Virchows Arch A [Pathol Anat] (1986) 410:43–48

*Virchows Archiv A*


# Experimental staphylococcal endocarditis and aortitis

## Morphology of the initial colonization

**D.J.P. Ferguson [1], A.A. McColm [2], D.M. Ryan [2], and P. Acred [2]**

[1] Electron Microscope Unit, Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford

[2] Chemotherapy Department, Glaxo Group Research Ltd., Greenford, Middlesex, Great Britain

**Summary.** The initial colonization, by *Staphylococcus aureus,* of the catheter damaged aortic valve and aorta of the rabbit, was examined by light and electron microscopy at 15 min, 3 h and 24 h post inoculation (PI). At 15 min PI, the majority of bacteria (80%) were located on the lateral surfaces of the thrombic vegetations while 20% were attached directly to the connective tissue of the aortic valve and aorta in areas where the endothelial lining was disrupted. By 3 h the bacteria on the thrombic vegetations were covered by fibrin. At this time, the bacteria both within the vegetations and on the surface of the vasculature were undergoing multiplication to form small groups. The precipitation of thrombus around the bacteria attached to the surface of the aorta to form microscopic infected vegetations had occurred by 24 h PI. The colonizing bacteria did not elicit any phagocytic response. The colonization of the cardiovasculature by *Staph. aureus* did not necessarily require pre-existing vegetations.

**Key words:** Endocarditis – Staphylococcus – Colonisation – Morphology – Ultrastructure

## Introduction

In man, acute bacterial endocarditis is still a serious condition which, despite the use of modern antibiotics, is difficult to cure and has a mortality rate of approximately 15%. The majority of cases are the result of infection with either streptococcal or staphylococcal bacteria. Recently, there have been changes in the epidemiology of the disease with an increase in the incidence of staphylococcal infections. This is particularly true in patients with no known predisposing abnormality and intravenous drug users (Freedman 1982). The reason for this increase is unknown but it could be related to differences in the ability of the bacteria to colonize the cardiovascular surfaces.

The initial colonization (first 24 h post inoculation) has been examined morphologically in the rabbit model of endocarditis using various species of Streptococci but some inconsistencies concerning the distribution of the bacteria and the period of multiplication have been reported (Durack 1975; Gutschik and Christensen 1978; McGowan and Gillett 1980). The initial colonization by *Staphylococcus* species has not been studied. In this report, the interaction between *Staph. aureus* and the host tissue was investigated by light and electron microscopy. In addition to pre-existing vegetations, the damaged surfaces of the aortic valve and aorta not associated with thrombi were examined for bacterial colonization.

## Materials and methods

The technique for producing staphylococcal endocarditis and aortitis in rabbits was as described previously (Durack and Beeson 1972; Ferguson et al. 1986). The surface of the aorta and aortic valve was traumatised by the insertion of a polythene catheter via the carotid artery 48 h prior to infection. A total of 12 rabbits were used in the study. Infection was instigated by the intravenous inoculation of either $6 \times 10^6$ colony forming units (CFU) of *Staphylococcus aureus* strain 853E and the rabbits autopsied after 3 (3 rabbits) or 24 h (3 rabbits) or $1 \times 10^{10}$ CFU with examination at 15 min (3 rabbits) or 3 h (3 rabbits) post inoculation (PI).

At autopsy, the tissue samples from the aorta and aortic valve were processed for histology and scanning (SEM) and transmission (TEM) electron microscopy by standard techniques as described previously (Ferguson et al. 1986). In addition, certain samples for TEM were stained with Ruthenium red (Luft 1971). This stain reacts with the mucopolysaccharides of the glycocalyx of bacteria and cells.

*Offprint requests to:* D.J.P. Ferguson at the above address

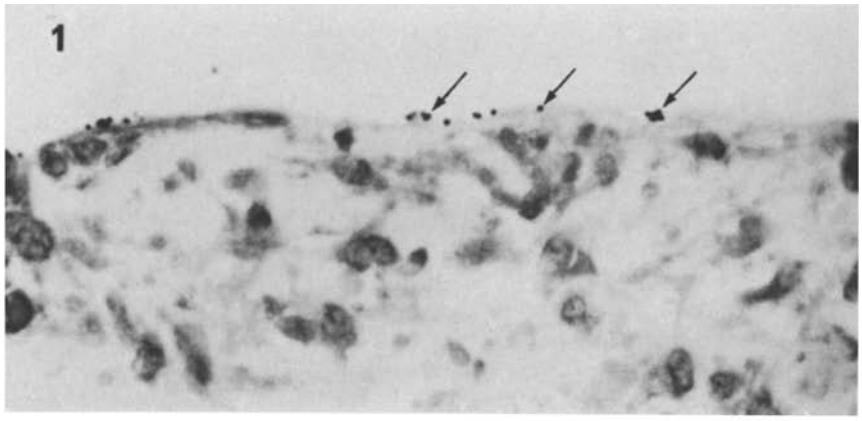



**Fig. 1.** Light micrograph showing bacteria (*arrows*) attached to the damaged surface of an aortic valve cusp 15 min postinfection. × 800

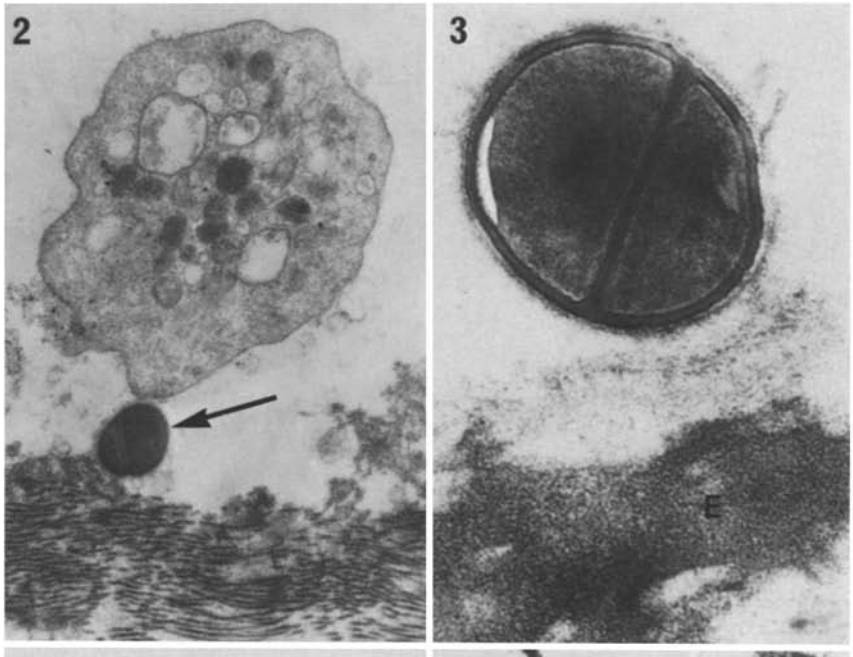



**Fig. 2.** TEM showing a bacterium (*arrow*) attached directly to the connective tissue of the valve leaflet with an overlying platelet 15 min postinfection. × 15,800

**Fig. 3.** TEM of the surface of the aorta showing a bacterium attached by its glycocalyx to the material adjacent to an elastic fibre (**E**) 15 min postinfection. × 55,000

**Fig. 4.** TEM from a sample stained with Ruthenium red showing a bacterium surrounded by a thick positive staining glycocalyx attached to the surface of a vegetation 15 min postinfection. × 40,000

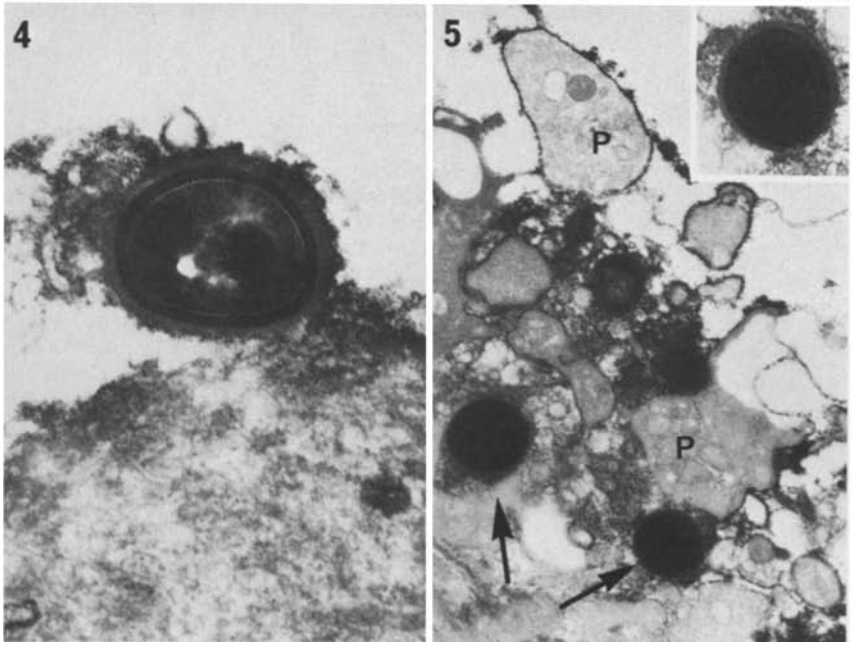



**Fig. 5.** Micrograph illustrating two bacteria (*arrows*) at the surface of a vegetation enclosed by overlying platelets (*P*) and fibrin 15 min postinfection/Ruthenium red staining (× 16,500). *inset*. Detail of one of the bacteria illustrating the positive staining glycocalyx. × 25,000

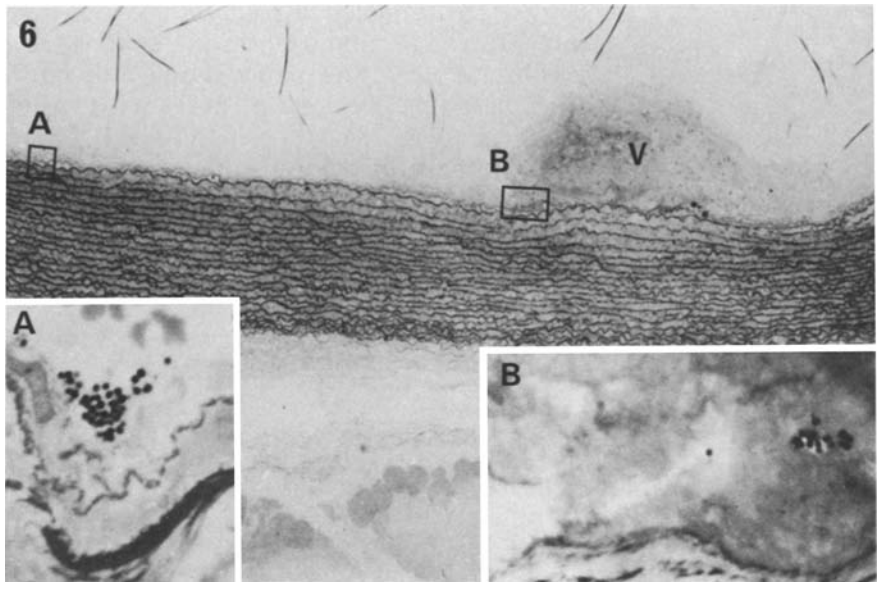



**Fig. 6.** Light micrograph showing a vegetation (*V*) attached to the wall of the aorta 3 h postinfection (×50): *inset A*, detail from the area marked *A* showing a group of bacteria on the surface of the aorta (×1,200); *inset B*, detail from the area marked *B* in which a small group of bacteria enclosed by fibrin is present on the lateral surface of the thrombus close to the junction with the aorta. ×800

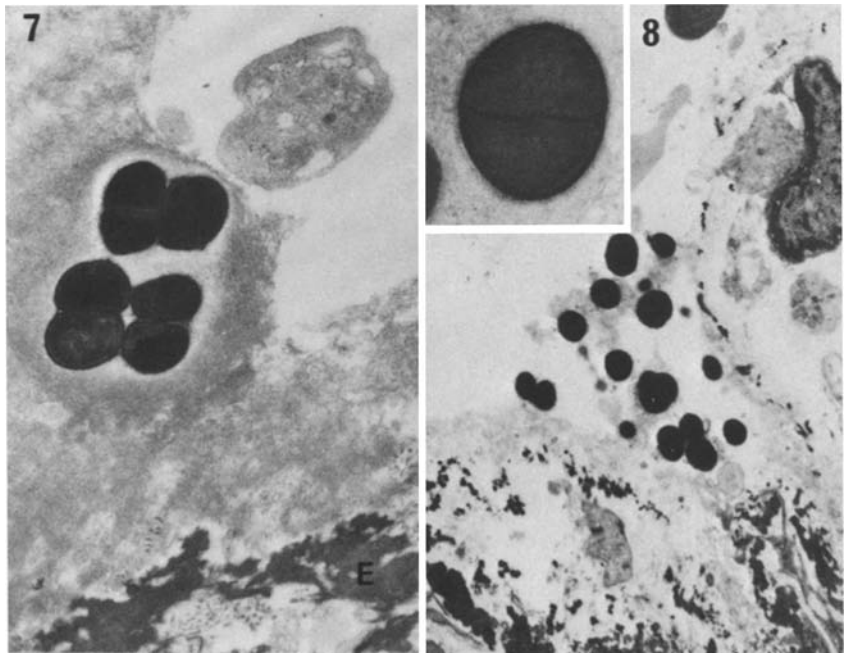



**Fig. 7.** TEM from an area similar to that shown in inset B of Fig. 4 showing a small group of bacteria undergoing division enclosed by fibrin. *E* – elastic fibre. 3 h postinfection. ×14,000

**Fig. 8.** TEM of the group of bacteria shown in inset A of Fig. 4 showing the bacteria directly colonising the surface of the aorta 3 h postinfection. ×6,000. *Inset*. Detail showing a dividing bacterium surrounded by amorphous material. ×30,000

*Bacterial distribution*

The SEM samples were examined using the criteria and techniques described by McGowan and Gillett (1980).

The light microscopic study was concentrated on those samples processed for TEM. This was because the 1 μm thick plastic sections stained with Azure A gave improved resolution with the dense blue staining bacteria being easily differentiated from platelets. When bacteria were observed ultrathin sections of suitable areas were cut for TEM.

The initial distribution of the bacteria on the vegetations and cardiovasculature was examined in the 15 min and 3 h PI groups of rabbits. The location of 70 sites of attachment were identified by light microscopy and in the majority of cases confirmed by TEM.

## Results

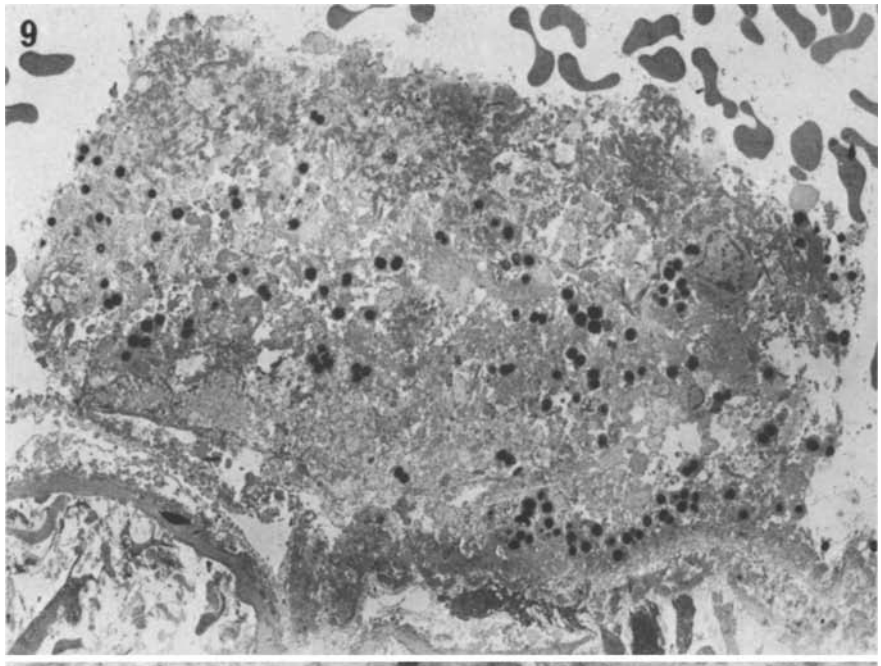
An extensive search of the surfaces of the aortic valve and the aorta was carried out using light and scanning electron microscopy. SEM allowed the examination of the entire surface of vegetations and the associated cardiovasculature (Fig. 1) with the resolution necessary to identify bacteria. Numerous structures within the size range of bacteria were observed attached to the surface of both vegetations and the damaged cardiovasculature. However, when these structures were reprocessed and examined by TEM it was found that the majority

**Table 1.** Comparison of the location of the bacterial attachment site

| Rabbit group | Bacterial attachment sites | |
|---|---|---|
| | Vegetation | Cardiovasculature |
| 15 min (PI) | 23 | 6 |
| 3 h (PI) | 33 | 8 |
| Total | 56 (80%) | 14 (20%) |

consisted of degranulated platelets and cell fragments. Thus, it was impossible to differentiate between bacteria and host material and little information on the initial colonisation was gained using SEM.

In an extensive study of rabbits infected with the lower inocula ($6 \times 10^6$ CFU) it was only possible to identify bacteria by light microscopy at 24 h post inoculation (PI). In the animals inoculated



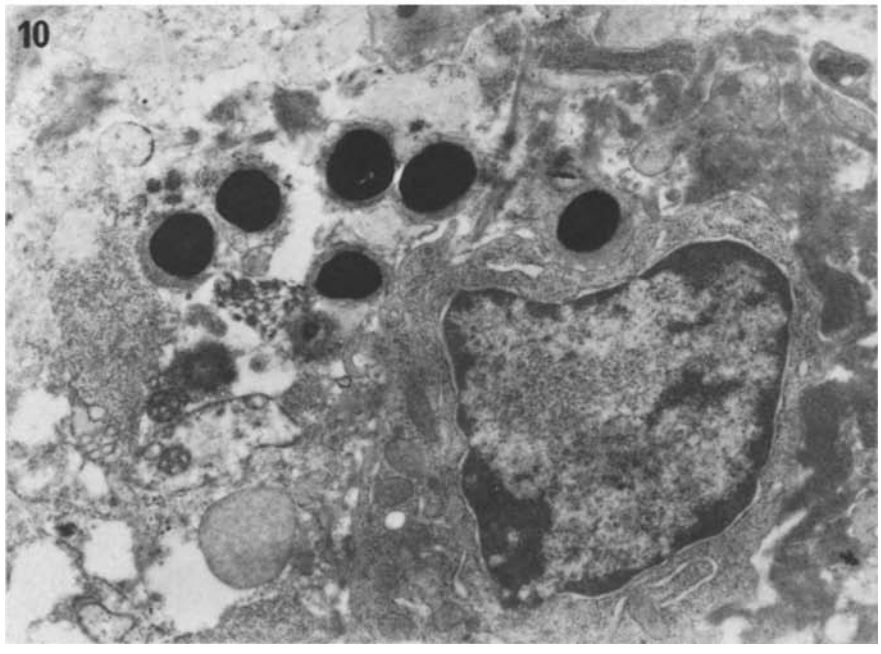




**Fig. 9.** TEM showing a small infected thrombus on the surface of the aorta 24 h postinfection. ×2,000

**Fig. 10.** Detail from Fig. 9 showing the bacteria with their glycocalyx surrounded by thrombus components (*fibrin and platelets*). *Note* the leucocyte does not appear to react to the adjacent bacteria. ×13,000

with the higher inocula ($1 \times 10^{10}$ CFU) bacteria were observed at both 15 min and 3 h PI.

At 15 min, individual bacteria were observed on the damaged surfaces of the cardiovasculature denuded of endothelial cells (Figs. 1, 2 and 3) and on the surface of vegetations (Fig. 4). Ultrastructurally, the bacteria were covered by a glycocalyx of amorphous material (Figs. 2, 3 and 4). This capsule which stains positively with Ruthenium red (Figs. 4 and 5) was present on the bacteria inoculated and was retained by the bacteria throughout the 24 h period studied. The bacteria appeared to be attached by the glycocalyx to the surface of the vegetation (Fig. 4) or to the exposed connective tissue of the damaged aortic valve and aorta (Figs. 2 and 3). We did not observe bacteria attached to the intact endothelium. On the surface of the vegetations, degranulated platelets were often observed clustered around the bacteria and even at 15 min PI, fibrin-like material and platelets were enclosing many of the bacteria (Fig. 5). There was no evidence of phagocytic leucocytes reacting to the attached bacteria. However, very rare examples (2) of macrophages containing phagocytised bacteria were observed.

The bacteria associated with vegetations at 3 h PI were embedded within the thrombus underneath a layer of cell-free fibrin (Figs. 6 and 7). At this and later stages no bacteria were observed on the surface of the vegetation. There was less evidence of the precipitation of material around the bacteria directly attached to the surface of the cardiovasculature although the bacteria were covered by a fine granular matrix (Fig. 8). The bacteria were undergoing multiplication as evidenced by the formation of transverse septae and had formed small colonies of between 2 and 10 organisms (Figs. 7 and 8).

The location of 70 separate sites of bacterial attachment were identified in the 15 min and 3 h PI groups of rabbits (Table 1). No differences were noted within or between the groups. It was observed that the majority (80%) of the sites were associated with the surface of the vegetations; particularly on the lateral surfaces close to the junction with the vasculature. The other 20% of attachment sites were located on the damaged surface of the cardiovasculature with the bacteria directly adherent to the exposed connective tissue.

At 24 h PI, small colonies of bacteria were observed within the vegetations and on the surface of the vasculature, although the initial inoculum was lower ($6 \times 10^6$ CFU). At this stage there was evidence of the precipitation of thrombus components around the bacteria attached to the vascular surfaces to form microscopic infected vegetations (Fig. 9).

An extensive search at 3 and 24 h PI failed to identify any phagocytic leucocytes containing bacteria. The colonizing bacteria did not appear to elicit any reaction from the leucocytes even when in close aposition (Fig. 10).

## Discussion

In the present study it was found that only by combining various morphological techniques could detailed information be obtained on the initial colonization of *Staph. aureus*. An attempt to use SEM to follow the initial distribution of the Staphylococci in a similar manner to that described for Streptococci (McGowan and Gillett 1980) was unsuccessful because of the difficulty of differentiating between bacteria and host cell fragments.

Therefore, although light microscopy is tedious and time consuming it provides information on the initial distribution and development of the bacteria which cannot be gained by other methods. By using material prepared for TEM it was possible to examine the same bacteria by both light and electron microscopy. In agreement with previous reports, it was possible to observe bacteria attached to the vegetations at 15 or 30 min PI, only by using a large bacterial inoculum (Durack 1975; McGowan and Gillett 1980). At 3 h PI, the presence of small groups of bacteria was similar to that reported for Streptococci (Durack 1975) and would be consistent with the progressive increase in the number of bacteria during the first 24 h of infection (Durack et al. 1973; Gutschik and Christensen 1978). Previous ultrastructural observations that bacteria do not undergo multiplication during the first 12 h (McGowan and Gillett 1980) are not supported either by infectivity data (Durack et al. 1973; Gutschik and Christensen 1978) or by the presence of dividing bacteria seen at 15 min and 3 h PI in the present study.

The increased incidence of bacteria attached to the lateral surfaces of the vegetations close to the junction with the vasculature has not been previously reported but could be related to disruption in the blood flow associated with the vegetation. It is possible that turbulence caused by the thrombus will result in slower moving currents around the base of the vegetation which may be more conducive to bacterial attachment.

The factors which affect bacterial attachment relate to the characteristics of both the bacteria and the surface to be colonised (reviewed, Freedman 1982). It is known that different species of

bacteria have different capabilities in initiating infections with *Staph. aureus* being more efficient than either *Strep. viridans* or *E. coli* (Freedman and Valone 1979). It has been shown that "stickiness" can be related to the production of the polysaccharide dextran which is present in the glycocalyx coating the bacteria (Scheld et al. 1978). The *Staph. aureus* used in the present study possessed a distinct glycocalyx although the specific factor responsible for adherence is unknown.

In the present study, platelets were often associated with newly attached bacteria which may be related to the observation that they enhance bacterial attachment (Scheld and Sande 1977). The mechanism of action of the platelets is unknown although from our observations, it could be proposed that the platelets are responsible for the rapid deposition of fibrin around the bacteria which will stabilise bacterial attachment.

The role of the phagocytic leucocyte in relation to the initial colonisation of *Staph. aureus* is unclear. The attached and colonizing bacteria did not elicit any reaction from the leucocytes nor were increased numbers of these cells associated with infected vegetations. The colonising *Staph. aureus* retained their glycocalyx and it has been shown that certain components of the glycocalyx can protect the bacteria from phagocytosis (reviewed Quie 1985). The observation of a few bacteria within phagocytes at 15 min PI but not thereafter, is similar to that reported by Durack (1975). However, we found no evidence for the involvement of these bacteria in the colonization process. It is possible that they represent a subpopulation of the bacteria inoculated which are not protected from the host phagocytes.

In the present study bacteria are observed colonizing directly the exposed *tunica intima* of the aorta and the connective tissue of the damaged aortic valve. This has not been previously reported in the studies on streptococcal infections (Durack 1975; Gutschik and Christensen 1978; McGowan and Gillett 1980). The apparent difference between Staphylococci and Streptococci in their ability to colonize the damaged cardiovasculature could explain the higher incidence of staphylococcal infection in human patients with no known predisposing valve abnormality. The subsequent precipitation of thrombus around the bacteria will result in infected thrombic vegetations whether or not thrombi were involved in the initial colonization.

*Acknowledgements.* The technical assistance of Miss E. Shelley, Mrs. M. Rychetnik and Mrs D. Harrison and the secretarial assistance of Miss L. Watts is gratefully acknowledged. We are indebted to Dr J-O. Gebbers for constructive criticism during the preparation of the manuscript.

## References

Durack DT (1975) Experimental bacterial endocarditis IV. Structure and evolution of early lesions. J Pathol 115:81–89

Durack DT, Beeson PB (1972) Experimental bacterial endocarditis I. Colonisation of a sterile vegetation. Br J Exp Pathol 53:44–49

Durack DT, Beeson PB, Petersdorf RG (1973) Experimental bacterial endocarditis III. Production and progress of the disease in rabbits. Br J Exp Pathol 54:142–151

Ferguson DJP, McColm AA, Savage TJ, Ryan DM, Acred P (1986) A morphological study of experimental Staphylococcal endocarditis and aortitis I. Formation and effect of infected and uninfected vegetations on the aorta. Br J Exp Pathol (in press)

Freedman LR (1982) Infective endocarditis and other intravascular infections. Plenum Medical Book Company, New York

Freedman LR, Valone J (1979) Experimental endocarditis. Prog Cardiovasc Dis 22:169–180

Gutschik E, Christensen N (1978) Experimental endocarditis in rabbits 2. Course of untreated *Streptococcus faecalis* infection. Acta Pathol Microbiol Scand (Section B) 86:223–228

Luft JH (1971) Ruthenium red and violet 1. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat Rec 171:347–348

McGowan DA, Gillett R (1980) Scanning electron microscopic observations of the surface of the initial lesion in experimental Streptococcal endocarditis in the rabbit. Br J Exp Pathol 61:164–171

Quie PG (1985) Mechanisms for evasion and survival of bacteria by perturbation of phagocytes. In: Jackson GG, Thomas H (eds) The pathogenesis of bacterial infections. Springer, Berlin Heidelberg New York Tokyo, pp 147–156

Scheld WM, Sande MA (1977) Bacterial adherence in the pathogenesis of endocarditis. Clin Res 25:492A

Scheld WM, Valone JA, Sande MA (1978) Bacterial adherence in the pathogenesis of endocarditis interaction of bacteria, dextran, platelets and fibrin. J Clin Invest 61:1394–1404

Accepted July 24, 1986