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SCANNING ELECTRON MICROSCOPY STUDIES OF STAPHYLOCOCCAL ADHERENCE TO HEART VALVE ENDOTHELIAL CELLS IN ORGAN CULTURE: AN *IN VITRO* MODEL OF ACUTE ENDOCARDITIS

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Abstract

Organ cultures of human heart valves were used as a model to study the initial pathobiology of acute infective bacterial endocarditis. We used *Staphylococcus aureus* isolated from a case of infective endocarditis to infect the *in vitro* culture of the heart valves. Using scanning electron microscopy, we assessed the initial damage, attachment to and invasion of the endothelial cell layer by staphylococci. Our results indicate there is initial damage to the endothelium prior to observation of staphylococci attaching to the endothelial cell. By 12 h post infection, there is significant attachment and damage. At 24 h after infection, destruction of the heart valve endothelium is complete. The attachment and destruction are progressive events and can be correlated quantitatively with bacterial numbers from the culture medium and those attached to the valves. This is correlated with increasing adherence ratios of the attaching staphylococci.

Keywords: Staphylococci, adherence, *Staphylococcus aureus*, organ cultures, endothelial cells, endocarditis, acute endocarditis.

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Introduction

Staphylococcus aureus causes an acute endocarditis which often involves previously normal valves. In a recent study covering a 25 year period (11) *S. aureus* endocarditis represents about 15% of the cases of infective endocarditis; the mortality of such cases is approximately 40%. The systemic toxicity is greater with *S. aureus* endocarditis and the clinical course is more active than in subacute bacterial endocarditis caused by viridans streptococci or enterococci. The ensuing valvular damage occurs rapidly and may not be initially apparent because previously normal heart valves may be involved.

Previous reports (3,6,13) have indicated that adherence to heart valve leaflets by bacteria associated with infective endocarditis may be one of the factors involved in the pathogenesis of endocarditis. Gould et al (3) reported that both gram positive and gram negative bacteria could adhere, *in vitro,* to the endothelium of normal human and canine aortic leaflets and that those microorganisms most frequently associated with endocarditis had the greatest amount of *in vitro* adherence. Several laboratories have implicated ligands or adhesins composed of bacterial surface components to be mechanisms by which microorganisms adhered to heart valves. Ligands composed of glucan have been implicated in *Streptococcus mutans* and *Streptococcus sanguis* attachment to heart valves (6). Beachy (1) showed that the lipoteichoic acid of group A streptococci was a suitable ligand for attachment to host cell epithelium. In similar studies Ramirez-Ronda et al. (7,8) implicated that staphylococcal teichoic acid was a potential ligand involved in staphylococcal attachment to damaged heart valves. These observations can be correlated with several studies (4,10,12,14) which suggest that the presence of high titers of anti-staphylococcal teichoic acid antibodies are compatible with staphylococcal endocarditis.

Although some information exists concerning the specific ligand-receptor interaction in the pathogenesis of staphylococcal endocarditis, relatively little is known about the basic events in the pathogenesis of the disease. This paper is directed toward understanding the initial events in the adherence of staphylococci to organ cultured heart valve leaflets such that the mechanisms of infective bacterial endocarditis can be better understood.

Materials and Methods

Bacteria

Three coagulase positive *S. aureus* strains, isolated from

human cases of infective endocarditis were used in these studies. The organisms were grown on trypic soy agar or broth (Difco Laboratories, Detroit, MI) and maintained at −70°C in 10% skim milk until used.

Standardized inocula were prepared as described by Gould et al. (3) and a dose of ~1 × 10^4 organisms was used to infect the heart valve leaflet organ cultures.

Heart Valve Leaflet Organ Cultures

Human aortic valves were obtained from the Departments of Pathology at St. John's Hospital and Memorial Medical Center, Springfield, IL. Canine and porcine valves were obtained from animals sacrificed for other purposes at the S.I.U. Laboratory Animal Resource Center. The following procedure was used to prepare heart valve organ cultures regardless of source. Heart valves were removed aseptically from the heart and placed in Hanks balanced salt solution (HBSS). Heart valve leaflets were removed from the HBSS and placed on a sterile mat of filter paper saturated with RPMI 1640 tissue culture media containing 25 mM HEPES (HEPES-RPMI 1640). Circular sections of the valve were removed with a 1 mm skin biopsy punch and sections were placed in a 10 × 30 mm tissue culture dish containing 1 ml of HEPES-RPMI 1640. These organ cultures were placed in an incubator containing humidified 5% CO_2 atmosphere at 37°C and incubated overnight. The following morning the cultures were examined for sterility and then infected with approximately 10^4 colony forming units (CFU) of staphylococci. Attachment of the staphylococci was monitored by quantitative measurements as well as scanning electron microscopy.

Quantitative Measurement of Bacterial Adherence to Heart Valve Leaflets

Four valve sections were placed in plastic tissue culture dishes and infected with approximately 10^4 CFU of staphylococci, and incubated with shaking at 37°C in a humidified 5% CO_2 atmosphere. At each time interval (0, 2, 4, 6, 12 and 24 h) four valve sections were removed from the culture media. Two of the sections were placed into a culture dish containing 3 ml of culture media. The tissue was washed by swirling through 3 changes of media in different culture dishes for 15 sec each. Each pair of sections was then placed in a homogenization tube containing 3 ml of culture media and homogenized using a Techmar tissue homogenizer (Techmar SDT) at maximum speed for 1 minute. Quantitation of viable staphylococci was performed by removing 0.1 ml of the bacterial suspensions in the original organ culture fluids as well as from the homogenized valve sections, serially diluting in HEPES-RPMI 1640 and plating 0.1 ml of appropriate dilutions on trypticase soy agar. Colonies were enumerated after overnight incubation at 37°C. The adherence ratio was defined as the proportion of bacteria in the original culture suspension that was recovered from the washed, homogenized valve sections. Adherence ratio = (viable bacteria recovered from the heart valves [CFU/ml])/(viable bacteria in the incubation media [CFU/ml]).

Electron Microscopic Examination of Attachment to the Heart Valve Endothelium

Tissues to be examined by scanning electron microscopy were washed (3×) in HEPES-RPMI 1640 and then fixed overnight at 4°C in 2.5% glutaraldehyde, pH 7.3, in 0.1 M sodium cacodylate. Post fixation was accomplished in the same buffer containing 1.5% osmium tetroxide for 1 h at ambient temperature. The samples were rinsed in 0.1 M sodium cacodylate buffer, dehydrated in a graded series of alcohol, and critical point dried with carbon dioxide in a Bomar SPC-900 critical point dryer. The intact organ culture was removed from the lens paper backing, mounted on colloidal carbon surfaced stubs, and coated with gold-palladium (40:60) in a Polaron E5000 sputter coater. Tissues were observed in a Hitachi S-500 scanning electron microscope and photographed on Polaroid SN55 film.

Results

Scanning Electron Microscopic Observations of Human Heart Valves and Staphylococcal Attachment

Both human aortic and mitral valves were used to prepare the heart valve organ cultures. The organ cultures had an intact endothelium at the initiation of the experiment as seen in Figures 1a and g. The culture conditions preserved the tissue in uniform endothelial cell layers throughout the experimental times as can be seen in a typical example of the 24 h non-infected controls (Figure 1b). It was evident that soon after the introduction of staphylococci into the organ cultures that there was significant disruption of the integrity of the endothelial cell layer. A typical example is seen in Figure 1c. Although the staphylococcal inoculum is too low (~10^4 CFU) and the time too soon after inoculation to see attachment, a primary and perhaps necessary event has taken place in which the endothelium has become traumatized. A typical example of the endothelial damage is seen 6 h after infection of the organ cultures in Figures 1d and h. Here most of the endothelium has been damaged and/or destroyed. A few remnants of damaged endothelial cells can be seen adhering to staphylococci (arrows). There appears to be attachment to and invasion of the fibrous subendothelium by the staphylococci. Further, there also appears to be the beginning of a micro colony here. Figure 1e depicts a focus of staphylococci in the denuded endothelial surface. Several remains of cells of the endothelial layer appear to overlay a large discrete group of staphylococci, further suggesting destruction of the single cell layer thick endothelium and invasion of the remaining valvular structure. The extent of endothelial destruction and amount of staphylococcal attachment is seen in Figure 1f. By this 24 h time frame, destruction of the endothelial layer of the heart valve is complete. This is in comparison to the undamaged uninfected control of the same time period (Figure 1b) in which the endothelium is uniform, undamaged and has complete integrity. Human mitral valves were also used in similar organ culture attachment experiments. It is of interest to note that initially these mitral valve endothelial cells had microvilli on their surfaces as is seen in control uninfected tissues (Fig. 2a). However, these microvilli do not appear to function as receptors since the staphylococci appear to attach to these endothelial cells without preference for the microvillar structures (Figure 2b). By 12 h post infection the cellular integrity is also lost and the microvilli are no longer in evidence (Figure 2c). In all other respects the attachment and damage to the mitral valves is identical to that seen in the human aortic valves.

It also appears that a tissue tropism is not associated with the staphylococcal attachment to heart valve endothelium. When similar experiments (data not shown) were performed using porcine and canine valves and staphylococci of human origin, there was similar destruction and damage to the endothelium as was seen in organ cultures of the human endothelium.

Table 1. Relationship between the concentration of bacteria in the culture media and the adherence ratio for *Staphylococcus aureus*.

Time (h)	Bacteria in the Culture Media[1] (CFU/ml)	± SEM	Bacteria Adherent to the Heart Valve Sections (CFU/ml)	± SEM	Adherence Ratio for Viable Bacteria (10^5)
0	2.5×10^2	2.6×10^1	0.7	0.5	264
2	8.8×10^3	2.3×10^2	2.8×10^1	5.5	318
4	1.2×10^3	1.8×10^2	1.7×10^1	4.1	1450
6	8.0×10^2	5.0×10^2	2.8×10^1	1.2×10^1	3475
12	1.1×10^6	6.0×10^5	1.1×10^5	2.7×10^4	991
24	1.7×10^7	1.0×10^6	1.1×10^6	2.1×10^5	6706

[1]Bacterial counts represent the mean of three experiments ± SEM.

Quantitation of Staphylococci in the Organ Culture Medium and Attachment to the Heart Valves

The heart valve organ cultures were infected with an inoculum of approximately 3.8×10^3 organisms per ml and the number of staphylococci in the culture medum and attached to the endothelial surfaces was determined over a 24 h period (0,2,4,6,12, 24 h). As is shown in Table 1, the mean number of staphylococci in the culture medium was 2.5×10^2 CFU/ml with essentially no attachment to the heart valve endothelium. Although staphylococci were present in both the culture medium and the heart valves there was a lag phase until about 12 h post infection. This lag was also noted in our SEM examination of the tissue. By 24 h there was maximum attachment to the tissue and presence in the culture medium. Further, there was an increasing adherence ratio over time with the greatest ratio occurring at 24 h.

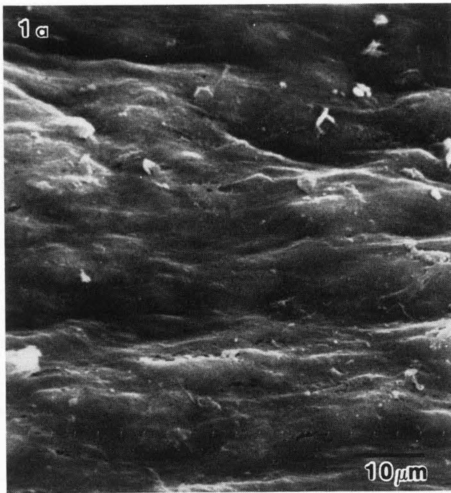
Discussion

Staphylococci cause approximately 20–30 percent of the cases of infective endocarditis and 80–90% of these are due to infection with coagulase-positive *S. aureus*. *S. aureus* is the etiological agent of acute infective endocarditis. This microorganism attacks normal heart valves (i.e. those with no clinically detectable disease) in about one third of the patients. The clinical course of such infection is frequently fulminant with widespread metastatic infection which results in death in approximately 40 percent of the cases (9).

Since the late 1800's attempts have been made to produce experimental endocarditis in animals. The most successful method was a direct damage to the heart valve followed by an intravenous injection of bacteria as described by Wyssokowitsch in 1886 (17). This is still the basic concept by which modern experimental endocarditis models are designed. A variation of this is the simple and reliable catheter induced endocarditis model described by Garrison and Freedman (2) in the early 1970's which depends on a deposition of platelets and fibrin on the endothelial surface followed by the development of a sterile vegetation. Following development of the vegetation, bacteria are introduced intravenously and infective bacterial endocarditis becomes established. When these models are used to study staphylococcal endocarditis, the pathophysiological investigations are affected by the influence of the indwelling catheter during the infectious process. Thus, there is a need for an experimental model without a retained catheter which could be used to gather basic information regarding the initiation, development and spontaneous course of the disease. Such a model would be useful for investigations on bacterial adherence and the virulence factors responsible for the severe and damaging consequences of the disease. Our heart valve model may prove useful in providing answers to these questions.

Gould et al. (3) first used heart valve leaflets as *in vitro* models of infective endocarditis. By the use of adherence ratios determined by two independent methods they were able to implicate a variety of bacteria, including staphylococci in adherence to heart valves. They found that the microorganisms which most frequently caused infective bacterial endocarditis were shown to adhere best to the heart valves. This observation suggested that adherence to valvular endothelium may be an important factor in the establishment of acute endocarditis in man. Our data confirmed and extended the observations of Gould et al. (3) in several ways: (1) We have shown that attachment of coagulase positive staphylococci to the heart valve endothelium receptors occurs and is a progressive event with time. Further the adherence ratios also increase with time and numbers. Gould et al. (3) looked only at a single event at a single time (24 h) (2). We have shown that uninfected heart valve cultures have an intact endothelium over the times studied (3). From Figure 1c it is evident that there is some event which is traumatic (toxic) to the endothelium which takes place early in the infective process. This event alters the endothelial cell surface and perhaps damages the endothelium such that direct staphylococcal attachment can and does proceed without the fibrin and thrombin deposition seen in subacute streptococcal endocarditis.

Several studies have attempted to explain the pathobiology of the interaction between bacteria, in particular streptococci, and the heart valve endothelium in both man and animals (5,15). Harasaki (5) studied the initial events in the pathogenesis of experimental streptococcal endocarditis in a rabbit model using both scanning and transmission electron microscopy of the heart valve endothelium. He found that a single i.v. injection of streptococci can damage the endothelium. Pinocytotic vesicles and intercellular junctions were widened after injection of streptococci. Then platelets and leukocytes adhered to form aggregates which in turn injured and denuded the underlying endothelial cells. He showed that in previously normal heart valves, streptococci can stick to the endothelial surface and cause endocarditis and that nonbacterial thrombic endocarditis is not always a prerequisite of bacterial endocarditis. Our studies with *in vitro*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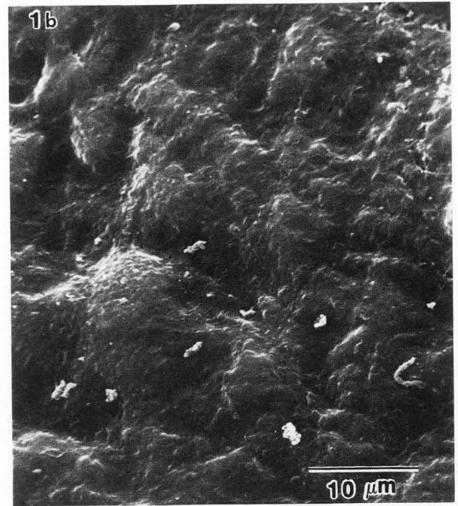
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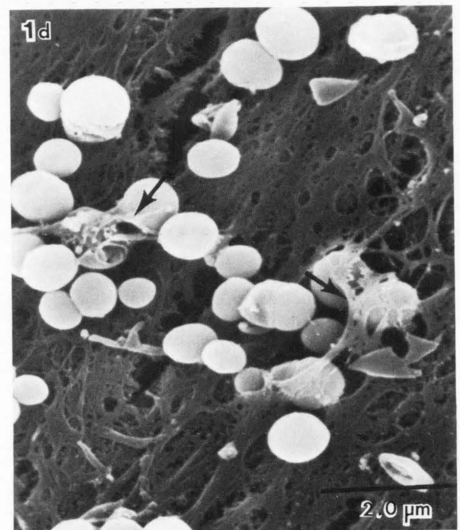
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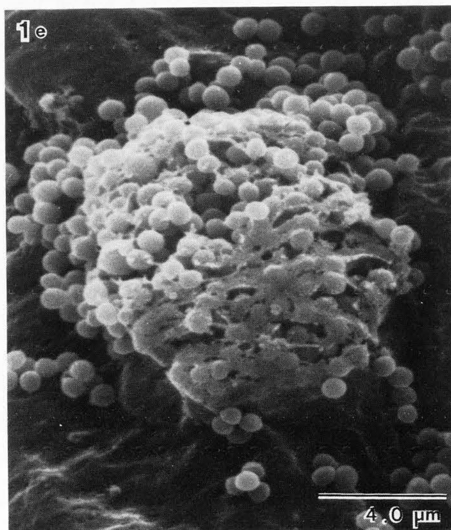
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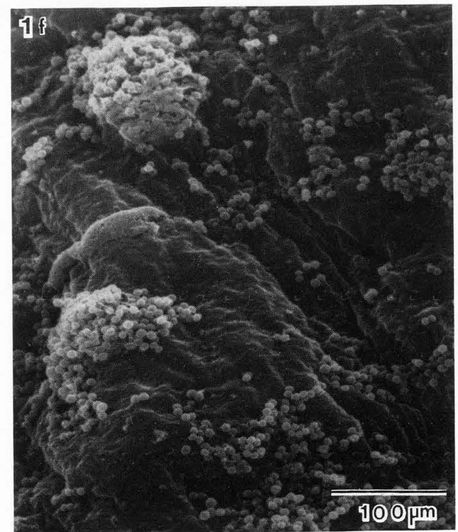
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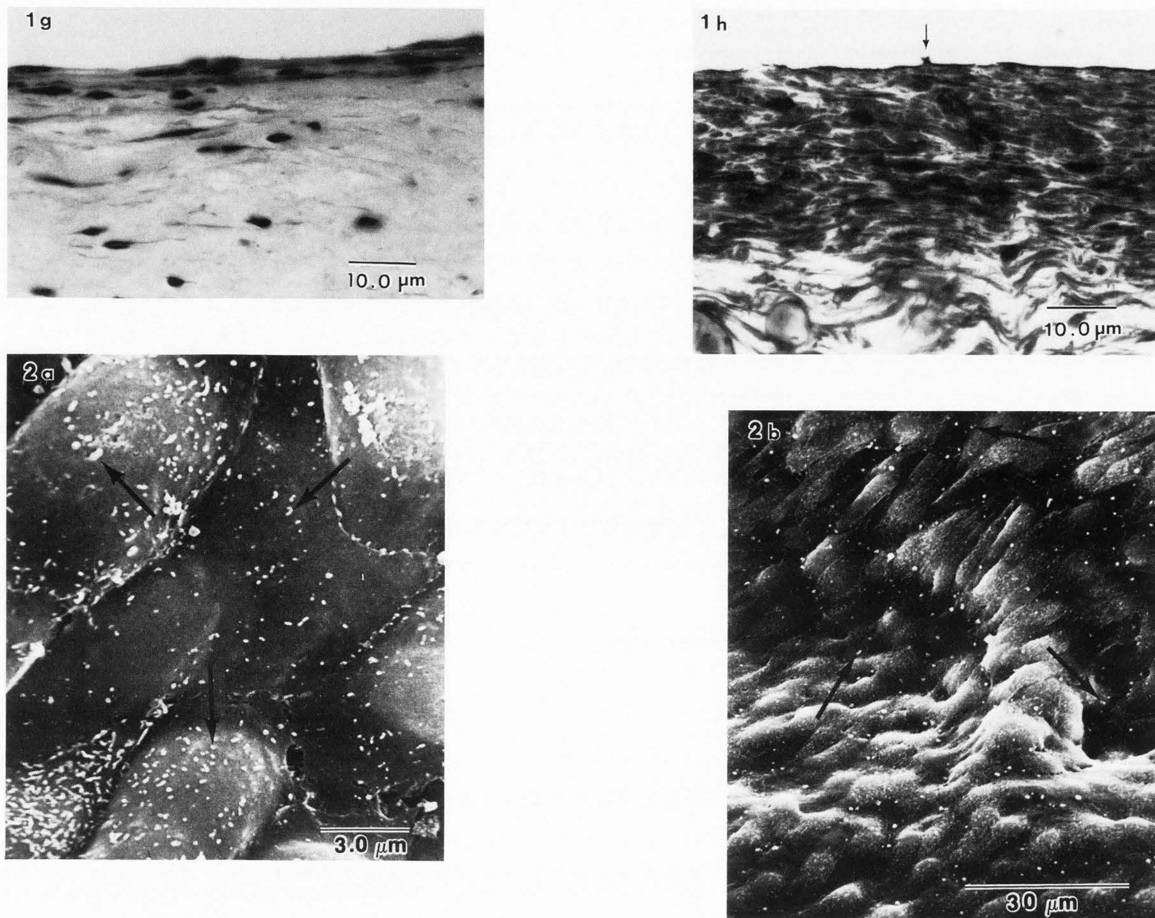

2c
40 μm

Fig. 1. Control and infected organ cultures of human aortic leaflets. *Figure 1a* shows the control normal heart valve endothelium at 0 h of the experiment. *Figure 1b* is of control endothelium after 24 h of culture. Note that there is still an intact uniform layer of endothelial cells. *Figure 1c* indicates the amount of damage and trauma to the endothelial surface within 15 min after introduction of staphylococci into the heart valve cultures. *Figure 1d* is an example of attachment and damage 12 h after infection. Note the remnants of endothelial cells (arrows) attached to the staphylococci. *Figure 1e* illustrates the damage to the endothelial cells at 24 h post infection. Note the appearance of a microcolony and its invasion of the endothelium and penetration of the subendoethelial tissue. *Figure 1f* illustrates the amount of attachment and damage to the endothelium at 24 h. *Figure 1g* is a light micrograph of uninfected aortic valve endothelium. *Figure 1h* is a light micrograph of an infected aortic valve (6 h). There is a lack of endothelium and staphylococci attached to the remaining matrix (arrow).

Fig. 2. SEM observations of staphylococcal attachment to human mitral valve cultures. *Figure 2a* illustrates microvillar structures (arrows) on the surface of the endothelial cells of mitral valves. *Figure 2b* illustrates the wide distribution of the microvilli and its lack of specificity as an attachment site for staphylococcal attachment. Staphylococci (arrows) are attaching randomly to the endothelial surface. *Figure 2c* shows that by 12 h, much of the microvillar structures have been lost and the ensuing damage.

staphylococcal infection of human aortic valves indicate a similar and more dramatic injury of the endothelial cells prior to attachment and invasion of the endothelium.

These data indicate that *in vitro* staphylococcal infection of human heart valve organ cultures provides a model which yields valuable information regarding the initial pathobiological events of acute infective bacterial endocarditis. These studies parallel those seen in *in vivo* experimental animals as well as some of the pathology seen in endocarditis studies on human cadavers (16). We have demonstrated that there is an initial insult (toxin or otherwise) to the normal endothelial cell which traumatized the cell surface and changed its topography. Following this action there occurred a direct attachment of the staphylococci to the endothelial cells and an invasion and destruction of the endothelium. These events were not species specific because human staphylococcal endocarditis isolates would attach to and damage the endothelial cells of both porcine and canine aortic valves. Further, there appeared to be a morphological difference between the endothelial cells of aortic and mitral valves in that the mitral valve endothelial cells have microvillar like structures while aortic cells do not. The microvillar structures do not appear to have particular receptors for staphylococcal attachment since there is random attachment over the entire cell surface. The evidence presented here shows the potential of the model for answering questions regarding attachment ligands and endothelial receptors for microorganisms involved in acute infective bacterial endocarditis.

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References

1. Beachey EH. (1981). Bacterial adherence: adhesion receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**, 325–345.

2. Garrison K, Freedman LR. (1970). Experimental endocarditis. I. Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of the heart. Yale J. Biol. Med. **42**, 394–410.

3. Gould K, Ramirez-Ronda CH, Holmes RK, Sanford JP. (1975). Adherence of bacteria to valves *in vitro*. J. Clin. Invest. **56**, 1364–1370.

4. Gronstrom M, Julander IG, Hedstrom S, Mollby R. (1983). Enzyme linked immunosorbent assay for antibodies against teichoic acid in patients with staphylococcal infections. J. Clin. Microbiol. **17**, 640–646.

5. Harasaki H. (1977). Studies of the pathogenesis of bacterial endocarditis. The Ryumachi **17**, 285–297.

6. Ramirez-Ronda CH. (1978). Adherence of glucan-positive and glucan-negative streptococcal strains to normal and damaged heart valves. J. Clin. Invest. **62**, 805–814.

7. Ramirez-Ronda CH, Gutierrez-Nunez JJ. (1980). Effect of antiteichoic acid serum on the adherence of *Staphylococcus aureus* CR-100 to damaged heart valves *in vitro*. Clin. Res. **28**, 377A.

8. Ramirez-Ronda CH, Gutierrez-Nunez JJ, Bernude RH. (1980). Effects of teichoic acid on adherence of staphylococci to heart valves *in vitro*. Clin. Res. **28**, 377A.

9. Scheld WM, Sande MA. (1985). Endocarditis and intravascular infections. In: Principles and Practice of Infectious Diseases, GL Mandell, RG Douglas, JE Bennett (Eds.), 2nd edition, John Wiley and Sons, New York, pp. 504–529.

10. Tennenbaum MJ, Archer GL. (1980). Prognostic value of teichoic acid antibodies in *Staphylococcus aureus* bacteremia: a reassessment. S. Med. J. **73**, 140–145.

11. Thompson RL. (1982). Staphylococcal infective endocarditis. Mayo Clin. Proc. **57**, 106–114.

12. Tuazon CU, Sheagren JN. (1976). Teichoic acid in the diagnosis of serious infections with *Staphylococcus aureus*. Ann. Intern. Med. **84**, 543–546.

13. Vercellotti GM, Lussenhop D, Peterson PK, Furcht LT, McCarthy JB, Jacob HS, Moldow CF. (1984). Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. J. Lab. Clin. Med. **103**, 34–43.

14. Wheat J, Kohler RB, Garten M, White A. (1984). Commercially available (ENDO-STAPH) assay for teichoic acid antibodies: Evaluation in patients with serious *Staphylococcus aureus* infections and in controls. Arch. Intern. Med. **144**, 261–264.

15. Wright AJ, Wilson WR. (1982). Experimental animal endocarditis. Mayo Clin. Proc. **57**, 10–14.

16. Wright JP, Kirschner RH. (1979). Scanning electron microscopy of infective endocarditis. Scanning Electron Micros. 1979: III:793–799.

17. Wyssokowitsch W. (1886). Beitrage zur Lehre von der Endocarditis. Arch. Pathol. Anat. **103**, 301–332.

Discussion with Reviewers

A.R. Wechezak: In order to support the observation that endothelial damage occurs prior to bacterial adherence, did the authors perform any experiments in which extracts or diffusible products from the bacteria were incubated with the valve preparations?

Authors: In order to minimize the effect of carry over extracellular products the bacteria were washed several times immediately prior to their use as an infecting inoculum. However, purified toxins or extracellular products were not used in our model to assess damage to the valve preparations.

A.R. Wechezak: Does bacterial attachment occur because the endothelium is damaged? Is adherence presented on intact healthy endothelium?

Authors: We believe that bacterial attachment occurs as the endothelium is damaged. We have observed staphylococci on areas of undisrupted endothelium but we have not observed attachment of the staphylococci to the valve preparation without damage (disruption) of the endothelium in an adjacent area. Clearly, valves with damaged endothelial cells have large numbers of staphylococci in and around them and thus give the impression of damage and attachment being associated with one another.

A.R. Wechezak: *In vivo*, the valve leaflets would be in constant motion. Do the authors have any observations on how valve dynamics would affect bacterial attachment?
Authors: We have not made any observations on how dynamics affect bacterial attachment.

R.M. Albrecht: Is the "trauma" seen in figure 1c "patchy" or does it cover the entire surface? How soon after introduction of the bacteria does the damage occur? What do the authors feel causes the early but extensive damage to the endothelial cell layer? To what extent is the damage a function of the culture system; would similar changes be expected to occur *in vivo* where volume, flow, growth conditions and media (blood) are considerably different than in the *in vitro* system?
Authors: The damage seen on the endothelial cells after introduction of the staphylococci is fairly uniform over the valvular surface. This damage is initiated within the first 15 minutes or so after bacterial exposure. We believe that the damage to the endothelium is most likely caused by one of the extracellular products excreted by actively metabolizing staphylococci. However, we do not have direct evidence to support this hypothesis. The effects that we see may to some extent be a product of the culture system. The organ culture system is an explant and as such is not continually flushed with changing flow and growth conditions. This is obviously a concentration effect on the toxic extracellular products within our system which may or may not be representative of *in vivo* attachment to heart valves. However, from pathological specimens and from our *in vitro* attachment studies, once the staphylococci attach, there is ensuing damage to the endothelium and the valve.

R.M. Albrecht: Do the authors expect the adherence seen in the shaking culture approximates the adhesion which occurs in the *in vivo* flow situations?
Authors: The shaking cultures were performed to ensure the maximal amount of contact between bacteria and organ culture material and not in an attempt to duplicate *in vivo* flow situations.

R.M. Albrecht: Are the microvilli on mitral control tissue endothelial cells still present after 12 and 24 h of culture in the absence of bacteria?
Authors: Yes.

J.M. Riddle: What are the mechanisms by which the *Staphylococcus aureus* adhere to the subendothelial components, i.e. basement membrane and/or fibrous structures?
Authors: We are not certain of all the mechanisms which staphylococci use to adhere to endothelial cells and subendothelial structures. We have evidence to implicate staphylococci teichoic acid as a ligand which is involved in attachment to these structures.

J.M. Riddle: Have you used cultures of only endothelial cells to further understand the "toxic events" which occur between these cells and the staphylococcus?
Authors: We are presently using cultured endothelial cells to attempt to better understand the toxic events between staphylococci and the endothelial cell.

J.M. Riddle: Does the disappearance of the endothelium after contact with the staphylococcus really mean that the endothelial cells are destroyed or do they just detach from the subendothelium?
Authors: We have seen fragments of endothelial cells as well as detached cells from the subendothelial matrix. However, these detached cells appear damaged.