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Proteus mirabilis biofilms and the encrustation of urethral catheters

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Summary. Bacterial biofilms were observed on 69 of 75 catheters taken from patients undergoing long-term bladder management. Ten catheters were colonized by pure cultures of *Proteus mirabilis*. In each of these cases the bacteria formed layers on the catheter surface, underlying encrustations of struvite and hydroxyapatite which partially or completely occluded the catheter lumen. Encrustation was also apparent on catheters colonized by *P. mirabilis* plus other species, but was rarely seen on catheters colonized by non-urease-producing species. These observations support the hypothesis that catheter encrustation is brought about by the activity of urease-producing biofilms and confirms that the main target in the control of catheter encrustation should be *P. mirabilis*.

Key words: Bacterial biofilms – Catheter encrustation – Urethral catheters

A major complication of long-term urethral catheterization is the formation of encrustations on the surfaces of the catheter [6, 10]. These encrustations can cover the surface of the retention balloon, and obstruct the eyelet and the lumen of the catheter, thus blocking the drainage of urine from the bladder. The hard crystalline deposits can cause trauma to the bladder mucosa and to the urethra on withdrawal. The blockage results in incontinence with urine by-passing the catheter, or urine retention in the bladder with the associated acute pain and distress. Bacteriuria in the presence of blockage may culminate in episodes of fever, sepsis and shock [13].

The encrustations consist of a mixture of struvite (ammonium magnesium phosphate hexahydrate) and a poorly crystalline form of calcium phosphate (hydroxyapatite) [3, 8]. The scanning electron microscopy study of encrusted catheters by Cox et al. [4] showed the presence of large numbers of bacteria associated with the catheter encrustations, and substantiated the view that the aetiology of catheter encrustations is similar to that of infection-induced urinary stones [1]. Cox et al. [4] thus proposed that encrustation is initiated by the colonization of the catheter with urease-producing bacteria. The urease in the biofilm then hydrolyses urea to produce ammonia and the resulting alkaline environment facilitates the crystallization of struvite and hydroxyapatite. The biofilm matrix serves to bind the crystals together, stabilizing the growing encrustation that eventually blocks the catheter. Cox et al. [4], however, did not identify the bacteria colonizing encrusted catheters.

Scanning electron microscopy has revealed that the luminal surfaces of long-term indwelling bladder catheters are commonly colonized by bacterial biofilms [16, 18, 19]. These biofilms are formed by bacterial cells attaching themselves via their hair-like fimbriae or extracellular polysaccharide onto catheter surfaces that have probably been conditioned in vivo by films of proteinaceous material [7, 18]. The attached organisms divide to form thick layers of cells embedded in a polysaccharide matrix [2]. In this mode of growth the bacteria are more resistant to antiseptics, antibiotics and host defence mechanisms than cells growing in suspension [11, 17, 21].

We have previously reported on the composition of the bacterial communities in biofilms on 50 catheters from patients undergoing long-term catheterization [5]. We have now extended this study and in this paper we report our observations on the structure of the biofilms on the catheters colonized by the active urease producer *Proteus mirabilis,* and examine the hypothesis of Cox et al. [4] on the mechanism of catheter encrustation.

Materials and methods

Catheters

Silicone or silicone-coated Foley catheters, freshly removed from patients undergoing long-term catheterization in local hospitals, nursing homes and in the community, were transported to the laboratory for examination.

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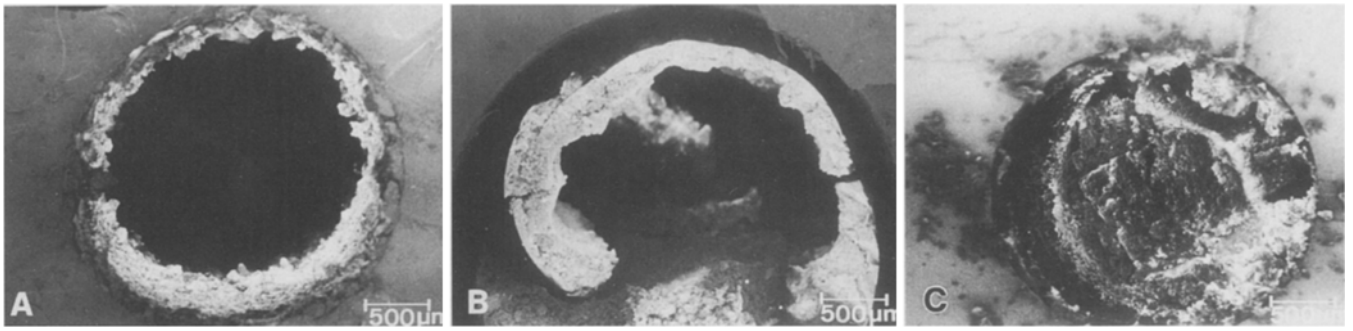

Fig. 1. Freeze-fractured, freeze-dried preparations showing cross sections of catheters colonized by pure cultures of *Proteus mirabilis*

Characterization of the biofilm communities

To characterize the bacterial communities colonizing the lumen of the catheters, sections (1 cm long) were cut from the region of the catheter within the retention balloon. The sections were gently rinsed once in 10 ml of buffer (Hanks-HEPES pH 7.4) and then placed in 10 ml of nutrient broth (Oxoid). Disruption of the luminal biofilm was achieved by sonication for 5 min (Transsonic Water Bath, Camlab, UK) followed by vortex mixing for 2 min. Samples of the broth suspension were then plated out on CLED agar (Oxoid) and incubated for 24 h at 37°C. The resulting isolates were characterized using the appropriate identification kits (API, UK).

Scanning electron microscopy

Sections of catheters (1 cm in length) were taken from the region adjacent to the retention balloon on the side away from the catheter tip. These were rapidly plunged into liquid-nitrogen-cooled propane and transferred to liquid nitrogen. Cross sections were produced by freeze-fracturing samples in a specially designed copper block which held the catheter and a blade in position and facilitated the production of reproducible cross sections. The samples were then freeze-dried for 24 h at −80°C, mounted fractured surface uppermost onto aluminium stubs, sputtered with gold and examined in a JEOL JSM5200 scanning electron microscope. Sections of catheters were also plunged into liquid nitrogen, freeze-dried, mounted onto carbon stubs and carbon coated for X-ray microanalysis of the crystalline formations.

To observe the nature of the surfaces of the biofilms, longitudinal sections of each catheter were prepared from the region adjacent to the retention balloon. The sections were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and then washed overnight in the phosphate buffer before being post-fixed in Millonig's phosphate-buffered osmium tetroxide (1.0%) for 1 h. The samples were dehydrated in a graded series of aqueous ethanol solutions (30–100%). Following dehydration the samples were critical-point-dried using liquid CO_2. Finally the samples were mounted on aluminium stubs, sputtered with gold and examined in the scanning electron microscope.

Results

Seventy-five catheters that had been indwelling for periods ranging from 3 to 83 days were examined using scanning electron microscopy. Biofilms were clearly visible on the luminal surfaces of 69 of the catheters. Bacteriological analysis revealed that 18 catheters were colonized by *Proteus mirabilis*. Ten of these biofilms were composed of pure cultures of the *Proteus* species and 8 contained up to three additional species. The organisms found in association with *P. mirabilis* were *Enterococcus faecalis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* and *Providencia stuartii*.

The catheters colonized by *P. mirabilis* had been in situ for periods ranging from 2 to 42 days (mean 27 days). At the time of catheter removal only 2 of the 18 patients were receiving antibiotic treatment (1 gentamicin and 1 tri-

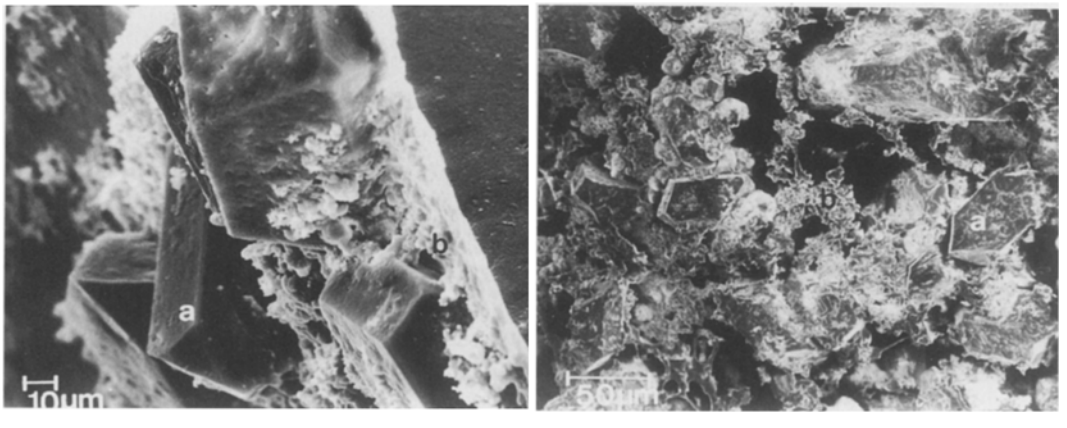

Fig. 2. Freeze-dried preparations of catheters colonized by *Proteus mirabilis* showing large coffin-shaped crystals typical of struvite (*a*) and small amorphous particles characteristic of hydroxyapatite (*b*)

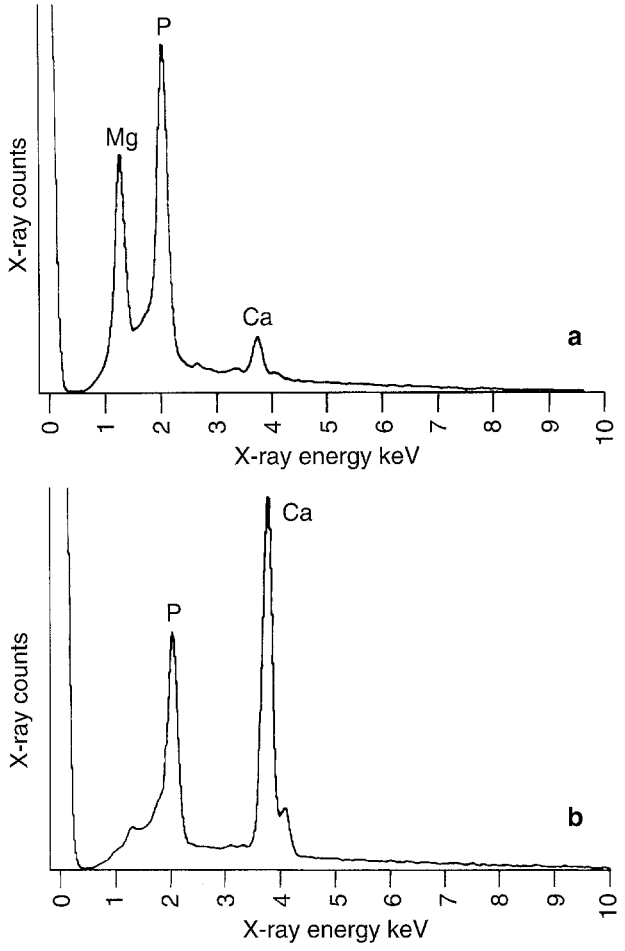

Fig. 3a, b. X-ray microanalytical spectra of the large crystals (**a**) and small amorphous particles (**b**)

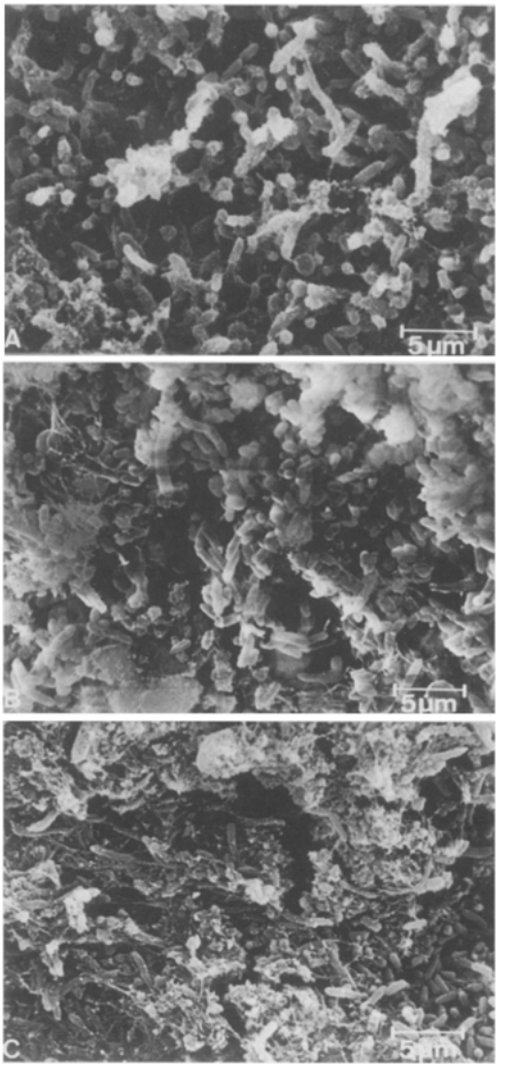

Fig. 4A–C. Surface views of *Proteus mirabilis* biofilms (**A, B**) and a mixed community biofilm of *P. mirabilis, Pseudomonas aeruginosa* and *Escherichia coli* (**C**). The preparations were fixed, post-fixed and critical-point-dried

methoprim); 1 patient was undergoing daily bladder washouts with Suby G solution. Urine samples were available from 8 of these 18 patients. In 6 cases *P. mirabilis* was isolated from both the catheter and the urine. In 2 cases this organism was not recovered from the urine although it was growing on the catheter. The pH of urine from patients with catheters colonized by *P. mirabilis* ranged from 6.0 to 9.0.

The catheter biofilms composed of pure cultures of *P. mirabilis* were characteristically crystalline in structure (Fig. 1). In all 10 cases these biofilms contained crystals that were partially or completely occluding the catheter lumen. The structure and X-ray microanalytical spectra of the crystals are shown in Fig. 2 and 3. The specimens prepared by critical-point-drying demonstrate that in each case layers of bacilli are also present in the encrustations (Fig. 4).

Crystal formation was observed in biofilms populated by *Morganella morganii* (Fig. 5A, B), *Pseudomonas aeruginosa* (Fig. 5C), and in 1 case by a mixed community of *Escherichia coli* and *Enterobacter cloacae* (Fig. 5D). Most of the non-*Proteus* biofilms, however, were devoid of crystalline material (Fig. 6).

Discussion

Kunin [14] used the term "blockers" to characterize patients who consistently and repeatedly develop extensive encrustations on their urinary catheter. In contrast "non-blockers" are patients who do not form encrustations even when the catheter is left in place for many weeks. In populations of elderly patients up to half have been reported to be blockers [15]. Kunin [14] found that there were no significant differences among blockers and non-blockers with respect to age, activities of daily living and mental status. Examination of the constituents of urine found no significant differences among the two groups as regards protein, sodium, calcium, potassium, chloride, uric acid, oxalate, osmality and volume. Examination of the microbial flora of the urine, however,

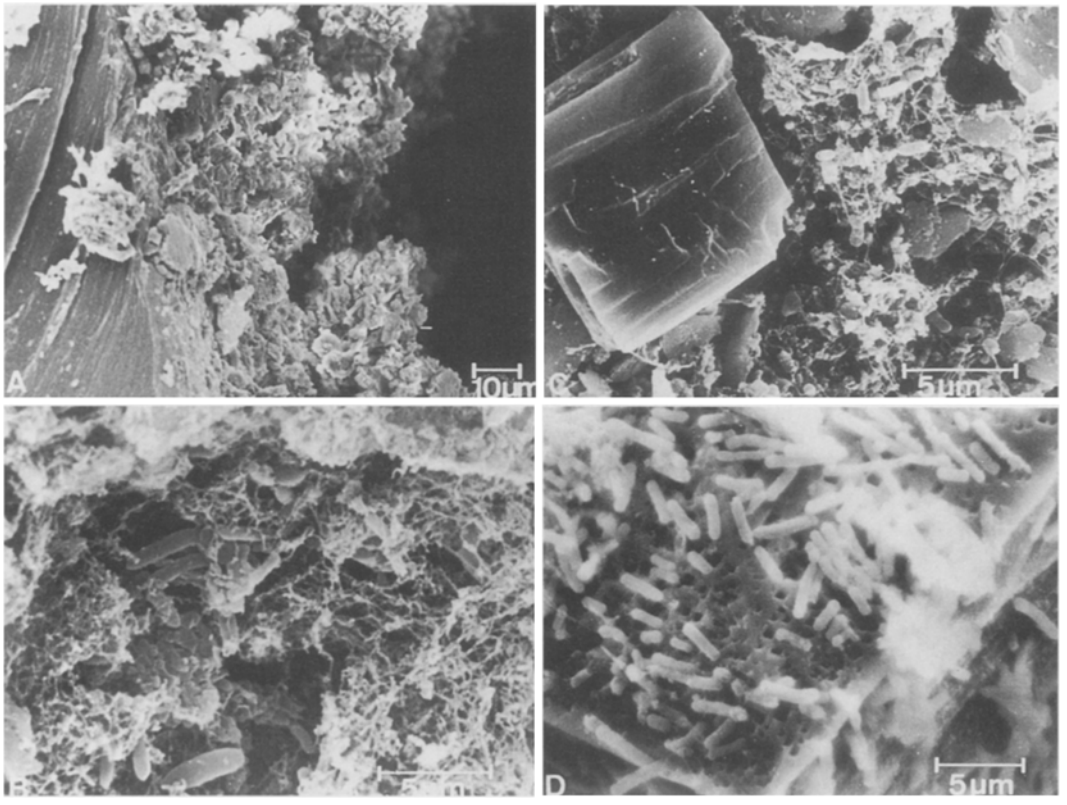

Fig. 5A–D. A freeze-dried (**A**) and a critical-point-dried preparation (**B**) of a *Morganella morganii* catheter biofilm, critical-point-dried preparations of a *Pseudomonas aeruginosa* biofilm (**C**) and a biofilm composed of *Escherichia coli* and *Enterobacter cloacae* (**D**)

revealed that all blockers were infected with at least one of the urease-producing *Proteus* group of bacteria. An extremely interesting observation was made on two patients who were long-term blockers and then converted to non-blockers. Examination of their charts showed that they had both received antibiotics which eradicated a urease producer and replaced it with an antibiotic-resistant *Pseudomonas aeruginosa*. While other workers have noted that urease-producing organisms and alkaline urines are commonly associated with catheter encrustation [7], the bacterial composition of the encrusting biofilm has not previously been examined. In this study of 75 catheters, it is clear that the most extensive encrustation was present on those catheters colonized by pure cultures of the urease producer *Proteus mirabilis* (Fig. 1).

The observations on the urine samples taken from 8 of the patients whose catheters were colonized by *P. mirabilis* suggest that catheter biofilms can generate alkaline microenvironments which can cause the crystallization of calcium and magnesium salts in the presence of acidic or neutral urine.

The X-ray microanalysis was performed on the crystalline formations present on 7 of the encrusted catheters. In each case the large crystals with the typical structure of struvite produced spectra which confirmed the presence of magnesium and phosphorus, while the analysis of the smaller amorphous particles characteristic of hydroxyapatite revealed the presence of calcium and phosphorus (Fig. 2, 3). In Fig. 4 it is apparent that the preparation of the specimens by fixing, washing, dehydrating and critical-point-drying has removed much of the encrustation from the catheter surface and revealed the underlying layers of bacteria.

Figure 5 reveals that encrustation can be generated by *Morganella morganii,* and *Pseudomonas aeruginosa* (both urease producers) but also by a mixed culture of organisms that do not produce this enzyme. In all these cases, however, the encrustation observed was not extensive. Most bacterial species found in catheter biofilms were apparently not capable of generating encrustation. It is clear from Fig. 6, for example, that biofilms of *Enterobacter cloacae* or *Citrobacter diversus* alone, or mixed populations such as *Escherichia coli, C. diversus* and *Enterococcus faecalis* are not crystalline. These results support the mechanism for catheter encrustation proposed by Cox et al. [4] and emphasize the important role of the active urease producer *Proteus mirabilis.*

The control of catheter encrustation is commonly attempted by the instillation of acidic solutions, but the rational basis of this treatment is not clear [20]. A recent clinical trial in elderly female patients showed that acidic bladder washouts performed twice weekly for 3 weeks had no demonstrable effect in preventing catheter encrustation [12]. In our experience the practice of simply replacing blocked catheters leads to their rapid recolonization and repeated blockage, and results in patients being designated as "blockers". We wish to suggest that in these cases it would be worth while investigating the effect of targeting *P. mirabilis* with antibacterials at the time of catheter change. In this way it might be possible to prevent or delay recolonization of the catheter with the urease producer that is mainly responsible for encrustation.

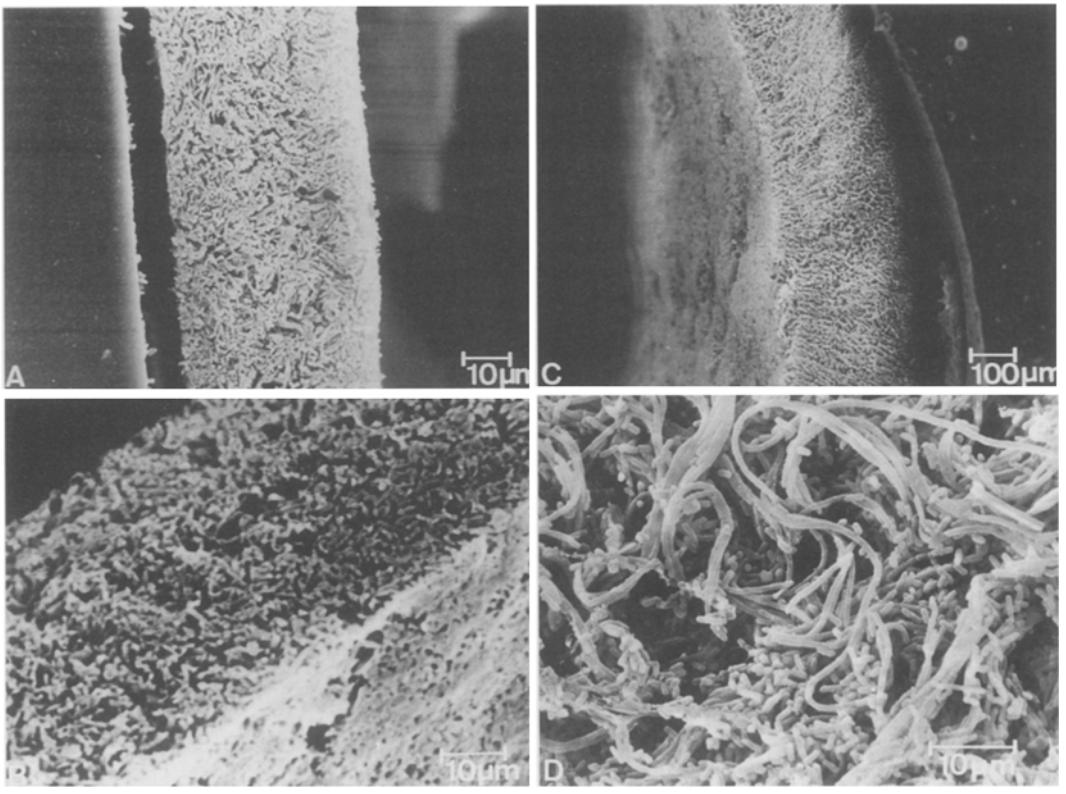

Fig. 6A–D. Biofilms showing no signs of encrustation: freeze-dried preparations of catheters colonized by **A** *Enterobacter cloacae*, **B** *Citrobacter diversus* and **C** *Escherichia coli*, *C. diversus* and *Enterococcus faecalis*; **D** a critical-point-dried preparation of the catheter biofilm shown in **C**

References

1. Clapham I, McLean RJC, Nickel JC, Downey J, Costerton JW (1990) The influence of bacteria on struvite crystal habit and its importance in urinary stone formation. J Crystal Growth 104:475
2. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ (1987) Bacterial biofilms in nature and disease. Annu Rev Microbiol 41:435
3. Cox AJ, Hukins DWL (1989) Morphology of minerals deposits on encrusted urinary catheters investigated by scanning electron microscopy. J Urol 142:1347
4. Cox AJ, Hukins DWL, Sutton TM (1989) Infection of catheterized patients: bacterial colonization of encrusted Foley catheters shown by scanning electron microscopy. Urol Res 17:349
5. Ganderton L, Chawla JC, Winters C, Wimpenny J, Stickler D (1992) Scanning electron microscopy of bacterial biofilms on indwelling bladder catheters. Eur J Clin Microbiol Infect Dis 11:789
6. Getliffe KA, Mulhall AB (1991) The encrustation of indwelling catheters. Br J Urol 67:337
7. Gristina AG (1987) Biomaterial-centred infection: microbial adhesion versus tissue integration. Science 237:1588
8. Hedelin H, Eddeland A, Larsson L, Pettersson S, Ohman S (1984) The composition of catheter encrustations, including the effects of allopurinol treatment. Br J Urol 56:250
9. Hedelin H, Larsson L, Eddeland A, Pettersson S (1985) Factors influencing the time long-term indwelling Foley catheters can be kept in situ. Eur Urol 11:177
10. Hukins DWL, Hickey DS, Kennedy AP (1983) Catheter encrustation by struvite. Br J Urol 55:304
11. Jensen ET, Kharazmi A, Lam K, Costerton JW, Hoiby N (1990) Human polymorphonuclear leukocyte response to *Pseudomonas aeruginosa* grown in biofilms. Infect Immun 58:2383
12. Kennedy AP, Brocklehurst JC, Robinson JM, Faragher EB (1992) Assessment of the use of bladder washouts/instillations in patients with long-term indwelling catheters. B J Urol 70:610
13. Kunin CM (1987) Detection, prevention and management of urinary tract infections, 4th edn. Lea and Febiger, Philadelphia, p 265
14. Kunin CM (1989) Blockage of urinary catheters: role of microorganisms and constituents of the urine on formation of encrustations. J Clin Epidemiol 42:835
15. Kunin CM, Chin QF, Chambers S (1987) Formation of encrustations on indwelling urinary catheters in the elderly. J Urol 138:899
16. Nickel JC, Gristina AG, Costerton JW (1985) Electron microscope study of an infected Foley catheter. Can J Surg 28:50
17. Nickel JC, Ruseska I, Wright JB, Costerton JW (1985) Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 27:619
18. Ohkawa M, Sugata LT, Sawaki M, Nakashima T, Fuse H, Hisazumi H (1990) Bacterial and crystal adherence to the surfaces of indwelling urethral catheters. J Urol 143:717
19. Ramsay JWA, Garnham AJ, Mulhall AB, Crow RA, Bryan JM, Eardley I, Vale JA, Whitfield HN (1989) Biofilms, bacteria and bladder catheters. B J Urol 64:395
20. Roe BH (1989) Use of bladder washouts: a study of nurses recommendations. J Adv Nurs 14:494
21. Stickler DJ, Clayton CL, Chawla JC (1987) The resistance of urinary tract pathogens to chlorhexidine bladder washouts. J Hosp Infect 10:219