Scanning and Transmission Electron Microscopy of In Situ Bacterial Colonization of Intravenous and Intraarterial Catheters

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Intravenous and intraarterial catheters were examined microbiologically and morphologically. Bacteria or yeasts were recovered from 38 of the 63 catheters examined, and *Staphylococcus epidermidis* was present on 29 of the 38 colonized catheters. Examination of unused Teflon catheters (Jelco; Surgikos, Inc., Peterborough, Ontario, Canada) showed surface irregularities, and the examination of colonized intravascular catheters recovered from patients showed very extensive amorphous accretions on both their lumenal and external plastic surfaces. Detailed scanning electron microscope examination of the accretions on vascular catheters from which *S. epidermidis* had been isolated showed (ca. 0.8 μm) coccoid bacteria within confluent biofilms, in which they were enveloped by amorphous material. Transmission electron microscope examination of these same accretions revealed coccoid cells (ca. 0.8 μm) with a gram-positive cell wall structure living in fibrous matrix-enclosed microcolonies in spaces between squamous epithelial cells. *Staphylococcus aureus* biofilms were seen to contain coccoid cells (ca. 1 μm) in a very extensive amorphous matrix, and a *Candida parapsilosis* biofilm contained very large numbers of large coccoid cells (ca. 4.3 μm) in a fibrous matrix resembling fibrin. Cells of a *Corynebacterium* species appeared to form much less extensive matrix-enclosed microcolonies on the colonized plastic surface. These data indicate bacteria and yeasts colonize intravascular catheters by an adherent biofilm mode of growth on these plastic surfaces.

Catheter-associated sepsis is one of the most serious complications of intravenous therapy (9, 10). Such infections are related to the duration of time that the catheter is in place (2). A wide variety of catheters and infusion sites are used to deliver fluid and nutrition to seriously ill patients. Peripheral veins are most commonly used. Central veins such as the subclavian are used for total parenteral nutrition. Catheters are also inserted into peripheral and central arteries to monitor arterial pressure.

In vitro studies have shown that bacteria in a liquid medium multiply on the surfaces of catheters, and various amounts of glycocalyx are evident, dependent upon the species of microorganism studied (T. J. Marrie and J. W. Costerton, submitted for publication). In contrast, organisms swabbed onto the surface of these catheters do not multiply on the surface and do not form a glycocalyx. In this study, we examined the surfaces of various intravenous and intraarterial catheters which became infected in vivo because we anticipate that an adherent microcolonial mode of growth has an effect on both the recoverability of these organisms by conventional microbiological methods and on their inherent susceptibility to antibiotics.

MATERIALS AND METHODS

Intravenous or intraarterial catheters were collected when they were removed. The Jelco catheters were rolled over a blood agar plate (5% sheep blood in Trypticase soy agar [BBL Microbiology Systems, Cockeysville, Md.]) according to the method of Maki et al. (10). Microorganisms were identified according to standard laboratory techniques.

Swan-Ganz catheters and central venous lines were cultured as above except the distal 8 to 10 cm only was submitted to the laboratory. The intravenous and intraarterial catheters were made from Teflon (Jelco; Surgikos, Inc., Peterborough, Ontario, Canada), as were the subclavian and total parenteral nutrition lines (subclavian jugular Infuset; Sorenson Research Co., Salt Lake City, Utah). We do not know the composition of the Swan-Ganz catheters (Edwards Laboratories, Inc., Santa Ana, Calif.).

Scanning electron microscopy. Sections of catheters were placed in a fixative solution consisting of 5% glutaraldehyde in cacodylate buffer (0.067 M, pH 6.2) with 0.15% ruthenium red for 24 h at 20°C immediately after removal from the patient. The catheter was then bisected and "metallized" with osmium tetroxide and thiocarbohydrazide (11), dehydrated in ethanol and Freon 113 before critical point drying (4), and examined with a Hitachi S450 (Hitachi, Rexdale, Ontario, Canada) scanning electron microscope.

Transmission electron microscopy. A sterile scalpel blade was used to scrape material from the surface of some of the Jelco intravenous catheters immediately after their removal from the patient. This material was fixed in 5% glutaraldehyde in cacodylate buffer (0.067 M, pH 6.2) with 0.15% ruthenium red for 24 h at 20°C. The material was then washed five times in the buffer, postfixed in 2% osmium tetroxide in buffer, washed five more times in the buffer, and dehydrated through a series of acetone washes. All of the solutions used in processing the specimen, from the wash after glutaraldehyde fixation to dehydration with the 70% acetone solution, contained 0.05% ruthenium red. (Ruthenium red was omitted from the 90 and 100% acetone solutions because of its limited solubility in these solutions.) After further dehydration in propylene oxide, the specimen was embedded in Spurr (15) low-viscosity embedding resin (Electron Microscopy Sciences, Ft. Washington, Pa.), sectioned.

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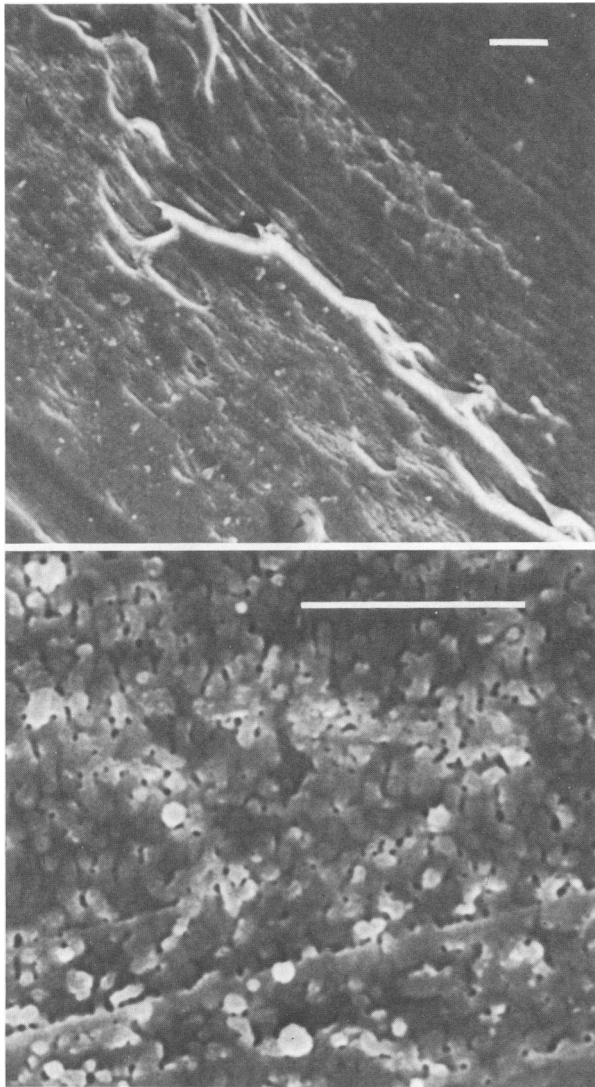
FIG. 1. Scanning electron micrographs of the surface of a Teflon Jelco intravenous catheter. This catheter had never been used. Note the surface irregularities. Both bars, 5 μm.
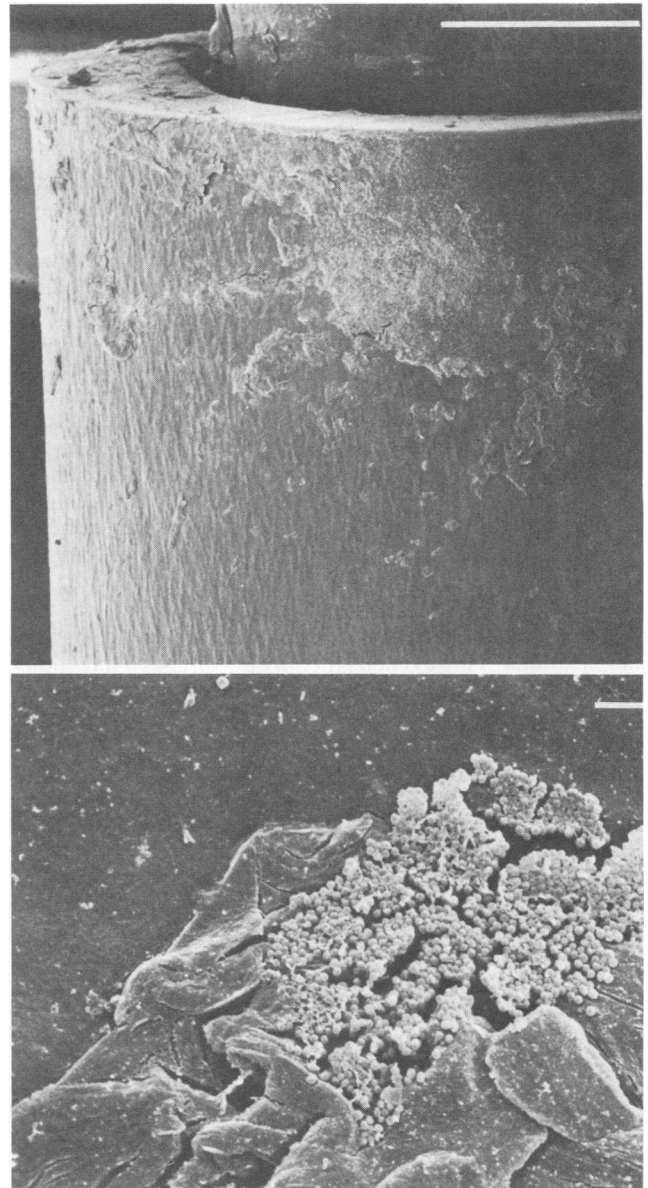

FIG. 2. (Top) Outer surface of a Teflon Jelco intravenous catheter recovered from a patient. Flakes of material are evident on the surface. Bar, 500 μm. (Bottom) Higher magnification shows clusters of coccoid cells intimately associated with these flakes. Note that in some areas the cocci are coated by slime (condensed glycocalyx). *S. epidermidis* was grown in culture. Bar, 5 μm.
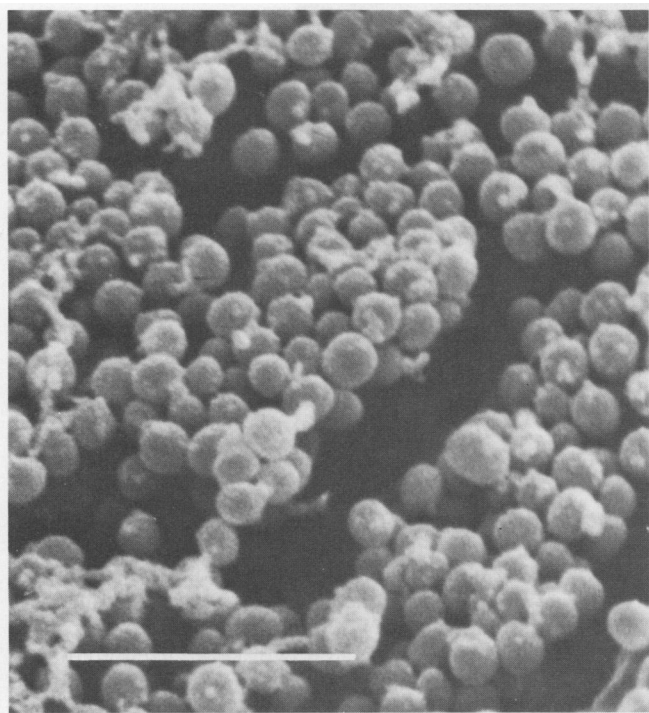
FIG. 3. A higher magnification of the cocci shown in Fig. 2, bottom. Note the strands of material interconnecting cells and also note the flocculent material on the surface of some of these cells. This probably represents exopolysaccharide which has condensed during the dehydration process. Bar, 5 μm.
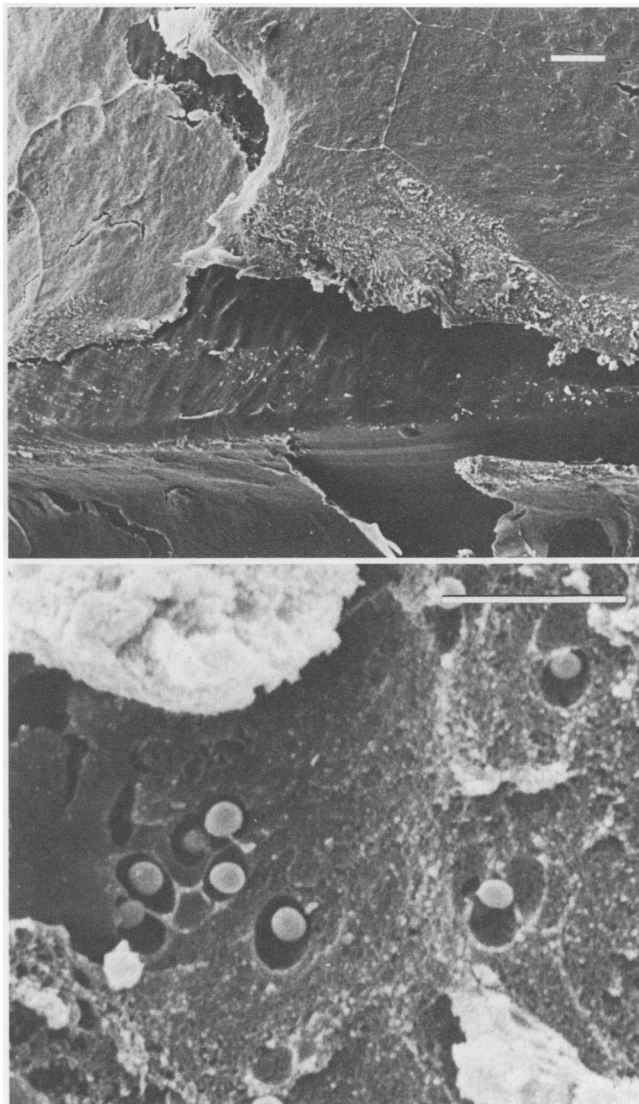

FIG. 4. Scanning electron micrograph of a Jelco catheter which had been in a radial artery for 3 days. (Top) Large cakes of material are seen. Bar, 50 μm. (Bottom) At higher magnification, cocci are seen embedded in an amorphous material. Bar, 5 μm. *S. epidermidis* and enterococci were isolated.
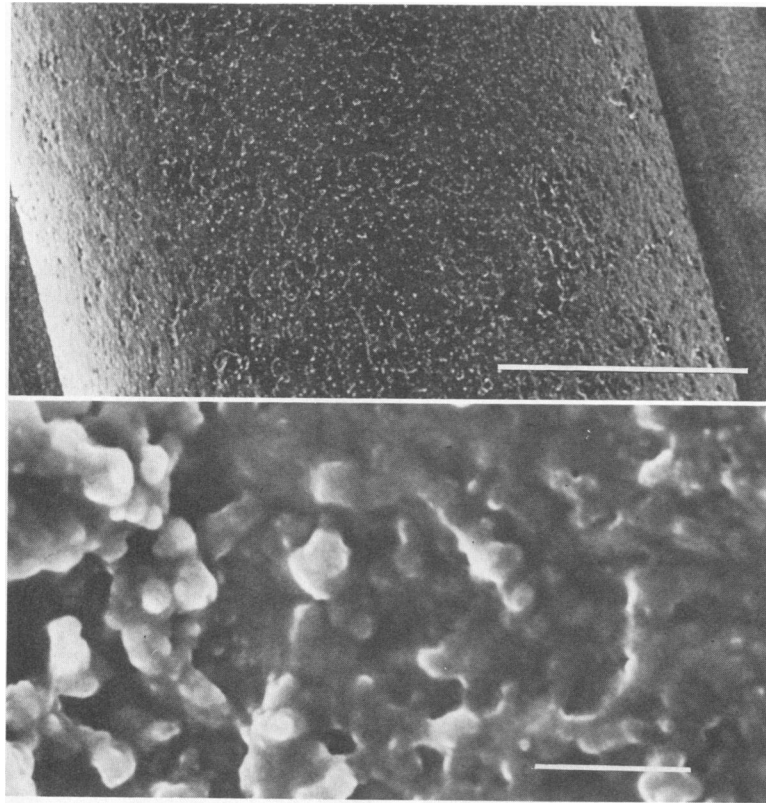
FIG. 5. Scanning electron micrographs of the surface of a central line. (Top) Low magnification view shows an uneven surface which at higher magnification (bottom) is covered by amorphous material within which profiles suggestive of coccoid bacteria are seen. Top bar, 500 μm. Bottom bar, 5 μm.

stained with uranyl acetate and lead citrate (13), reinforced with evaporated carbon, and examined with a transmission electron microscope (model 801; Associated Electrical Industries) at an acceleration voltage of 60 kV.

RESULTS

The number of each type of catheter studied and the results of cultures are shown in Table 1.

Figure 1 shows scanning electron micrographs of a sterile, unused intravenous catheter. Note the surface irregularities. Flakes of material were evident on many of the recovered intravenous catheters at relatively low magnification (Fig. 2, 4, 6, 8, top). Such material often had microorganisms embedded in it (Fig. 4, 6, 8, bottom) or intimately associated with it (Fig. 2, bottom). Frequently, both *Staphylococcus epidermidis* and *Staphylococcus aureus* were covered by an extensive matrix (Fig. 3 and 6, bottom), which we suggest is partially composed of bacterial glycocalyx (6) that has undergone condensation during the dehydration process (16). In other instances, the surface was covered by amorphous debris (Fig. 5, top) that had outlines suggestive of bacterial cells deeply buried in amorphous material (Fig. 5, bottom). We can only speculate whether such material represents excessive slime production by microorganisms as described by Christensen et al. (3). The spectrum of material that one may find on the surface of intravenous catheters is exemplified by the diatom (Fig. 7). The only catheter with *Candida parapsilosis* infection showed a very extensive biofilm on the plastic surface (Fig. 8, top), and this biofilm was seen to be composed of large numbers of *Candida* cells

intimately associated with a fibrous material resembling fibrin (Fig. 8, bottom).

When we examined material scraped from the surface of *S. epidermidis*-infected intravenous catheters by transmission electron microscopy, we found very large numbers of bacterial cells closely associated with keratinized epithelial cells (Fig. 9). These organisms had a gram-positive cell wall architecture (5) and were seen to form large microcolonial aggregates in the interstitial spaces between epithelial cells. Within these aggregates, and in pockets in the epithelial cell surface (Fig. 9, white arrowhead), the bacterial cells were seen to be extensively surrounded by an anionic fibrous material (Fig. 9, M) that stained with ruthenium red and showed a pattern of association with these cells that indicated that it is a bacterial product. Venous catheters from which *Corynebacterium* species were recovered were not found to be extensively colonized by bacteria, but small aggregates that appeared to contain bacterial cells in an amorphous matrix were regularly observed (Fig. 10).

DISCUSSION

The morphology of bacterial adherence to intravascular catheters in vivo contrasts markedly with in vitro results (submitted for publication). To a large extent, this reflects the interplay of host and microorganisms. The observation that bacteria grow in the interstitial spaces of skin (Fig. 9) that was scraped, with the bacterial biofilm, from the transcutaneous portion of these catheters indicates that autochthonous skin organisms may play an important role in the colonization of a cutaneous tract (10) associated with these

FIG. 7. Scanning electron micrograph of the inner surface of a Jelco intravenous catheter. Strands of fibrin are present. In addition, a diatom (arrow) and several coccoid bacterial profiles (white arrowheads) are seen. *S. epidermidis* was isolated from this catheter.
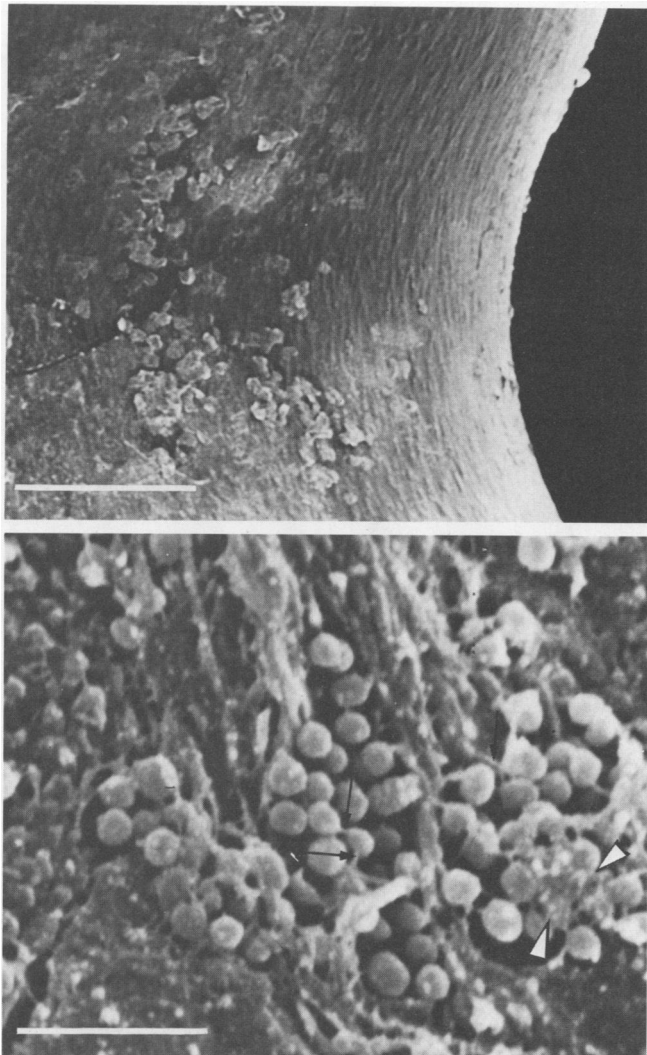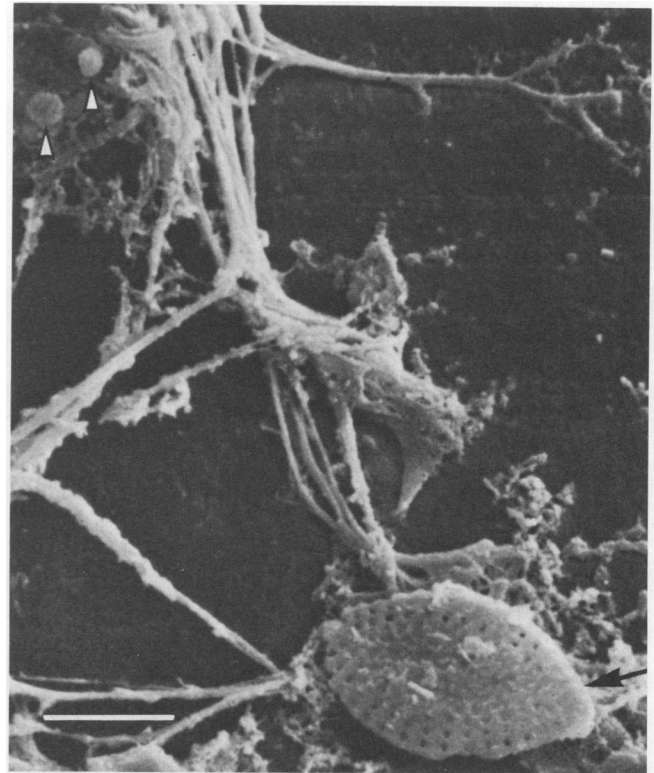

FIG. 6. Scanning electron micrographs of the outer surface of a Jelco intravenous catheter. (Top) Flakes of material are present on the surface. (Bottom) At higher magnification, coccoid bacterial cells are readily evident. These cells adhere to one another by intercellular strands (arrows). There is a dense background matrix, which in some areas seems to be covering coccoid cells (white arrowheads). *S. aureus* was isolated on culture. Top bar, 500 μm. Bottom bar, 5 μm.

TABLE 1. Number and type of intravenous and intraarterial catheters studied and microorganisms isolated from each

Type of catheter and organism isolated[a]	No. of catheters
Intravenous and intraarterial (Jelco) (11)	
S. epidermidis	15
Corynebacterium sp.	1
S. epidermidis + Corynebacterium sp.	1
S. epidermidis + enterococcus	1
S. aureus	1
Central venous	
Total parenteral nutrition lines (1)	
C. parapsilosis	1
S. epidermidis	2
Proteus sp.	1
Corynebacterium sp.	1
Subclavian lines (7)	
S. epidermidis	6
S. aureus	1
C. albicans	1
Enterobacter sp.	1
Swan-Ganz catheters (6)	
S. epidermidis	3
S. epidermidis + S. aureus	1
C. albicans	1

[a] Numbers in parentheses represent the number of catheters from which no organisms were isolated.
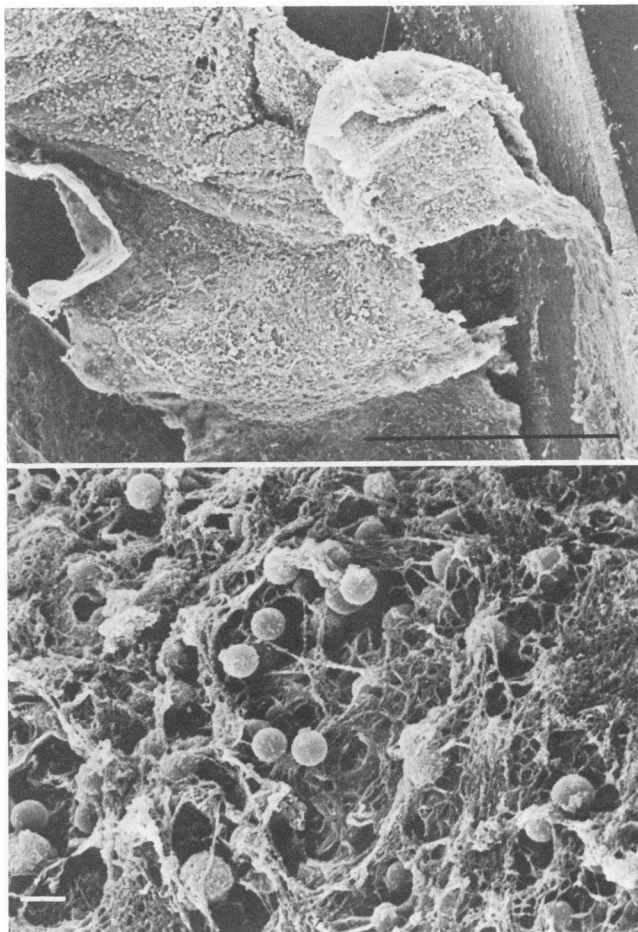
FIG. 8. Scanning electron micrographs of the inner surface of a catheter used for total parenteral nutrition. (Top) A large amount of biofilm material is evident. This has become detached from the catheter wall during the fixation process. (Bottom) At higher magnification, large cells (ca. 4.3 μm in diameter) are seen in a fibrous matrix. *C. parapsilosis* was cultured from this catheter. Top bar, 500 μm. Bottom bar, 5 μm.

prostheses. In addition, *Candida* cells appeared to be associated with fibrin. Others have shown that surface mannan may play an important role in the adherence of *Candida* species to fibrin-platelet matrices (14). *C. albicans* and *C. tropicalis* adhere better to intravenous catheters made from polyvinylchloride than to those made from Teflon (7).

The surfaces of intravascular catheters are imperfect, as shown in Fig. 1. Such imperfections may be due to protruding material, scratches, troughs, scales, lacunae, or adhering particles (1). Furthermore, infusates can leave residue on the surface of the catheters (12). It is not surprising then that we found a film of material on most of the catheters that we examined. When bacteria are present, we term this a biofilm. In such instances, some bacteria produce an extensive glycocalyx (Fig. 3), whereas others (Fig. 10) do not. Similar variations in slime production have also been described in vitro (8). The design of intravascular catheters that incorporate antibacterial components and thus resist bacterial colonization has to be a research priority if we are to reduce the hazard of intravascular line sepsis.

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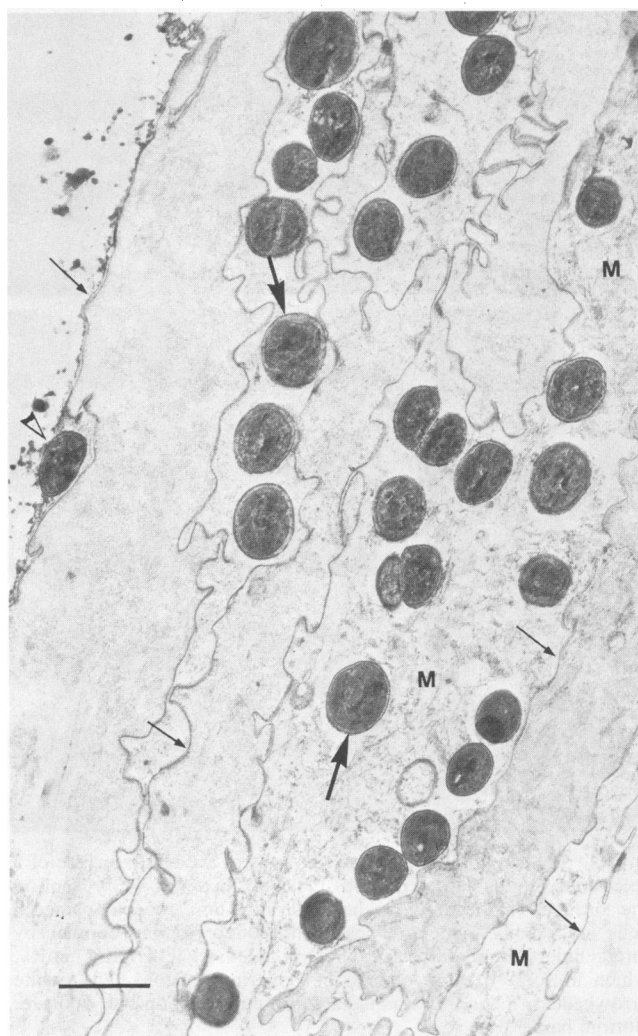

FIG. 9. Transmission electron micrograph of material scraped from the outer surface of a Jelco intravenous catheter. Several stratified squamous epithelial cells are present (small black arrows). In between these squamous epithelial cells are bacterial cells with gram-positive cell walls (large black arrows), and similar bacteria are seen in "pits" in the surfaces of epithelial cells (white arrowhead). There is also a fine fibrillar matrix (M) evident in the intercellular spaces. Bar, 1 μm. *S. epidermidis* was recovered on culture.
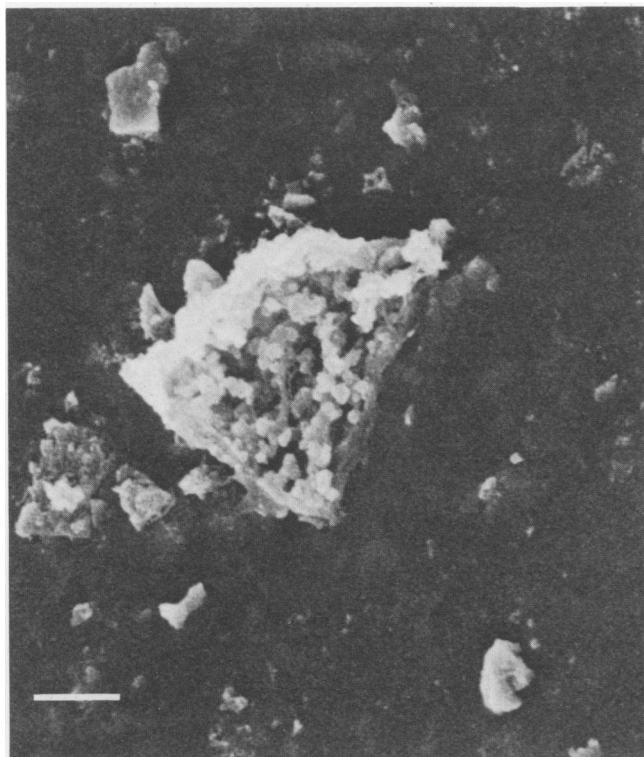
FIG. 10. Scanning electron micrograph of the inner surface of a Jelco intravenous catheter from which *Corynebacterium* species was isolated in culture; note the particulate matter on the surface. In the center of this micrograph is a small microcolony of bacterial cells in an amorphous matrix. Bar, 5 μm.

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