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Staphylococcal Adherence to Polyvinyl Chloride and Heparin-Bonded Polyurethane Catheters Is Species Dependent and Enhanced by Fibronectin

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Intravenous hyperalimentation has improved the survival of premature infants. However, long-term placement of intravenous catheters may result in the development of catheter-related sepsis. Fibronectin in plasma contains binding sites for staphylococcal species as well as marked affinity for inert plastics and therefore may provide a substrate for bacterial adherence to indwelling catheters. We determined the adherence of labeled ([^{3}H]leucine) coagulase-positive (CPS) and coagulase-negative (CNS) staphylococci to untreated and fibronectin-coated polyvinyl chloride (PVC) and heparin-bonded polyurethane (HBP) catheter segments and quantitated the binding of ^{14}C-labeled, purified fibronectin to these catheters. PVC catheter segments bound significantly more CNS than CPS ($P < 0.05$), while HBP catheters bound more CPS than CNS ($P < 0.05$). Fibronectin significantly increased the adherence of CPS to PVC catheters ($P < 0.05$) and CNS to HBP catheters ($P < 0.05$). PVC catheters bound more fibronectin ($P < 0.0001$) than did HBP catheters. Catheter composition may influence the spectrum of nosocomial pathogens to which infants are susceptible through different bacterial adherences and interactions with adhesive proteins.

Hyperalimentation through indwelling central venous catheters is an accepted method of providing nutrition for low-birth-weight infants. The long-term use of intravenous catheters has been associated with serious complications (thrombosis, perforation, etc.). Recent attention, however, has focused on the occurrence of catheter-related sepsis due to coagulase-positive and coagulase-negative staphylococci (3, 6, 20, 23, 29). Although many factors are thought to contribute to the high incidence of sepsis in newborn infants with indwelling catheters, the effect of catheter composition on the adherence of microorganisms has received little attention (18, 21, 22). The recently introduced heparin-bonded polyurethane (HBP) catheters are purported to decrease the incidence of thrombosis (13). Heparin, however, may also provide a substrate for factors in serum, such as fibronectin (FN), which can modulate bacterial adherence.

In this study, we quantified and compared the adherence of *Staphylococcus aureus* and *Staphylococcus epidermidis* to polyvinyl chloride (PVC) and HBP catheters and determined the effect of exogenous FN on this adherence.

MATERIALS AND METHODS

Catheter preparation. Size five French PVC catheters (Argyle Co., St. Louis, Mo.) and HBP catheters (Bard Co., Lombard, Ill.) were divided into segments (surface area, 0.50 to 0.95 mm^2) and cut longitudinally before use.

To determine the effect of FN on bacterial adherence, preweighed and longitudinally cut catheter segments were preincubated (18 h, 4°C) in 1 ml of Tris buffer (0.05 M Tris hydrochloride [pH 9.2]) containing 0.5 μg of FN per ml. The purity of the FN preparation was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 5 to 15% gradient under reduced conditions (16). Catheter segments were washed continuously for 2 min in phosphate-buffered saline (PBS; pH 7.4) before use. Control catheter segments were incubated in Tris buffer (pH 9.2, 18 h, 4°C).

Preparation of radiolabeled bacteria. Three protein A positive *S. aureus* isolates (CHP101, CHP102, and CHP103) and three slime-producing *S. epidermidis* isolates (CHP201, CHP202, and CHP203) were tested. Bacterial strains were identified by the Clinical Microbiology Laboratory, The Children's Hospital of Philadelphia. All bacteria were isolated from blood cultures and identified by catalase testing followed by coagulase evaluation, and finally a final identification was made with Staph Trac (Analytab Products, Plainview, N.Y.). Slime production was detected by the method described by Christensen et al. (4).

To prepare radiolabeled bacteria, tritiated leucine (0.25 ml; specific activity, 57.4 Ci/mmol); Amersham Corp., Arlington, Heights, Ill.) was added to 10 ml of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) containing 10^2 CFU of a single bacterial strain per ml and incubated overnight (37°C). The following morning the bacteria were washed twice in PBS and suspended in 10 ml of PBS, and 300 μl was placed in vials containing 10 ml of scintillation fluid (ACS II, Amersham) and counted in a beta counter (LS7500; Beckman Instruments, Inc., Palo Alto, Calif.). Before each experiment the radiolabeled bacteria were quantified by plating out serial dilutions.

Bacterial adherence to catheter segments. Preweighed and longitudinally cut catheter segments that were either untreated (Tris buffer; pH 9.2, 18 h, 4°C) or preincubated in FN (0.5 μg/ml diluted in 0.05 M Tris buffer; pH 9.2, 18 h, 4°C) were washed vigorously in PBS (room temperature) for 2 min and added to 300 μl of radiolabeled bacteria diluted in PBS (37°C). At six time points (0, 0.25, 0.5, 1, 2, and 4 h) two catheter pieces were removed, washed vigorously in PBS, and counted. To exclude the possibility that FN was exerting a nonspecific effect on bacterial adherence, PVC and HBP catheter segments were preincubated in human serum albumin (0.5 μg/ml; Alpha Therapeutic Corp., Los Angeles,

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TABLE 1. Binding of *S. aureus* and *S. epidermidis* to HBP and PVC catheters

Catheter	Time (h)	CFU (10^3)/mm^2 of catheter (mean ± SD)	
		S. aureus CHP101	*S. epidermidis* CHP201
HBP	0	48 ± 23	65 ± 83
	0.25	104.6 ± 45[a]	24 ± 12
	0.5	185 ± 69[a]	30 ± 14
	1	260.8 ± 135[a]	39.6 ± 23
	2	331 ± 160[a]	50.5 ± 22
	4	523.9 ± 378[a]	86.7 ± 42
PVC	0	22.8 ± 20.6	39.4 ± 32
	0.25	71.8 ± 42	73.0 ± 62
	0.5	94.5 ± 54.7	76.2 ± 68
	1	178.1 ± 131.5	161.4 ± 201
	2	208.3 ± 134	300.8 ± 275.8[a]
	4	396.4 ± 264[a]	515.5 ± 716[a]

[a] Significant increase ($P < 0.05$) in adherence versus time zero.

Calif.). Adherence of *S. aureus* to the albumin-treated PVC catheter segments at 2 h and *S. epidermidis* to albumin-treated HBP catheter segments at 4 h was compared with adherence of catheter pieces that were preincubated in Tris buffer or FN at identical concentrations. To address the possible quenching effect contributed by the various geometric configurations of the catheter specimens, control vials were prepared containing known levels of radioactivity and catheter segments which were up to five times the size used in the test procedure. No significant quenching was observed with either catheter material. All experiments were repeated on four occasions, and results were expressed as CFU per square millimeter of catheter surface area. In preliminary experiments it was demonstrated that the bacteria suspended in PBS remained viable but did not replicate significantly over the 4-h time period.

Binding of radiolabeled FN to HBP and PVC catheters. Preweighed and longitudinally cut catheter segments were suspended in 1 ml of Tris buffer (pH 9.2) containing 0.5 μg of ^{14}C-labeled, methylated FN (specific activity, 2.9 μCi/mg; Amersham). Following overnight incubation at 4°C the segments were removed, washed in PBS, and counted. Results were expressed as micrograms of FN per 10 mm^2 of catheter surface area.

Electron microscopy. Unused catheters were cut into segments (length, 3 to 4 mm), attached to stubs with conducting paint, sputtered with platinum palladium, and observed by using a electron microscope (super three scanning; International Scientific Instruments) (11).

Statistical analysis. The data were analyzed by using a statistical software package supplied by Hewlett-Packard (Fort Collins, Colo.) for the HP-9845B computer by using one-way and two-way analysis of variance and Student's *t* test.

RESULTS

Adherence of staphylococcal strains to HBP and PVC catheters. There was a significant increase over time in the adherence of *S. aureus* and *S. epidermidis* to the PVC ($P < 0.05$) and *S. aureus* to the HBP ($P < 0.05$; Table 1) catheter segments. HBP catheter segments bound significantly more *S. aureus* than *S. epidermidis* ($P < 0.05$; Fig. 1A). There was preferential binding of *S. epidermidis* to the PVC catheter segments ($P < 0.05$; Fig. 1B).

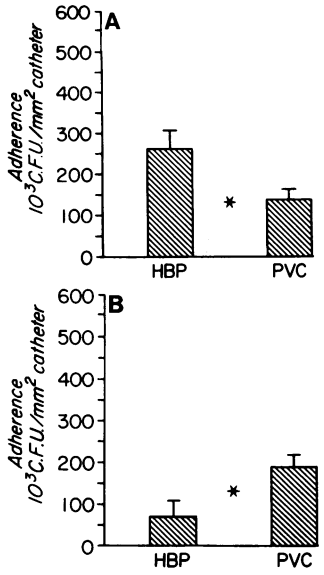

FIG. 1. Values represent the mean ± standard error of the mean adherence of *S. aureus* CHP101, CHP102, and CHP103 (A) and *S. epidermidis* CHP201 to CHP203 (B) at the 4-h time point to HBP and PVC catheter segments. For panel A, $P = 0.02$ (*); for panel B, $P = 0.034$ (*).

Effect of FN on bacterial adherence. Preincubation of catheter segments in FN significantly increased the binding of *S. aureus* and *S. epidermidis* to HBP catheter segments and that of *S. aureus* to PVC catheter segments (Fig. 2). Neither albumin nor Tris buffer alone increased the adherence of the bacteria to the catheter segments. For the PVC catheter, incubation in Tris buffer for 2 h yielded 6.6×10^3 CFU/mm^2; incubation in albumin for 2 h yielded 5.2×10^3 CFU/mm^2. For the HBP catheter, incubation in Tris buffer for 4 h yielded 10.05×10^4/mm^2; incubation in albumin for 4 h yielded 12.5×10^4/mm^2).

Binding of radiolabelled FN to HBP and PVC catheters. PVC catheter segments bound significantly ($P < 0.0001$) more FN than did HBP segments (0.20 ± 0.04 μg of FN per

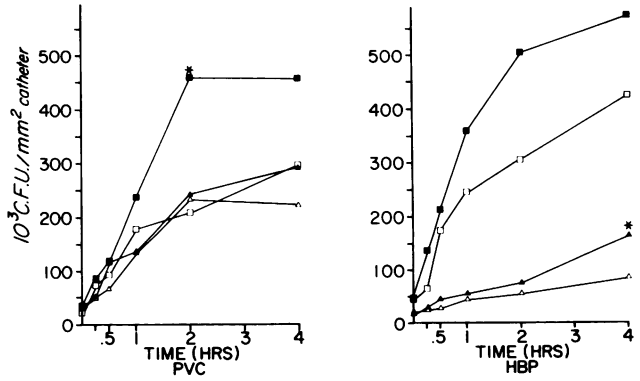

FIG. 2. Symbols: ■, *S. aureus* (catheters preincubated with FN); □, *S. aureus* control; ▲, *S. epidermidis* (catheters preincubated with FN); △, *S. epidermidis* control. Effect of FN on adherence. Values represent the mean adherence of *S. aureus* CHP101 and *S. epidermidis* CHP201 to HBP and PVC catheter segments at times of 0, 0.25, 0.5, 1, 2, and 4 h. Catheter segments were preincubated in FN (0.5 μg/ml) or PBS (control). $P < 0.05$ (*) for catheters with FN versus controls.

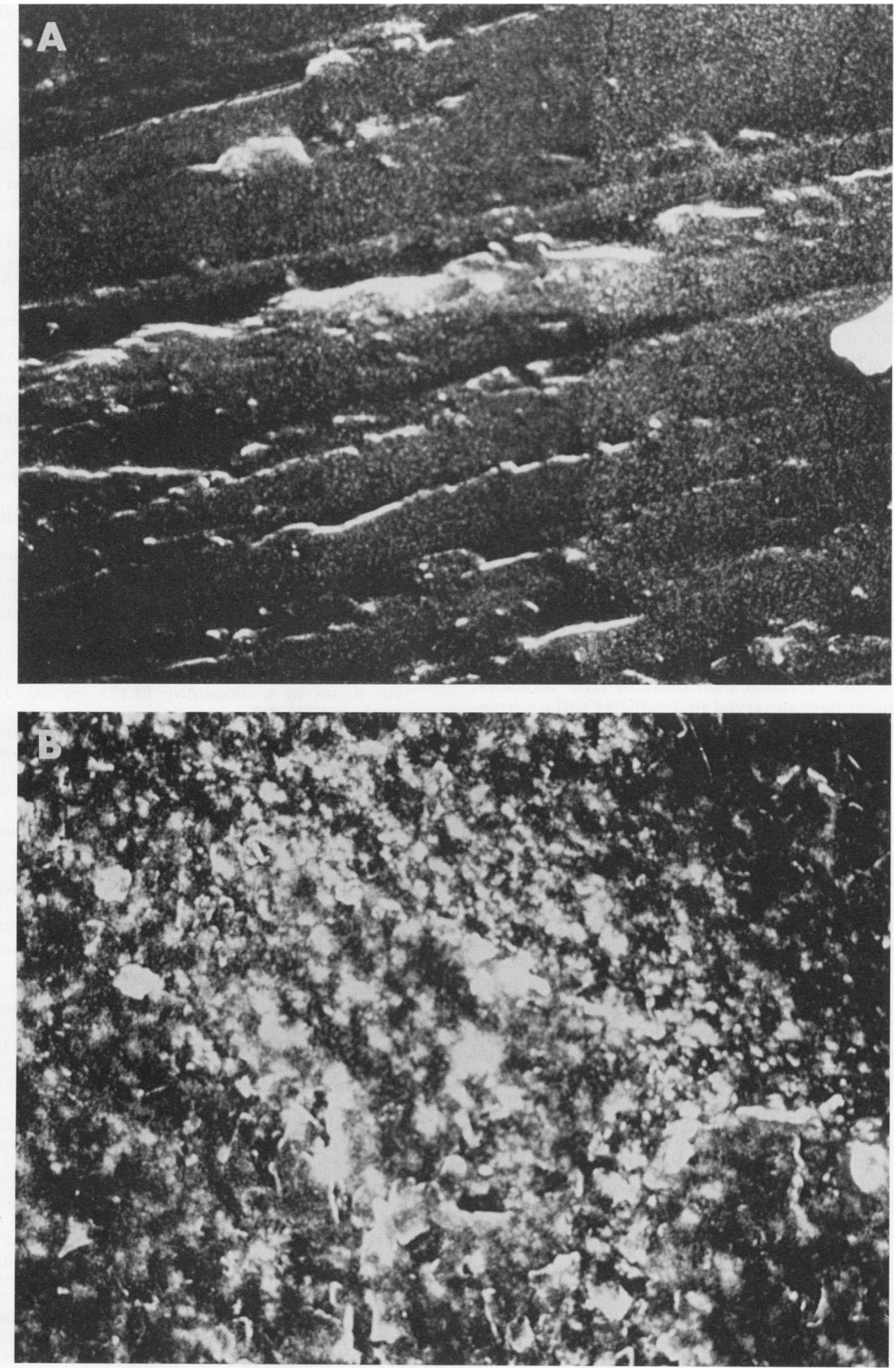

FIG. 3. Electron micrograph of new HBP (A) and PVC (B) catheter segments. Magnifications, ×1,000.

10-mm^2 catheter surface area versus 0.07 ± 0.01 μg of FN per 10-mm^2 catheter surface area, respectively).

Electron microscopy. Electron photomicrographs of the external surface of HBP and PVC catheters are shown in Fig. 3A and B, respectively (magnification, ×1,000). The outer surfaces of the PVC catheters demonstrated numerous irregularities that were not evident on the HBP catheter outer surfaces.

DISCUSSION

Results of this study demonstrate that *S. epidermidis* and *S. aureus* bind both to HBP and PVC catheters. Although only three strains of each bacterial species were tested, *S. aureus* preferentially bound to HBP catheter segments and *S. epidermidis* preferentially bound to PVC catheter segments. FN adhered to both kinds of catheters (PVC and HBP) and modulated bacterial attachment.

Results of previous studies (1) have demonstrated that up to 30% of indwelling intravenous catheters become colonized with bacteria. While all the contributing factors have not been identified, the incidence of colonization and septicemia have been associated with the duration of insertion, the experience and technique of the personnel introducing the catheter, the length of the catheter that is inserted, the occurrence of clots, and the use of topical or systemic antibiotics (6).

The surfaces of intravascular catheters are imperfect, which may be due to protruding material, scratches, troughs, scales, lacunae, or adhering particles (17). In this study electron microscopy demonstrated that the surfaces of PVC catheters are more irregular than those of HBP catheters. Peters et al. (18) have observed that catheter surface irregularities appear to be the preferential sites for the attachment of *S. epidermidis*. Colonizing bacteria adhere to the irregularities on the catheter surfaces by means of polysaccharide fibers or branching sugar molecules that extend from the bacterial surface and that form a glycocalyx surrounding an individual cell or colony of cells (7). Colonization appears to increase over time and originates as a single layer of microorganisms progressing to multiple layers (18).

S. epidermidis, the organism most frequently cited in neonatal catheter-related infections, is known to secrete a slimy material which increases in volume over time and which is thought to facilitate adherence. Results of previous studies (18) have demonstrated a correlation between the production of staphylococcal slime and the number of cells adhering to the catheter surface. Animals that have been challenged with slime-producing strains exhibit an increased susceptibility to infection (4, 5).

In this study, FN increased the adherence of staphylococcal species to PVC and HBP catheters. FN binds to many kinds of substances, and there is great variation in the affinity of FN to inert plastics (14). FN has also been shown to increase the attachment of cells and microorganisms to these materials and has a strong binding site for *S. aureus* (8, 9. 12, 14, 15, 19, 24, 26–29) and variable affinity for *S. epidermidis* (8, 28). Conversely, FN in plasma may act as an opsonin that promotes the clearance of FN-bound organisms (2). Although we have not stained indwelling catheters for the presence of FN, this glycoprotein should bind to catheters in vivo, either directly to the plastic material or via fibrinogen or fibrin, which are known to coat catheters after a short time interval following insertion (12, 25).

In conclusion, catheter composition and bacterial adherence characteristics may modulate the spectrum and degree of intravenous catheter colonization and may influence the incidence of nosocomial sepsis. FN and perhaps other adhesive proteins may be involved in the process of catheter colonization by modulating bacterial adherence.

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