, 1978.
4. Fowler JE Jr, Stamey TA. Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence. J Urol **117**:472–476, 1977.
5. Parsons CL, Anwar H, Stauffer C, Schmidt JD. In vitro adherence of radioactively labeled *Escherichia coli* in normal and cystitis-prone females. Infect Immun **26**:453–457, 1979.
6. Kivisto AK, Vasenius H, Lindberg A, Sandholm M. Desorption assay. A functional in vitro test for measuring the adhesion of *E. coli* on the urinary tract epithelium. Invest Urol **15**:412–415, 1978.
7. Parsons CL, Greenspan C, Mulholland SG. The primary antibacterial defense mechanism of the bladder. Invest Urol **13**:72–76, 1975.

8. Aronson M, Medalia O, Schori L, Mirelman D, Sharon N, Ofek I. Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl-D-manno-pyranoside. J Infect Dis **139**:329–332, 1979.
9. Davis CP, Balish E, Mizutani K, Uehling DT. Bladder response to *Klebsiella* infection. A scanning electron microscopy study. Invest Urol **15**:227–231, 1977.
10. Fader RC, Avots-Avotins AE, Davis CP. Evidence for pili-mediated adherence of *Klebsiella pneumoniae* to rat bladder epithelial cells in vitro. Infect Immun **25**:729–737, 1979.
11. Gillenwater JY, Cardozo NC, Tyrone NO, Mulholland SG, Hughes DH, Foster EA. Antibacterial activity of rat vesical mucosa. J Urol **104**:687–692, 1970.
12. Uehling DT, Barnhard DD, Seastone CV. Antibody production in induced canine urinary bladder infections. Invest Urol **6**:211–216, 1968.
13. Uehling DT, Mizutani K, Balish E. Effect of immunization on bacterial adherence to urothelium. Invest Urol **16**:145–147, 1970.
14. Uehling DT, Wolf L. Enhancement of the bladder defense mechanism by immunization. Invest Urol **6**:520–526, 1969.
15. Parsons CL, Mulholland SG, Anwar H. Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan (heparin). Infect Immun **24**:552–557, 1979.
16. Parsons CL, Pollen JJ, Anwar H, Stauffer C, Schmidt JD. Antibacterial activity of bladder surface mucin duplicated in the rabbit bladder by exogenous glycosaminoglycan (sodium pentosanpolysulfate). Infect Immun **27**:876–881, 1980.
17. Svanborg Eden C, Eriksson B, Hanson LA. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect Immun **18**:767–774, 1977.
18. Svanborg EC, Hanson LA, Jodal U, Lindber U, Akerlund AS. Variable adherence to normal human urinary-tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary-tract infection. Lancet **11**:490–492, 1976.
19. Svanborg EC, Hansson HA. *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. Infect Immun **21**:229–237, 1978.
20. Svanborg EC, Jodal U. Attachment of *Escherichia coli* to urinary sediment epithelial cells from urinary tract infection-prone and healthy children. Infect Immun **26**:837–840, 1979.
21. Svanborg EC, Larsson P, Lomber H. Attachment of *Proteus mirabilis* to human urinary sediment epithelial cells in vitro is different from that of *Escherichia coli.* Infect Immun **27**:804–807, 1980.
22. Schaeffer AJ, Amundsen SK, Schmidt LN. Adhesion of *Escherichia coli* to human urinary tract epithelial cells. Infect Immun **24**:753–759, 1979.
23. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med **44**:301–307, 1954.
24. Colleen S, Myhrberg H, Mardh PA. Bacterial colonization of human urethral mucosa. 1. Scanning electron microscopy. Scand J Urol Nephrol **14**:9–15, 1980.
25. Ramphal R, Small PM, Shands JW Jr, Fischlschweiger W, Small PA Jr. Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect Immun **27**:614–619, 1980.
26. Ramirez-Ronda CH. Adherence of *Pseudomonas aeruginosa* to normal and damaged canine aortic valves. Clin Res **26**:404A, 1978.
27. Woods DE, Straus DC, Johanson WG Jr, Bass JA. Factors influencing adherence of Pseudomonas aeruginosa to mammalian cell surface. Abstr Annu Meet Amer Soc Microbiol p20, 1979.
28. Marrie TJ, Harding JKM, Ronald AR, Dikkema J, Lam J, Hoban G, Costerton JW. J Infect Dis **139**:357, 1979.
29. Woods DE, Straus DC, Johanson WG Jr, Berry VK, Bass JA. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect Immun **29**:1146–1151, 1980.

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