A, then a SCAN might consist of n stations that are evenly distributed over that area. If hypocentral depths are assumed to be negligible, then the mean P wave travel time $\overline{T}$ from a uniform random distribution of epicenters to the closest station is

$$\overline{T} = \frac{0.3825}{C}\sqrt{\frac{A}{N}} \quad (10)$$

If we assume an area of 3×10^5 km^2, 500 seismometers, and a P wave velocity of 6 km/sec, the mean P wave travel time to the closest station is 1.56 seconds.

The model just presented is a very simple one. Ground motions are considered to be a simple function of distance and magnitude. However, the standard deviation of data on ground motions for actual earthquakes with respect to these functions is typically of the order of 50 percent. Furthermore, little or no data exist for sites at small distances from large earthquakes, and the predicted ground motions for such situations vary greatly with the technique used to extrapolate these functions into a region of sparse data. Other simplifications were made in the computation of warning times. For example, the model does not include the effect of the finite depth of hypocenters, nor does it account for the distance between the first reporting seismometer and the epicenter. Also, a delay is introduced by data processing and telemetry. If these effects are included, then it is likely that the warning times shown in Figs. 3 and 4 would be reduced by several seconds. Because shaking from the initial P wave may be weak, particularly if the epicenter is at a great distance, warning times in Figs. 3 and 4 are given for the S wave from the closest point on the rupture. However, in many instances appreciable shaking may occur before this time, and the exact definition of warning time becomes uncertain. If the warning times in Figs. 3 and 4 are computed for the arrival time of hypocentral P waves instead of S waves from the closest point on the rupture, then warning times are decreased by about a factor of 2.

Southern California is only one of the regions that might benefit from a SCAN. Large subduction earthquakes are a threat to much of the circum-Pacific region, and evidence suggests that they might constitute a threat in the northwestern United States (*7*). Great shallow earthquakes in subduction zones sometimes have very long rupture lengths. For instance, the rupture length of the 1964 Alaskan earthquake probably exceeded 400 km. Because of the potential for large rupture dimensions, a SCAN could provide substantial warning times for shallow subduction earthquakes.

Intraplate earthquakes, such as the New Madrid earthquakes in 1811 and 1812 or the 1886 Charleston earthquake, also constitute a threat. Although the mechanisms of these earthquakes are poorly understood, these events probably do not involve large rupture dimensions (*8*). Nevertheless, the felt areas of these earthquakes were larger than those for the largest of the California earthquakes (*9*). It is generally thought that the principal reason for this phenomenon is a lesser degree of attenuation of seismic waves east of the Rocky Mountains. Because rupture lengths of great earthquakes in the central and eastern United States may be less than 50 km, the regions of strongest shaking that lie adjacent to the rupture zone are not likely to receive large warning times. However, other models (*7*) indicate that Rossi-Forel intensities of IX and VIII may have extended to epicentral distances of 100 and 200 km, respectively, for the 1811 to 1812 New Madrid earthquakes. This means that a SCAN could still provide large warning times for areas shaken strongly enough to cause great damage.

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10. I thank W. Gates and W. Iwan for helpful discussion and for originally suggesting this topic to me and R. Page and J. Dieterich for review of the manuscript.

27 November 1984; accepted 27 February 1985

Adherent Bacterial Colonization in the Pathogenesis of Osteomyelitis

Abstract. *Direct scanning electron microscopy of material obtained during surgical debridement of osteomyelitic bone showed that the infecting bacteria grew in coherent microcolonies in an adherent biofilm so extensive it often obscured the infected bone surfaces. Transmission electron microscopy showed this biofilm to have a fibrous matrix, to contain some host cells, and to contain many bacteria around which matrix fibers were often concentrated. Many bacterial morphotypes were present in these biofilms, and each bacterium was surrounded by exopolysaccharide polymers, which are known to mediate formation of microcolonies and adhesion of bacteria to surfaces in natural ecosystems and in infections related to biomaterials. The adherent mode of growth may reduce the susceptibility of these organisms to host clearance mechanisms and antibiotic therapy and thus may be a fundamental factor in acute and chronic osteomyelitis.*

Adherence of bacteria in extensive microcolonies appears to be a fundamental step in the development of certain infectious disease states (*1*). Adherence may be mediated by highly hydrated, anionic exopolysaccharide polymers of the infecting bacteria which bind to the teichoic acid polymers of the Gram-positive cell wall (*2, 3*) and to the distal portions of the lipopolysaccharides of the Gram-negative cell wall (*2*). They often surround the bacterial cell in a fibrous matrix 0.5 to 1.0 μm thick.

Adherent bacteria have been demonstrated on compromised bone, infected orthopedic prostheses and methyl methacrylate, and tissues adjacent to those foreign substances (*4–7*). Such infections tend to resist treatment and to persist until the biomaterial and the infected adjacent tissue are removed (*4–8*); they are sometimes polymicrobial (*8–10*), and the adherent bacteria are difficult to detect unless special recovery and culture techniques are used (*4, 9, 10*). The natural mode of growth of many adherent bacteria in nature and in some diseases is polymicrobial, involving symbiotic mixed forms of aerobes and anaerobes (*2, 4, 11, 12*) coexisting in a biofilm held together by a ruthenium red–staining matrix (*13*). These structured biofilms occasionally shed bacteria into the ambient milieu or, in an infection, into the adjacent tissue fluids and circulatory system (*4–7*). The shed bacteria are not necessarily representative of the adherent colonies, but they are the bacteria that are recovered by conventional sampling techniques.

Our interest in the mechanism of bacterial adhesion in orthopedic diseases began when we observed that bacteria use biomaterials as a substrate or scaffold along which they propagate into sterile regions (*14, 15*). In 1979 Gristina and colleagues (*6, 7*) identified microbes in a polysaccharide biofilm adhering to the surface of a metallic device removed from an infected human surgical wound. The passive surface of this biomaterial was ideal for colonization. As we will show here, bacteria also find fragments of dead tissue and bone to be susceptible substrates for colonization. These fragments are no longer protected by normal intact eukaryotic membranes, but they bear remnants of glycoproteins on their surfaces (*16, 17*). They are nonviable and defenseless, making them similar to biomaterials.

Osteomyelitis shares characteristics with other infections caused by adherent bacteria: traumatized, dead, or compromised tissue is frequently involved (*10, 12, 18*); the disease is resistant to antibiotic therapy and to natural host defense mechanisms (*5, 10, 12*); polymicrobial infections are often present (*5, 9, 10, 12*); and the disease tends to persist until dead tissue or sequestra are removed (*10, 12*). With these features in mind, we investigated bacterial adherence on bone and surrounding tissues in resistant cases of osteomyelitis.

Culture specimens, acquired during surgical debridement from three patients with chronic osteomyelitis and one patient with acute osteomyelitis, consisted of one or more of the following: bone, necrotic tissues, pus, and debris. All the specimens were cultured aerobically and anaerobically by routine methods (*5*), and the cultures were examined by standard microbiological techniques in the hospital laboratory and our orthopedic laboratory. Materials to be studied by scanning electron microscopy (SEM) were processed routinely (*5*) and examined with a Hitachi S450 scanning electron microscope at an accelerating voltage of 20 kV by a technician who knew neither the diagnosis nor the condition of the patient involved. All available surfaces (usually about 2 cm^2 per specimen) were scanned and representative areas were photographed. Areas affected by preparation techniques, such as excision and sawing, were avoided.

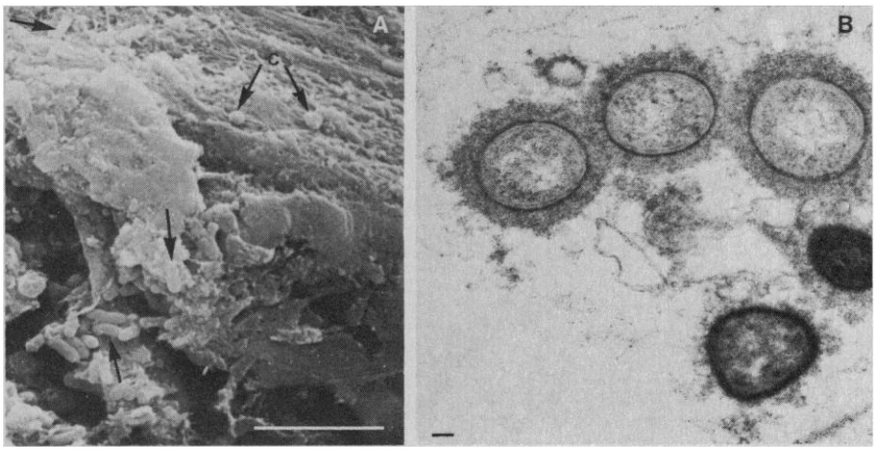

Fig. 1. (A) Scanning electron micrograph of amorphous biofilm covering the medullary surface of bone removed from an infected tibia. The film contains cocci (C) and rod-shaped bacteria (arrows); more bacteria are buried deeper within the biofilm. Scale bar, 5 μm. (B) Transmission electron micrograph of biofilm scraped from the cortex of the infected tibia, showing discrete microcolonies of Gram-positive bacteria. Scale bar, 1 μm.

Materials to be examined by transmission electron microscopy (TEM) were prepared routinely, except that they were stained with ruthenium red to visualize any polysaccharides (*2, 5*). Sections (~50 nm) were cut at random and examined with an AEI EM801 transmission electron microscope at an accelerating voltage of 60 kV. Complete examination of 100 sections was considered to cover approximately 3 mm^3 of the specimen.

Table 1 summarizes the characteristics of the bacterial infections that were identified by culturing, SEM, and TEM. Figure 1A shows bacteria in the biofilm covering the medullary surface of an infected tibia. The film, which is so thick it obscures the haversian canals, contains both cocci and rod-shaped bacteria. Figure 1B shows bacteria in biofilm from the tibial cortex. There are microcolonies of two different morphotypes of Gram-positive bacteria, which have formed an aggregate adherent to a host cell. The bacteria are surrounded by an extensive fibrous matrix that mediates their adhesion to each other and to the host cell. Figure 2 shows medullary cavity material containing Gram-positive and -negative bacteria of several different

Table 1. Summary of data of four consecutive cases of osteomyelitis.

Diagnosis	Culture	SEM	TEM
	Male, age 20		
Posttraumatic hematogenous osteomyelitis of left distal tibia	*Peptostreptococcus productus, Peptostreptococcus anaerobius, Bacteroides fragilis, Bacteroides nodosus*	Rods and cocci in extensive biofilm	Many types of Gram-positive cells in microcolonies in extensive biofilm
	Male, age 36, first admission		
Posttraumatic chronic osteomyelitis of right tibia	Enterococcus, *Serratia marcescens, Pseudomonas aeruginosa*	Not done	Not done
	Same male, fourth admission (34 months later)		
	Staphylococcus aureus, Staphylococcus epidermidis, Serratia marcescens, Pseudomonas aeruginosa, gamma *Streptococcus*	Cocci and occasional rods in thick biofilm	Rods and cocci, many lacking cell walls, in extensive biofilm
	Female, age 56		
Chronic osteomyelitis of distal left femur	*Staphylococcus aureus*	Intermittent biofilm containing cocci and rods	Not done
	Male, age 71		
Open fracture of left tibia and fibula, dead bone fragments	*Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescens*	Thick biofilm with a few bacteria showing at the surface	Gram-negative rods and Gram-positive cocci in thick biofilm

morphotypes, and many bacteria whose outer cell walls appear to be missing so that they are bounded only by their cytoplasmic membranes. These bacteria are surrounded by a ruthenium red–staining fibrous matrix.

In general, osteomyelitis presents as an acute disease characterized by bone destruction and septicemia or as a chronic indolent infection of osseous structures featuring recurrent purulent drainage from resistant foci, which are often centered on residual dead bone or sequestra. There are reports of unique bacterial forms and of site-specific (*10, 12, 19*) and patient-specific (*10, 20–22*) clinical presentations. The etiology of acute, chronic, and regional osteomyelitis is certainly multifactorial, and all variables interlock to provide specific clinical sequences. At the initiation of each sequence an inoculum of bacteria and a susceptible focus are required. Microbial delivery may be provided by direct contamination (as in trauma or an operation) or by immediate or delayed hematogenous or contiguous spreading. The concept of site specificity of the bacteria would appear to apply in trauma (if trauma is etiological) (*9, 18*), in the presence of compromised tissue (*10, 18, 22, 23*) or biomaterials (*9, 10, 12, 22*), and in certain patient groups.

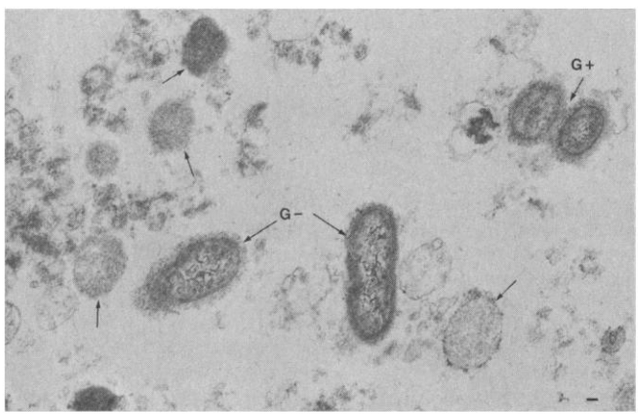

Fig. 2. Transmission electron micrograph of material from the medullary cavity of the infected tibia, showing Gram-positive cocci (G+), Gram-negative bacteria (G−), and many bacteria (arrows) whose outer cell walls appear to be missing. Scale bar, 1 μm.

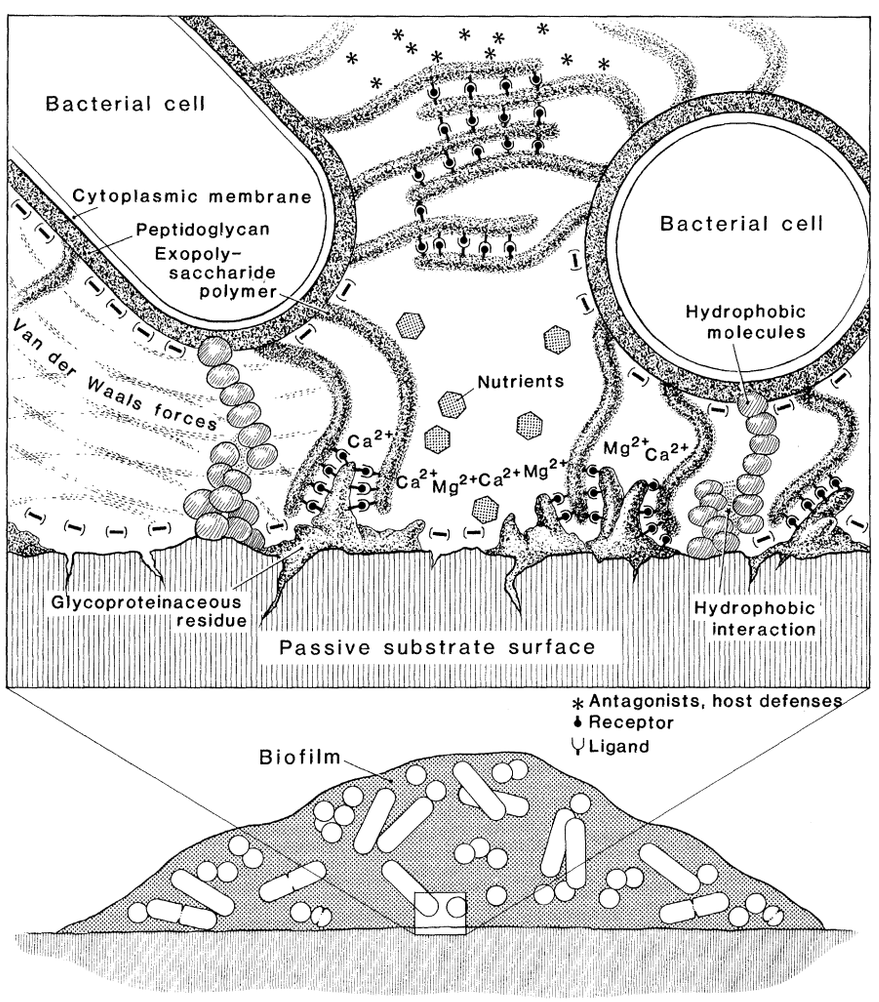

Fig. 3. Mechanism of bacterial adherence. At specific distances the initial repelling forces between like charges on the surfaces of bacteria and substrate are overcome by attracting van der Waals forces, and there are hydrophobic interactions between molecules. Under appropriate conditions there is extensive development of exopolysaccharide polymers, allowing ligand-receptor interaction and pertinacious bonding of the bacteria to the substrate.

The mechanism of bacterial adherence in osteomyelitis is suggested by information on marine ecosystems (*11, 13*), dental caries (*16*), certain prokaryotic and eukaryotic cell diseases (*1, 24*), and resistant infections centered on biomaterials (*4–7, 14, 15*). The surfaces of bacteria arriving in the vicinity of a substrate are polyanionic, as is the surface of the substrate. The like charges initially repel the bacteria; however, within a certain range, the initial repulsion is overcome by van der Waals forces created by fluctuating dipoles in the molecules of each surface (Fig. 3) (*24*). Juxtaposition at a critical distance allows hydrophobic binding from both surfaces to tether the prokaryotic cell to the substrate. Tethering positions some bacteria near the substrate long enough for the exopolysaccharides produced by the bacterial cells to begin forming strong bonds with residual glycoproteins of the substrate. Once the bacteria are firmly attached to the substrate, the exopolysaccharide polymers proliferate and form additional bonds with the substrate as well as between prokaryotic cells. These eventually coalesce to form a microbial biofilm in which each bacterium has access to suitable nutrients and a useful physiological "rapport" with its neighbors. Under favorable conditions, exponential growth soon establishes an extensive bacteria-laden biofilm.

Various factors influence the establishment of bacterial biofilms on favorable substrates. These include flow rates of the extracellular fluid, the surface configuration of the substrate, tensions between fluid and substrate (*13*), the glycoproteinaceous residua on surfaces (which provide exopolysaccharide bonding sites) (*13, 16, 17, 24, 25*), the availability of nutrients and cations, the temperature of the milieu, the stage of growth (*13*) of each prokaryotic cell, and competition by other organisms (*13*). The effect of surface area of substrates

such as biomaterials or devitalized bone remains to be investigated. It seems logical to assume that, all other factors being equal, random contact between bacterial cells and substrate increases as substrate area increases.

The matrix formed by the exopolysaccharide polymers serves not only as an adhesive mechanism but also as a nutrient-trapping ion-exchange material (*11*), and it allows the bacteria to resist engulfment by phagocytic cells. It appears to be virulence-related (*1, 4–7*), and it has been shown to confer resistance to host defense mechanisms such as surfactants (*26*) and antibodies (*27*) and to the effective penetration of antibiotics (*27, 28*). The stability of this biofilm appears to be a major factor in the persistence of many chronic orthopedic infections (*4–7*).

This study suggests that compromised, dead, or sequestrated bone is a suitable substrate for bacterial colonization and biofilm formation. There are indications that the adherent state mediated by the exopolysaccharide polymers enhances the virulence of the bacteria in the biofilm. In such states progressive microcolonial habitation occurs with development of the complex consortia seen in chronic osteomyelitis. We believe that the adherent form of infection is natural in acute and chronic osteomyelitis and that adherence explains in part the resistance of this disease to antibiotic therapy and its persistence until all dead bone and compromised tissues have been removed.

The infections in the cases presented here share some qualities of the adherent state in nature and in certain diseases and biomaterial-related infections. There appears to be no contradiction in these shared qualities, but rather a compelling unity that suggests common causal mechanisms for all adherent bacterial infections.

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24 September 1984; accepted 10 January 1985

Pancreatic Secretion by Nonparallel Exocytosis: Potential Resolution of a Long Controversy

Abstract. *The idea that pancreatic digestive enzyme secretion can occur in a nonparallel manner has been controversial because of its presumed incompatibility with the exocytosis secretory mechanism. Correlation and regression analysis of enzyme output by the rabbit pancreas after it is stimulated with cholecystokinin and chymodenin revealed that digestive enzymes are secreted in a highly linked fashion, compatible with exocytosis and with nonparallel secretion. Thus, exocytosis and nonparallel secretion are not contradictory processes, but rather nonparallel secretion is due to exocytosis from heterogeneous sources within the pancreas.*

The physiological and cell biological phenomena underlying the secretion of digestive enzymes by the mammalian pancreas have been the subject of an intense ongoing controversy for over a decade. Palade, in his 1975 Nobel Prize address (*1*), summarized the process of synthesis, segregation, storage, and final exocytotic expulsion of the several digestive enzyme species from the pancreatic acinar cell. Rothman (*2*) criticized the exocytosis hypothesis of enzyme secretion as being based on incomplete and insufficient evidence and contradictory to the observed phenomena of nonparallel or enzyme-specific secretion of digestive enzymes. In place of the exocytosis hypothesis, Rothman (*2, 3*) offered as an alternative model of cellular secretory protein routing the "equilibrium hypothesis." In this model, secreted proteins cross directly through the membranes of the cell in a regulated fashion, rather than being totally segregated from the cytosol and stored within the membranes. The controversy has continued to the present, with laboratories in several countries contributing evidence favoring one side of the argument or the other.

Observations from various laboratories have shown rapid nonparallel or enzyme-specific secretion of digestive enzymes after administration of diverse hormones, neurotransmitters, metabolites, or digestive stimuli to various whole-pancreas preparations both in vitro and in vivo (*4*). According to the exocytosis hypothesis, distinct species of digestive enzymes are synthesized, processed, segregated from the cytosol, and stored in the zymogen granules, in parallel with each other, within 45 minutes to 1 hour (*1, 5*). Further, the zymogen granules appear in electron micrographs to be homogeneous, even when their contents are assessed by immunohistochemical methods (*6*). Thus, either the observations of nonparallel secretion are incorrect [which has been the claim of some cell biologists (*7*)] or exocytosis is not a satisfactory mechanism to explain the secretory phenomena (*2*).

We explored the possibility that the rabbit pancreas in vitro and in situ might secrete enzymes in a nonparallel fashion, and we simultaneously determined whether the enzyme species were secreted in groups, as expected of exocytosis, or independently, as expected of an equilibrium-type mechanism. The existence of nonparallel secretion was explored by regression analysis of the secretion of paired digestive enzymes under several different conditions of stimulation. Sig-