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EXPERIMENTAL OSTEOMYELITIS DUE TO *STAPHYLOCOCCUS AUREUS* OR *PSEUDOMONAS AERUGINOSA*: A RADIOGRAPHIC–PATHOLOGICAL CORRELATIVE ANALYSIS

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Summary.—A previously-described experimental model of bacterial osteomyelitis was used to investigate systematically the sequential radiographic and histopathological changes in the tibias of rabbits infected with either *Staphylococcus aureus* or *Pseudomonas aeruginosa*. The radiographic changes induced by both organisms were progressive, increasing in severity from the first to the fourth week after infection. The severity and extent of radiographic changes, especially that of bone destruction, were significantly greater for tibias infected with *S. aureus*. Histopathologically, staphylococcal disease was a severe, rapidly progressive purulent infection which led to extensive destruction of marrow and cortical bone, formation of sequestra, and frequent extraosseous extension. Disease due to *P. aeruginosa* was more indolent and less destructive, leading to earlier healing and no extraosseous extension. The sequential radiographic and pathological changes observed with this experimental model closely resemble those described in man and suggest that this model may be useful for future investigations of pathogenesis and therapy.

AN EXPERIMENTAL MODEL of osteomyelitis caused by *Staphylococcus aureus* has been used extensively by Norden (1970, 1971, 1975, 1978) to study the efficacy of various antibiotic programmes for treatment of this disease. Experimental osteomyelitis caused by *Pseudomonas aeruginosa* has also been described by van Wingerden, Lolans and Jackson (1974) and by Norden and Keleti (1980) and used to measure similar parameters of therapy. Both models employ similar techniques to produce the disease state; it was observed by Norden and Keleti (1980) that osteomyelitis caused by *P. aeruginosa* appeared less severe with respect to mortality, clinical illness of the animal, and formation of sequestra than did osteomyelitis caused by *S. aureus*.

These preliminary observations led to a more intensive study of the sequential pathological and radiographic changes that occur in animals with experimental osteomyelitis caused by either *P. aeruginosa* or *S. aureus*. In addition, the changes produced in the rabbit were compared with the pathological and radiographic changes reported in humans with osteomyelitis in an attempt to determine more carefully how closely the experimental model mimics human disease.

MATERIALS AND METHODS

Production of osteomyelitis.—This technique has been described previously in detail by Norden (1970). Briefly, New Zealand white rabbits (weight 4 lb) received an intramedullary injection into the proximal tibia of sodium morrhuate and either 3×10^6 colony-forming units of *S. aureus* or a similar number of colonies of *P. aeruginosa*. Both strains have been described previously. As controls, certain rabbits

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received injections of sodium morrhuate only or buffer only, or either strain of bacteria only.

Culture of bone.—Animals were killed 7, 14 or 28 days after infections. All infections were documented by cultures which were obtained by techniques described previously by Norden (1978).

Pathological evaluation.—Specimens of tibia were immersed in 100 ml of 10% neutral phosphate-buffered formalin. Fixation was carried out at room temperature for at least 1 week. The entire specimen was then decalcified in 100 ml of 15% nitric acid with "Aerosol" wetting agent (Fisher Scientific) for 48 h. Four transverse sections were then prepared from each tibia one of which represented the proximal metaphysis, 2 sections the mid-shaft, and one the distal metaphysis. Approximately 20% of the bone length was included in these sections. The remaining tissue was not further processed or examined.

The tissue fragments were processed using routine histological procedures and embedded in paraffin. Each paraffin block was sectioned at 6 μm and stained with haematoxylin and eosin. Those blocks having marked acute inflammation were re-sectioned and stained with the Brown and Brenn (tissue Gram) technique of Luna (1968). Thus each bone was evaluated at 4 representative levels *via* a single transverse section from each of those levels.

Specimens were evaluated by one observer (RLM) without prior knowledge of the infecting strain or the time since inoculation. The following histological parameters were evaluated for each specimen and graded on a subjective scale of 1+ (mild) to 4+ (severe): Acute medullary inflammation with abscess formation, chronic intramedullary inflammation and fibrosis, subperiosteal new bone formation (involucrum), and extent of disease (one or more of the sections examined). Three other histological parameters were also quantitatively evaluated: presence or absence of sequestrum, bacterial colonies, and extraosseous extension of infection into soft tissues.

Radiographic evaluation.—Roentgenograms of the injected bones were performed shortly after killing. All roentgenograms were read by one observer (CWN) without knowledge of the infecting agent or the duration of infection. Four radiographic parameters (sequestrum formation, presence of periosteal new bone, presence of bone destruction, and the extent of involvement) were determined for each bone. Using the criteria detailed in Table I, a numerical score was assigned to each variable and the 4 scores were added together to give an overall ranking for radiographic severity.

Statistical methods.—Comparisons between mean radiographic severity scores were tested by a two-tailed Student's t test.

RESULTS

Radiographic findings

A sequential increase in severity of disease was observed in tibias infected with either organism as the interval between injection and killing was extended (Table II). The overall score for

TABLE I—*Roentgenographic criteria*

	Variable	Definition	Point score
I	Sequestrum formation	+ = present	1
		− = absent	0
II	Periosteal new bone formation	+ = present	1
		± = equivocal	0·5
		− = absent	0
III	Destruction of bone	+ + = severe, multiple areas involved	2
		+ = moderate, only one area involved	1
		± = mild, only one area involved	0·5
		− = no destruction	0
IV	Extent of disease: involvement of each of three areas (proximal, mid, and distal tibia)	+ = present	1
		± = equivocal	0·5
		− = absent	0

Overall score = sum of scores for Variables I, II, and III, plus sum of scores for each area of involvement (Variable IV). The maximum score attainable is 7.

TABLE II.—*Results of radiographic evaluation*

Material inoculated	No. animals studied	Days after inoculation	Radiographic parameters: Sequestrum formation +	Sequestrum formation −	Periosteal new bone +	Periosteal new bone ±	Periosteal new bone −	Bone destruction 2+	Bone destruction +	Bone destruction ±	Bone destruction −	Extent of disease* Prox.	Extent of disease* Mid.	Extent of disease* Dist.	Overall score (mean ± s.d.)
S. aureus	5	7		5	3	1	1		3	2		5/5	4/5	1/4	3·1 ± 1·1
+ morrhuate	3	14		3	3			1	2			3/3	3/3	2/3	4·8 ± 0·6
	6	28	4	2	5	1		4	2			6/6	6/6	6/6	6·3 ± 1·2
P. aeruginosa	4	7		4	3		1		1	3		4/4	2/4	0/4	2·1 ± 1·3
+ morrhuate	4	14		4	4				3	1		4/4	3/4	1/4	3·6 ± 1·2
	7	28	4	3	6	1			6	1		7/7	7/7	3/7	4·7 ± 1·2
Buffer only	2	7		2			2				2			2	0
Morrhuate only	2	7		2			2				2			2	0
S. aureus only	2	7		2			2				2			2	0
P. aeruginosa only	2	7		2			2				2			2	0

* Number with (+) or (±) over total tested.

rabbits infected with staphylococci was significantly greater for those killed 28 days after injection than those killed 7 days after injection ($P < 0{\cdot}01$); there was also a trend, which does not achieve statistical significance, present by 14 days (Fig. 1). Similarly, the overall score for rabbits infected with *P. aeruginosa* is significantly greater for animals killed 28 days after injection than for those killed 7 days after injection ($P < 0{\cdot}01$; Fig. 2). The observation that the severity of the disease increased with time was least obvious for the variable of periosteal new bone formation; it was more clearly present for sequestrum formation, bone destruction, and the extent of bone involved.

A comparison of the overall scores for the 2 infecting organisms shows a significantly higher overall score for rabbits infected with staphylococci and killed 28 days after injection when compared to rabbits infected with *P. aeruginosa* and killed 28 days after injection ($P = 0{\cdot}025$). The overall scores for animals infected with staphylococci and killed at either 7 or 14 days after injection are higher than the respective scores for animals infected with *P. aeruginosa* and killed at comparable times, but do not achieve statistical significance ($P > 0{\cdot}1$) for either time. The frequency of sequestrum formation is comparable for the 2 infecting organisms and was generally not present in roentgenograms taken 7 or 14 days after injection. By 28 days after injection, the majority of animals infected with either organism demonstrated sequestra. The development of periosteal new bone formation was also similar for the 2 infecting organisms. The major roentgenographic differences, which account for the increased severity observed with staphylococci, were the presence of more severe bone destruction and the greater extent of bone destruction with more frequent involvement of the mid and distal shaft of the tibia in rabbits infected with staphylococci.

All control animals, injected with either sodium morrhuate, buffer, staphylococci or *P. aeruginosa* only, and killed 7 days

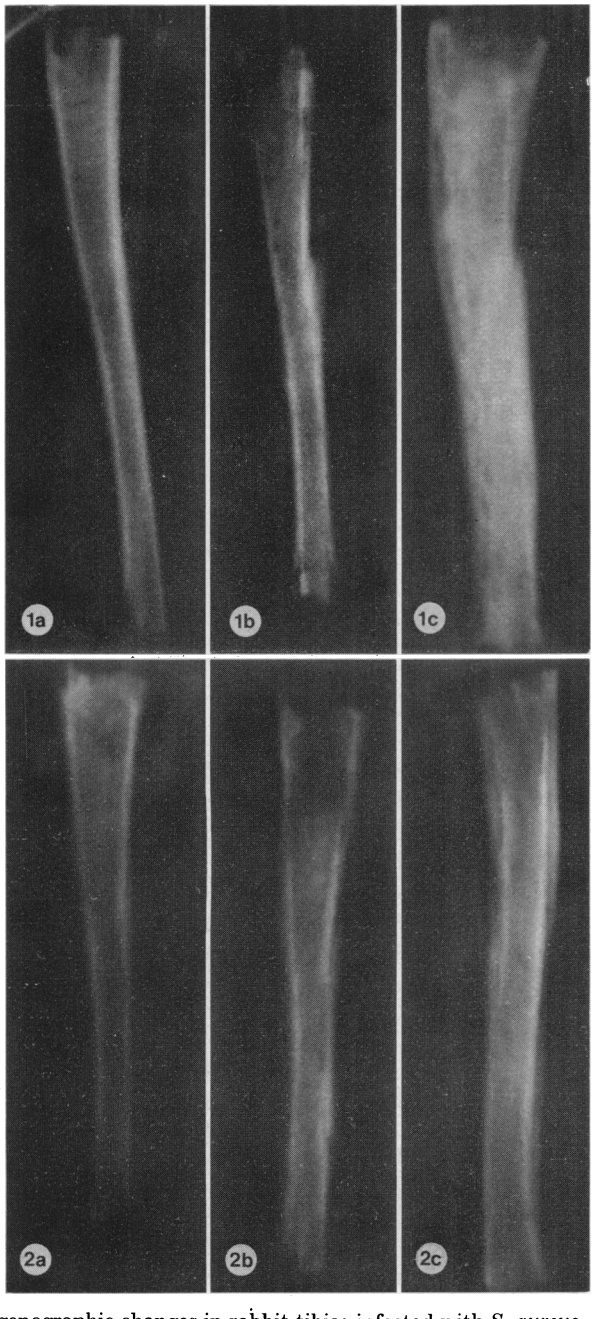


FIG. 1.—Serial roentgenographic changes in rabbit tibias infected with *S. aureus*. An uninfected bone is shown for comparison in (a). The specimen examined at 7 days after injection (b) shows moderate bone destruction and periosteal new bone formation involving the proximal and mid-shaft. At 28 days after injection (c), extensive sequestrum formation is easily seen as well as severe bone destruction and new bone formation involving the entire shaft.

FIG. 2.—Serial roentgenographic changes in rabbit tibias infected with *P. aeruginosa*. At 7 days after injection (a) mild bone destruction and periosteal new bone are seen predominantly in the proximal tibia. The specimen examined at 14 days after injection (b) shows somewhat more severe bone destruction involving the proximal and mid-shaft. At 28 days after injection (c) sequestrum formation and moderate bone destruction involving the proximal and mid-shaft are present.

after injection, showed no roentgenographic changes.

Pathological findings

Tibias infected with staphylococci all developed extensive acute destructive osteomyelitis. At 7 days, large intramedullary abscesses were evident in the proximal tibia near the inoculation site (Fig. 3) already associated with periosteal new bone formation at that level and clusters of Gram-positive cocci within the abscesses and within the haversian canals of adjacent cortical bone (Fig. 4). Extraosseus extension and sequestra were each visible in 2 of 7 specimens. By Day 14, intramedullary acute inflammation and periosteal new bone formation was present at all levels of the tibia (proximal and distal metaphysis plus mid-shaft). Extensive destruction of cortical bone had occurred with sequestrum formation in all 5 specimens (Fig. 5). Large clusters of Gram-positive cocci were seen within and around the sequestra. Extraosseous extension into soft tissues was also apparent in 2 of 5 specimens. At Day 28, medullary inflammation was still acute in most areas and almost the entire cortical bone was destroyed in all 3 specimens. The thickness of the periosteal new bone was markedly increased (Fig. 6). Extraosseous extension was apparent in 2 of 3 specimens. The proximal and distal metaphysis showed no evidence of fibrosis (healing), although foci of fibrosis were seen within sections from the mid-shaft area.

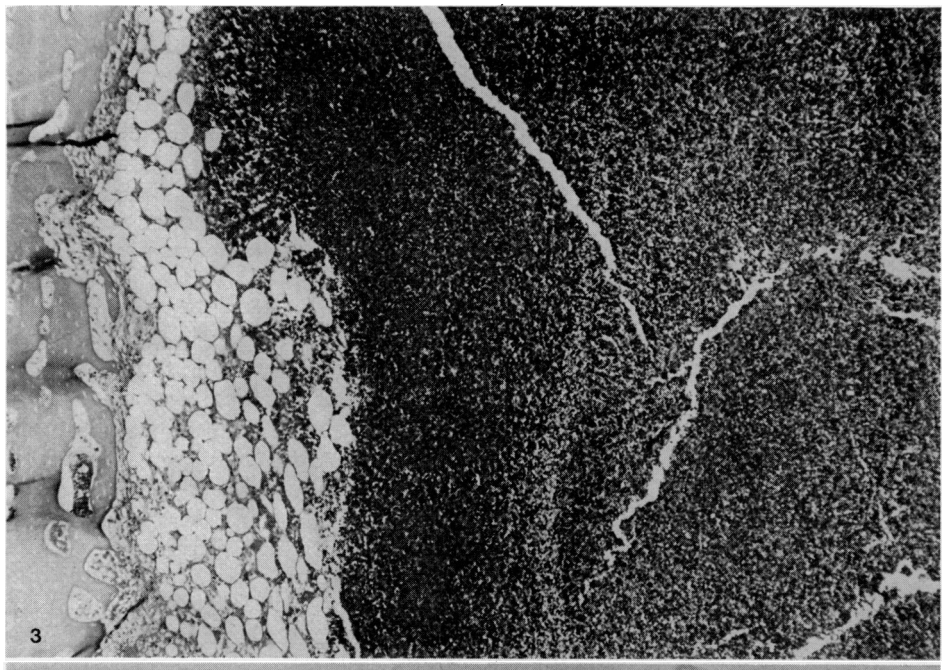
In contrast to tibias infected with staphylococci, those infected with *P. aeruginosa* showed a more indolent, less destructive and less extensive form of osteomyelitis. At 7 days, only mild endosteal acute and chronic inflammation with minimal periosteal new bone was evident in the proximal tibia near the inoculation site in all 4 specimens. Most of the marrow fat was still preserved at this time and no abscess was apparent. By the 14th day, the infecting process involved most of the proximal and distal tibias. Again, the inflammation was a mixture of acute and chronic inflammatory cells located exclusively around the endosteum with preservation of marrow elements in the central areas of the medulla. Periosteal new bone associated with extensive chronic inflammation and granulation tissue of the periosteum (Fig. 7) was apparent in most sections. Although the entire circumference of cortical bone was surrounded by endosteal and periosteal inflammation, actual sequestrum formation was only present in 2 of 5 specimens. At 28 days, all 4 specimens showed extensive medullary fibrosis and periosteal new bone formation (Fig. 8). Small foci of residual chronic active inflammation were apparent in some metaphyseal sections. In contrast to tibias infected with staphylococci, those infected with *P. aeruginosa* never demonstrated extraosseous extension and no Gram-negative bacilli were seen in numerous sections stained by the Brown and Brenn method.

The differences in histopathological alterations between tibias infected with *S. aureus* as compared to those infected with *P. aeruginosa* were so striking that a correct prediction of the infecting agent was possible in virtually every specimen without prior knowledge of which agent had been used and without observing the Brown-and-Brenn-stained sections.

The control tibias inoculated with only buffer or sodium morrhuate or *S. aureus* or *P. aeruginosa* showed no evidence of active inflammation. Those inoculated with sodium morrhuate or *P. aeruginosa* showed a small focus of medullary fibrosis at the inoculation site.

DISCUSSION

The results of this patho-radiographic correlative study indicate that experimental osteomyelitis due to *S. aureus* is a severe, rapidly progressive purulent infection which leads to extensive destruction of marrow and cortical bone with large amounts of sequestrum formation and frequent extraosseous extension. Experimental osteomyelitis due to *P. aerugi-*



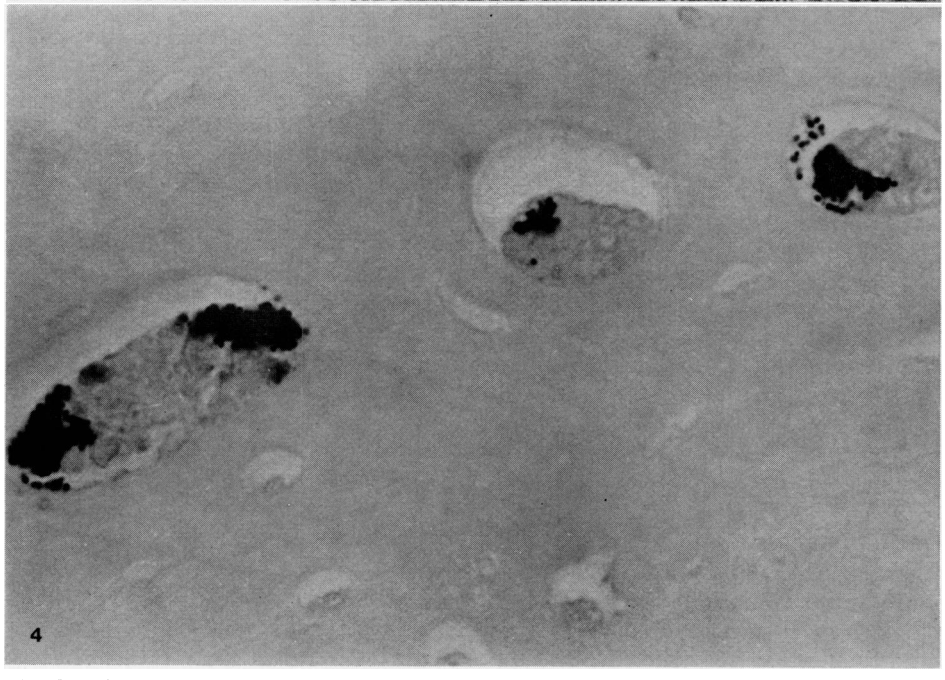


FIG. 3.—Proximal tibia infected with *S. aureus* at 7 days after injection. Almost the entire medulla is replaced by a large suppurative abscess. (Haematoxylin and Eosin, × 36.)

FIG. 4.—Cortical bone of a proximal tibia infected with *S. aureus* at 7 days after injection. Gram positive cocci in clusters are present within haversian canals. (Brown and Brenn, × 600.)

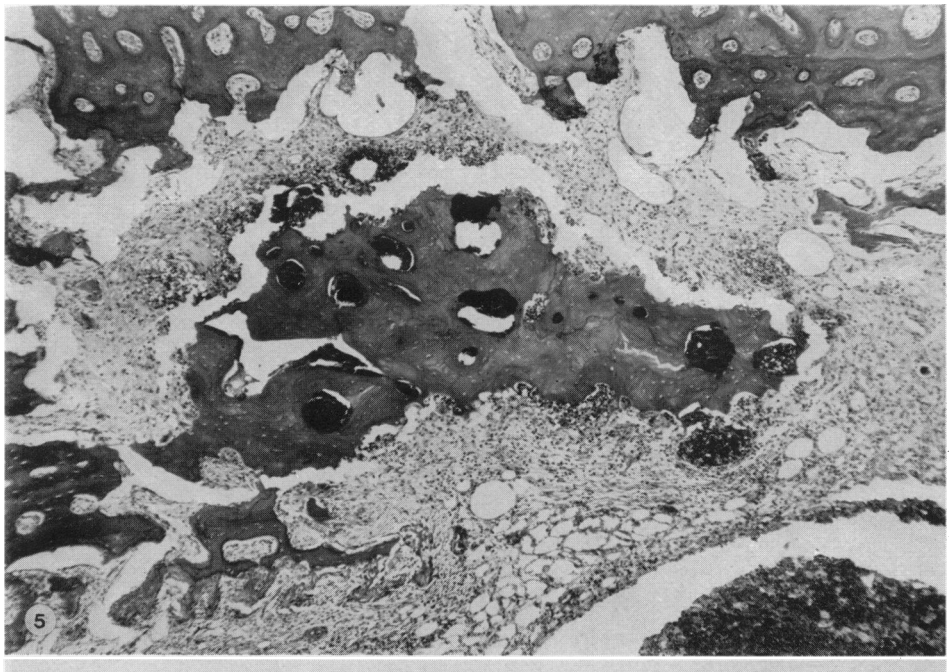


FIG. 5.—Distal tibia infected with *S. aureus* at 14 days after injection. A large fragment of necrotic cortical bone (sequestrum) is present beneath the periosteal new bone and is surrounded by chronic active inflammatory infiltrate. (Haematoxylin and Eosin, × 36.)

FIG. 6.—Transverse section of a proximal tibia infected with *S. aureus* at 28 days after injection. Note the markedly thick periosteal new bone (involucrum) which surrounds the complete circumference of bone. (Haematoxylin and Eosin, × 10.)

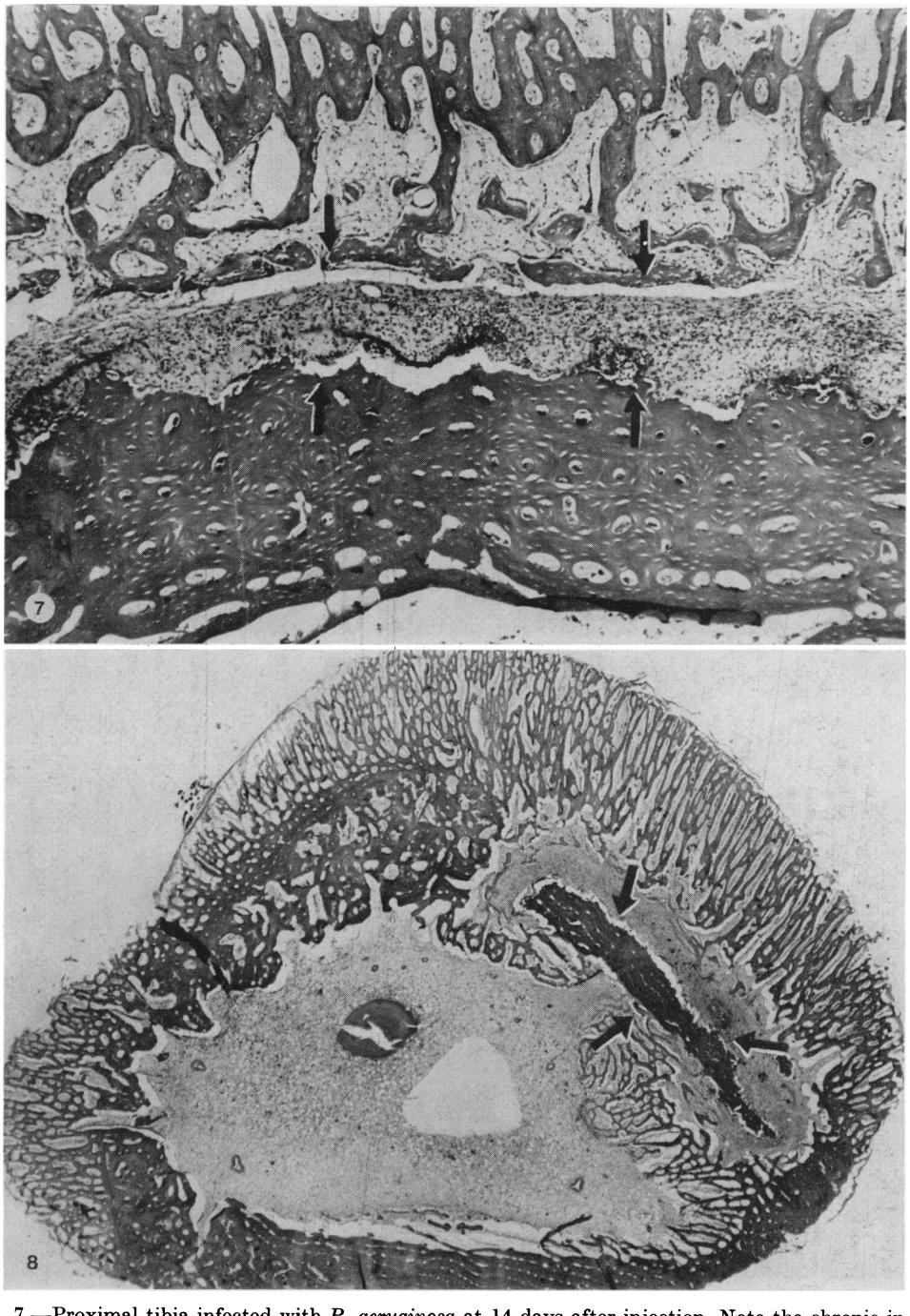


FIG. 7.—Proximal tibia infected with *P. aeruginosa* at 14 days after injection. Note the chronic in-inflammation and granulation tissue between the periosteum and cortical bone (arrows). (Haematoxylin and Eosin, × 36.)

FIG. 8.—Transverse section of an entire tibia infected with *P. aeruginosa* at 28 days after injection. Extensive fibrosis is present within the medulla and surrounds a focus of necrotic cortical bone (sequestrum—arrows). (Haematoxylin and Eosin, × 10.)

nosa, on the other hand, is a more indolent and less destructive infection which leads to earlier healing and never results in extraosseous extension.

Experimental osteomyelitis, produced in the rabbit by the injection of a sclerosing agent and bacteria, has been used as a model by Norden (1971, 1975, 1978) to test the efficacy of various antibiotic regimens and by Mader *et al.* (1978) to test hyperbaric oxygen. Although some previous studies by van Wingerden *et al.* (1974), Crane *et al.* (1977), Scheman, Janota and Lewin (1941) and Stevens (1966) have described the radiographic changes noted in the present study, and one study by Crane *et al.* (1977) has described some detailed histological changes, there has been no previous attempt to examine systematically the sequential radiographic and pathological changes which occur in this model when 2 distinctly different organisms (*S. aureus* and *P. aeruginosa*) are used. Clear differences emerged in the radiological severity produced by the 2 organisms; significant differences in pathological appearance allowed the observer to determine correctly the infecting agent in all cases without prior knowledge of the organisms injected.

The radiographic changes showed progressive increase in severity as the period between infection and killing extended from 7 to 28 days. At each time period, the X-ray picture seen with *S. aureus* was more severe than that produced by *P. aeruginosa*. For both organisms, sequestrum formation was not seen radiographically until after 14 days and the earliest change noted was periosteal new bone formation. The major difference in severity in the X-ray patterns produced by the 2 organisms was the increased amount of bone destruction seen with *S. aureus* and the greater extent of bony involvement. By 28 days, essentially all tibias infected with *S. aureus* showed bone destruction involving the entire length of the tibia while *P. aeruginosa* involvement of the distal tibia occurred in less than half.

The histopathological observations closely paralleled those made by radiography. Sequestrum formation occurred at 7 days in 2 of 7 tibias infected with *S. aureus* despite the absence of this change in radiographs at this time. Otherwise, the sequential changes seen by radiography correlated with virtually identical changes by histopathology. The pathogenesis of spread of osteomyelitis from an initial focus to other areas of an involved bone is probably related to contiguous intramedullary spread by direct extension. However, the observation that clusters of Gram-positive cocci were present in haversian canals may indicate that proximal and distal spread in a long bone *via* these canals within cortical bone may be an alternate method of spread. Jaffe (1972) has suggested that intraosseous spread *via* haversian canals may be an important mechanism in human osteomyelitis.

If an experimental model is to have relevance to human disease, it should resemble the clinical entity seen in man. The sequential changes seen on the X-ray beginning with periosteal new-bone formation and proceeding to bone destruction and ultimately sequestrum formation closely parallel the changes seen in man as described by Edeiken and Hodes (1973). The histological changes found in human osteomyelitis are also seen in the rabbit model. Of the 2 major elements, suppurative destructive necrosis is beautifully demonstrated with infection due to staphylococci and the second element of fibrous and bony repair is seen in the sections taken 28 days after infection with *P. aeruginosa*. Thus, by both radiographic and histological criteria, the experimental model of osteomyelitis, due either to *S. aureus* or *P. aeruginosa*, closely mimics human disease and offers an opportunity to study both pathogenesis and therapy and, hopefully, to extrapolate data from this model to clinical situations.

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