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Ultrastructural Studies of Adherence of *Staphylococcus aureus* in Experimental Acute Hematogenous Osteomyelitis

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A model of acute hematogenous osteomyelitis initiated by injection of *Staphylococcus aureus* into 29-day-old chickens was used. Transmission electron microscopy showed that the bacteria adhered to exposed cartilage matrix in the metaphyseal region of long bones but not to adjacent vascular linings or to erythrocytes. It is proposed that the combination of exposure of growth plate cartilage during normal bone growth and the ability of *S. aureus* to adhere to this cartilage is the mechanism for initiation of infection which proceeds to an osteomyelitic abscess.

The most common causative organism of acute osteomyelitis is *Staphylococcus aureus*. Antibiotic therapy has reduced mortality from this disease to almost zero, but the acute disease can become chronic, resulting in life-long crippling disability. Workers in our laboratory have developed a reproducible experimental model of acute osteomyelitis in chickens which closely mimics the human disease (3). Previous models of osteomyelitis have not been characteristic of the disease in children (1, 6, 8), and trauma to the bone was often required before induction of the disease. Our model requires no trauma to the limb site. A histopathological study of the natural history of the disease has been previously reported (2). That study suggested a specific tropism of the bacteria for the initial infection site, the growth plate cartilage of the long bone. Studies of avian long bones have been performed with light microscopy (10, 11) and more recently at the ultrastructural level (4, 5). These have revealed many similarities between avian and mammalian growth plates, particularly in the cytological sequence of the cartilaginous cells. Furthermore, it has been shown that during the growth of long bones the endothelium at the tips of the metaphyseal vessels is discontinuous, exposing the cartilage in this region (5). An ultrastructural investigation of the growth plate during active acute osteomyelitic infection has not been reported previously. This ultrastructural study of the growth plate of avian long bones harboring staphylococcal abscesses was conducted to advance our knowledge of the pathogenesis of the disease.

One-day-old male broiler chickens were raised on a standard diet of "chick starter crumbles" and tap water. At 29 days of age, infection was induced by injecting a suspension of *S. aureus* into a wing vein. The organism used was a strain isolated from a spontaneous case of chicken tenosynovitis. Primary cultures from bone swabs were stored at −70°C in brain heart infusion broth with 10% glycerol. Bacteria were grown in brain heart infusion broth with shaking for 6 h at 37°C, centrifuged (1,400 × *g*) for 10 min, and suspended in 10 ml of phosphate-buffered saline (pH 7.2). Quantitative cultures consistently showed 10^9 to 10^{10} CFU per ml. Chickens were injected with 0.1 ml of this suspension and then were anesthetised with halothane after 12 h. The proximal tibiae were exposed and removed into 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) containing 0.15% ruthenium red. Thin vertical sections were then sliced under fixative, and those containing abscesses were retained.

Antiserum was produced by the use of an antigen prepared from a 12-h broth culture grown in brain heart infusion broth with 1% sucrose and glucose. The broth was centrifuged, and the bacteria were suspended in 0.15 M NaCl, vortexed for 2 min, and repelleted. The pellet was discarded, and the supernatant was heated to 56°C for 1 h. Precipitates were removed by centrifugation, and the supernatant was dialyzed against phosphate-buffered saline (pH 7.2) for 24 h at 4°C. The antigen was then freeze-dried. Before inoculation, the antigen was redissolved in distilled water. A New Zealand white rabbit at age 3 months was given weekly intravenous injections of the antigen for a 6-week period. Five days after the last injection, the blood was collected and clarified and the serum fraction was stored at −70°C.

For antiserum treatment, exposed tibiae were sliced into thin vertical sections, and those containing abscesses were placed into undiluted antiserum and incubated at 37°C for 1 h. After incubation, the slices were placed into fixative. After initial fixation, the tissues were placed in fresh fixative for 12 h, rinsed three times in 0.05 M cacodylate buffer (pH 7.4) containing 0.05% ruthenium red, and postfixed in 1% OsO_4 in the cacodylate buffer. Tissues were dehydrated in ascending ethanol concentrations containing 0.05% ruthenium red and then were embedded in araldite after extra dehydration in propylene oxide. Sections were cut on a LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips 201 transmission electron microscope at an accelerating voltage of 60 kV.

No abscesses were detected in the lungs, liver, or kidneys. Lesions were evident histologically in metaphyseal vessels within the cartilage matrix of the growth plate of the proximal tibiae within 24 h after bacterial injection. These lesions presented as a bacterial deposit occluding the blood vessel (Fig. 1). Adjacent uninfected metaphyseal vessels retained normal vascular structure, but this was absent around the bacterial deposit. Smaller secondary deposits of bacteria were found in nearby vessels which subsequently produced further microabscesses within the same growth plate.

Transmission electron micrographs of growth plate cartilage from infected chickens showed that circulating bacteria were in direct contact with the cartilage matrix in this region. The bacteria were found to concentrate against the cartilage matrix (Fig. 2). They were not found bound to erythrocytes

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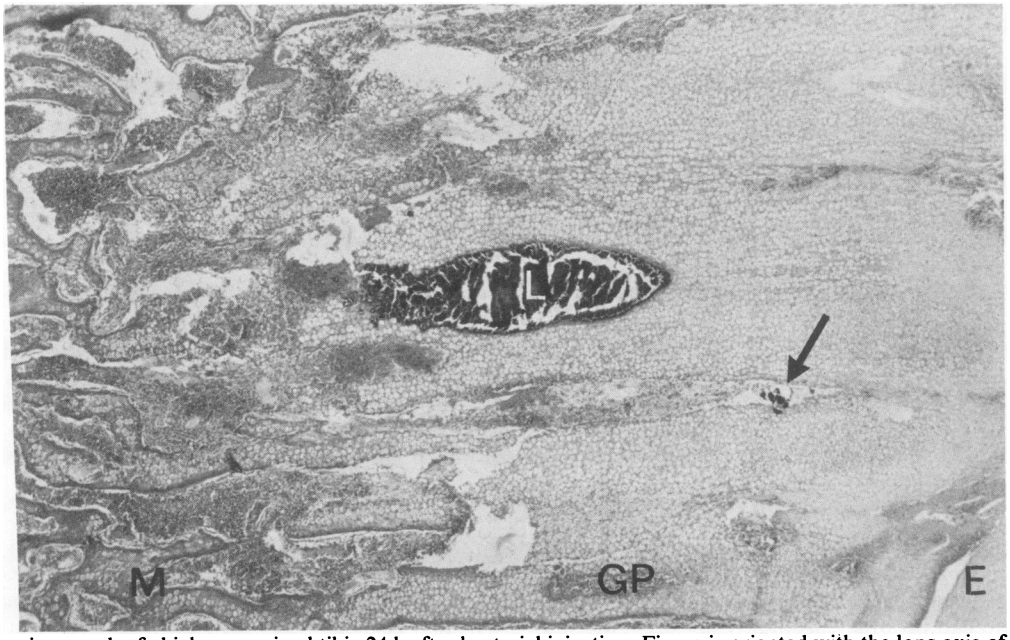

FIG. 1. Light micrograph of chicken proximal tibia 24 h after bacterial injection. Figure is oriented with the long axis of the bone running from left to right. Within the growth plate (GP), a metaphyseal tunnel is totally occluded by bacteria (L). A secondary focus of infection is also present in an adjacent vessel (arrow). E, epiphyseal cartilage; M, metaphysis. Hematoxin and eosin stain. Magnification, ×40.

or to endothelial cells. Thin sections of the growth plate region from infected chickens not treated with antiserum showed the cell wall of the bacteria to be in direct contact with the cartilage matrix (Fig. 3A). Antiserum treatment of specimens from infected chickens showed the bacteria and cartilage to be separated in these specimens, the resultant gap between the bacterial cell wall and the cartilage matrix being traversed by the glycocalyx (Fig. 3B). This observation was made for each bacterium associated with the cartilage matrix.

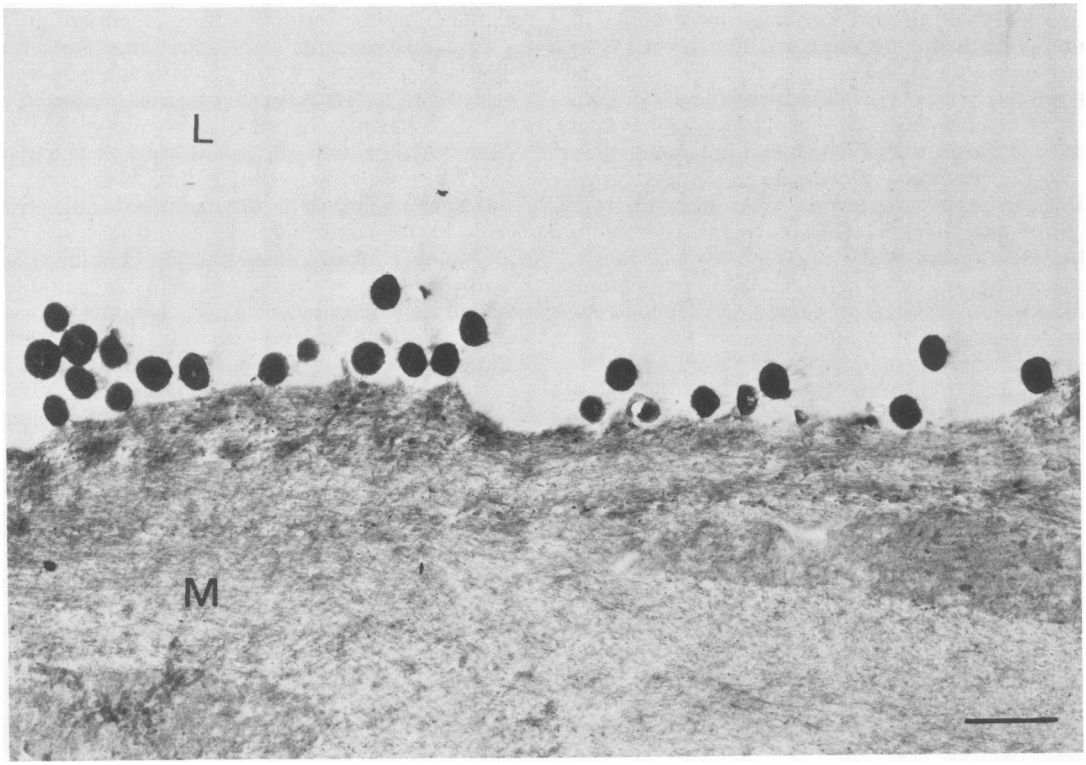

FIG. 2. Transmission electron micrograph of a metaphyseal tunnel in growth plate cartilage 12 h after bacterial injection. Circulating bacteria have deposited against the exposed cartilage matrix (M). No bacteria are seen free in the lumen (L) of the vessel. Bar, 2 μm.

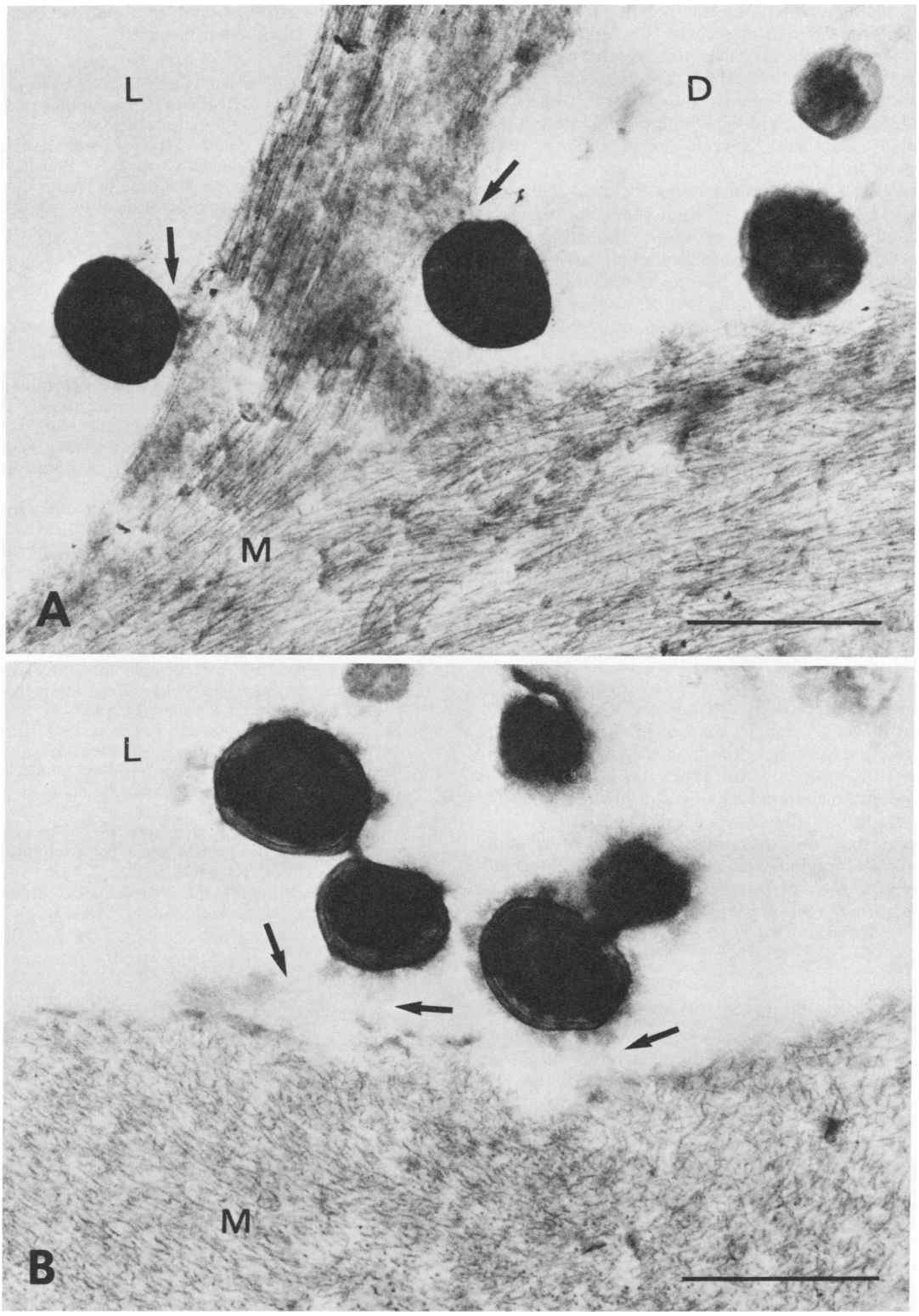

FIG. 3. Electron micrographs of metphyseal tunnels in growth plate cartilage 12 h after bacterial injection. (A) Sample untreated with specific antiserum. Arrows indicate the bacterial cell walls in direct contact with the cartilage matrix (M). (B) Sample treated with antiserum. Arrows indicate the bacterial glycocalyx extending between the bacterial cell wall and the cartilage matrix. L, Lumen of metaphyseal tunnel; D, lacuna of degenerated chondrocyte. Bars, 1 μm.

The initiation of acute osteomyelitis in chickens simply by production of a septicemia indicates a specificity for bacterial localization in the metaphyseal region of long bones, since lesions did not appear elsewhere. The endothelium of the metaphyseal vessels is generated by the division of endothelial cells some distance from the advancing capillary tips, resulting in a discontinuous endothelium at these tips (5). This situation, in which blood elements can interact with non-endothelial structures, appears to be unique to the growth plates of long bones.

It is known that a major predisposing factor in the development of *S. aureus* infections is trauma to tissues, exposing collagen, basement membrane proteins, and clots to the bloodstream (7). The loss of an endothelial barrier during normal growth processes may be one explanation for the observed tropism and may explain why acute hematogenous osteomyelitis of childhood has a different pattern from that of adult life (9).

The use of transmission electron microscopy of thin sections in the first 24 h after infection has shown the circulating bacteria passively carried to the growth plate region associated with the exposed cartilage matrix but never with the adjacent endothelial cells or erythrocytes. The adhesion of one bacterium to the cartilage surface appeared sufficiently strong to anchor several other bacteria. This resulted in a series of microcolonies deposited along sections of exposed cartilage which were probably the initial sites of infection. This situation was transformed by 24 h to a bacterial plug occluding the lumen of the vessel. The colonization of exposed cartilage surfaces in adjacent capillaries then occurred, resulting in secondary foci of infection. Subsequent spread of infection resulted in a metaphyseal lesion 24 h after infection, easily visible by the naked eye (2). Initial transmission electron microscopic studies of the bacteria revealed that a glycocalyx existed in various stages of condensation. By treatment with an anticapsular serum and staining with ruthenium red, an extensive glycocalyx was found to completely surround each bacterium. This capsule may play a role in the adhesive process.

We propose that the initiation of infection in acute hematogenous osteomyelitis may result from specific binding of *S. aureus* to the cartilage surfaces exposed during the formation of normal metaphyseal blood vessels. Once the pathogens adhere to the cartilage, their subsequent growth and spread results in complete occlusion of the metaphyseal vessel and the establishment of a metaphyseal lesion recognized as an osteomyelitic abscess.

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