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Source: Journal of Wildlife Diseases, 17(2) : 277-287

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-17.2.277

PATHOBIOLOGY OF SEPTIC ARTHRITIS AND CONTIGUOUS OSTEOMYELITIS IN A LEATHERBACK TURTLE *(Dermochelys coriacea)*[1]

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Abstract: Analysis of a case of presumed hematogenous septic arthritis and osteomyelitis involving the elbow, distal humerus, and proximal radius and ulna in a leatherback turtle *(Dermochelys coriacea)* showed the chondro-osseous response to be similar to the diseases in skeletally immature humans and terrestrial mammals (both spontaneous and experimental). This particular reptile has bone that is similar to mammalian bone. The infection had partially destroyed the distal humeral, proximal ulnar and proximal radial joint surfaces and epiphyseal cartilages. The elbow was filled with a fibrovascular pannus that had caused a partial ankylosis of the joint.

INTRODUCTION

In the human, and presumably in other mammalian species, osteomyelitis affecting the developing (immature) skeleton is an extremely different disease from that which affects the adult (mature) skeleton.[2,9,12,13] While some studies are available discussing the pathobiology and histologic changes of the various stages of osteomyelitis and septic arthritis in the human, comparable data in other mammals are basically limited to some early experimental studies based on a nonphysiologic inoculation of bacteria into the marrow cavity after sclerosis with sodium morrhuate.[10,13] Recently, two of the authors have succeeded in reproducing hematogenous osteomyelitis in skeletally immature rabbits and dogs by the injection of *Staphylococcus aureus* into either the aorta or the iliac artery.[6] This strongly suggests that hematogenous spread, rather than direct inoculation, may be an etiologic mechanism in most skeletally immature mammalian species, just as it is in the human. However, studies of naturally occurring osteomyelitis in immature or mature mammals, especially wild mammals are virtually nonexistent. Lack of appreciation of the disease mechanism and the subsequent, often devastating effect on skeletal growth may lead to misinterpretation of skeletal deformities. For example, while osteoarthritis was the alleged problem in a coyote × dog hybrid,[7] the severe changes of destruction of the femoral head, ankylosis of the knee, and severe hypertrophic overgrowth and ankylosis involving multiple foot joints in a limb presumably chewed free from a trap suggest hematogenously disseminated osteomyelitis and septic arthritis could also have caused some of the skeletal changes.[5]

[1] Supported in part by N.I.H. grants R01-HD10854 and K04-AM00300.

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From ongoing studies in our comparative skeletal develpment laboratory, one species of marine reptile, the leatherback turtle *(Dermochelys coriacea)*, has been found to be unique among turtles, and perhaps among living reptiles, in possessing a type of chondro-osseous morphology which closely parallels certain mammalian patterns.[5,15,18,19] A unique feature of this species' skeletal morphology is the presence of extensive vascular canals within large epiphyseal cartilages. These vessels communicate freely with the metaphyseal circulation across the growth plate, a feature found in no other extant turtle or reptile, and apparently limited primarily to larger mammals, and present for only a limited time (birth to 18 months) in humans.[5,11,19] A non-mammalian, but typical chelonian feature retained by the leatherback turtle is the presence of continued endochondral growth throughout life, primarily by maintaining large cartilaginous epiphyses which never form the secondary ossification center necessary for physiologic growth cessation (epiphyseodesis).

While processing a series of stranded sea turtles, we have had the unique opportunity to study an example of naturally occurring septic arthritis of the elbow and contiguous osteomyelities of the distal humerus and proximal radius and ulna in an adult leatherback turtle. Interestingly, the overall appearance of the disease, particularly the chronic response, was very similar to the disease process in the skeletally immature human.[9-15]

MATERIALS AND METHODS

This ca 250 kg leatherback turtle *(Dermochelys coriacea)* with a carapace length of 135 cm was obtained by the Marine Mammal Stranding and Study Center. The animal apparently died when hit in the head by a boat propeller. External flipper morphology of both the pectoral and pelvic limbs showed no recent or old external injuries. The flipper skin and the majority of the muscles were removed to expose the underlying skeleton of both pectoral limbs. No purulent material or soft tissue abcesses were found among the muscles. The elbow joint was disarticulated on the left side and found to be a perfectly normal joint. However, on the right side there was extensive scar formation and fibrous pannus completely replaced the joint, and established a fibrous ankylosis that permitted minimal motion. Each distal humerus, as well as the entire radius and ulna were sectioned longitudinally in the sagittal plane (Figures 1, 2). Examination of the split sections failed to discern any active infection or pockets of purulent material.

The normal and infected humeri, radii and ulnae were fixed in 10% formaldehyde, decalcified in 10% formic acid and embedded in paraplast. Sections were made at 6 to 10 μm and stained with hematoxylin and eosin (Figure 3). In addition selective bacterial and fungal stains were done.

RESULTS

The chondroepiphysis of the distal humerus was extensively eroded, especially on the trochlear side, with loss of epiphyseal cartilage, and gross extension of this loss down to the level of the physis in some areas (Figure 1B). An area about one cm^2 showed erosion of the infection into the metaphyseal bone (Figure 1D). Similarly, there was extensive loss of the chondroepiphyses of both the proximal radius and proximal ulna.

The metaphyseal bone of the distal humerus did not exhibit any gross overgrowth compared to the opposite side. However, the proximal metaphysis of both the radius and ulna showed extensive reactive overgrowth. Interestingly, this appeared to be both endochondral as well as membranous

overgrowth, which would make this reactivity to the infection somewhat different than in the human, in whom the primary response is usually membranous bone formation when the periosteum is elevated by the accummulation of subperiosteal purulent material.

The histologic findings were most revealing of the extent of chondro-osseous destruction (Figure 3). Virtually the entire cartilaginous proximal ulna, including articular cartilage, epiphysis, and physis, were replaced by the fibrovascular pannus filling the joint. Less severe destruction was evident in the radial epiphysis, with no extension of infection to the physis. The distal humerus showed invasion of the cartilage canals and extension of the infection at the margins of destruction. Segments of the physis were completely destroyed by direct extension.

After a week of culturing in aerobic, anaerobic and fungal media, there was no growth. Specific cultures for mycobacteria were not performed. Selective bacterial, acid fast and fungal stains failed to reveal any microorganisms. It was not possible to identify the microorganism responsible for this infection.

DISCUSSION

The occurrence of hematogenous dissemination of bacterial infections in reptiles and turtles is well documented.[8,17] The reptilian skeletal system is generally felt to be frequently exposed to disease,[16] yet neither osteomyelitis nor septic arthritis has previously been described in a turtle. In fact, we find no reports in the literature describing the pathobiology of osteomyelitis in any reptile, and only one previous description of septic arthritis occurring in a lizard *(Tupinambis teguixin)* caused by *Serratia marcescens*.[1]

Characteristically, bacteria affecting the developing skeleton reach either the bone or the joint by a hematogenous route, usually from a focus elsewhere in the body, such as a cutaneous infection, a tonsillitis, or a tooth abscess.[9,10,13] Bacteria normally found as bowel flora may occasionally enter the blood stream in sufficient quantity to cause osteomyelitis or septic arthritis, even in an otherwise normal individual.[11] Children unable to mobilize adequate cellular defense mechanisms may have significant problems with skeletal and joint bacterial infection.[2] Direct inoculation of microorganisms is an unusual etiology of septic arthritis or osteomyelitis in the skeletally immature human, although, in distinct contrast, it is the predominant etiology after skeletal maturity is attained.

Certain microorganisms have a characteristic pathobiology in the human. For example, *Staphylococcus aureus* normally reaches the metaphyseal sinusoids where the primary spongiosia is being formed from the growth plate (physis). It creates a focus of infection and then spreads from this area in a latitudinal fashion across the juncture between metaphysis and physis, and then goes through the porous metaphyseal cortex to reach either the subperiosteal space, or, in certain joints such as the hip, to enter the joint itself and create a contiguous septic arthritis. In contrast, *Hemophilus influenza* primarily infects developing joints, creating a septic arthritis and may subsequently invade the epiphysis or metaphysis to create a secondary osteomyelitis.

Certain microorganisms may inhabit certain species, often in a completely symbiotic fashion, and yet be pathogenic in another species. The bacterium, *Arizona hinshawii*, which is related serotypically to *Salmonella*, may cause osteomyelitis, but usually only in children debilitated by diseases such as sickle cell anemia. Interestingly, the

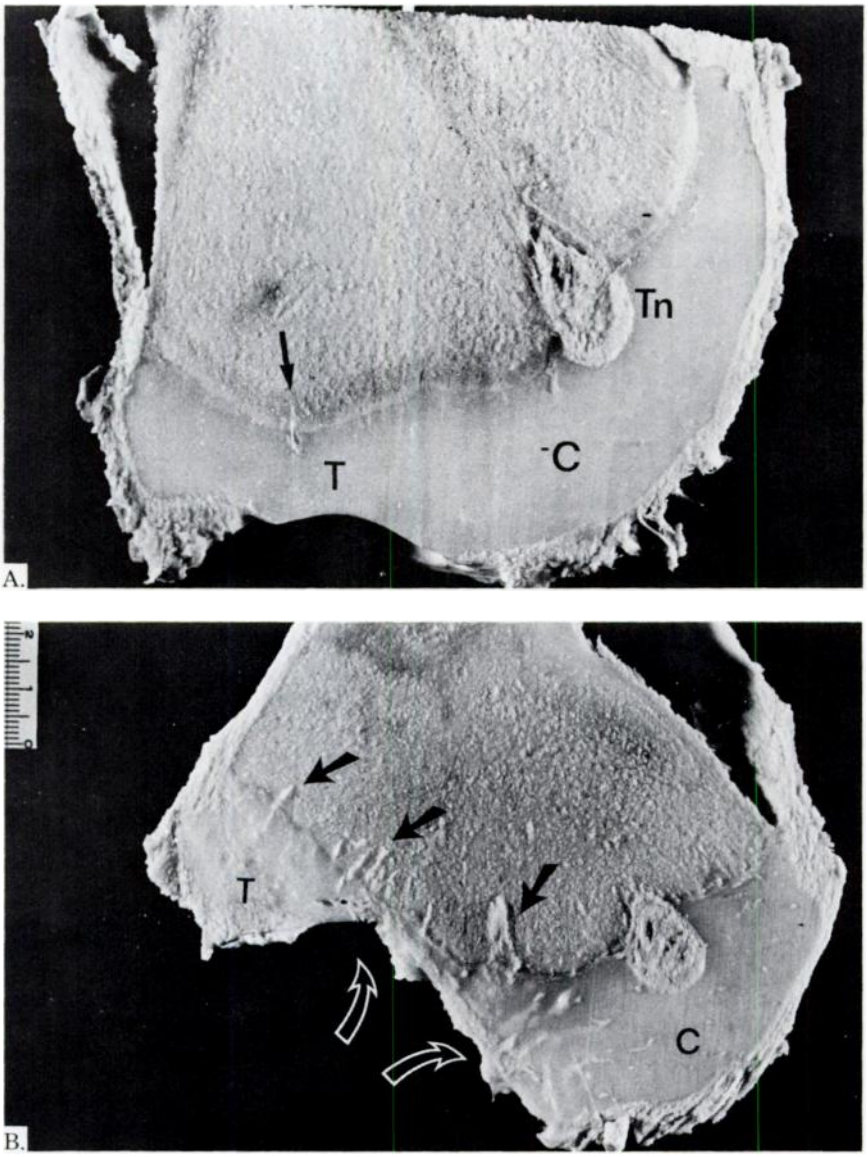


FIGURE 1. A. Normal left distal humerus showing contour of trochlea (T) and capitellum (C). A typical transphyseal canal can be seen in the trochlear region (arrow). Tn-tendinous tissue in foramen. B. Right side (reversed for comparison to the normal side) showing destruction of mid-portion of the trochlea (white arrows), while the capitellum is less severely involved. The vascularity crossing the physis is significantly increased (black arrows). The articular and epiphyseal cartilages have been destroyed in the trochlear mid-section, and replaced by fibrous tissue (removed

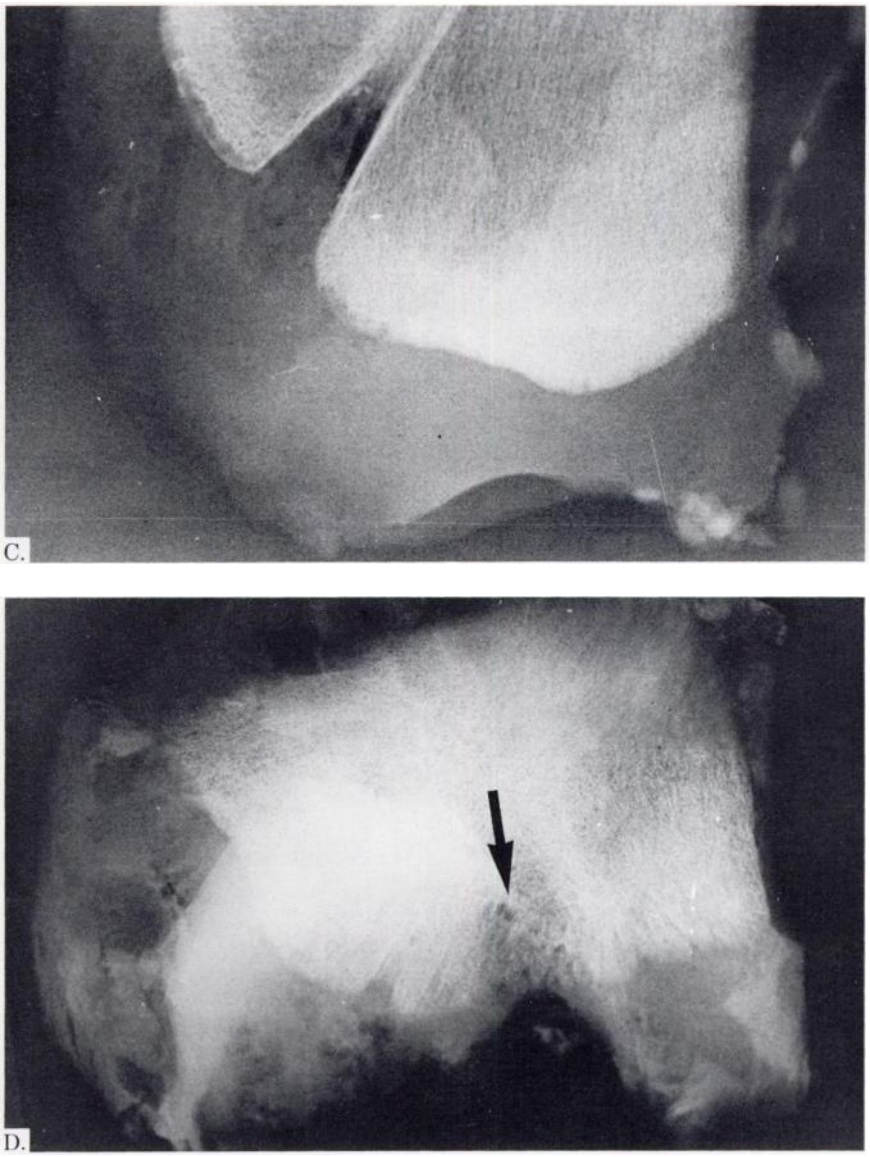


FIGURE 1. (continued)
by dissection for this photograph). There has been a growth difference (slowdown) directly adjacent to the infectious process, implying trochlear physeal destruction. In the mid-portion, where the major vascular invasion across the physis has occurred, there has been destruction of the physeal cartilage. C, D. Roentgenograms of the normal and infected distal humeri. The arrow in D indicates extension of the infection into the metaphyseal bone.

primary reservoirs for this particular microorganism are fowl and reptiles.[12]

Since there were no lesions on the flipper suggesting a direct inoculation from an open wound (we could not absolutely rule out such an injury which had healed), and since the bone and cartilage are quite similar in morphology (and presumably basic blood flow characteristics) in mammals and this unique species of sea turtle, a similar hematogenous route is likely in this animal. The presumed mechanism was an initial septic arthritis with destruction of articular cartilage, subsequent invasion of epiphyseal hyaline cartilage, and finally invasion into the epiphyseal cartilage canals with slow progression across the growth plate to involve some of the metaphysis. However, the bulk of the destruction was to the joint and the cartilage within the joint, with minimal secondary osteomyelitis. The bulk of bone formed was reactive bone in response to the contiguous infection. Some of this may have been a vascular-mediated response of increased endochondral ossification secondary to increased blood supply (a phenomenon that is not uncommon in infected joints in mammals).

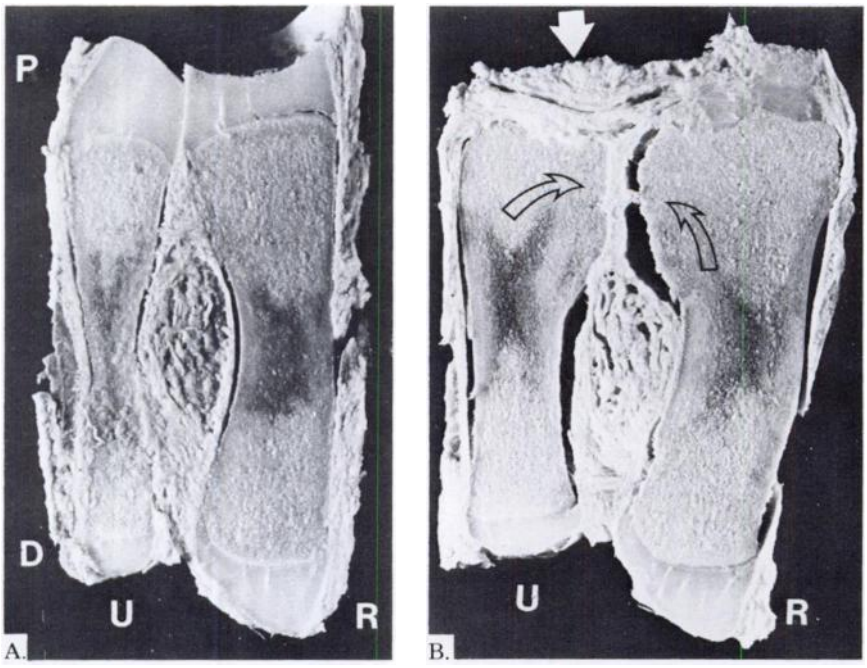


FIGURE 2. A. Normal radius and ulna of the left side showing well-formed epiphyseal and physeal cartilage at both the proximal (P) and distal (D) ends. Also note the centrally located, deeply pigmented (black) trabecular bone formed by the periosteum (membranous ossification), in contrast to the non-pigmented trabecular bone of the proximal and distal endochondral cones. Transphyseal vessels are readily evident proximally and distally. B. Radius and ulna of the infected right side showing almost total destruction of the proximal ulnar and radial epiphyses in this section. Notice that the metaphyses of both are extensively flared and overgrown in response

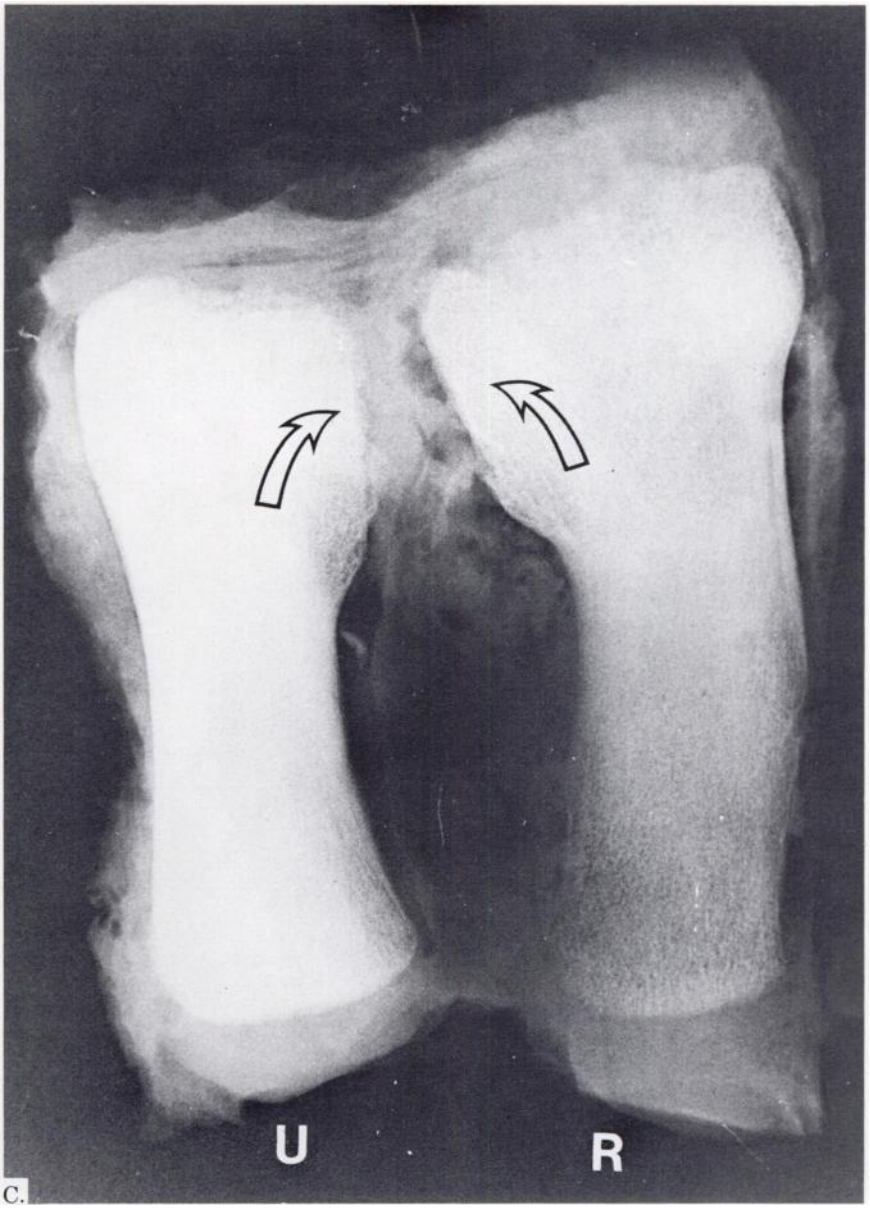
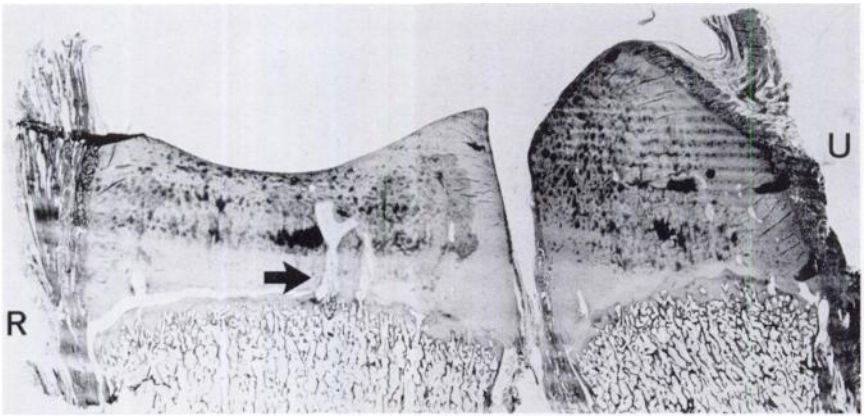
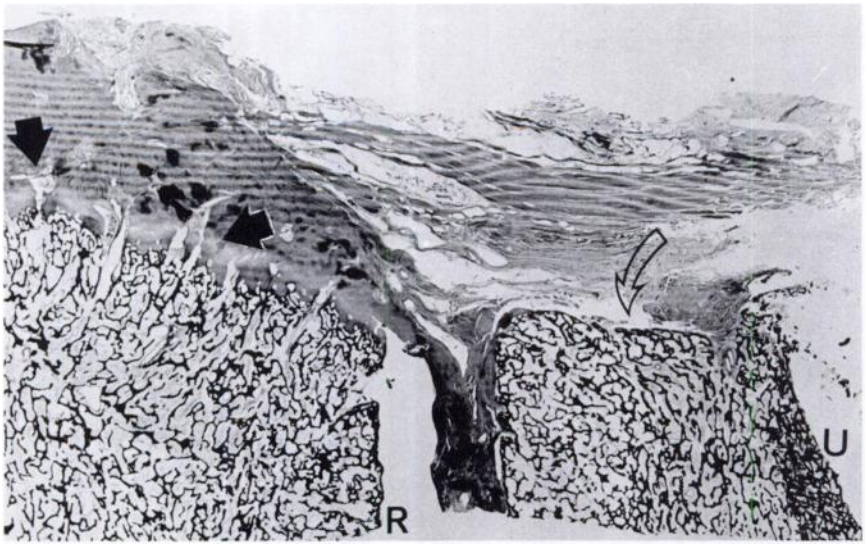


FIGURE 2. (continued)
to the infection (open arrows). The white tissue (solid arrow) across the joint represents the intra-articular fibrous pannus that has created the fibrous ankylosis. C. Roentgenogram of infected radius and ulna showing the latitudinal overgrowth of the distal end (arrows).

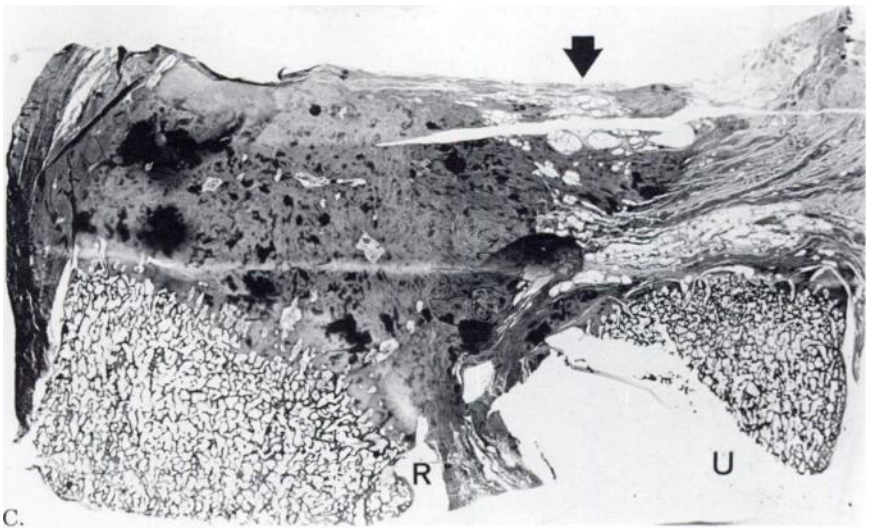


A.



B.

FIGURE 3. A. Histologic section of proximal radius and ulna showing well-formed epiphyses (3×). A large transphyseal vessel is evident (arrow). Because endochondral growth is relatively slow, there is irregular ossification extending from the metaphysis into the physis. B, C. Serial sections, 6 mm apart, from mid-section of infected radius and ulna. In both there is relatively complete destruction of the ulnar epiphysis and physis (U). In B a transversely oriented trabecular pattern is evident separating the ulna from the fibrous pannus. However, in the deeper section (C), this is not as evident, and there is an increased amount of trabecular bone, undoubtedly in response to the hyperemia. Also note the extremely vascular nature of the pannus contiguous to the ulna (arrow, in C). The radius in B shows markedly increased transphyseal vascularity into a partially destroyed epiphysis (solid arrows). Note the



C.

FIGURE 3. (continued)
pannus adherent to and invading the epiphysis. There is increased trabecular bone formation. In C fibrous pannus invasion of the radius is also evident. However, a functional physis still exists in this region.

Following the primary invasion of the joint, the most likely subsequent mechanism was elaboration of various enzymes, particularly hyaluronidases, which can be secreted by many bacteria, and which lead to the destruction of at least the superficial layers of cartilage, particularly the articular cells, allowing subsequent progressive destruction and invasion into the epiphysis.[5,4] Interestingly, the gross appearance of the epiphyseal cartilage was quite different on each side. The normal side was an opaque white, whereas the involved side, both in the distal humerus as well as the proximal radius and ulna, had a translucent appearance. This presumably relates to alteration of the extra-cellular component of the cartilage, probably the the destructive nature of the hyaluronidases or similar enzymes secreted by the bacteria and by white cells in response to the infection. These changes have also been described in human osteomyelitis.[9,10,13]

The mechanisms of articular cartilage destruction in skeletally mature animals have been explored by Curtiss and Klein.[3,4] Of particular importance is the appearance of enzymes, the presence and activity of which appears to show a positive correlation with intraarticular white blood cell concentration. These enzymes are contained particularly within the polymorphonuclear leukocytes. The synovial cells also contain lysosomal granules and acid phosphatases. Fibrin deposition initiated by altered clotting mechanisms, which are not present in non-infected joints, may also be a factor in the joint destruction, inasmuch as the adherence of these deposits to the articular cartilage could (a) impair the entrance of nutritional material into the cartilage from the synovial fluid, and (b) could impede clearing of toxic metabolites from the articular cartilage. This adherence of fibrin and formation of a large fibrovascular pannus was quite evident

in the elbow joint. Fibrin chemotactically may attract leukocytes, since these cells phagocytize fibrin as well as particulate matter (i.e., bacteria). Degranulation of leukocytes and release of enzymes into the joint may then perpetuate and worsen initial bacterial destruction. Probably these enzymes also directly attack intercellular matrix, especially after initial destruction of the specific articular layer.

The histologic changes within the joint with the formation of excess inflammatory tissue again are most compatible with a septic process and not with any type of degenerative disease. The opposite elbow joint was perfectly normal.

The absence of bacteria, either by culture or specific stains, and the absence of purulent tissue destruction were not totally unexpected. First, the animal eventually may have been able to control the infection by normal cellular defense mechanisms. Such mechanisms are present in turtles. Second, many microorganisms, especially anaerobic, facultatively anaerobic and gram-negative ones, do not form large areas of purulence, as do *Staphylococcus, Pneumococcus* and *Hemophilus* species. In addition, reptiles do not usually develop purulent lesions. Thirdly, it is not always possible to get a positive culture in acute hematogenous osteomyelitis in the human, and the incidence of a positive culture decreases significantly in subacute and chronic cases.

This particular species of sea turtle maintains large vascularized cartilaginous epiphyses throughout its life span. Thus, the animal would be susceptible to an infection pattern comparable to the current one, no matter what its age, in contrast to mammals, where changes of skeletal maturation create situations in which septic arthritides and osteomyelitides have very characteristic appearances at different ages based on stages of chondro-osseous maturation. The vascular cartilage canal system in this animal is very well developed. The infection could easily spread from one area to another by dissecting along the undifferentiated mesenchymal tissue that comprises much of the perivascular tissue in these canals.

Other species of turtles, including the other marine forms (e.g. *Chelonia, Caretta,* etc.) have thin, avascular epiphyseal cartilages and probably would be less susceptible to this pattern of arthritis and contiguous osteomyelitis. The same is true for crocodilians. Lizards and tuataras have vascularized epiphyses, but these vessels do not cross the growth plate as in *Dermochelys*. The patterns of arthritis and osteomyelitis are therefore different, with lizards[1] showing greater similarity to older human children in that the growth plate serves as a relative barrier to spread of infection. *Dermochelys* appears unique among extant reptiles in the pathobiology of septic arthritis and contiguous osteomyelitis being very similar to human neonatal patterns.

Acknowledgements

We wish to thank R.C. Schoelkopf and the Marine Mammal Stranding Center, Atlantic City, New Jersey, for obtaining this and other sea turtle specimens.

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Received for publication 15 September 1980