Adhesion of two uropathogens to silicone and lubricious catheters: influence of pH, urea and creatinine

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The adhesion of a hydrophilic *Escherichia coli* and a hydrophobic *Staphylococcus epidermidis* was significantly higher to silicone-coated latex than to a hydrophilic lubricious-coated catheter after 24 h incubation. Time-course studies showed a steady increase in viable *E. coli* adhesion to the hydrophilic catheter over 24 h. However, in contrast to thermodynamic modelling predictions, *S. epidermidis* adhered well to the hydrophilic catheter within 30–60 min. By 18 h the adherent *S. epidermidis* were non-viable, apparently demonstrating the presence of an unidentified antibacterial factor on this catheter. A range of creatinine concentrations had some effect on the adhesion. Bacterial binding was significantly higher in low urea concentrations (< 200 mM) and was significantly affected by variation of pH from 3 to 9. There was some correlation between the suspending fluid surface tension, ΔF^{Adh}, and the experimental adhesion.

1. Introduction

The use of biomaterials in urology is expanding at a high rate. These materials are designed for size, shape, utility and ease of use, and they range from urinary and peritoneal catheters and implants to polymers for drug delivery and *in vitro* investigation [1]. However, progress is hindered by infectious complications, initiated by microbial adhesion [2–4]. It has been estimated that 20% of the many millions of US patients who are catheterized become infected after implantation [5]. Invariably, indwelling catheters must be removed once infection occurs [6], emphasizing the need to understand the pathogenesis and to implement alternative management procedures.

It is known that coagulase-negative staphylococci and *E. coli* are the major colonizers of urinary catheters, and the most common infecting agents [7]. Previous *in vitro* studies have demonstrated how Tamm Horsfall protein as well as the urinary, bacterial and substrata surface tensions can influence bacterial adhesion to polymers *in vitro* [8–11]. However, it is still not clear how urinary components interplay with commercial devices which comprise proprietary structures that are often irregular in shape [11] and contain undisclosed surface properties.

The aim of this study was to investigate the influence of bacterial and commercial catheter surface properties as well as suspending fluids on *E. coli* and *S. epidermidis* adhesion. Rather than measure adhesion by microscopy imaging as previously [10, 11], it was decided to determine the actual viable counts of attached bacteria, as it is these viable organisms that subsequently colonize the bladder and cause infection.

2. Materials and methods

2.1. Bacteria

Two uropathogenic strains used extensively in previous studies [8–11] were selected for *in vitro* adhesion experiments. These strains were representative of the two species most commonly isolated from catheter-associated urinary tract infections. In addition, almost all uropathogenic *E. coli* express type 1, and this along with *P. fimbriae* are regarded as the key virulence factors in adhesion of these organisms to cells. The strains used were *E. coli* Hu734, and *S. epidermidis* 1938. They were cultured at 37 °C from stock in brain heart infusion yeast extract broth, and stationary-phase cultures were used for adhesion experiments, after being harvested, washed and resuspended in phosphate-buffered saline (PBS) pH 7.1.

2.2. Suspending fluids

The range of values used for pH, creatinine and urea were those found in the urine of normal adult subjects [12]. The suspending fluids were prepared as follows: urea (Difco, Detroit, Michigan, USA) was made up in PBS (pH 7.1) to concentrations of 0, 200, 400, 600 and 800 mM. Creatinine (BDH Ltd, Poole, UK) was made up in PBS (pH 7.1) to concentrations of 0, 4, 8, 12 and 16 mM. Solutions were also made up using buffered solutions of pH 3, 4, 5, 6, 7, 8 and 9 [13].

2.3. Biomaterials

Two sterile-packed urinary catheters were used as-received: silicone-impregnated latex Foley (TFX

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Medical, New Jersey, USA) and Lubricath Lubricious Foley (Bard, Covington, Georgia, USA).

2.4. Bacterial adhesion assay

Sections of catheters (2 cm) were incubated in 10 ml microbial suspensions (10^8 bacteria ml^{-1}) at 37 °C for 24 h. Unattached organisms were removed by submerging and rinsing in buffer, and adherent organisms were quantitated by first sonicating the catheters in an ultrasonic water bath, then dilution plating on agar. Preliminary experiments had shown that 1 min sonication was optimal for recovery of bacteria, and microscopy of Gram-stained specimens verified removal of all bacteria. The technique provided a measure of viable organisms that had adhered.

2.5. Catheter, suspending fluid and bacterial surface tensions

Contact angle measurements reflect the overall hydrophobic characteristics of a surface [2], and this technique was used to determine the relative hydrophobicity of the Bard lubricious catheter and bacteria. Similar measurements could not be undertaken for the silicone-coated latex catheter, due to size and flexibility restrictions. Contact angle values were obtained by techniques referred to as axisymmetric drop shape analysis contact diameter (ADSA-CD), as described in [14–16]. The method calculates contact angles by solving the Laplace equation, using the surface tension of the liquid, the drop volume and the contact diameter of the water drop as input parameters.

A sessile drop was placed on the flattened section of the catheter material, especially prepared for us by Bard. The volume of the sessile drop was obtained using a micrometer syringe (Gilmont Canlab, Toronto, Canada) that delivers drops with an accuracy of 0.02 µl. The surface tension of distilled water was determined by the Wilhelmy technique [17]. The contact diameter of the drop was determined by computerized digitization of the drop periphery on an image taken from above. The drop image was obtained using a stereomicroscope (M7S Zoom; Wild Heerbrugg, FRG). The surface tension values were calculated using an equation of state for interfacial tension [18, 19].

Suspending liquid surface tension values were obtained before the adhesion experiments were performed by means of axisymmetric drop shape analysis – profile (ADSA-P) using a pendant drop. This technique calculates the surface tension of fitting the Laplace equation of capillarity to an arbitrary array of co-ordinate points selected from the drop profile. The Laplace equation of capillarity is

$$\Delta P = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$

where γ is the interfacial tension, R_1 and R_2 represent the two principal radii of curvature and ΔP is the pressure difference across the interface. The profile co-ordinates were found and analysed by an automatic digitization technique described in detail in [20, 21]. The pendant drop was formed inside a quartz cuvette at the tip of a Teflon capillary. To maintain the vapour pressure in equilibrium and prevent evaporation of the pendant drop during the experiment, the cuvette was half filled with the sample and then sealed.

The reported contact angles of *E. coli* Hu734 and *S. epidermidis* 1938 were measured with a telescope fitted with a goniometer eyepiece [10, 11].

2.6. Electron microscopy

Selected specimens were examined for adherent bacteria under scanning electron microscopy, using the techniques described in [22].

3. Results

3.1. Presence of viable uropathogens on catheter surfaces

The *E. coli* strain Hu734 was found to be hydrophilic, with a contact angle of 12°, whereas the *S. epidermidis* was hydrophobic with contact angle 30°. The adhesion of viable *E. coli* to two commercial urinary catheters was higher than that of *S. epidermidis* under the conditions tested, as determined from an analysis of the data presented in Figs 1–3. Statistical analyses were carried out using three- and two-factor repeated-measures analysis of variance [23]. Adhesion to the TFX catheter was significantly higher than to the Bard device ($P < 0.0001$). Viable *E. coli* adhesion was significantly higher than *S. epidermidis* adhesion ($P < 0.0001$).

Viable *E. coli* adhesion to the hydrophilic catheter increased with time (Fig. 4), and single-factor repeated-measures analysis of variance showed this to be significant ($P < 0.0001$). Controls showed that this was not due to multiplication, as the concentration of the bacterial suspension was maintained at 1.1–1.4 $\times 10^8$ organisms over 24 h. Attempts to depict this increase in adhesion using electron microscopy did not provide convincing results, although a few adherent *E. coli* were seen at 1 and 24 h on an uneven, irregular Bard catheter surface (Fig. 5). The time-course experiments for *S. epidermidis* provided unexpected results. Bacteria were found adherent within the 30 and 60 min time-points, but no viable bacteria were found after 18 h. This experiment was repeated six times with similar results ($P < 0.0001$; Fig. 4). A precipitate was observed in the suspensions and around the test tubes after 18 h, but no attempt was made to identify this substance and confirm its anti-staphylococcal activity.

The Bard catheter becomes instantly slippery when wet, and this hydrophilicity was indicated from contact angle measurements of 0° (or 72.58 mJ m^{-2}) as soon as water was deposited on the surface and at all time-points after 1–24 h incubation in buffer. When the surface was allowed to dry, the subsequent contact angle was 53°. The uncoated section of this catheter had a contact angle of 138°. Electron microscopy analysis showed that the device had a very uneven surface (Fig. 5), and this probably increased the contact angle value of the uncoated polyurethane base.

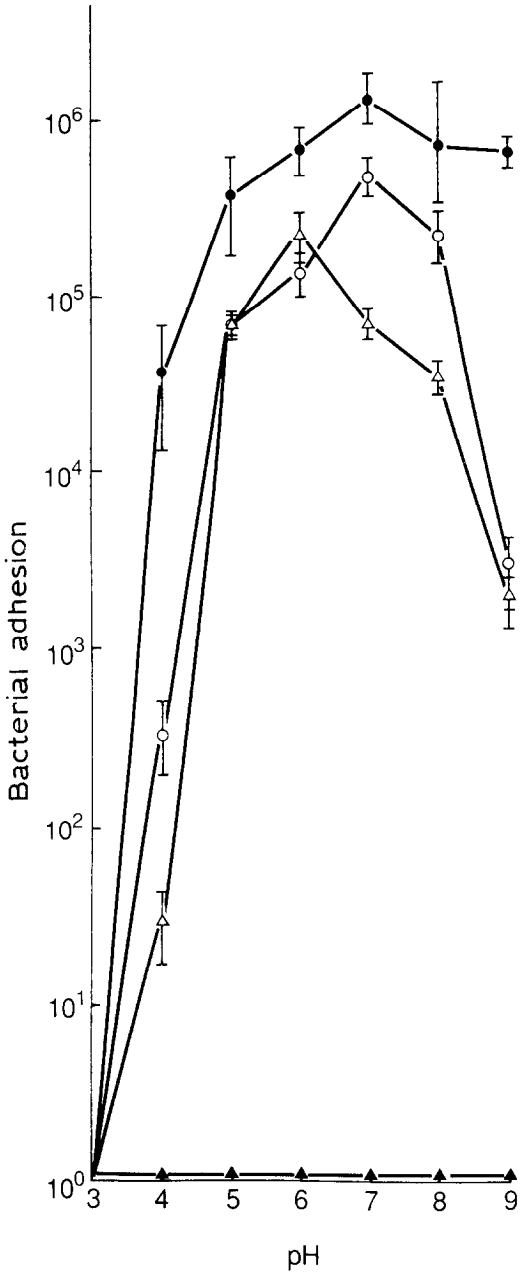

Figure 1 The effect of pH on adhesion of *E. coli* Hu734 to (●) TFX silicone latex and (○) Bard lubricious urinary catheters, and for *S. epidermidis* 1938 to the same (△ and ▲, respectively).

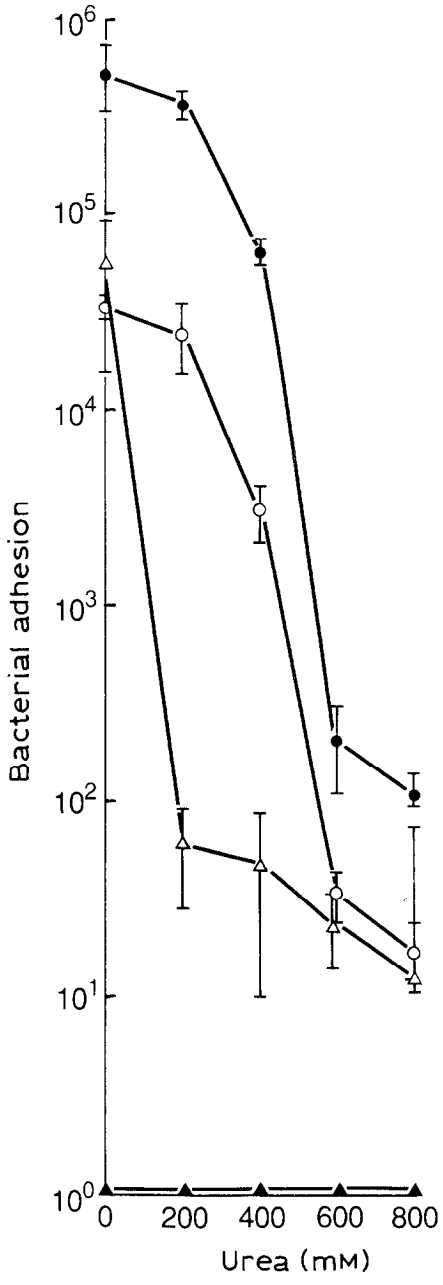

Figure 2 The effect of urea concentration on adhesion of *E. coli* Hu734 to (●) TFX silicone latex and (○) Bard lubricious urinary catheters, and for *S. epidermidis* 1938 to the same (△ and ▲, respectively).

3.2. Influence of pH, urea and creatinine

Two of the three urinary parameters tested were found greatly to influence bacterial adhesion. For pH the difference in adhesion varied according to the catheter ($P = 0.0003$), pH dosage ($P = 0.0003$) and organism ($P = 0.0017$; Fig. 1). There appeared to be greater adhesion at pH 5–8. For urea there was a significant difference in adhesion, dependent on the concentration ($P = 0.0001$), organism ($P = 0.0004$) and catheter type ($P = 0.0003$; Fig. 2). Adhesion was higher in low urea concentrations, whereas at 800 mM the organisms were found to be mostly dead. Although examination of Fig. 3 suggests that there was no difference in adhesion caused by the creatinine concentration, statistical analyses indicate that the dosage did affect the adhesion ($P = 0.0136$). This was probably due to the 4 mM creatinine level. The adhesion trend for creatinine was dependent on the organism ($P = 0.0143$), with *E. coli* more adherent than *S. epidermidis*. The dosage difference was not caused by the catheters *per se* ($P = 0.21$).

3.3. Correlations between experimental adhesion and free energy of adhesion

The free energy of adhesion is a numerical value that shows the likelihood of a micro-organism interacting with a given substratum in a particular fluid [2]. According to thermodynamic modelling predictions (Table I), *E. coli* would be expected to adhere better than *S. epidermidis* to the hydrophilic catheter. The adherence at 30 and 60 min (Fig. 4) showed an opposite correlation to the predictions, assuming the organisms "saw" a hydrophilic coat and not the hydrophobic polymer underneath.

Free energy values were calculated from the surface

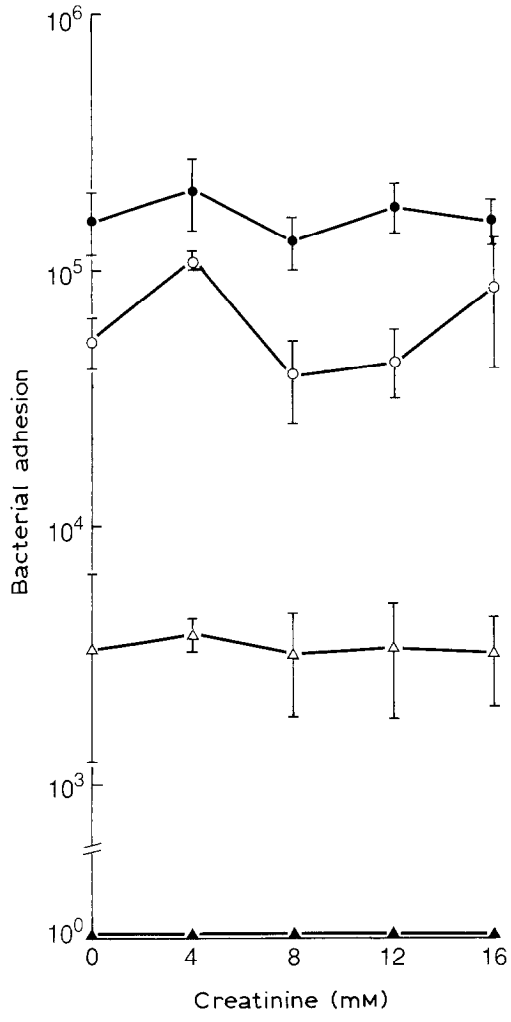

Figure 3 The effect of creatinine concentration on adhesion of *E. coli* Hu734 to (●) TFX silicone latex and (○) Bard lubricious urinary catheters, and for *S. epidermidis* 1938 to the same (△ and ▲, respectively).

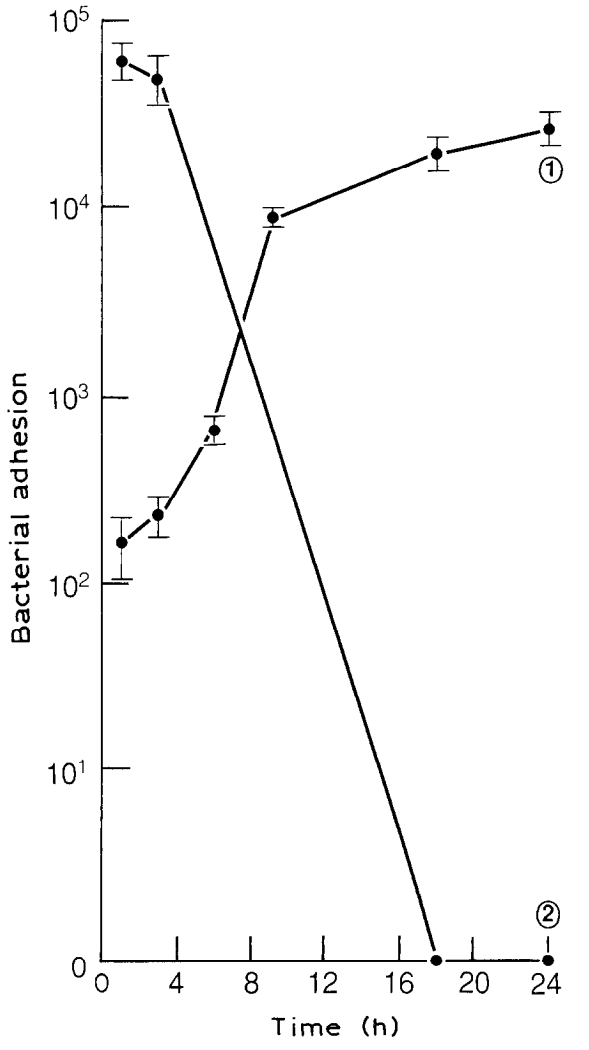

Figure 4 Time-course study on the adhesion of (1) *E. coli* Hu734 and (2) *S. epidermidis* 1938 to the Bard lubricious catheter over 24 h.

tension measurements to examine whether the surface tension values of the parameters tested could influence the relative adhesion to the lubricious catheter. The surface tension of *E. coli* Hu734 was 71.4 mJ m^{-2} and that of *S. epidermidis* was 63.9 mJ m^{-2}. The fluid surface tensions were found to be 67.72 ± 0.55 mJ m^{-2} at pH 3, 68.72 ± 0.4 mJ m^{-2} at pH 7, 70.32 ± 0.21 mJ m^{-2} at pH 9, 70.50 ± 1.0 mJ m^{-2} for 200 mM urea, 70.81 ± 0.36 mJ m^{-2} for 400 mM urea, 72.55 ± 0.177 mJ m^{-2} for 8 mM creatinine and 72.72 ± 0.215 mJ m^{-2} for 16 mM creatinine. As expected, the highest adhesion to the hydrophilic catheter was predicted for hydrophilic *E. coli* (negative ΔF^{Adh}; Table I).

There appeared to be a correlation between the free energy of adhesion and the experimental results. The more-negative ΔF^{Adh} at pH 7 corresponded to a higher experimental adhesion count of 4.8×10^5 compared with 2.9×10^3 for pH 9. Although the surface tension values for 200 and 400 mM urea were similar, the predicted and experimental adhesion values were higher for 200 mM. In addition, although the 8 and 16 mM creatinine surface tension values were similar, the predicted value and experimental value for 16 mM were higher.

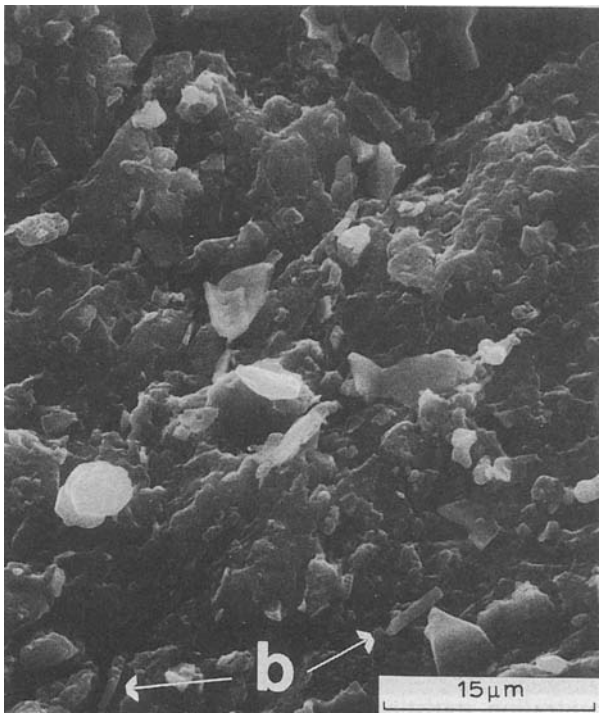

Figure 5 SEM micrograph showing a very few *E. coli* Hu734 (b) adherent to the Bard urinary catheter after 1 h. A similar result was obtained at 24 h.

TABLE I Free energy of adhesion results for *E. coli* Hu734 (surface tension 71.4 mJ m^{-2}) and *S. epidermidis* 1938 (surface tension 63.9 mJ m^{-2}) to the Bard lubricious catheter (72.58 mJ m^{-2}) in various suspending fluids.

Fluid, surface tension (mJ m^{-2})	*E. coli* ΔF^{Adh}	*E. coli* adhesion count	*S. epidermidis* ΔF^{Adh}
pH 3, 67.72	0.75	0[a]	0.76
pH 7, 68.72	− 0.44	4.8×10^5	0.77
pH 9, 70.32	− 0.10	2.9×10^3	0.60
200 mM urea, 70.50	− 0.08	2.5×10^4	0.57
400 mM urea, 70.81	− 0.04	3.3×10^3	0.51
8 mM creatinine, 72.55	0.002	3.9×10^4	0.01
16 mM creatinine, 72.72	− 0.01	8.8×10^4	− 0.05

[a] Bacteria dead.

4. Discussion

The latest findings demonstrated that the uropathogenic *E. coli* strain tested here was hydrophilic and highly adherent to two commercial urinary catheters after 24 h incubation at 37 °C. This adhesion of viable bacteria was significantly higher to the TFX catheter and was significantly higher than for *S. epidermidis*. The finding of adhesion to a lubricious catheter disagrees somewhat with a previous report which showed that hydrophilic and hydrophobic fimbriated *E. coli* did not adhere to hydrophilic urinary catheters [24]. One possible explanation is that those authors used scanning electron microscopy (SEM) to detect adherent organisms. Our use of this technique failed to illustrate reasonably large numbers of adherent bacteria after 24 h (as expected from viability counts), suggesting that the fixation and processing method for SEM in some way caused detachment of the coating and removal of the bacteria before examination.

The actual mechanisms of adhesion were not investigated, but without the presence of specific receptors, such as mannose and glycosphingolipids (for the uropathogenic *E. coli*), these are more likely to have involved hydrophobic interactions, influenced possibly by the presence of fimbriae on the *E. coli* [25]. The finding that the *S. epidermidis* strain was relatively hydrophobic agrees with previous studies [24, 26], but the correlation between specific hydrophobic surface components and adhesion was not made.

According to the concept of the interfacial free energy, cell adhesion is favoured when interfacial tension between the solid, the particle and the suspending liquid is reduced [27]. In relation to the critical surface tension for wetting, the fewest numbers of bacteria have been found to adhere to substrates with surface energies in the 20–30 mJ m^{-2} range, with highest adhesion at 45 mJ m^{-2} after 7 and 14 days incubation [28]. This agrees with the finding that *E. coli* adhered to the Bard catheter whose surface tension was basically that of water (72.5 mJ m^{-2}). However, the viable adhesion counts for the *E. coli* and *S. epidermidis* strains at 30 and 60 min time-points did not agree with thermodynamic modelling predictions (Fig. 4). Thus, the findings could not simply be explained on the basis of contact angles and free energy of adhesion.

Three urinary components were found to influence the ability of bacteria to adhere to urinary catheters. Bacterial adhesion appeared to be optimal at pH 5–8, suggesting perhaps that if urinary pH could be altered, less uropathogens might infect the urinary tract of catheterized patients. The fact that the urinary pH is usually in the 5.0–6.0 range presumably indicates inherent susceptibility to bacterial adhesion once a catheter is put in place. The increased concentrations of urea resulted in fewer viable adherent organisms being recovered. Again, if urologists and nephrologists could influence this concentration, it might provide a method of reducing the risk of infection. Creatinine concentrations found in human urine did not greatly influence the viable adhesion of *E. coli* Hu734 or *S. epidermidis* 1938, except for the 4 mM concentration which resulted in higher adhesion. Viable *E. coli* adhered to a much greater extent than *S. epidermidis* in the creatinine.

The thermodynamic modelling calculations showed an interesting correlation between the predicted and experimental values for viable adhesion of *E. coli* to the Bard catheter in various suspending fluids. Although the surface tension of the suspending fluids is probably not the only factor influencing the process, there is evidence to suggest that it may play a role.

Based on these latest findings, it is concluded that neither of the urinary catheters tested was capable of resisting adhesion of two commonly found uropathogens. It could be that a more important parameter for selecting a catheter for clinical use is the biocompatibility of the prosthetic device. It is this latter characteristic that may provide the hydrophilic Bard catheter with an advantage by causing less trauma upon insertion.

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