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Bacterial Adherence to Human Endothelial Cells In Vitro

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Differences in the ability of bacteria to adhere to normal valvular endothelium may account for the predominance of particular species as pathogens in acute endocarditis. An in vitro adherence assay was developed to simulate the host surface encountered in acute bacterial endocarditis by using confluent monolayers of human endothelial cells. Adherence of 32 gram-positive and -negative blood culture isolates to this surface was compared. All five *Staphylococcus aureus* strains tested were highly adherent to endothelial cells, as was one gram-negative strain (*Serratia marcescens*). The remaining gram-positive and -negative isolates, including four viridans streptococci, were relatively nonadherent. Transmission electron microscopy demonstrated attachment of *Staphylococcus aureus* and invagination of the underlying endothelial cell membrane at 1 h followed by engulfment of large numbers of bacteria after 3 h. The intracellular bacteria appeared to be contained within vacuoles. Preferential attachment of some strains of bacteria, in particular *Staphylococcus aureus*, to human endothelial cells occurred in vitro, suggesting that adherence is an important determinant of bacterial pathogenicity in acute endocarditis. Active uptake of bacteria by endothelial cells may help account for the virulence of *Staphylococcus aureus* in endovascular infections and for the ability of this organism to establish multiple metastatic foci of infection.

Acute infective endocarditis is a fulminant, life-threatening disease of endocardial surfaces caused by *Staphylococcus aureus* and other virulent bacteria. In contrast to the indolent course of subacute endocarditis, acute endocarditis can result in the rapid destruction of infected valves, metastatic bacterial dissemination, and death in up to 60% of cases (6, 7, 15, 25). Intravenous drug abusers and patients with indwelling vascular devices, who usually do not have underlying cardiac disease, constitute the major risk groups (3, 6, 7, 17, 25). The importance of this infection is underscored by the fact that, in some series, *Staphylococcus aureus* is the leading cause of endocarditis, even surpassing viridans streptococci, the predominant pathogen in subacute endocarditis (15).

Bacteremia followed by attachment of bacteria to an endocardial surface precedes the establishment of endocarditis (2, 6, 25, 26). Earlier work by Gould et al. (9) demonstrated higher adherence by gram-positive species than by enteric gram-negative bacilli to sections of human and canine heart valves in vitro. These findings, coupled with the predominance of gram-positive cocci in endocarditis seen in clinical practice, suggest that the enhanced ability of these bacteria to adhere to normal endothelial surfaces contributes to the pathogenesis of this infection (25).

Detailed investigation into the role of tissue tropism in the pathogenesis of acute endocarditis has been hindered by the limited availability of the target tissue, namely, viable human endothelium. However, it is now possible to maintain human endothelial cells derived from umbilical veins, major arteries, and heart valves in tissue culture (8). Growth of these cells in uniform monolayers provides a convenient substrate for the detailed study of bacteria-endothelial cell interactions and simulates the surface encountered by bacteria in endocarditis.

The purpose of the present study was to investigate the pathogenesis of acute endocarditis by examining bacterial adherence to endothelial cells in vitro. We measured the adherence of different bacterial species to cultured human endothelial cells. *Staphylococcus aureus*, the most adherent species, was selected for more detailed, ultrastructural studies of this interaction.

MATERIALS AND METHODS

Endothelial cell tissue culture. Endothelial cells harvested from human newborn umbilical cords with 0.1% collagenase (Sigma Chemical Co., St. Louis, Mo.) were maintained at 37°C under 5% CO_2 in Medium 199 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% newborn calf serum (GIBCO) and a partially purified growth factor extracted from bovine brain (8). The cells were split at a 1:3 ratio every 7 days. Confluent monolayers were prepared in 12-well plastic trays (Linbro; Flow Laboratories, McLean, Va.) on a 0.2% gelatin (Sigma) substrate. The cells from two wells per tray were collected with 1% trypsin for cell counts with a hemacytometer after trypan blue exclusion for viability. Cell counts ranged from 220,000 to 400,000 per well at confluence.

Fresh autopsy specimens of human heart valves provided a second source of endothelial cells. The valves were obtained within 12 h of death, washed in HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Calbiochem-Behring, La Jolla, Calif.) buffer and treated with 0.1% collagenase. The cells were collected by centrifugation and maintained in a fashion similar to that used for the umbilical vein cells, except that: (i) 25 μg of heparin (Sigma) per ml was added to the culture medium, (ii) 10% human and 20% fetal calf serum (GIBCO) were used instead of newborn calf serum, and (iii) 1.5% gelatin was used in the well sets. There were 160,000 to 250,000 cells per well at confluence. Both types of endothelial cells were tested for von Willebrand factor antigen by immunofluorescent staining; all cells expressed the antigen (10).

Bacterial strains. Clinically important blood culture iso-

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lates were obtained from the microbiology laboratory of Montefiore Medical Center, Bronx, N.Y. Two of the 32 strains collected were from endocarditis patients. Strains were stored in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) at −70°C until just before use, when they were subcultured onto blood agar plates.

Preparation of bacteria for radioisotopic studies. Radiolabeled bacteria were used for the initial set of experiments, which were performed on umbilical vein endothelial cells. Bacterial strains were grown overnight at 37°C in Todd-Hewitt broth with 5 μCi of [^{3}H]thymidine (Amersham Corp., Arlington Heights, Ill.) per ml and used in the stationary phase of growth. *Pseudomonas aeruginosa* strains were labeled with [^{3}H]uracil (Amersham). Labeled bacteria were collected by centrifugation, washed five times with 0.9% NaCl, and suspended in Medium 199. The last saline wash was filtered through a membrane filter (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.) and counted to determine the amount of unincorporated label. Bacterial aggregates were dispersed by repeated aspiration through a 25-gauge needle followed by filtration through a membrane filter (pore size, 5 μm; Nuclepore Corp., Pleasanton, Calif.). Visual inspection under light microscopy confirmed a predominance of single organisms or short chains. The final concentration of bacteria was adjusted to an optical density between 0.1 and 0.3 at 620 nm. The average bacterial inoculum, determined by viability counts on agar pour plates, was $2.2 \times 10^8 \pm 0.5 \times 10^8$ CFU per well (mean ± standard deviation).

Adherence assay. Initially, two methods were used to quantitate the number of bacteria adherent to umbilical vein endothelial cells. The results from both assays were compared. In the first method, a modification of the method of Davison and Sanford (5), 0.5-ml aliquots of each radiolabeled bacterial suspension were applied to confluent monolayers of endothelial cells and gently agitated on a rotary shaker (Junior Orbit shaker; Lab-Line Instruments, Melrose Park, Ill.) at 50 rpm and 37°C for 2 h. Each strain was done in triplicate. After the bacterial supernatant was discarded, the endothelial cells were washed, treated with 1 N NaOH, and collected, and a sample was removed for scintillation counting. Adherence, expressed as bacteria per endothelial cell, was calculated as [(counts per minute per milliliter in well − background)/counts per minute per bacterium in initial inoculum]/number of endothelial cells.

In a second method, 2 ml of Todd-Hewitt broth was added to each well in place of NaOH at the end of the incubation period. The endothelial cells and adherent bacteria were collected by using a rubber policeman, diluted serially, and plated in heart infusion agar (Difco Laboratories, Detroit, Mich.). Colony counts were divided by the number of endothelial cells per well to arrive at the number of bacteria per endothelial cell.

When bacterial adherence to valvular endothelial cells and umbilical vein cells was compared, the simpler pour plate method was used by itself. In addition, the assay was modified as follows: (i) the bacterial inoculum was lowered to 10^7 CFU/ml to reduce the potential variability in results from the high bacterial inoculum/endothelial cell ratio, and (ii) the incubation period was shortened to 1 h after timed studies showed little change in measured adherence from 1 to 2 h of incubation.

Adherence to background. Confluent monolayers were used to minimize background adherence. This was confirmed by direct visualization and by cell counts which exceeded 200,000 per well for umbilical cells and 160,000 per well for valvular endothelial cells. When *Staphylococcus aureus* and *Escherichia coli* were incubated with preconfluent monolayers, only minimal bacterial adherence to background was seen in a modified Giemsa stain. Scanning electron microscopy also confirmed that *Staphylococcus aureus* was predominantly cell associated, with few bacteria in the background (Fig. 1).

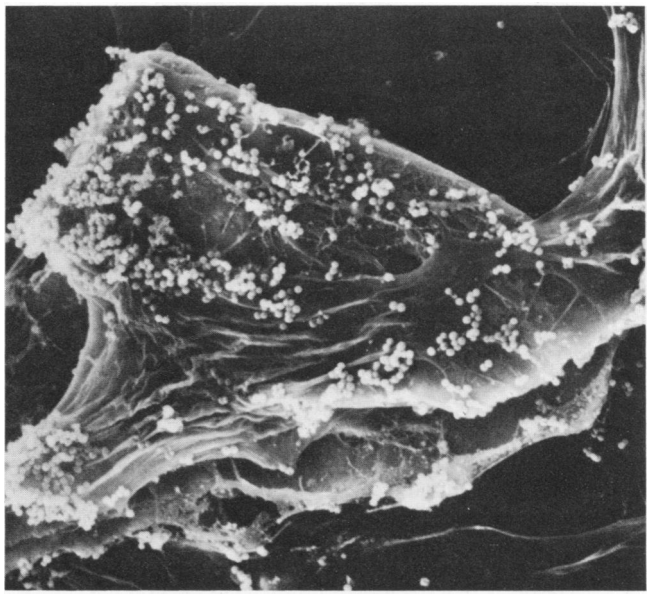

FIG. 1. Scanning electron micrograph of *Staphylococus aureus* adherent to a human endothelial cell following a 1-h incubation. The bacteria are randomly distributed across the cell surface. Magnification, ×1,500.

Bacterial adherence of six representative strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa*) to the gelatin substrate on which the endothelial cells were grown was measured. There was minimal adherence (less than 3% of the original inoculum) except with the *Staphylococcus aureus* strain, which adhered 21%. High adherence (>20%) to gelatin was also seen with two other strains of *Staphylococcus aureus*.

Radiolabel versus agar pour plate methods. The correlation coefficient between the radiolabel method and the pour plate technique for the 29 strains tested by both methods with umbilical vein endothelial cells was 0.84. Therefore, the simpler pour plate technique was selected for subsequent studies comparing adherence to umbilical vein endothelial cells with adherence to valvular endothelial cells.

Statistical analysis. An analysis of variance was performed on the large study comparing the adherence of 32 strains to umbilical vein endothelial cells (see Fig. 4). Because bacterial colony counts often follow a Poisson distribution, normal statistical tests were applied to the square roots of the total bacterial colony counts (27). Multiple pairwise comparisons were performed by the Student-Newman-Keuls technique.

Electron microscopy. Endothelial cells were grown to confluence on carbon-coated cover slips for transmission electron microscopy. Bacterial suspensions of adherent *Staphylococcus aureus* isolates were incubated with the cells for 1 and 3 h at 37°C. After washing with serum-free media, the cells were fixed in 1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h, postfixed in 1% OsO_4 in

0.1 M cacodylate buffer, enbloc stained with 1% aqueous uranyl acetate, dehydrated in a graded ethanol series, and embedded in LX 112 (Ladd Research Industries Inc., Burlington, Vt.). Thin sections were stained with uranyl acetate and lead citrate.

For scanning electron microscopy, one of the same *Staphylococcus aureus* isolates, as well as an *E. coli* isolate, was used. Bacterial suspensions were added to the endothelial cells grown on cover slips and incubated for 1 h at 37°C. The cells were fixed in 2% glutaraldehyde with 0.1 M sucrose for 15 min, postfixed in 1% OsO_4, dehydrated in ethanol, and critical point dried with 100% ethanol. The cells were sputter coated with gold.

RESULTS

Bacterial adherence over time and varying bacterial density. Adherence to umbilical vein endothelial cells over time was measured with representative gram-positive and -negative strains by the radiolabel method (Fig. 2). The inocula used ranged from 7.7×10^7 to 3.1×10^8. Adherence by the *Staphylococcus aureus* strain increased significantly over the first hour of the 2-h incubation period. Adherence by *P. aeruginosa* also increased over the 2-h period. The hierarchy of adherence among strains remained the same from 30 min to 2 h.

The effect of bacterial density on adherence to umbilical vein cells after a 2-h incubation with inocula from 10^2 to 10^8 CFU/ml was studied with the same four strains used in the timed study above (Fig. 3). The number of adherent bacteria per cell increased with the inoculum size for all strains and

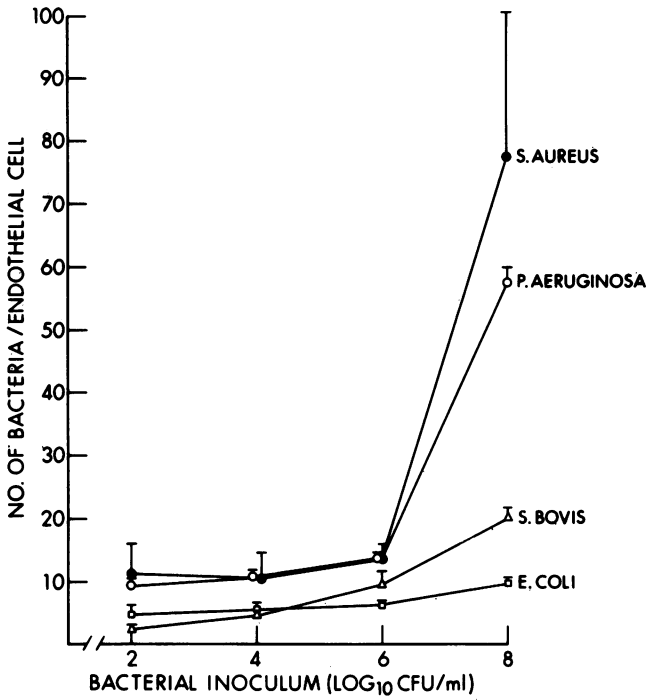

FIG. 3. Effect of bacterial density on the adherence of four bacterial species to endothelial cells. Adherence to umbilical vein endothelial cells was determined at bacterial inocula of $\log_{10}$ 2, 4, 6, and 8 CFU/ml after a 2-h incubation. Each point represents the mean ± standard deviation of three determinations obtained by the radiolabel technique. These strains are the same as in Fig. 2.

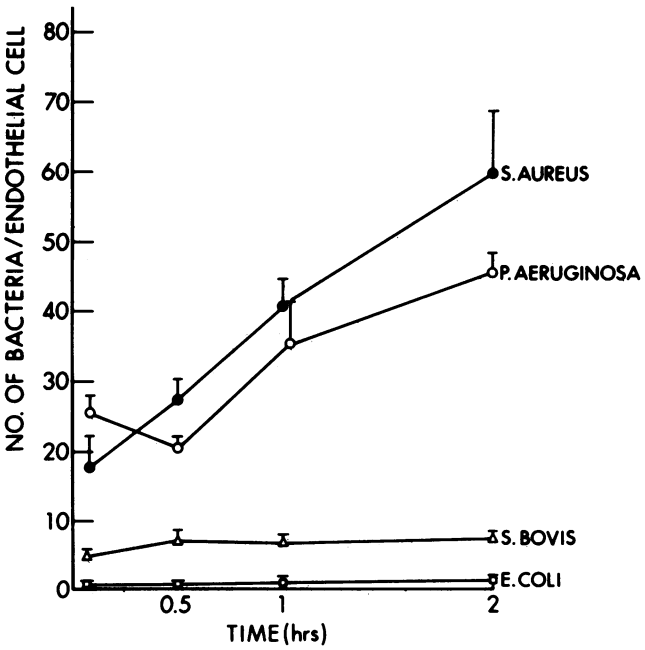

FIG. 2. Effect of time of incubation on adherence for four bacterial strains. Adherence to umbilical vein endothelial cells was determined at 5 min and at 1/2, 1, and 2 h at 37°C. Results are expressed as the number of bacteria per endothelial cell. Each point represents the mean ± standard deviation of three determinations obtained by the radiolabel technique. Inoculum sizes (CFU per milliliter): *Staphylococcus aureus*, 3.1×10^8; *P. aeruginosa*, 7.7×10^7; *Streptococcus bovis*, 1.3×10^8; *E. coli*, 1.8×10^8.

was most noticeable between 10^6 and 10^8 CFU/ml, with the largest increments occurring with *Staphylococcus aureus* and *P. aeruginosa*.

Bacterial adherence to umbilical vein endothelial cells. The results of studies comparing the adherence of 32 blood culture isolates (1 *Staphylococcus aureus*, 1 *Streptococcus bovis* from endocarditis patients) to confluent umbilical vein endothelial cells are shown in Fig. 4. *Staphylococcus aureus* strains demonstrated the highest adherence when compared with all other species. Other gram-positive species as well as gram-negative species were less adherent. A notable exception was one strain of *Serratia marcescens* with adherence values in the range of those seen with *Staphylococcus aureus*. An analysis of variance demonstrated significant differences among species, i.e., at least two groups within the comparison that differed significantly from each other ($P < 0.001$). Due to the relatively small numbers of strains studied within each species, the specific differences were not identified by multiple pairwise comparisons made with the less powerful Student-Newman-Keuls test. Nonetheless, *Staphylococcus aureus* stood out as the most adherent species.

In summary, in experiments with confluent umbilical vein endothelial cells, *Staphylococcus aureus* consistently demonstrated high adherence. Isolates typically associated with subacute endocarditis, such as viridans streptococci and enterococci, consistently demonstrated low adherence. An isolated strain of *Serratia marcescens* was also highly adherent. The endocarditis isolates adhered to the same degree as the nonendocarditis isolates of the same species.

Simultaneous comparison of adherence to valvular and umbilical vein endothelial cells. Six representative strains

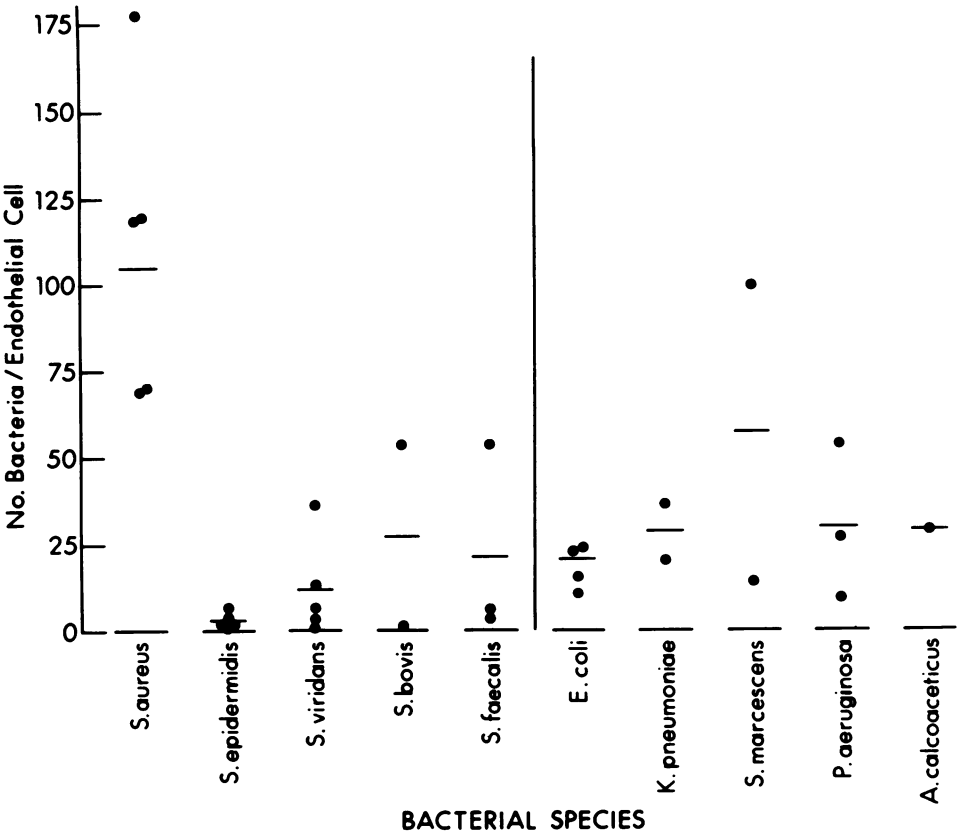

FIG. 4. Adherence of 32 gram-positive and -negative bacterial blood culture isolates to umbilical vein endothelial cells after a 2-h incubation at 37°C. Adherence is expressed as the number of bacteria per endothelial cell. Each point represents the mean of three determinations per strain, obtained by the radiolabel technique. The average inoculum was 2.2×10^8 CFU/ml.

were run simultaneously on both valvular and umbilical vein endothelial cell monolayers of comparable cell density by using the pour plate technique (Table 1). Different bacterial species adhered to both cell types in the same relative order after 1 h of incubation. The results correlated to a high degree ($r = 0.92$).

TABLE 1. Comparison of bacterial adherence to umbilical vein versus valvular derived endothelial cells[a]

Bacterial species	No. of adherent bacteria per cell	
	Umbilical vein	Valvular
Staphylococcus aureus	61.5 ± 31.7	55.6 ± 29.8
Streptococcus bovis	50.3 ± 16.3	31.2 ± 3.3
Streptococcus faecalis	4.7 ± 1.1	16.0 ± 4.4
Klebsiella pneumoniae	27.4 ± 2.1	34.3 ± 11.1
Pseudomonas aeruginosa	4.5 ± 1.3	5.7 ± 0.3
Serratia marcescens	5.7 ± 1.0	11.4 ± 0.8

[a] The bacteria and cells were incubated at 37°C on a rotary shaker at 50 rpm for 1 h. Each assay was performed in triplicate by the pour plate technique. Inocula were as follows (CFU per milliliter): *Staphylococcus aureus*, 4.7×10^7; *S. faecalis*, 1.8×10^7; *K. pneumonia*, 1.2×10^7; *Serratia marcescens*, 3.0×10^7. The endothelial cells in these comparisons were grown to confluence and were in the same transfer (T6 to T8). Comparison of strains was performed on the same day. Average endothelial cell counts were 2.4×10^5 for the umbilical vein well sets and 2.2×10^5 for the valvular cell well sets. Correlation between the two groups was 0.92.

Transmission and scanning electron microscopy. Scanning electron microscopy demonstrated *Staphylococcus aureus* closely associated with endothelial cells (Fig. 1). In contrast, only a few bacteria were seen both cell associated and in the background with *E. coli* (not shown). Staphylococci were seen in a patchy distribution over the entire surface of the cells, with a few concentrated areas where organisms were clumped together. Transmission electron microscopy after 1 h of incubation revealed invagination of the endothelial cell membrane underlying the adherent staphylococci (Fig. 5). The 3-h sections showed numerous staphylococci enclosed within vacuoles distributed throughout the cytoplasm of the endothelial cell (Fig. 6). Engulfment of large numbers of bacteria could be seen on the periphery. Bacterial remnants also appeared to be present within some of the intracellular vacuoles, suggesting bacterial lysis. Similar findings were noted with a second adherent *Staphylococcus aureus* strain.

DISCUSSION

Virulent bacteria such as *Staphylococcus aureus* and, to a lesser extent, *Serratia marcescens* and *P. aeruginosa* predominate as pathogens in acute endocarditis. This contrasts with the relatively avirulent gram-positive bacteria associated with subacute endocarditis. Although still subject to debate, it has been estimated that acute endocarditis involves normal heart valves in at least 50% of cases, while the subacute form develops most often in patients with underlying valvular abnormalities (2, 3, 25, 26). This difference in the host target tissue underscores the importance of using viable, undamaged endothelial cell surfaces as the substrate

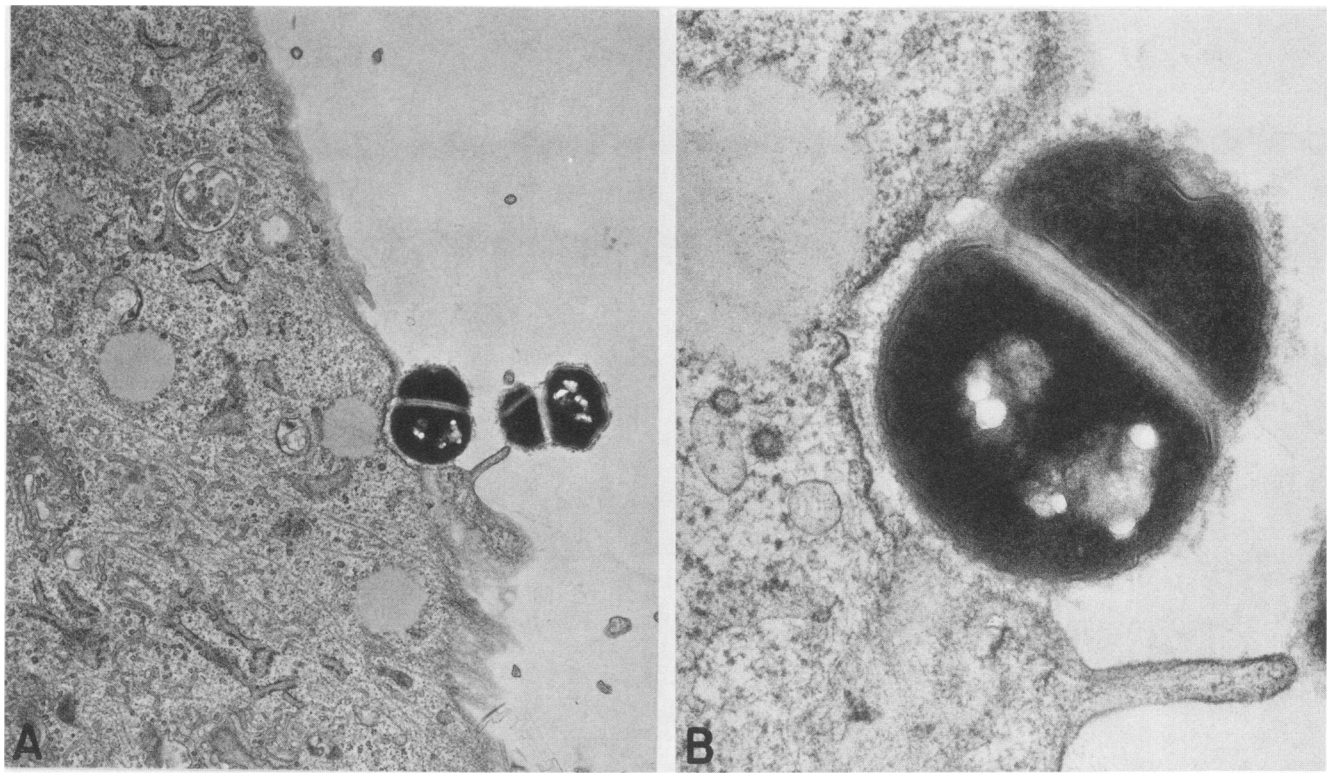

FIG. 5. Transmission electron micrograph demonstrating the attachment of *Staphylococcus aureus* to a human valvular endothelial cell after a 1-h incubation. (A) Invagination of the endothelial cell at the site of staphylococcal attachment is shown. (B) At higher magnification, fibrillar-like structures are visible on the surface of the *Staphylococcus aureus* cell. Magnification, ×12,500 (A) and ×57,000 (B).

in experimental models of acute endocarditis. For this reason, we chose confluent monolayers of human endothelial cells in tissue culture as an analog of the host surface encountered in vivo. Our studies demonstrate the preferential adherence of *Staphylococcus aureus* and a single strain of *Serratia marcescens*, compared with that observed with other gram-positive and -negative strains, and suggest that selective adherence of bacteria to normal endothelium may help explain the predilection of these organisms for causing acute endocarditis.

Bacterial adherence to endocardial surfaces has been studied previously. Gould et al. (9) found that strains of enterococci, viridans streptococci, *Staphylococcus aureus*, and *P. aeruginosa* (in descending order) were more adherent than were *E. coli* and *K. pneumoniae* to sections of aortic valve leaflets. Vercellotti et al. (22) examined bacterial adherence to human umbilical vein endothelial cells in tissue culture and found that *Staphylococcus aureus* and *Streptococcus sanguis* were more adherent than were gram-negative bacilli.

We studied the adherence of 32 blood culture isolates, including two strains from patients with endocarditis, to human umbilical vein or valvular endothelial cells or both in tissue culture. The study was limited to blood culture isolates which, by definition, have caused invasive bloodborne infections and therefore had the potential to infect endothelial surfaces. *Staphylococcus aureus* strains consistently adhered best. One gram-negative strain of *Serratia marcescens* adhered to nearly the same degree. Two of three *P. aeruginosa* strains were moderately adherent in several experiments, but these results were not as consistent as those obtained with other species.

Viridans streptococci were not highly adherent, in contrast to the findings of the previously mentioned studies (9, 22). This may be related to several factors. Earlier work has shown that bacterial strains isolated from different sites may differ in their adherence properties (1); the isolates used in the present study were limited to blood culture isolates, while other studies included streptococci from throat cultures, blood cultures, and laboratory strains. The type of endothelial substrate may also be important. The trauma of procurement and the limited viability of endothelium obtained from cadavers may have affected streptococcal adherence in those studies (9, 20, 26), although some have argued that anatomical relationships are less disturbed in tissue sections (13).

The uniformly high adherence of all *Staphylococcus aureus* strains tested in this study is suggestive of a specific mechanism for the binding of *Staphylococcus aureus* to human endothelial cells. One possible mediator of adherence is fibronectin, a serum glycoprotein produced by various cells, including endothelial cells. Fibronectin binds to some bacteria, including *Staphylococcus aureus*, and may play a role as a mediator of gram-positive bacterial attachment to cells (14, 16, 18, 19, 22).

Scanning and transmission electron microscopy illustrated the close association between endothelial cells and *Staphylococcus aureus* after 1 h of incubation. Timed studies showed engulfment of large numbers of bacteria at 3 h. Mammalian cells (other than leukocytes and macrophages) capable of phagocytizing bacteria include HeLa cells, which can actively ingest *Salmonella typhimurium* (11, 12). Endothelial cells can also actively ingest soluble material by pinocytosis (4, 23) and large particles such as *Rickettsia*

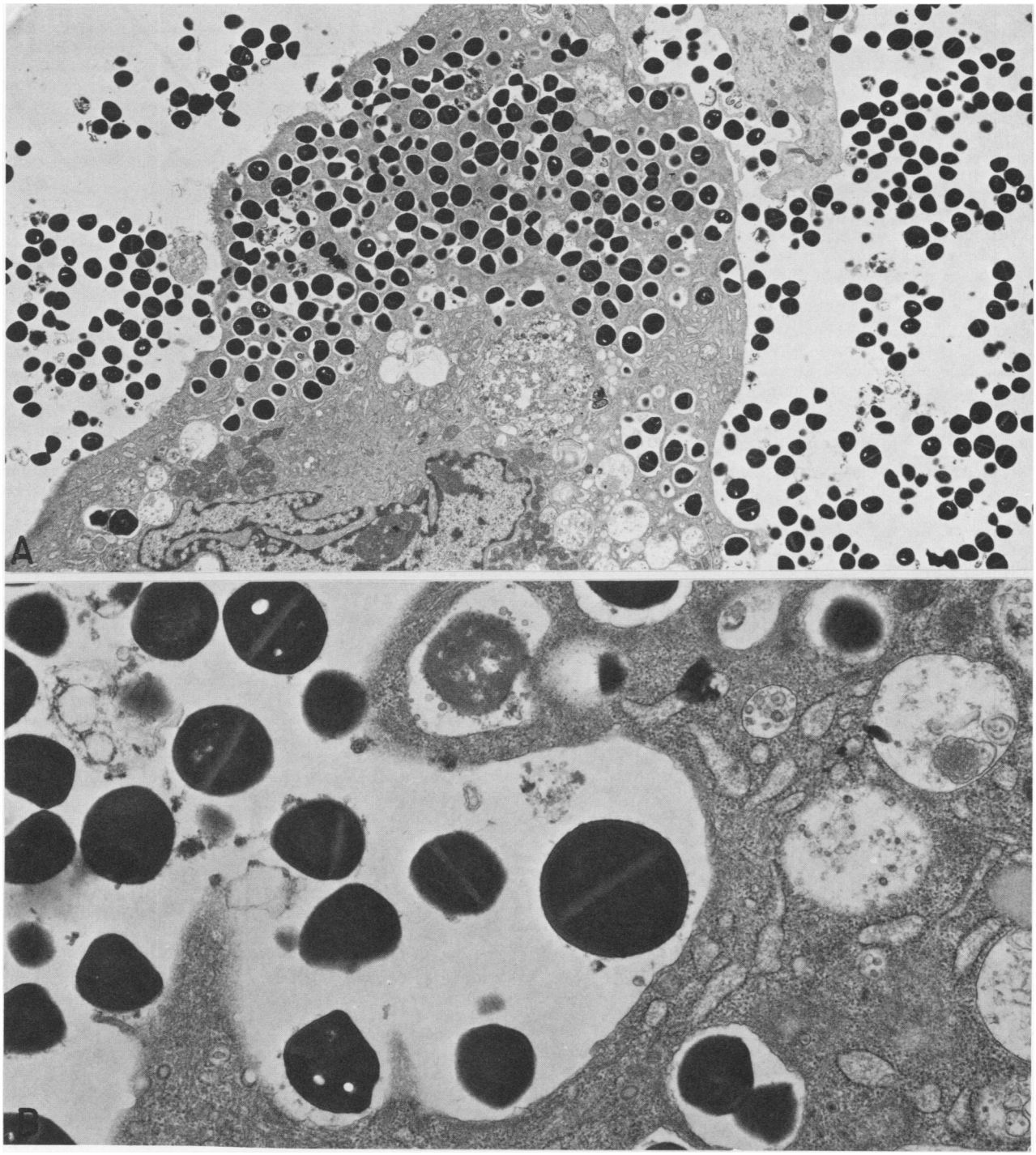

FIG. 6. Transmission electron micrograph demonstrating endocytosis of the *Staphylococcus aureus* after a 3-h incubation. (A) Large numbers of intracellular *Staphylococcus aureus* enclosed within vacuoles are shown. (B) Endocytosis of several *Staphylococcus aureus*. Magnification, ×4,200 (A) and ×21,000 (B).

prowazekii by phagocytosis (24). Other microorganisms such as *Candida albicans* may assume a more active role in penetrating endothelial cells by disrupting cell surfaces soon after attachment in a process that may involve enzymatic degradation (13).

Uptake of *Staphylococcus aureus* by endothelial cells, as shown in the ultrastructural studies, has not been previously described and offers a novel explanation for the frequency of staphylococcal infections of endothelialized surfaces such as heart valves, vascular grafts, and catheterized blood vessels. In the present study, the distinction between bacterial invasion by *Staphylococcus aureus* and endocytosis by endothelial cells was not resolved. However, findings typical of endocytosis, including invagination of the endothelial cell,

inclusion of the bacteria within vacuoles, and occasional fusion of the vacuoles with what appear to be lysosomes, were present (21).

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LITERATURE CITED

1. **Alkan, M., I. Ofek, and E. H. Beachey.** 1977. Adherence of pharyngeal and skin strains of group A streptococci to human skin and oral epithelial cells. Infect. Immun. **18**:555–557.
2. **Angrist, A. A., and M. Oka.** 1963. Pathogenesis of bacterial endocarditis. J. Am. Med. Assoc. **4**:249–252.
3. **Cohen, P. S., J. H. Maguire, and L. Weinstein.** 1980. Infective endocarditis caused by gram-negative bacteria: a review of the literature, 1945–1977. Prog. Cardiovasc. Dis. **4**:205–242.
4. **Davies, P. F., and R. Ross.** 1978. Mediation of pinocytosis in cultured arterial smooth muscle and endothelial cells by platelet-derived growth factor. J. Cell. Biol. **79**:663–671.
5. **Davison, V. E., and B. A. Sanford.** 1981. Adherence of *Staphylococcus aureus* to influenza A virus-infected Madin-Darby canine kidney cell cultures. Infect. Immun. **32**:118–126.
6. **Durack, D. T., and P. B. Beeson.** 1980. Pathogenesis of infective endocarditis, p. 1–53. *In* S. H. Rahimtoola (ed.), Infective endocarditis. Grune and Stratton, Inc., New York.
7. **Garvey, G. J., and H. C. Neu.** 1978. Infective endocarditis—an evolving disease. A review of endocarditis at the Columbia-Presbyterian Medical Center, 1968–1973. Medicine **67**:105–127.
8. **Gordon, P., I. I. Sussman, and V. Hatcher.** 1983. Long term growth of human endothelial cells. In Vitro **19**:661–671.
9. **Gould, D., C. H. Ramirez-Ronda, R. K. Holmes, and J. P. Sanford.** 1975. Adherence of bacteria to heart valves *in vitro*. J. Clin. Invest. **56**:1364–1370.
10. **Jaffe, E. A., L. N. Hoyer, and R. L. Nachman.** 1973. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J. Clin. Invest. **52**:2757–2764.
11. **Kihlström, E., and S. Latkovic.** 1978. Ultrastructural studies on the interaction between *Salmonella typhimurium* 395 M and HeLa cells. Infect. Immun. **22**:804–809.
12. **Kihlström, E., and G. Söderlund.** 1981. Endocytosis in mammalian nonprofessional phagocytes, with special reference to the pathogenesis of invasive microorganisms. Monogr. Allergy **17**:148–170.
13. **Klotz, S. A., D. J. Drutz, J. L. Harrison, and M. Huppert.** 1983. Adherence and penetration of vascular endothelium by *Candida* yeasts. Infect. Immun. **42**:374–384.
14. **Mosher, D. F.** 1980. Fibronectin. Prog. Hemostasis Thromb. **5**:111–151.
15. **Pelletier, L. L., Jr., and R. G. Petersdorf.** 1977. Infective endocarditis: a review of 125 cases from the University of Washington Hospitals, 1963–1972. Medicine **56**:287–313.
16. **Proctor, R. A., D. F. Mosher, and P. J. Olbrantz.** 1982. Fibronectin binding to *Staphylococcus aureus*. J. Biol. Chem. **257**:14788–14794.
17. **Reisberg, B. E.** 1979. Infective endocarditis in the narcotic addict. Prog. Cardiovasc. Dis. **22**:193–204.
18. **Ruoslahti, E., E. Engvall, and E. Q. Hayman.** 1981. Fibronectin: current concepts of its structure and functions. Collagen Relat. Res. **1**:95–128.
19. **Rydén, C., K. Rubin, P. Speziale, M. Höök, M. Lindberg, and T. Wadstrom.** 1983. Fibronectin receptors from *Staphylococcus aureus*. J. Biol. Chem. **258**:3396–3401.
20. **Scheld, W. M., J. A. Valone, and M. A. Sande.** 1978. Bacterial adherence in the pathogenesis of endocarditis. Interaction of bacterial dextran, platelets and fibrin. J. Clin. Invest. **21**: 1394–1404.
21. **Silverstein, S. C., R. M. Steinman, and Z. A. Cohen.** 1977. Endocytosis. Annu. Rev. Biochem. **46**:669–722.
22. **Vercellotti, G. M., D. Lussenhop, P. K. Peterson, L. T. Furcht, J. B. McCarthy, H. S. Jacob, and C. F. Moldow.** 1984. Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. J. Lab. Clin. Med. **103**:34–43.
23. **Vlodavsky, I., P. E. Fielding, C. J. Fielding, and D. Gospodarowicz.** 1978. Role of contact inhibition in the regulation of receptor-mediated uptake of low density lipoprotein in cultured vascular endothelial cells. J. Cell Biol. **75**:356–360.
24. **Walker, T. S.** 1984. Rickettsial interactions with human endothelial cells in vitro: adherence and entry. Infect. Immun. **44**:205–210.
25. **Weinstein, L., and J. J. Schlesinger.** 1974. Pathoanatomic, pathophysiologic and clinical correlation in endocarditis. N. Engl. J. Med. **291**:832–837, 1122–1126.
26. **Wright, A. J., and W. R. Wilson.** 1982. Experimental animal endocarditis. Mayo Clin. Proc. **57**:10–14.
27. **Zar, J. H.** 1974. Biostatistical analysis, p. 187. Prentice Hall, Englewood Cliffs, N.J.