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Xeroradiographic, Bacteriologic, and Pathologic Studies in Experimental Staphylococcus Osteomyelitis[1] (39926)

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Introduction. Prompt recognition of and appropriate therapy for osteomyelitis determines the subsequent course of this still common and difficult infection. Animal models of osteomyelitis are useful in order to explore newer diagnostic and therapeutic approaches. In this regard, Norden described a readily available model of experimental staphylococcus osteomyelitis in the rabbit tibia (1). This model simulates its human counterpart resulting from trauma (2). Norden and Dickens have subsequently used this model to assess possible effectiveness of several antibacterial regimens (3–5). In this report, we employ Norden's model to present detailed sequential bacteriologic, pathologic, and xeroradiographic findings which describe progressive disease more fully than before.

Methods. Organism. Carl W. Norden kindly sent us *Staphylococcus aureus,* strain SMH. Strain SMH belongs to phage type 52/52A/80, is coagulase-positive, forms a yellow pigment on blood agar, and elaborates α-hemolysin (1). *S. aureus* SMH was kept on blood-agar slants. Fresh cultures were prepared by inoculating 5 ml of trypticase soy broth with the organism and incubating overnight at 37°. We diluted these cultures (1:6) in phosphate buffer (PBS), pH 7.4 and 0.1 ml (containing about 3×10^6 colony-forming units) was promptly injected into rabbit tibiae.

Experimental osteomyelitis. We obtained 45 young New Zealand White rabbits, weighing about 2 k. After cleansing the ventro-medial aspects of the proximal tibia with alcohol, we passed an 18-gauge needle into the marrow of the unanesthetized animal and inserted 0.1 ml of 5% sodium morrhuate,[5] followed by 0.1 ml of the staphylococcus suspension. Finally, we instilled 0.1 ml of sterile phosphate-buffered saline (PBS) through the needle. The right leg of each rabbit was injected with strain SMH and the left served as its uninjected control. Additionally, both tibiae of five other rabbits were inoculated with sodium morrhuate and PBS.

On the 2nd, 5th, 10th, 15th, and 20th days following injections, both legs of five infected animals were examined by xeroradiography. Immediately thereafter, animals were sacrificed by an overdose of intravenous pentobarbital, and bacteriologic and pathologic studies were done. Morrhuate-injected control animals were similarly examined on each experimental day. The latter group were all sacrificed on Day 20.

Preparation of bone. Infected and positive (morrhuate) control and negative (noninjected) control tibiae were dissected from surrounding tissues and cleansed with alcohol. Material from each proximal and distal marrow was aspirated through a 20-gauge needle, carefully avoiding sites of injection and obvious abscesses. Marrows were streaked onto blood-agar plates.

Bones were fixed in 10% formalin for 48–72 hr, decalcified for 72 hr in EDTA,[6] and cut longitudinally. Paraffin sections

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[5] Eli Lilly Company, Indianapolis, Ind.

[6] Decalcifying solution, Scientific Products, McGraw Park, Ill.

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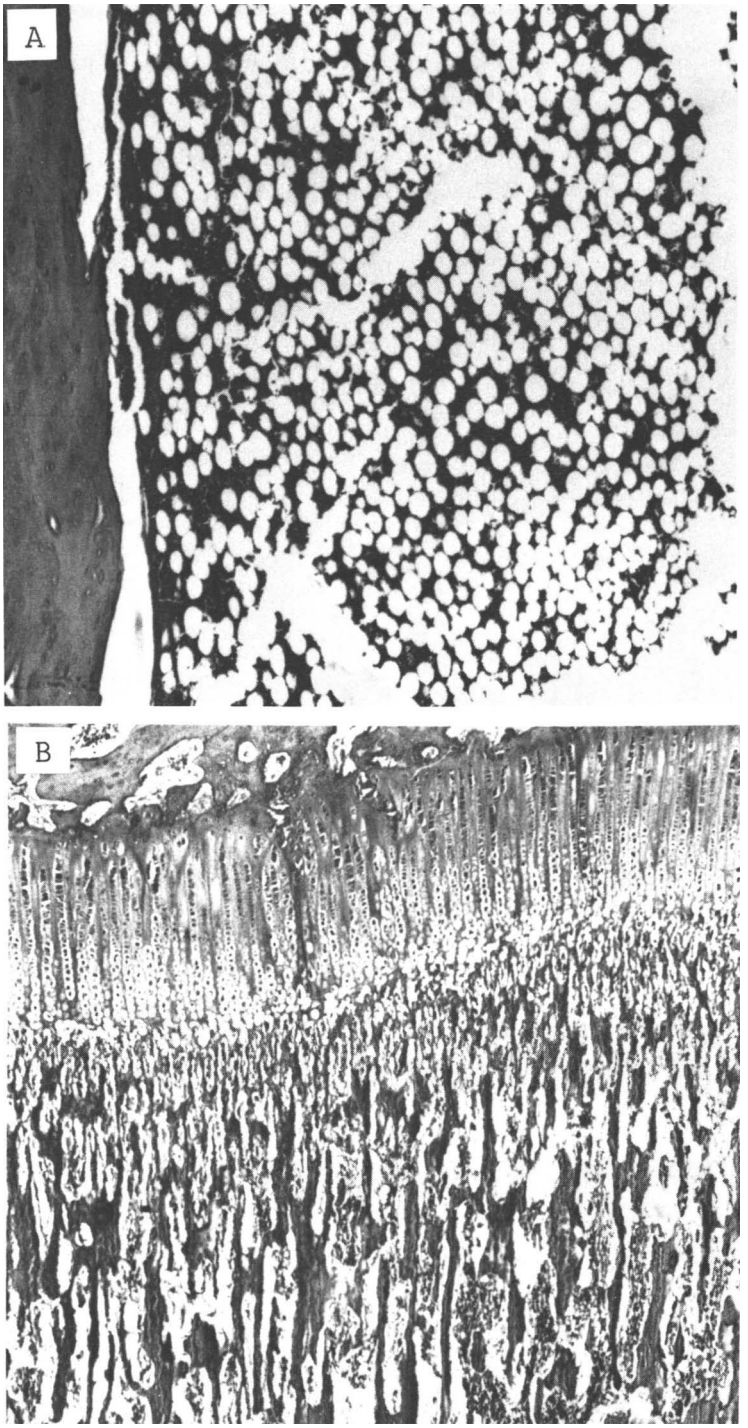


PLATE 1. Histologic sections (hematoxylin and eosin) of control marrows of rabbits are shown. (A) A normal portion of uninjected tibia (negative control) exhibiting hematopoiesis at mid-shaft is shown. ×15. (B) A normal proximal tibia in a young rabbit is shown (negative control). The epiphysis is at the upper and the metaphysis at the lower portion of this section. ×15. (C) The medullary cavity of this proximal tibia was injected with sodium morrhuate 20 days earlier (positive control). Trabeculae are necrotic and dense sclerosis (arrow) has replaced normal marrow. ×10.

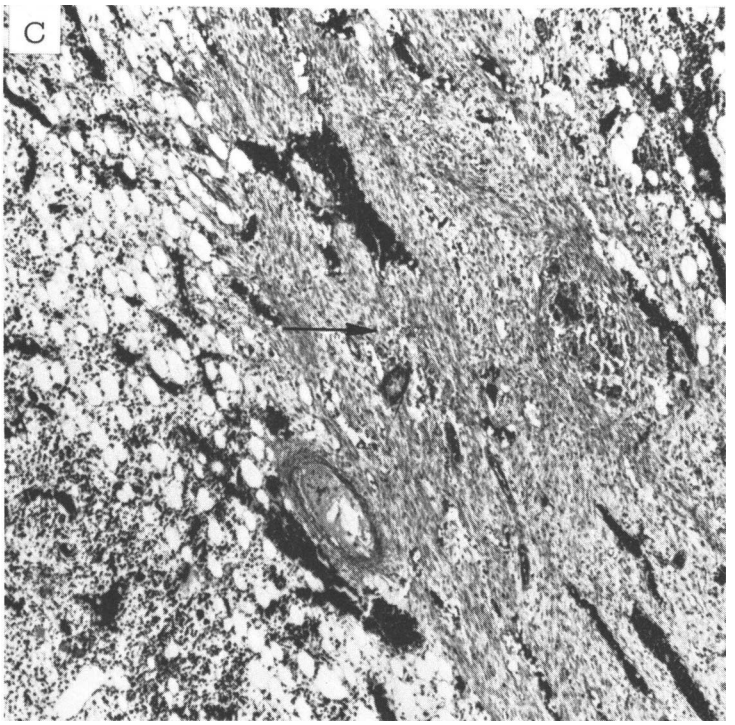


PLATE 1C

were prepared and stained with hematoxylin and eosin.

Roentgenographic techniques. Xerograms of restrained animals were made using an X-ray unit having a focal spot of 2 mm with 120-kV peak, 25 mA, contrast B, density C, and negative mode.

Images were read independently by two of us (C. C. K. and J. N. W.). Each reviewer used eight criteria, grading their severity 0–4 +. They were: (1) swelling of soft tissues, (2) periosteal reaction, (3) osteoporosis and lytic changes, (4) cortical thickening, (5) joint effusion, (6) obliteration of the medullary cavity, and formation of (7) sequestra and (8) involucra.

Statistics.[7] On each designated day, roentgenographic scores of the eight criteria were added, and the totals were examined by an analysis of variance. At each point of observation (i) there was a decreasing exponential relationship between the probability (P_i) that a criterion was present.

$$\hat{m} = \frac{\Sigma X_i Y_i}{X_i^2}$$

[X_i = 2, 5, 10, 15, or 20 days and $Y_i = \log (1 - P_i)$].

The expected value ($E[Y]$) in days at which a finding was first seen is determined by the following equation:

$$E[y] = \sum_{0}^{\infty} /10 \frac{m_i (i - 1)}{2}.$$

Results. Of the 45 rabbits inoculated with *Staphylococcus aureus,* strain SMH, seven died, apparently from sepsis, before completion of the study. Rabbits died on the second (2×) or 10th to 16th days (5×) after infection. In each of these animals staphylococci were recovered from the tibiae and subcutaneous abscesses which were often present at sites of injection (2×). We excluded these seven rabbits from our analysis and they were not studied further. Osteomyelitis did not develop in 13 other rabbits. In the latter group, X rays were unchanged and cultures of the marrow were sterile. Therefore, 25 rabbits (all confirmed

[7] Professor Andre G. Laurent (Department of Mathematics, Wayne State University) and David M. Laurent did these calculations with us.

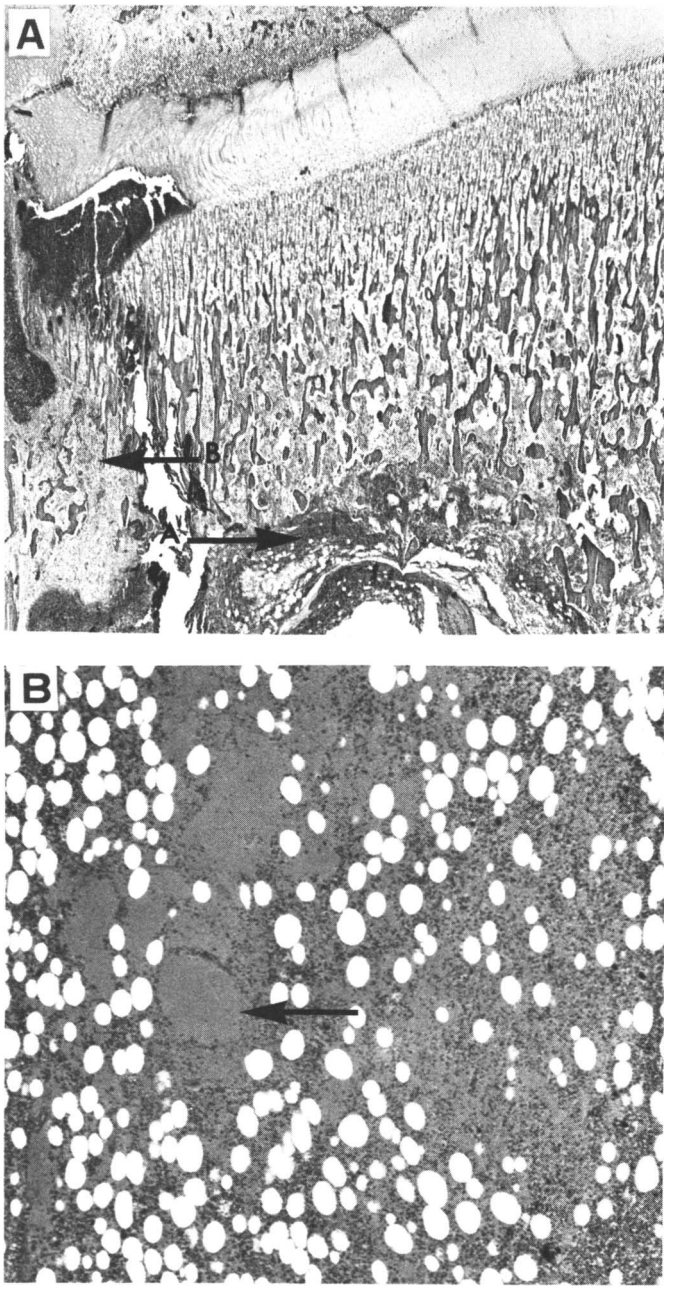
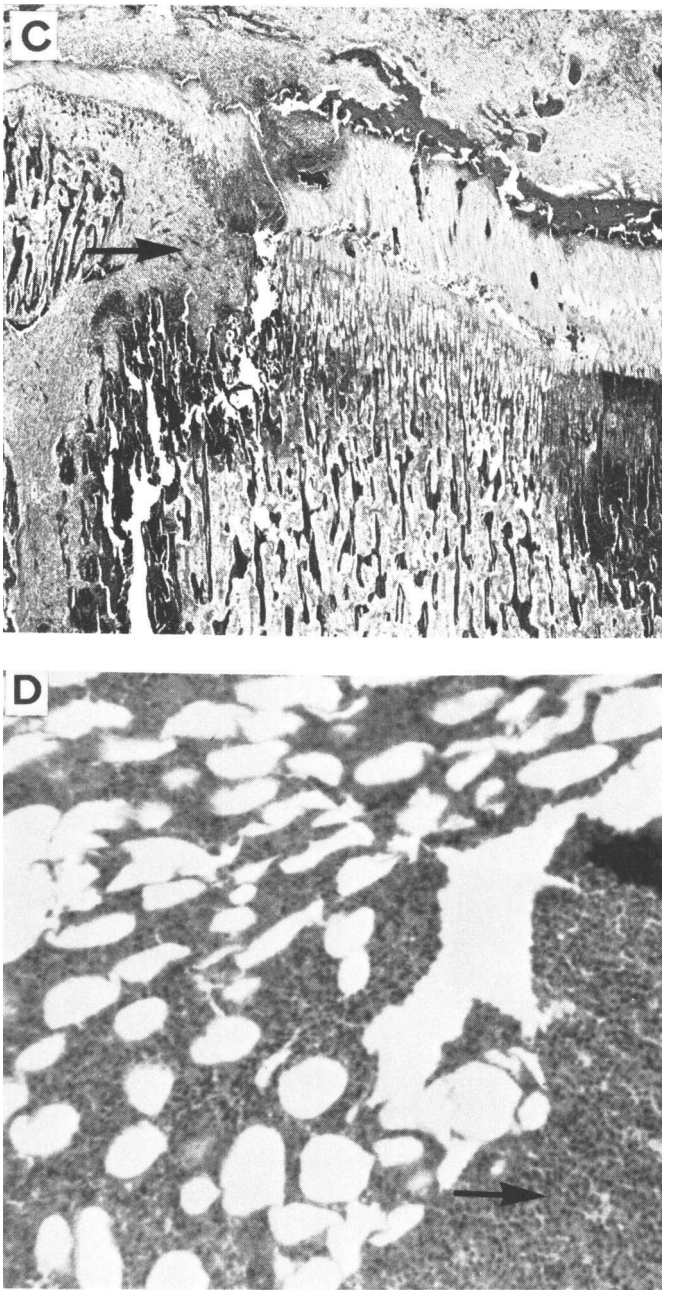


PLATE 2. Histologic sections (hematoxylin and eosin) of changes in the tibia in experimental *Staphylococcus aureus* osteomyelitis are shown. (A) On the fifth day after infection, microabscesses at the site of injection (arrow A) and at the lateral aspect of the epiphysis (arrow B) distort bony architecture. ×10. (B) Acellular zones of edema (arrow) with adjacent areas of inflammation distort the marrow of the mid-shaft of the tibia on the 10th day after infection. Staphylococci were isolated from inflammatory but not edematous zones. ×15. (C) By the 15th day of infection, erosion of the lateral portion of the epiphyseal plate (arrow) is complete and microabscesses have spread over both the secondary ossification center and metaphysis. ×10. (D) On the 15th day, the distal shaft of the tibia shows replacement of fatty marrow by inflammatory infiltrates and microabscesses (arrow). ×30. (E) On the 20th day of infection, complete distortion of the epiphysis and metaphysis by abscesses has occurred. A large sequestrum is present (arrow). ×7.5.



PLATES 2C AND D

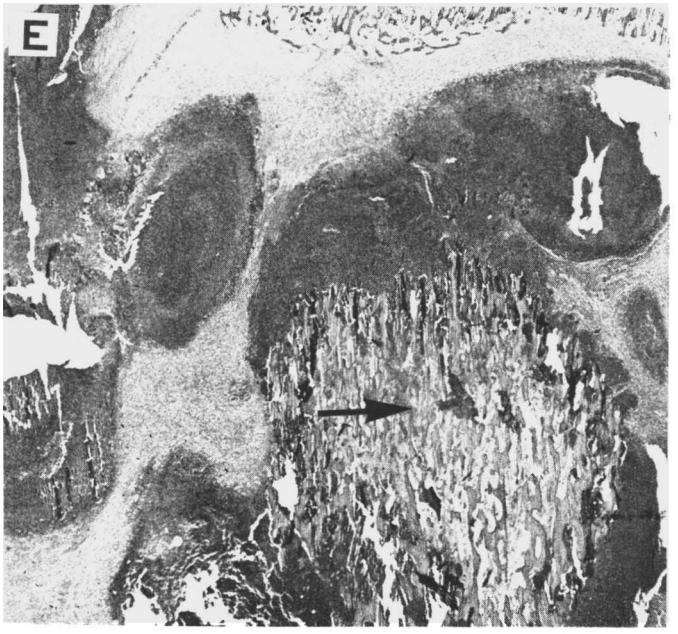


PLATE 2E

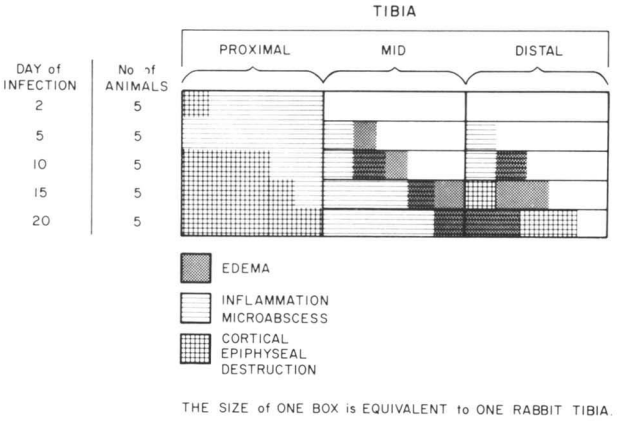


FIG. 1. Infection began with inflammation and microabscesses at sites of inoculation in proximal tibiae. By the second day, there was destruction of the bone of the epiphyseal cortex. Zones of edema preceded changes in the shaft of the tibia. By the 20th day, every portion of proximal, mid-, and distal tibiae was affected.

by isolations of staphylococci from tibiae) comprise this study.

Bacteriologic and pathologic findings. At aspirations from infected proximal tibiae, *Staphylococcus aureus* was always isolated from the 2nd to the 20th day. On the other hand, distal tibiae from each of five rabbits sacrificed on both the second and fifth days of infection were negative. By the 10th and 15th days, two of five distal aspirates of each of these groups were positive for staphylococci. On Day 20, distal tibiae from four of five rabbits yielded staphylococci.

When sodium morrhuate alone was injected into the medullary cavity, trabeculae degenerated and dense sclerosis had replaced normal fatty marrow by Day 20 (Plates 1A-C). After sodium morrhuate and *Staphylococcus aureus* SMH, microabscesses appeared at the sites of injection, distorting normal architecture (Plate 2A). Exudates, predominantly of polymorpho-

nuclear leukocytes, surrounded abscesses, and the former, in turn, were preceded by advancing zones of eosinophilic, acellular edema (Plate 2B).

Even in infected legs, normal-appearing bone was sterile (12×). Only occasionally did aspirates from areas of edema yield staphylococci (1 of 5×). On the other hand, inflammatory exudates and microabscesses always contained staphylococci. Infection proceeded by direct extension both proximally and distally. Eventually, the entire length of the tibia was affected. Later the zones of edema were replaced by exudates and abscesses (Fig. 1). By the fifth day, large areas of proximal tibiae showed destruction of the epiphyseal cortex, and 10 days later, although less extensive, these changes could be seen in the distal epiphysis (Fig. 1). At this time (Day 15) erosions of the lateral epiphyses were usually complete (Plates 2C and D). By the 20th day epiphyses were often completely destroyed and large sequestra were common (Plate 2E).

Radiographic findings. No radiographic changes were seen in any of the noninjected control tibiae. Minor lytic changes were seen in the proximal tibiae of sodium morrhuate-injected controls, but pathologic scores were low, and a progressive increase in their severity did not ocur after the fifth day (Table I).

TABLE I. MEAN PATHOLOGIC SCORES BY XERORADIOGRAPHY IN RABBIT TIBIAL OSTEOMYELITIS.

Day of infection	Number of animals	Number of criteria examined	Mean pathologic score	
			Infected rabbits	Control rabbits
2	5	8	2	1.0
5	5	8	4.6	1.2
10	5	8	10.4	1.2
15	5	8	14	1.2
20	5	8	18.2	1.2

On the other hand, pathologic scores among infected tibiae increased daily (Table I). Lytic changes, sequestrum, periosteal reaction, obliteration of the medullary cavity, cortical thickenings, joint effusion, soft tissue swelling, and involucrum appeared, one following the next (Table II).

The appearance of progressively worsening radiologic changes correlated with sequential bacteriologic, gross, and microscopic findings (Table II; Fig. 1). On Day 2, the lytic changes in infected extremities at injection sites could not be distinguished from their morrhuate controls. By Day 5, most xeroradiographs showed extension of the lytic process and periosteal elevation at the proximal tibia. Joint effusion was frequently present (Plate 3A). Extensive obliteration of the medullary cavity and periosteal elevation were easily seen by xeroradiographs on Day 10 (Plate 3B). The extent of medullary involvement correlated with the extent of the zone of inflammatory edema which was seen at anatomic examination (Fig. 1). By Days 15 and 20 marked sequestra and involucra, pathologic fractures, and periosteal changes were present (Plates 3C and D). Distal tibiae were affected at xeroradiography and this was confirmed at autopsy in four of five animals by Day 20 (Fig. 1 and Plates 3C and D).

Discussion. We have used bacteriologic, pathologic, and xeroradiographic techniques to define further the progression of experimental staphylococcus osteomyelitis in the rabbit tibia. Originally, Norden found bacteremia to be limited to Day 1, but leukocytosis and an elevated rate of sedimentation of erythrocytes persisted for at least 20 days (1).

The present studies indicate that a microabscess formed in the proximal tibia at the site of injection of *Staphylococcus aureus* SMH with the sclerosing agent sodium morrhuate. Acellular edema followed by

TABLE II. EARLIEST RECOGNITION TIME BY XERORADIOGRAPHY IN RABBITS WITH TIBIAL OSTEOMYELITIS.

Pathologic finding							
Lytic changes	Sequestrum	Periosteal reaction	Obliteration of medulla	Cortical thickening	Joint effusion	Soft tissue swelling	Involucrum
1[a]	3.5	3.6	3.8	3.9	4.1	5.1	5.4

[a] Values are mean numbers of days.

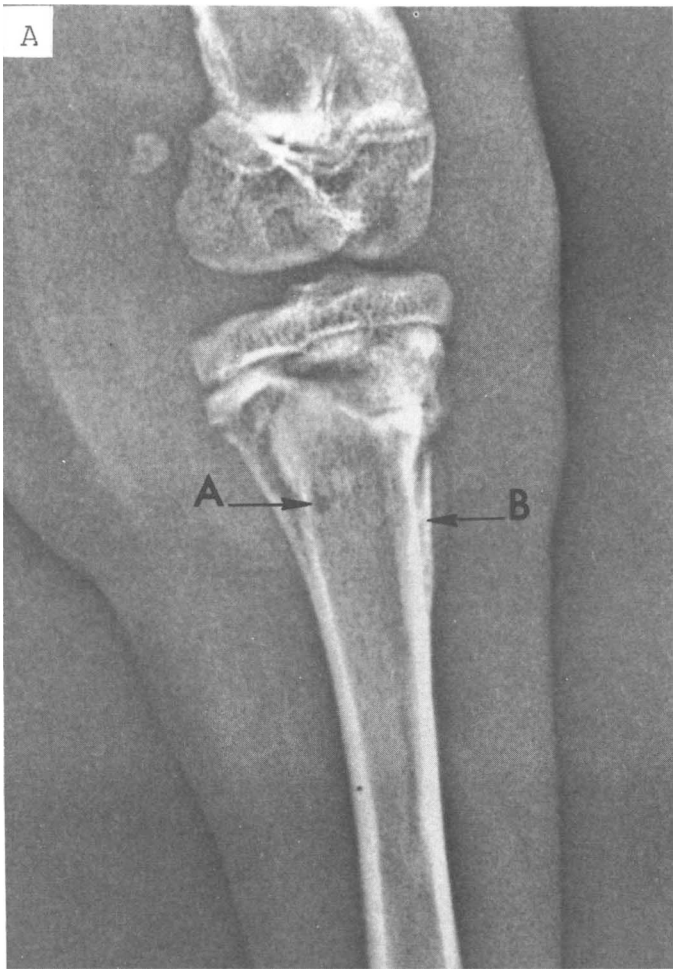


PLATE 3. *Staphylococcus aureus*, strain SMH, osteomyelitis of the rabbit tibia is demonstrated by xeroradiography on the 5th, 10th, 15th, and 20th days after infection. Although changes were noted on day 2 of infection, they were minimal (Table II). (A) Day 5 of *Staphylococcus aureus* infection of the proximal tibia is shown. Periosteal and lytic changes are easily seen. Joint effusion is present. The pathologic score is 11. The site of inoculation of sodium morrhuate in staphylococci in the proximal tibia is indicated by arrow A. Periosteal elevation is indicated by arrow B. (B) On Day 10 of infection joint effusion is still present. Sequestra (e.g., medial superior tibia), lytic changes, periosteal reactions, soft tissue swelling, joint effusion, and obliteration, along with extensive disease within the medullary cavity, are evident. Changes in the distal medullary cavity are noted. The pathologic score is 14. A sequestrum at the proximal tibia (arrow A) and the extent of disease in the distal tibia (arrow B) are indicated. Microabscess and inflammation were found in the autopsied distal tibia (Fig. 1). (C) On Day 15 of infection a large sequestrum is present at the metaphysis (arrow A). There is a cortical sequestrum along the medial aspect of the distal one-third of the diaphysis and a marked periosteal reaction just above (arrow B). A pathologic cortical fracture is outlined by xeroradiogram alone (arrow C). Erosion of the lateral portion of the epiphyseal plate is evident, and was confirmed at autopsy. The involvement of the medullary cavity along its entire length is obvious. The pathologic score is 12. (D) Day 20 of infection is shown. Periosteal new bone formation (involucrum at proximal tibia, arrow A) and elevation of the periosteum by subjacent abscesses (arrow B) are remarkable at the middle one-third of the shaft in this totally involved bone. The pathologic score is 27.

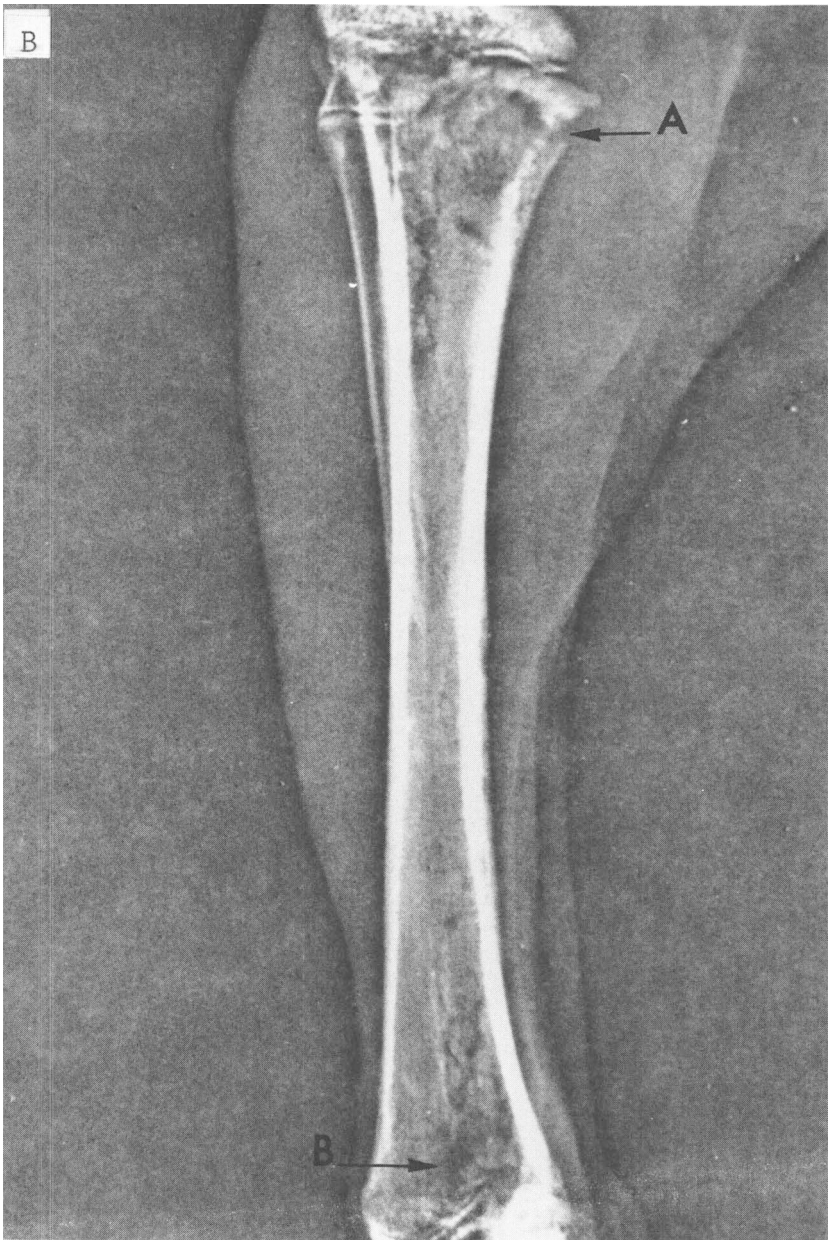


PLATE 3B

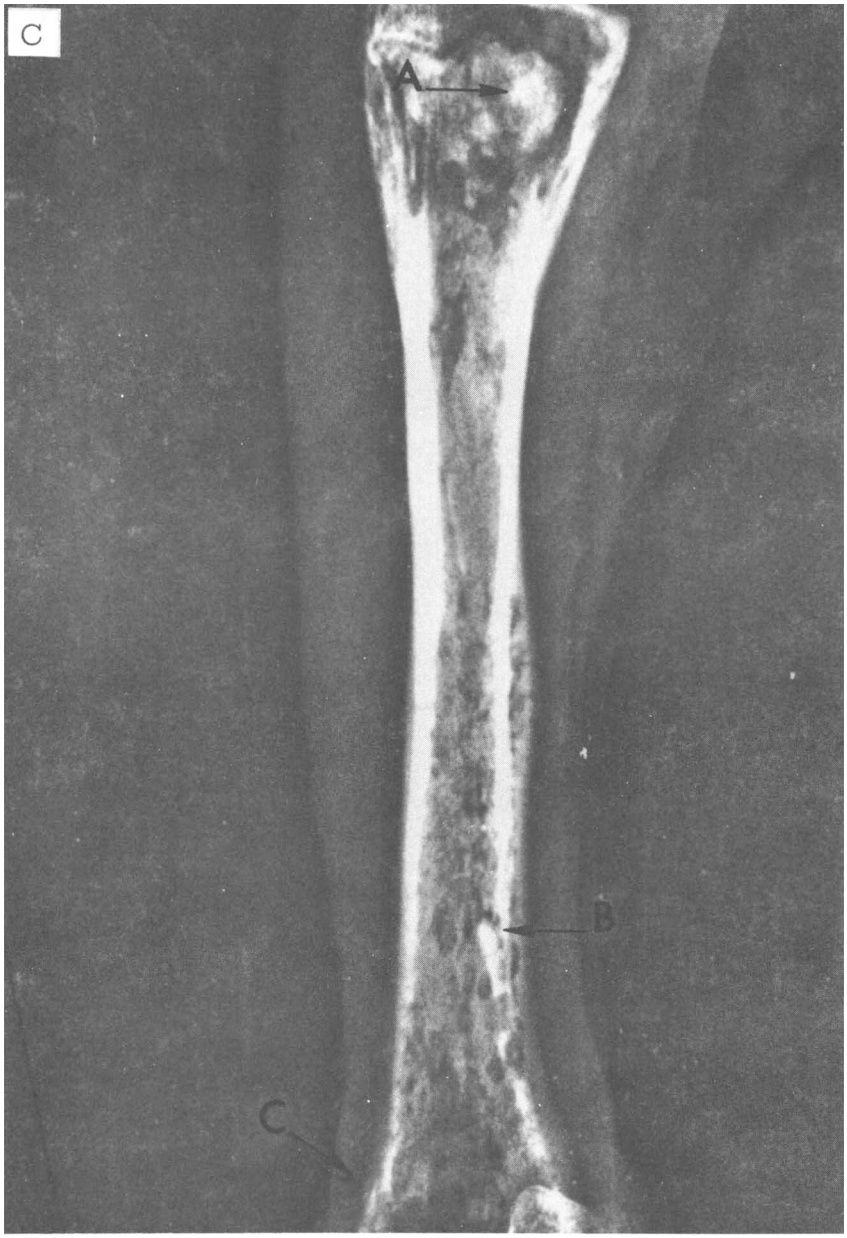


PLATE 3C

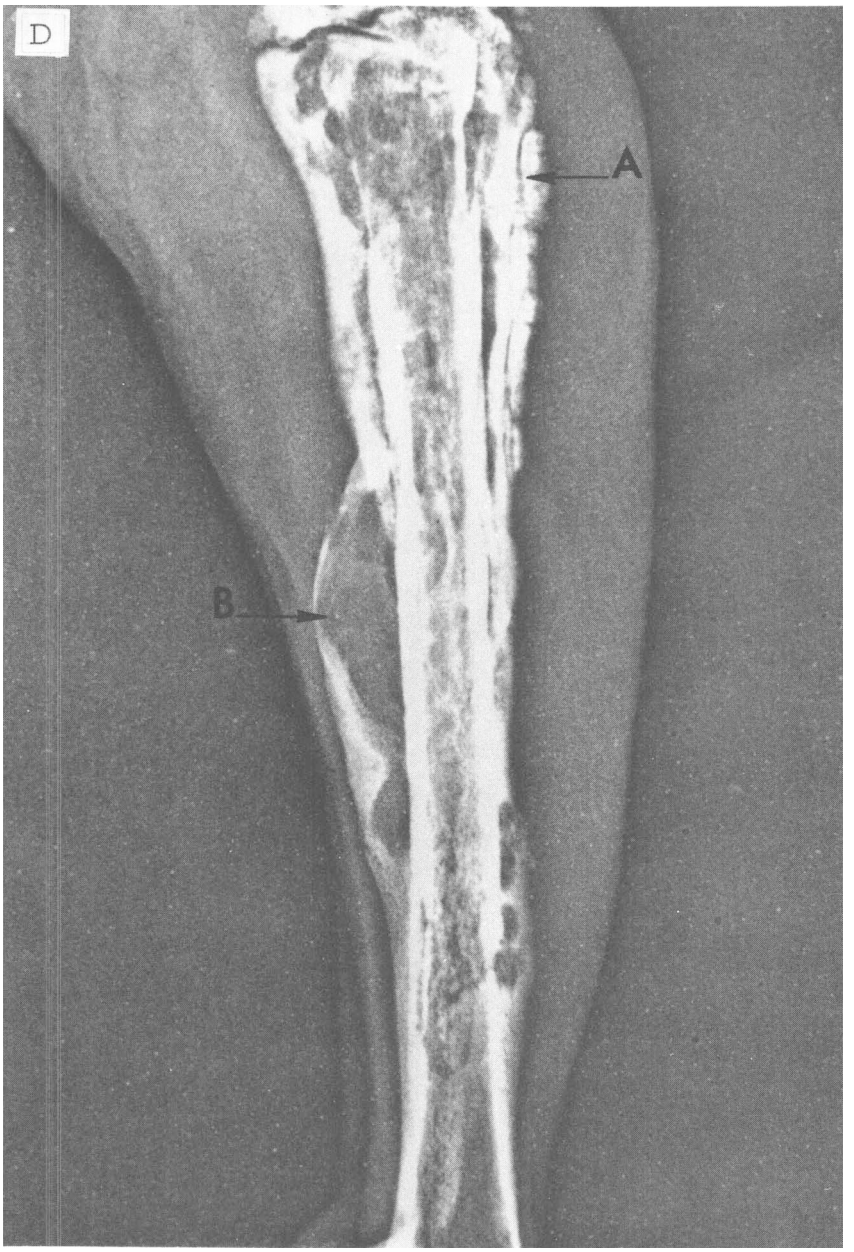


PLATE 3D

polymorphonuclear exudates and, finally, abscesses spread contiguously toward the metaphysis and down the shaft. Edematous areas were sterile, but exudates and abscesses contained staphylococci. On the fifth day after infection disease was limited to the proximal tibia, but 5 days later two-thirds of the length of the tibia was affected. Ultimately (Day 20) most of this long bone was replaced by abscesses interspersed with involucra and sequestra.

Norden did not study radiographic findings systematically, but he observed changes of osteomyelitis in this model by use of X ray on the 14th day after infection. Sequestra were observed on Day 40. We noted definite radiographic changes of osteomyelitis within the first week of disease and correlated microbiologic and autopsy findings. Radiologic and serial pathologic scoring as done here is a useful means of assessing progression or response of this disease to antibiotic therapy in this model (3–5).

Summary. Detailed roentgenographic, pathologic, and bacteriologic findings of a widely used rabbit model of staphylococcus osteomyelitis have not been reported. We induced post-traumatic staphylococcus tibial osteomyelitis in 25 young New Zealand rabbits. Abscesses formed at sites of inoculation, and infection spread contiguously, both superiorly and caudally to eventually involve the entire tibia. An outer advancing zone of edema was followed by an inflammatory mid-zone and a proximal zone of abscess. Progressive bony destruction produced pathologic fractures, sequestra, and involucra. By the 20th day of infection, the entire bony architecture was replaced by contiguous abscesses. Serial xeroradiographs correlated well with progressing pathologic findings. A definitive radiographic diagnosis was possible on the fifth day of infection.

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