Adherence to Uroepithelial Cells of *Providencia stuartii* Isolated from the Catheterized Urinary Tract

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The long-term catheterized urinary tract appears to offer a niche for *Providencia stuartii*, otherwise an unusual clinical isolate. *P. stuartii*, the most frequent and persistent isolate from the urine of 51 long-term catheterized patients, was recovered from 761 of 1230 (62%) weekly urine specimens. To test the hypothesis that prevalence of this species may be due to adherence properties of the organism, 20 selected strains from 14 patients at two nursing homes, representing six distinct serotypes and harbouring combinations of nine different plasmid species, were tested for adherence to uroepithelial cells (UEC). Optimal conditions were determined for differentiating strains on the basis of *in vitro* adherence to UEC. These strains, grown in nutrient broth, were incubated with UEC isolated from the urine of a healthy adult female (10^8 bacteria per 10^5 cells). Washed UEC, retained on 8 µm pore diameter filters, were transferred to slides, fixed and stained; bacteria were counted on each of 40 cells. Fourteen of the 20 strains were defined as adherent to UEC by comparison of mean adherent bacteria and percentage of uroepithelial cells with more than 10 bacteria. Adherence was compared to that of a P-fimbriated strain of *Escherichia coli*. It was not inhibited by 50 mM-mannose. We conclude that the majority of *P. stuartii* isolates are adherent to UEC *in vitro* and suggest that this may play a role in the persistence of this organism in the catheterized urinary tract.

INTRODUCTION

There are currently many more nursing home beds than acute care hospital beds in the USA (Hing, 1977). Within these nursing homes, urinary incontinence may be a problem for up to 35% of the patients (Hing, 1977). If other methods are unsuccessful, an indwelling urinary catheter is used to manage incontinence. Bacteriuria, usually polymicrobial, is a universal complication of long-term indwelling catheters. *Providencia stuartii* is increasingly recognized as a frequent isolate from the urinary tract of chronically catheterized patients, whereas this species is rarely recovered in the acute care setting (Garibaldi *et al.*, 1981; Mobley *et al.*, 1985a; Nyren *et al.*, 1981; Warren *et al.*, 1982; Warren, 1986; Wibell *et al.*, 1980). *P. stuartii* is prevalent not because of more frequent entry into the catheterized urinary tract but because it is a very persistent inhabitant. Once in the urinary tract, it persists significantly longer than all other bacterial species with the single exception of *Escherichia coli* and may be isolated for weeks and months after the initial identification (Warren *et al.*, 1982). We can hypothesize that the persistence of *P. stuartii* may be related to adherence properties of the organism. To test this hypothesis it is necessary to be able to differentiate adherent from non-adherent *P. stuartii* strains.

Tissue tropism is often defined by a lectin-like interaction between bacterium and mammalian cell. *In vitro* assays have, in many instances, been found to correlate with *in vivo* tissue specificity. *Shigella flexneri* binds to cells of the descending colon in guinea pigs (Izhar *et*

Abbreviation: UEC, uroepithelial cell(s).

al., 1982), *Bordetella pertussis* to hamster ciliated epithelium (Muse *et al.*, 1977), *Pasteurella multocida* to rabbit squamous nasopharyngeal cells (Glorioso *et al.*, 1982), *Moraxella bovis* to corneal epithelial cells (Jackman & Rosenbusch, 1984), *Neisseria meningitidis* to human nasopharyngeal cells (Salit & Morton, 1981), *Pseudomonas aeruginosa* to human nasal and tracheal cells (Neiderman *et al.*, 1983), and enterotoxigenic *E. coli* to human duodenal enterocytes (Deneke *et al.*, 1983; Knutton *et al.*, 1985). Specificity for the urinary tract has been elegantly documented for some *E. coli* which bind specifically to a digalactoside moiety on the surface of uroepithelial cells via an adhesin encoded by the *pap* (pyelonephritis-associated pili) operon (Uhlen *et al.*, 1985). The expression of the 'Gal-Gal' binding (mannose-resistant) adhesin is strongly correlated with *in vitro* uroepithelial adherence and with potential to establish infection of the kidney (Svanborg-Eden *et al.*, 1976; Lomberg *et al.*, 1983; Tullus *et al.*, 1984; O'Hanley *et al.*, 1985). *Proteus mirabilis*, commonly isolated from the urinary tract, has also been found to adhere to uroepithelial cells *in vitro* (Svanborg-Eden *et al.*, 1980).

In this report we describe experimental conditions under which strains of *Providencia stuartii* isolated from the urine of chronically catheterized patients can be differentiated in an *in vitro* uroepithelial cell adherence assay. Definitions of 'adherent' and 'non-adherent' are proposed and comparisons of the adherence of these *P. stuartii* isolates with that of a uropathogenic strain of *E. coli* are made. A preliminary report of this work has appeared (Mobley *et al.*, 1985b).

METHODS

Bacterial strains and growth conditions. Strains of *P. stuartii* were isolated from weekly urine specimens obtained over a one-year period from 51 nursing home patients with urinary catheters in place. The patients resided at two geographically separate Baltimore chronic care facilities. Isolates were identified by the Minitek numerical taxonomy system (BBL), and differentiated from other *Providencia* species by fermentation of adonitol, trehalose and erythritol (Hickman-Brenner *et al.*, 1983). Strains were stored at -70°C in trypticase soy broth (BBL) supplemented with 15% (v/v) glycerol.

E. coli SH1 (a Lac^{-} derivative of the P and type 1 fimbriated strain J96) was kindly provided by Drs S. and R. Hull, Baylor College of Medicine, Houston, Texas, and was used as a positive control in adherence assays (Hull *et al.*, 1981). *E. coli* HB101 (*pro leu rpsL recA lacYI*) was used as a non-adherent control.

Bacteria were recovered from storage by growth on trypticase soy agar (BBL), and subcultured in a variety of media. Liquid cultures were grown with aeration (200 r.p.m.) for 18 h and statically for 24 or 48 h; agar (1.5%, w/v) plates were incubated for 18 h. All cultures were incubated at 37°C . Urine agar was prepared by mixing three parts filter-sterilized human urine with one part 6% (w/v) agar (Difco). These plates were sealed before incubation. Nutrient Broth, Brain Heart Infusion Broth and Heart Infusion Broth were purchased from Difco. Luria broth and minimal salts medium were prepared according to Miller (1972).

Bacteria grown in liquid media were harvested by centrifugation at $6000g$ for 10 min at 4°C . Supernatants were aspirated, cell pellets were well drained, and bacteria were gently suspended in PBS (0.145 M-sodium chloride, 0.05 M-sodium phosphate, pH 7.2) with a Pasteur pipette to OD_{550} 0.1 (1 cm light path) (approximately 2×10^8 bacteria ml^{-1} as determined by colony counts) unless otherwise indicated. Bacteria from agar plates were gently scraped from the surface with a sterile swab and suspended in a similar fashion.

Haemagglutination assays. Haemagglutination and tannic acid treatment of erythrocytes were done by the method of Old & Adegbola (1982). Guinea-pig erythrocytes were obtained from Pel-Freez Biologicals (Rogers, Arkansas), ox erythrocytes from Cleveland Scientific (Cleveland, Ohio) and human erythrocytes from the first author.

Uroepithelial cells (UEC). First morning urine of healthy adult females (ages 22–36 years) was transported on ice within 2 h of collection and UEC were isolated by centrifugation ($4000g$, 10 min, 4°C), washed three times with cold PBS, pH 7.2, resuspended in 1 ml PBS, quantified in a haemocytometer, and adjusted to a concentration of 2×10^5 cells ml^{-1} unless otherwise indicated. The mean number of UEC collected from 100 ml urine was $4.7 \times 10^5 \pm 3.3 \times 10^5$ (SD, $n = 24$). The mean area that UEC occupied on the microscope slide was determined by calibrating an ocular grid with a stage micrometer; the areas of 40 UEC were measured (see legend to Fig. 1).

Quantification of adherence. Assays were done according to the optimal assay conditions described in Results. Slides were observed under oil immersion ($1000\times$, total magnification). Single rod-shaped bacteria that touched the border of or rested within the boundary of the UEC were counted on each of the first 40 epithelial cells observed, starting at the top left of the cell imprint. An epithelial cell was counted if it: (1) excluded viability stain completely; (2) had a well-defined border (no overlapping cells); (3) was not folded back onto itself; (4) was in a field free of background bacteria; and (5) was not visibly fragmented. Assays were done in triplicate. Background bacteria are defined as patches of organisms not associated with UEC. Endogenous bacteria are defined as those organisms associated with UEC that were derived from the urinary tract of the UEC donor.

Plasmid isolation. Plasmid DNA was isolated from 0.5 ml of overnight aerated Luria-broth-grown cultures by extraction with alkaline sodium dodecyl sulphate as described by Birnboim & Doly (1979). The DNA was electrophoresed on 0.7% agarose (BRL) gels, stained with ethidium bromide (Sigma) and visualized on a UV transilluminator. Patterns were recorded on Polaroid type 47 film through an orange filter (Wratten filter no. 22A, Kodak). Plasmid sizes were estimated by comparing electrophoretic mobilities to plasmids of known molecular mass.

Electron microscopy. A drop of bacterial cell suspension was placed on a carbon-coated Formvar film on a 200-mesh copper grid and negatively stained with 1% (w/v) sodium phosphotungstate (pH 6.8). Specimens were examined in a Siemens IA electron microscope at an accelerating voltage of 80 kV.

Serotyping. *P. stuartii* isolates were serotyped by Dr John Penner (University of Toronto, Canada) according to his O-serotyping scheme (Penner *et al.*, 1976).

RESULTS

Optimization of adherence assay

P. stuartii was recovered from 761 of 1230 (62%) weekly urine specimens. Adherence assays were done with 20 isolates of *P. stuartii* (Table 1) according to conditions established for *E. coli* by Svanborg-Eden *et al.* (1977). To provide a basis for differentiating adherent from non-adherent isolates, these data (not shown) were used to select one isolate, AB189, as a strongly adherent strain and one isolate, RO7305, as a poorly adherent strain. These isolates were selected as representatives of adherent and non-adherent, respectively. Strain AB189 agglutinated tannic-acid-treated (but not untanned) ox erythrocytes, was heavily fimbriated as revealed by transmission electron microscopy of negatively-stained specimens, and was not flagellate. Strain RO7305 did not agglutinate tanned or untanned ox erythrocytes, did not express fimbriae, but was flagellate.

To select a single donor of UEC for the assay, adherence assays were done using UEC obtained from first morning urine from six healthy adult females (ages 22–36). Day-to-day reproducibility of mean number of adherent bacteria per UEC for *P. stuartii* strains AB189 and RO7305 and *E. coli* SH1 was determined, and numbers of endogenous bacteria were measured. One donor was selected whose cells provided consistent adherence values and had acceptable levels of endogenous bacteria (<10 bacteria per UEC). UEC collected from first morning urine of the selected donor were harvested by centrifugation, washed, adjusted to 2×10^5 cells ml⁻¹ in PBS and incubated at 37 °C. Samples were taken over time and tested for exclusion of the viability stain erythrosin B (Sigma). After 30 min incubation, 50.0% (100/200) of the UEC were viable; after 60 min, 20.5% (41/200) excluded stain. Viability remained at that level (21.0%; 42/200) in samples taken at 180 min.

Washed UEC were mixed with 0, and from 10^5 to 10^9 , nutrient-broth-grown bacteria, incubated for 60 min, filtered through 8 µm filters, washed, transferred by pressing onto microscope slides, fixed and stained. Bacteria were counted on each of 40 UEC; the results of triplicate experiments are shown in Fig. 1. *P. stuartii* AB189 adhered more avidly at all ratios than strain RO7305. The diamonds represent the filter background (mean number of bacteria per 1820 µm², the mean area of UEC from the donor used in this study). Filter background was unacceptably high when 10^9 bacteria ml⁻¹ were added in the assay. At 10^8 bacteria ml⁻¹ adherence values for strains AB189 and RO7305 were significantly different ($P < 0.025$). For these reasons, a ratio of 10^8 bacteria per 10^5 UEC was chosen for the assay.

P. stuartii AB189 and *E. coli* SH1 showed peak adherence 10–20 min after mixing (Fig. 2). *P. stuartii* RO7305 showed little adherence above the control to which no bacteria were added.

Adherence assays were done in triplicate with bacteria cultured in eight media under conditions of aeration (18 h) and static growth (24 and 48 h), in tubes of broth and on agar plates (18 h). Aerated nutrient broth and statically grown urine cultures gave the highest mean adherence values, 27.8 and 22.0 bacteria per UEC, respectively. Statically grown Brain Heart Infusion Broth cultures yielded the lowest values, 9.4 bacteria per UEC. An endogenous value of 8.9 bacteria per UEC was determined. Cultures in minimal salts supplemented with glycerol grown statically and on agar yielded high values but organisms derived from these cultures clumped and were difficult to suspend. Nutrient broth, as for *E. coli* (Svanborg-Eden *et al.*, 1977), yielded optimal adherence for the *P. stuartii* test strain.

Table 1. *Adherence of selected P. stuartii isolates*

<i>P. stuartii</i> isolate	Serotype*	Estimated plasmid size (kb)†	MR/K haemag- gluti- nation‡	Adherence to UEC (bacteria per cell)§	Percentage of UEC with > 10 bacteria	<i>P</i>
Nursing home A						
D14761	O:24	110	—	15.9	48.3	<0.001
BA418	O:24	180, 130, 92, 60	+	11.0	31.7	NS
BE1814	O:25	150, 96, 79	+	13.4	45.0	<0.005
BE5653	O:25 (O:43)	150	—	15.0	41.7	<0.025
BE3304	O:43	150	—	19.3	41.7	<0.025
PE4390	O:43	—	—	17.4	52.7	<0.001
HA41	O:43	—	—	13.2	36.7	NS
TA1144	O:43	110	—	16.0	37.5	NS
PL22	O:43	92	—	19.7	39.2	NS
PL4954	O:52	—	—	9.8	25.0	NS
BA3512	NT ^R	110	—	21.4	61.7	<0.001
RO7305	NT ^S	—	—	9.2	27.5	NS
Nursing home B						
WE2020	O:4	—	—	17.3	50.8	<0.001
AB189	O:25	—	+	23.2	60.8	<0.001
AB454	O:25	—	+	17.2	49.2	<0.001
EII130	O:25	92	+	30.9	67.5	<0.001
FE4361	O:25	—	+	15.2	45.8	<0.005
KE2974	O:25	—	+	29.5	64.2	<0.001
EI219	NT ^R	—	+	20.9	54.2	<0.001
SI3211	NT ^R	92, 58	—	24.7	54.2	<0.001

* NT^R, rough, non-typable; NT^S, smooth, non-typable.

† —, No detectable plasmids.

‡ Haemagglutination of tannic-acid-treated ox erythrocytes.

§ Values are means of three experiments; mean number of endogenous bacteria = 9.6; percentage of UEC with > 10 endogenous bacteria = 26.7.

|| By comparison to the percentage of UEC with > 10 bacteria for endogenous bacteria by chi-squared test; NS, not significantly higher than endogenous levels or that of strain RO7305.

The effect of washes and centrifugations on adherence of *P. stuartii* AB189 was measured (Fig. 3). One wash reduced adherence by 10% and two washes diminished it by 50%. *E. coli* SH1 was similarly affected by the washing procedure. Therefore, for the adherence assay, bacteria were centrifuged, the pellets well drained, and the bacteria suspended to the desired concentration and assayed with no additional centrifugations.

Adherence assays were done using either PBS, pH 7.2, or filter-sterilized urine. In triplicate assays, the mean value of adherence for *P. stuartii* AB189 in PBS was 20.9 per UEC in both PBS and filter-sterilized urine. Because there was no difference in adherence values, the less offensive PBS was used as the incubation buffer of choice from this point on.

The final assay conditions, optimized by varying a number of factors as described above, were as follows. Equal volumes (0.2–0.5 ml) of bacterial suspension (2×10^8 cells ml⁻¹) and UEC suspension (2×10^5 cells ml⁻¹) were mixed in glass tubes (10 × 75 mm) in the presence of 50 mM-mannose (Sigma) to prevent mannose-sensitive adherence (Old & Scott, 1981) to uromucoid (Chick *et al.*, 1981), sealed with silicone rubber stoppers and incubated at 37 °C on a test-tube rotator at 8.5 r.p.m. (Roto-Mix, Fisher Scientific). After 20 min, 0.1 ml erythrosin B (0.8%, w/v, in PBS) was added and incubation was continued for 10 min. The suspension was then filtered through nitrocellulose filters (8 µm pore diameter; Millipore) under 103.5 kPa vacuum and washed three times with 5 ml ice-cold PBS. The filters were removed and UEC were transferred to carefully cleaned glass microscope slides by gentle rolling finger pressure; the filters were peeled from the slide and discarded. The slides were air dried, fixed for 5 min in 70% (v/v) ethanol, stained with freshly diluted Giemsa stain (1:50, v/v) for 10 min, rinsed with

Fig. 1

Fig. 2

Fig. 1. Effect of ratio of bacteria to UEC on adherence. UEC (0.5 ml, 10^5 cells) were incubated with various concentrations of bacteria in 0.5 ml for 60 min at 37 °C. Suspensions were filtered through 8 μ m pore diameter nitrocellulose filters and washed three times with 5 ml PBS, pH 7.2. Cells were transferred to slides, dried, fixed, and adherent bacteria on each of 40 UEC were counted. Filter background was estimated by counting bacteria within 40 random fields of 1820 μ m² (mean area of a uroepithelial cell) that contained no epithelial cells. Results are the mean of three experiments. ●, *P. stuartii* AB189; ■, *P. stuartii* RO7305; ◆, filter background.

Fig. 2. Effect of incubation time on bacterial adherence to UEC. Bacteria (10^8 per 0.5 ml) were mixed with UEC (10^5 per 0.5 ml) and incubated for various times at 37 °C. Cells were filtered, washed and prepared for microscopy as described in Methods, and bacteria were counted on each of 40 UEC. Results are the mean of three experiments. ●, *P. stuartii* AB189; □, *E. coli* SH1; ▲, *P. stuartii* RO7305; ■, no added bacteria.

distilled water, and air dried. Control slides of (1) no added bacteria (endogenous), (2) adherent *E. coli* SH1 and (3) non-adherent *E. coli* HB101 were included with each run.

Distribution of adherent bacteria

To determine the distribution patterns of adherent bacteria to UEC, the results from all assays done under what were determined to be the optimal conditions were pooled. The percentage of total UEC bearing a given range of bacteria is shown in Fig. 4 for the adherent strain AB189, the non-adherent strain RO7305, the UEC control, and P-fimbriated *E. coli* SH1. Strain RO7305 and the control showed similar distributions of adherent bacteria and yielded means (arrows) that were not significantly different ($P > 0.1$). Strain AB189 showed a broad distribution of number of bacteria per UEC, quite distinct from RO7305, the control, and *E. coli* SH1. Despite the large standard deviation (represented by the bar extending to the right of the arrow) the mean of 27.3 bacteria per UEC was significantly different from that of RO7305, the control and *E. coli* SH1 (Table 2). The distribution pattern of AB189 was also found to be significantly different ($P < 0.001$) from that of RO7305, the epithelial cell control and *E. coli* SH1 when the data were analysed by the Kolmogorov-Smirnov Test (Sokal & Rohlf, 1981). When these data are reported as the percentage of UEC with more than 10 bacteria, the values for *P. stuartii* AB189 and *E. coli* SH1 are also significantly greater than those for *P. stuartii* RO7305 and endogenously adhering bacteria (Table 2). Values for *E. coli* were intermediate between those for *P. stuartii* AB189 and RO7305.

Adherence to squamous versus transitional epithelial cells

To determine whether *P. stuartii* preferentially adheres to squamous epithelium which lines the urethra or to transitional epithelial cells, found predominantly on the bladder wall, adherence values were obtained for both squamous and transitional cells. Cell types were

Fig. 3

Fig. 4

Fig. 3. Effect of washing of bacteria on adherence. Bacteria grown in nutrient broth with aeration at 37 °C were harvested by centrifugation at 6000 *g* for 10 min at 4 °C, suspended to a concentration of 10^8 per 0.5 ml and either mixed with UEC (10^5 per 0.5 ml) immediately, or subjected to additional washes before mixing. Suspensions were incubated for 30 min at 37 °C, filtered, transferred to slides, dried, fixed, stained, and bacteria on each of 40 UEC were counted. All experiments were done in triplicate. ■, *P. stuartii* AB189 (100% equals 23.2 bacteria per UEC); □, *E. coli* SH1 (100% equals 19.5 bacteria per UEC).

Fig. 4. Frequency distribution of numbers of bacteria per UEC. Results from all adherence assays done under the optimal conditions determined (see Results) were pooled; the percentage of UEC bearing a given number of bacteria is plotted for *P. stuartii* AB189, *P. stuartii* RO7305, endogenous bacteria and *E. coli* SH1. *n* is the number of UEC for which adherent bacteria were quantified. The arrow in each panel indicates the mean; the bar spans one standard deviation (see Table 2). *P. stuartii* strains were grown in nutrient broth; *E. coli* SH1 was grown on trypticase soy agar.

Table 2. *Adherence values for test strains*

Strain	Mean no. of bacteria per UEC ± SD	<i>P</i> *	No. of UEC with > 10 bacteria/ total UEC	<i>P</i> †
No added bacteria (endogenous bacteria)	8.2 ± 14.1	—	88/440 (20.0%)	—
<i>P. stuartii</i> RO7305	9.2 ± 11.0	> 0.1	102/360 (28.3%)	< 0.008
<i>P. stuartii</i> AB189	27.3 ± 32.8	< 0.0001	526/720 (73.1%)	< 0.0001
<i>E. coli</i> SH1	20.9 ± 29.4	< 0.0001	221/480 (46.0%)	< 0.0001

* Mean and standard deviation compared with that for endogenous bacteria by the *t*-test.

† Ratio compared with that for endogenous bacteria by chi-squared test.

distinguished strictly by morphology. Of viable cells obtained from urine of a single donor, 97.9% (2463/2515) were of squamous morphology and 2.1% (52/2515) were of transitional morphology. Using strain AB189, the mean adherence value for squamous cells was 26.8 bacteria per UEC (160 UEC, four assays) and that for transitional cells was 27.9 bacteria per UEC (27 UEC, four assays).

Adherence of selected P. stuartii isolates

To determine adherence properties for other isolates of *P. stuartii*, assays were done under optimal conditions for selected isolates. Twenty isolates from 14 patients at two chronic care facilities representing at least six serotypes and harbouring combinations of nine plasmids were assayed. The results (Table 1) show that 17 isolates had 13 or more bacteria per epithelial cell and six strains adhered with a mean of more than 20 bacteria per UEC. Three isolates had fewer than 13 bacteria per UEC. Within this group of strains there were no correlations between serotype and adherence values or plasmid content and adherence values.

DISCUSSION

We report that strains of *P. stuartii* isolated from the urine of chronically catheterized patients can be differentiated as adherent or non-adherent to UEC on the basis of a standardized *in vitro* adherence assay. This assay combines features from several previous reports (Chick *et al.*, 1981; Reid *et al.*, 1983; Schaeffer *et al.*, 1979; Svanborg-Eden *et al.*, 1977) and provides ease of sample preparation and accurate evaluation of adherent bacteria.

The decision to quantify adherent bacteria by direct visualization rather than by addition of radiolabelled organisms was based on the types of information that could be obtained. (1) By observing cells directly, viable cells could be singled out for counting and non-viable cells excluded. In addition, the percentage of viable epithelial cells could be assessed at the end of each experiment. (2) Endogenously bound and background bacteria could be accurately assessed. A high endogenous flora has been shown to protect against adherence of exogenously added bacteria (Chan *et al.*, 1984). The possibility of inhibition by endogenous bacteria would not be detected in the routine assay quantifying radiolabelled bacteria. (3) Direct observation also allows the investigator to avoid areas of non-specific background bacteria or adherence of organisms to cellular and non-cellular debris. (4) By direct visualization, we can quantify adherence to both squamous epithelial cells which originate primarily from the lining of the urethra, and transitional epithelial cells that line the bladder wall. In the catheterized urinary tract, we might speculate that adherence to transitional epithelium would establish a more important reservoir than adherence to squamous cells. (5) The distribution of bacteria on epithelial cells can be examined; that is, the number of bacteria on each cell can be quantified, which is not possible when radiolabelled bacteria are used.

During the assay, repeated washing of bacteria before incubation diminished adherence (Fig.

3). This observation is consistent with disruption of bacterial surface structures (Svanborg-Eden & Hanson, 1978), suggesting that fimbriae may be involved in *P. stuartii* adherence. Three kinds of haemagglutinins (MS, MR/K and MR/P) have been described for *P. stuartii* (Old & Scott, 1981; Old & Adegbola, 1982) and have been correlated with morphologically distinct fimbriae. In our assays, the addition of mannose had no effect on adherence values, suggesting that mannose-sensitive haemagglutinins did not play a role in adherence to UEC. Eight of 20 strains expressed the MR/K haemagglutinin (Table 1) and, although the value 'percentage of UEC with > 10 bacteria' tended to be higher for these strains than for those not expressing the MR/K haemagglutinin (52 versus 43, respectively), there was no significant difference between the groups ($P = 0.08$).

In most reports using *in vivo* adherence assays, values for adherent bacteria are reported as the mean number of bacteria per UEC. Many reports include adherence values with rather narrow standard deviation ranges about the mean as a result of individual assays (Bruce *et al.*, 1983; Reid *et al.*, 1983, 1984; Svanborg-Eden *et al.*, 1977). We observed a broad distribution pattern (Fig. 4), which was also reported for a limited number of observations by Mardh *et al.* (1979). This may be due to the large range in size of UEC, the mean area of which was found by us to be $1820 \pm 915 \mu\text{m}^2$. Moreover, the distributions of adherent bacteria (Fig. 4) were neither normal nor Poissonian, but were skewed, suggesting that the mean and standard deviation are not the ideal measure of adherence. We suggest that the value 'percentage of UEC with > 10 adherent bacteria' (Tables 1 and 2) may also prove valuable in assessing adherence. This value does not assume a normal distribution, can be compared with other values by the chi-squared test, and does not give undue weight to a few cells with large numbers of bacteria per UEC.

Although numerous reports present values for mean number of bacteria per UEC $\pm$ standard deviation, what can we consider to be 'adherent'? When we compare such values for *P. stuartii* AB189 and RO7305 with values obtained when no exogenous bacteria are added, we find that values for AB189 are significantly greater than endogenous values (Table 2), whereas values for RO7305 are not significantly higher than for endogenous organisms. By this definition we can therefore say that strain AB189 is adherent and strain RO7305 is not adherent. By adopting a significance level of $P = 0.01$ and using values determined experimentally (Table 2) for the non-adherent RO7305 and using mean adherence values $\pm$ standard deviation for triplicate assays (3×40 UEC), a cut-off point for statistically significant adherence can be calculated to be about 13 bacteria per UEC. Using this value, 17 of the strains listed in Table 1 can be called adherent while 3 are not. As a comparison, the P-fimbriated *E. coli* SH1 (derived from the pyelonephritogenic strain J96) adhered with values comparable to (Figs 2 and 3) or slightly less than (Fig. 4, Table 2) *P. stuartii* AB189. The literature cites examples of *E. coli* (Bruce *et al.*, 1983; Chan & Bruce, 1983; Chan *et al.*, 1984; Reid *et al.*, 1983; Svanborg-Eden *et al.*, 1976, 1977), *Klebsiella pneumoniae* (Bruce *et al.*, 1983) and *Pseudomonas aeruginosa* (Bruce *et al.*, 1983) strains adhering with values of more than 75 bacteria per UEC, but values of 12 and fewer bacteria per UEC (after subtraction of endogenous bacteria) are also reported (Chick *et al.*, 1981) for *E. coli* displaying mannose-resistant haemagglutination of human erythrocytes. One report of adherence of *Proteus mirabilis* (Svanborg-Eden *et al.*, 1980), the most closely related of these species to *P. stuartii*, described mean adherence values for groups of strains derived from blood, stool and urine ranging from 21 to 41 bacteria per UEC.

If we compare adherence of the isolates listed in Table 1 by 'percentage of UEC with > 10 bacteria', 14 of the 20 isolates demonstrated significantly higher values than endogenous levels or that of the non-adherent RO7305. These same isolates showed significant adherence by comparison of means and standard deviations of the numbers of bacteria per UEC.

In our studies we have observed *P. stuartii* to be the most common isolate in chronically catheterized individuals (Mobley *et al.*, 1985a; Warren *et al.*, 1982). Additionally, once the organism gains entrance to the urinary tract, it can be isolated persistently weeks or months after initial isolation (Warren *et al.*, 1982). It is our hypothesis that the persistence of *P. stuartii* is due to adherence to some component of the urinary tract, whether it be UEC as reported here, soluble glycoproteins such as Tamm-Horsfall protein (Ørskov *et al.*, 1980), or a foreign body such as a urinary catheter. We are pursuing this hypothesis.

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