# Contributions of paleorheumatology to understanding contemporary disease

## *Contributo della paleoreumatologia alla conoscenza della patologia osteoscheletrica dell'epoca contemporanea*

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**RIASSUNTO**

*La paleopatologia è una disciplina che, a partire da semplici osservazioni, oggi si è spinta verso speculazioni sempre più raffinate, in virtù di tecnologie avanzate, che hanno aperto la via a nuove conoscenze sulla paleontologia dei vertebrati.*

*In presenza di modificazioni strutturali e densitometriche dell'osso, l'analisi radiografica, ed in particolare la fluoroscopia – tecnica semplice ed economica – può fornire moltissime informazioni, in grado di documentare la risposta dell'osso stesso alle sollecitazioni meccaniche ed in corso di varie condizioni patologiche. Ancora, la radiografia consente di "sezionare", in maniera non invasiva, i campioni, definendo così i dettagli interni non altrimenti esplorabili. Il materiale può essere anche sezionato sopra un piano, e le porzioni così ottenute a loro volta ruotate, tanto da "aprire" l'immagine (radiografia tridimensionale).*

*Nei reperti fossili, inoltre, possono essere perfettamente conservate le microstrutture ed il corredo antigenico. Ad esempio, l'ottimo grado di conservazione della struttura molecolare ad elica, con sensibilità alla collagenasi, è stato dimostrato in fibre collagene di materiale biologico risalente a 10 milioni di anni fa. Antigeni sono stati estratti da reperti ossei di 100 milioni di anni fa e rilevati "in situ" su materiali di 11.000 anni fa. Grazie all'impiego di antisieri specifici per determinati antigeni, si possono visualizzare reazioni di immunofissazione. Minime tracce di DNA possono anche essere amplificate e quindi analizzate. In questo modo sono stati identificati acidi nucleici di mammuth (40.000 di anni fa), bisonti (17.000 di anni fa) ed insetti (25 milioni di anni fa).*

*Le più moderne tecnologie, applicate alla paleopatologia, dimostrano, anche in prospettiva, quanto sia comunque importante un approccio multidisciplinare e collaborativo.*

## INTRODUCTION

Accuracy of paleontologic perspectives, understanding disease states, hypothesis formation, and technology form the basis for this review. Moodie's (1) effort, in isolation, to attribute diagnoses to paleontologic observations were originally met with skepticism (Don Baird, personal and graphic communication) and have not stood the test of time (2, 3). Familarization with the technology should allow innovative approaches to mainstream paleontologic questions. Collaborative efforts should allow avoidance of some of the pitfalls of that technology. When all parties to such collaboration must convince each other of the appropriateness of their "pronouncements," confidence is generated in the conclusions.

Generating testable hypotheses is the goal of disease analysis. [Perhaps a better term might be wellness analysis, as the number of healthy (paleontologic) individuals far exceeds the few "sick" ones]. While in the clinical setting, it certainly is fulfilling to "pronounce" a patient healthy, it is the pathologic paleontologic alterations which appear most amenable to habitat and lifestyle analysis. If the variety of responses of bone to any environmental factor is reproducible across phylogeny and

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chronology, identifying the cause of variations (from the normal condition) should be feasible. De-lineating those physical conditions identifiable in bone, which are confirmable in etiology and dis-tinguishable from diagenesis and other artifact is the initial focus.

## PATHOLOGIC CONDITIONS AFFECTING BONE

Pathologic conditions amenable to identification in paleontologic specimens predominantly relate to bone and joint surfaces. While malignant bone tumors have been extremely rare (4, 5), hereditary forms of exostoses (bone overgrowths) have been found. Alterations in the outer layer of cortical bone (periosteal reaction) is rarely noted. While there are a multitude of causes for periosteal reac-tion, the nature and skeletal distribution may allow recognition of a specific diagnosis.

Diseases characterized by generalized alteration in bone density are difficult to assess because of the difficulty of controlling for diagenesis/taphon-omy (post-mortem changes). Disorders producing osteopenia are typically not distinguishable in the absence of soft tissues. The major characteristic distinguishing osteoporosis and osteomalacia, for example, is presence of uncalcified osteoid tissue (6,7). This is not generally assessable in paleon-tologic materials. However, osteopenia-producing disorders such as hyperparathyroidism may be as-sociated with other alterations, which may sug-gest the diagnosis. Osteodystrophy (secondary hy-perparathyroidism and osteomalacia related to re-nal failure) has been reported in non-human pri-mates (8).

## PATHOLOGIC CONDITIONS AFFECTING JOINTS

Identification of arthritis is based on the nature of bony (e.g., preservation of size and configuration, presence of erosion, reactive bone formation or re-modeling), as well as soft tissue alterations (2, 6, 7). While bony alterations represent only a subset of those parameters, "defleshed" bones are fre-quently all that is available to the paleontologist. Most of the 200 varieties of arthritis are not known to produce any bony alterations (2, 6, 7) and are therefore not amenable to paleontologic evaluation (with currently applied technology). This consid-
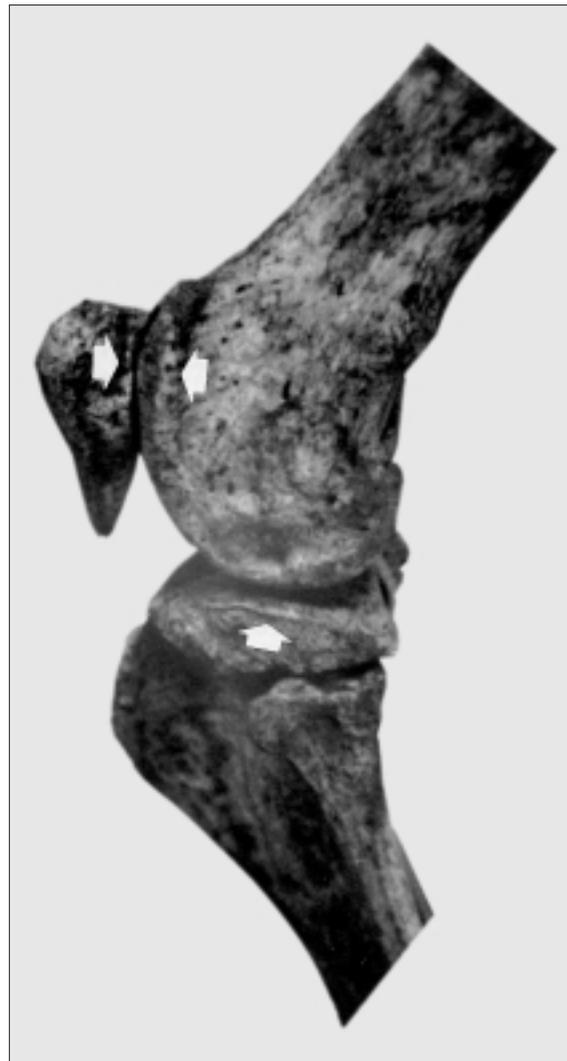


**Figure 1** - Lateral view of Smiledon knee. Tibial, femoral, and patel-lar osteophytes (arrows) are present.

erably narrows the list of diagnoses recognizable in paleontologic specimens.

Arthritis is typically classified as erosive or non-erosive. One form of non-erosive arthritis is os-teoarthritis. Bone remodelling occurs with spur (os-teophyte) formation (Fig. 1). The density of the subchondral bone is increased and metaphyseal cysts may form (as recognized by x-ray or cross-sectional view). With increasing severity, the ar-ticular surfaces may even become grooved and eburnated. Extremely rare in dinosaurs [only two known examples (3)], it is more commonly noted in Quaternary mammals. Its analysis provides in-triguing perspectives of "lifestyle" and habitat. The osteophytes of osteoarthritis must be distinguished

from those of spondylosis deformans, the vertebral centra spuring phenomena. As the disc spaces are not diarthrodial joints, the term osteoarthritis is not appropriate for spine spuring, exclusive of zygoapophyseal joints.

### *EROSIVE ARTHRITIS*

Erosive arthritis is recognized as disruption of cortical bone (Fig. 2), within or extrinsic to the joint capsule of diarthrodial joints (junction of hyaline cartilage-covered bone surfaces, at which movement occurs) (2, 6, 7). Intrinsic erosions (Fig. 3) are localized to subchondral (that portion of cortical bone to which articular cartilage is attached) or marginal regions (the metaphyseal cortical component located between cartilage-covered subchondral bone and the site of insertion of the joint capsule). As thirty to fifty percent of bone matrix must be lost before a lesion can be detected radiologically (7) most erosions recognized on gross examination of bones are not visible on specimen x-rays.

Artifacts must be excluded. Cracked and ragged edges, on the border of pseudo-erosions (artifacts) are readily discernable from the front of bone resorption more characteristic of erosive arthritis (9,10). Plant roots, bacteria, fungi, protozoans, insects, snails and mammals often produce focal breakage, destruction and disturbance (11-16). Gnawing, producing linear U-shaped bands of crushed cortex or broad grooves, is usually highly characteristic and not easily confused with true erosions. Punctures wounds present as circular or elongated depressions with a perpendicular zone of jagged-edged cortical fragments (17).

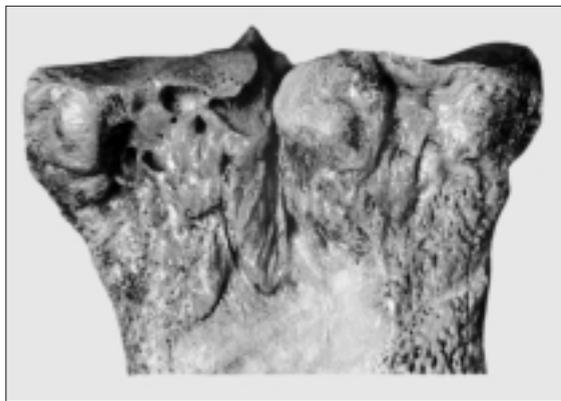A lytic lesion from which bone tissue has been removed by osteoclasts will present smooth, rounded edges of any surfaces within and at the boundaries of the lesion; transitions from one plane of bone tissue to another will be smoothed. The edges of all exposed trabeculae, as in metaphyseal lesions, will appear smoothed, while intersecting planes of dense cortical bone will meet at a rounded edge. Although inflammation initially may activate osteoclastic resorption of peri-lesional trabeculae, it subsequently activates osteoblastic deposition in the same region. Thus, any trabecular edges initially exposed at the lesion boundary by osteolysis would subsequently appear thicker than trabecular edges revealed by post-mortem processes in the same region.

### *Infectious arthritis*

Infectious arthritis is a form of erosive disease in which gross distortion of the joint surface and underlying bone (Fig. 4) is associated with reactive new bone formation (3, 6, 7). It is usually limited in distribution, typically affecting only one joint. If the latter has a filigree appearance often bridging joint surfaces, the diagnosis of infectious arthritis is virtually assured. A combined sclerot-
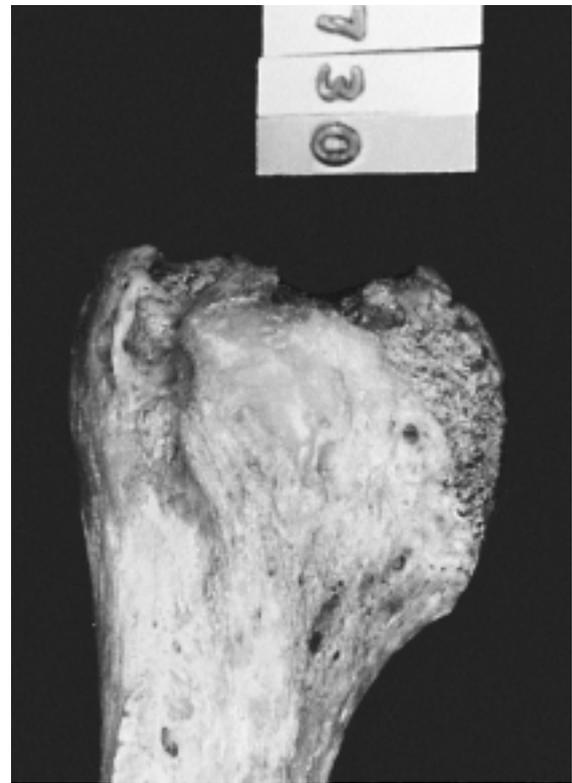


**Figure 2** - Posterior view of proximal aspect of Bison knee (partial view). Left tibial plateau subchondral erosion is prominent.



**Figure 3** - Lateral view of human proximal humerus. Severe erosive disease, predominantly affecting subchondral bone.

### Crystalline arthritis

Gout erosions, caused by urate accumulation, are also associated with reactive new bone formation (3, 6, 7). In contrast to the ill-defined margins of infectious arthritis, those of gout may be polyarticular and have a "punched out" appearance with a sclerotic margin. Overgrowth of peri-erosional bone forming an overhanging edge is considered pathognomonic (3, 6, 7, 19, 25). Identification of urate in an excavated skeleton (26) emphasizes the potential importance of the preparator. As that skeleton was only a few hundred years old, it is unclear if fossil materials could retain urate crystal deposits in a recognizable form. Classic gout erosions have been found in tyrannosaurs (27). Another form of crystalline arthritis, calcium pyrophosphate deposition disease is recognizable radiologically as giant subchondral geodes (cysts) and as deposition of calcium resulting in a sheet of calcium which parallels the articular surface or within intervertebral discs (3, 6, 7, 18).

### Spondyloarthropathy and Enthesopathy

Spondyloarthropathy or reactive arthritis are terms applied to a group of disorders characterized by tendency to reactive new bone formation, asymmetrical pauciarticular peripheral joint erosions and ankylosis, and axial (spine and sacroiliac) joint disease (3, 6, 7, 28-30). Both marginal and subchondral erosions occur. The erosive disease is often unassociated with loss of peri-articular bone density, enabling it to survive taphonic forces in a recognizable form. Population analysis is essential with this group of disorders, as axial joint disease is not universal and the peripheral arthritis is occasionally polyarticular. Zygoapophyseal joint erosion or fusion and ossification within the annulus fibrosus (Fig. 5) are pathognomonic. Evidence of spondyloarthropathy abounds in the literature of human skeletal disease (3, 16, 31, 32), and the precedent for its non-human occurrence, reported (33).

While rheumatoid arthritis appears limited to humans (34), spondyloarthropathy is clearly represented among contemporary animals and in the fossil record (35, 36). Actually, spondyloarthropathy has a very long history, with geometric increase in frequency over geologic time (36). Trackable to the Paleocene (35), classic zygapophyseal joint changes even have been documented in a dinosaur (37).

Enthesial bone formation (ossification of sites of

ic and resorptive picture is noted on cross-section or x-ray views. If the erosion appears to undermine subchondral cortical bone, without major destruction of the subchondral bone surface, tuberculosis is likely responsible (3, 6, 7, 18-21). Associated phenomena may facilitate diagnosis. The erosive lesions of syphilis are often associated with a characteristic spiculated periosteal reaction (3, 6, 22, 23).

Treponemal disease is recognized on the basis of periosteal reaction and osteitis. The appearance of individual bones and the nature and intensity of bone damage are similar in all three varieties known to afflict skeletal structures (22). However, the pattern of involvement is actually sufficiently disparate to allow distinguishing among them. Study of skeletal populations with yaws (in precontact Guam), bejel (in the 19th century Middle East) and syphilis (in 20th century Cleveland) allowed development of criteria, which have since proved reproducible in more than 40 archeologic populations (22, 24).
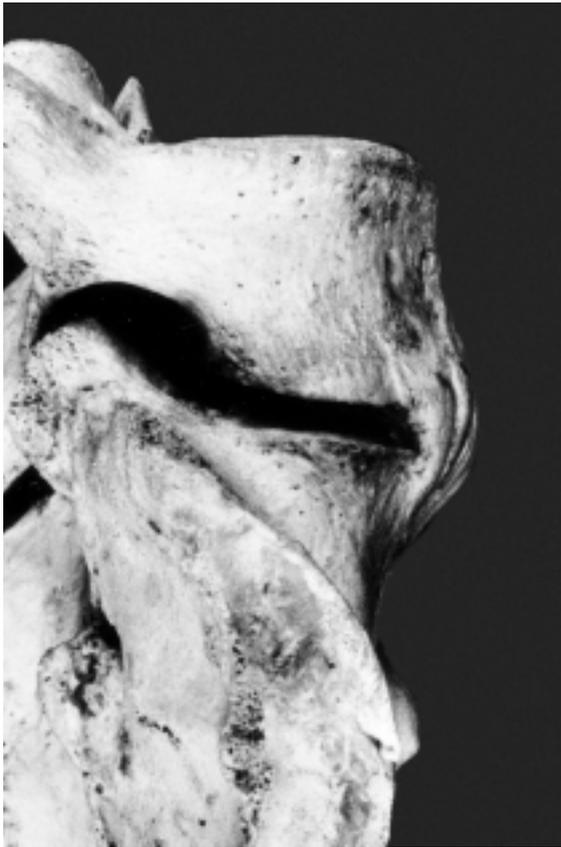
**Figure 5** - Lateral view of Gorilla spine. Osseous bridging through anterior aspect of anulus fibrosus.

tendon, ligament, and joint capsule insertions), is highly suggestive of spondyloarthropathy, but not diagnostic. Diffuse idiopathic skeletal hyperostosis (DISH), ubiquitous among many groups of dinosaurs and older mammals (2, 38), is also characterized by enthesial bone formation (3, 6, 7, 39). Flowing ossification of spinal ligaments is found in DISH, as well as spondyloarthropathy. However, DISH does not affect the zygoapophyseal joints

and sacroiliac joints, nor does it produce erosions. While DISH affects older mammals, other enthesial disorders [hypervitaminosis A (Fig. 6) and fluorosis] are not age dependent (40-42). Fluorosis is typically associated with diffuse osteosclerosis, irregular periosteal thickening and disorganization of osseous lamellar structure (in contrast to DISH which retains normal osteon relationships) (38, 43).

### *Rheumatoid arthritis*
Rheumatoid arthritis contrasts with spondyloarthropathy by affecting the skeleton so symmetrically, that any asymmetry is cause for a reevaluation of the diagnosis (3, 6, 7, 44). It further differs from spondyloarthropathy by the associated loss of periarticular bony trabeculae, lack of significant reactive new bone formation or bony ankylosis and by sparing of axial joints (44-46). The erosions are marginal in distribution (47). The disorder has been identified in Indians dating back 6500 years (48, 49). The frequency of joint affliction both grossly and radiologically varies little from that noted in contemporary populations (50-53). In contrast to spondyloarthropathy, rheumatoid arthritis has not been documented in any other animals.

## DIAGNOSTIC APPROACH AND HYPOTHESIS FORMATION

Diagnosis of diseases of fossil bone requires the development of new criteria for diagnosis (3, 33, 44, 48, 49). Utilization of such criteria (e.g., population frequency, sex ratio, radiologic appearance, pattern of involvement, nature of the lesions, and association with other disease features), often allows confirmation of a specific clinical diagnosis (3, 33, 44, 48, 49). For this reason, ossuaries are usually not
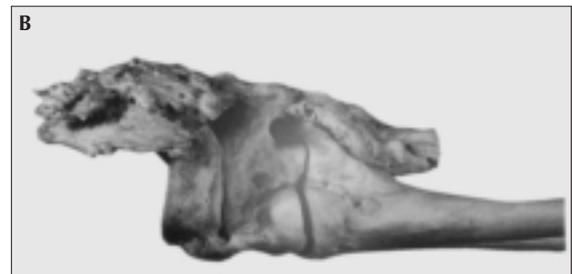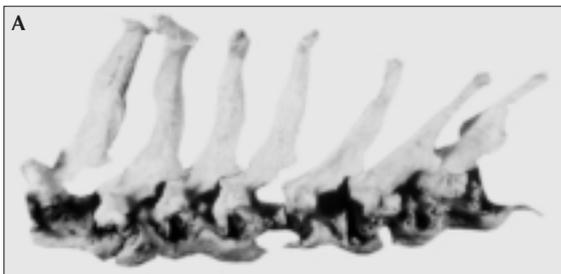


**Figure 6** - Hypervitaminosis A affecting a zoological tiger. a. Lateral view of spine. Bulky osseous bridging of vertebrae centra and spinous processes, sparing the zygoapophyseal joints. B. Lateral view of midfoot. Enthesial osseous reaction results in ligamentous joint fusion.

conducive to recognition of disease, only for its exclusion (dependent upon the completeness of skeletal representation). An exception is a ossuary study of osteoarthritis in fossil marsupials, in which osteoarthritis was limited in distribution to one joint (54). Although isolated examples of pathology are occasionally of value, placing them in perspective is enhanced through understanding the population they represent (55, 56).

Surveying for disease requires recognition of all changes which are not part of the normal condition of bone, and that each change be fully characterized (compatibility with the diagnosis being considered or does its presence infer an alternative diagnosis). Recording the presence and distribution of deviances from the normal state, instead of diagnosing them "in isolation", allows an epidemiologic approach. Examination of limited numbers of individuals from small sites similarly may be problematic, as disease mimicry can only be excluded on a population basis. Any population-based diagnostic effort must of course consider the the possibility that more than one disease may manifest in an individual or population. Comparison of occurrence of phenomena in different populations can proceed, only if the biases inherent in the deposit or acquisition of those populations can be identified.

The optimal technique for examination of specimens is non-destructive, easily accessible, and amenable to interpretation. Collaborative investigations by specialists in structure/function relationships (e.g., paleontologists) and specialists in disease (e.g., health care providers) should allow significant progress. While "pattern recognition" may document the diagnosis, more commonly a guess at the etiology will be the hypothesis to be proved or disproved. Given the large number of hypotheses amenable to testing, inability to test a hypothesis should perhaps stimulate its "back burner" position, until insight or technology provides a methodology for its testing.

## TECHNOLOGICAL ADVANCES FROM CHAIN SAWS TO RADIOLOGY

The "great Texas chain saw massacre" destructive approach (Sally Shelton, personal communication) to the study of fossils, as exemplified by ground sections (57), has been complemented by techniques allowing three dimensional reconstruction of the derived information and also by techniques which often eliminate the need for destruction of the specimen. The sections or peals can be laser-scanned to generate a digitalized image and regenerated from any angle. The data can be manipulated to "dissect away" portions. A three-dimensional image can even be generated of the structure of interest. Fiber optic light sources and optics can be passed through small apertures, allowing visualization of internal structures. The internal structures can also be visualized radiologically. Endocranial casts can be obtained without skull sectioning and sometimes even without specimen preparation! When sectioning is required, advances in microscopic technique minimize the requirement for thin grinding and allow more definitive identification of structures.

### *Immunology*
Preservation of fossil materials is sometimes quite remarkable. While replacement does take place, structural morphology and even the actual constituent protein may be retained. Bone collagen has been detected in 200 million year old dinosaur bones (58), in Pleistocene avian and mammalian fossils (59, 60), and in 1.8 million YBP (year before present) human fossils (61). Collagen extracted from a frozen *Elephas primigenius* tusk (dated at l0,000 to 15,000 years) even retained (X-ray diffraction) its helical structure and sensitivity to collagenase denaturation (59, 60), Weser et al. (62) even reported survival of enzymatically active superoxide dismutase in a 3,000 year old mummy. Immunologic preservation, similar to histologic preservation, may be quite variable (63).

Collagen preservation has even been utilized to determine the premorbid environmental temperature of fossil specimens (64). Purified Pleistocene collagen was hydrolyzed and subjected to automated amino acid analysis (65, 66). Collagen hydroxyproline content in cold environment fishes and other vertebrates was found to be lower than that of their warm environment contemporaries (67, 68). Ho (69) found that two thirds of the variation in body temperature of mammalian species was explainable on the basis of relative collagen hydroxyproline content. Body temperature could be predicted at l9.5 + 0.198 (hydroxyproline content per 1000 residues) or at 11.5 + 0.124 (iminoacid content per 1000 residues). The iminoacids measured reflected the combined hydroxyproline and proline content. Body temperature increased 1 degree Centigrade for each 5 hydroxyproline or

9 iminoacid increase per 1000 aminoacid residues. The predicted 35.3 degree temperature of *N. shastense* (ground sloth) is comparable with the 34.5 noted in *Choloepus hoffmanni* (contemporary two-toed sloth). The predicted temperature for *Canis dirus* (37.2) is lower than that of the contemporary timber wolf (*Canis lupus*) (40.5), but comparable to that of the modern dog (36.7 - 40.6) (71).

Albumin phylogenetic structural alterations provides an excellent tool for fossil studies, as it is resistent to denaturation, is present in high concentration, and has been defined structurally in 2000 extant species (71-76). The amount extracted from frozen *Mammuthus primigenius* muscle was approximately 50-fold less than that found on extraction of extant elephants (77). Antisera developed against it "reacted strongly" with albumin from extant elephants and only weakly with that from a sea cow. Postmortem albumin modification appeared responsible for the major differences, however.

## *Microscopy*

Routine light and polarizing microscopy allow recognition of normal structure and its disruption. Bacteria (e.g., cyanobacteria) have been recognized histologically in 3.3 billion year old sediment (78). The first histologic studies were actually of fish (12, 79-81). Preservation of microscopic structure in animal bones was recognized by Schaffer in 1889 (82). Haversian canals have been recognized in bone and even in ossified tendons (*Trachodon*) (83, 84). Examination of 40-million YBP amber-enclosed insects even revealed preservation of subcellular details (85, 86).

Immunologic techniques using specific antibodies allow recognition of intrinsic or extraneous antigens. Immunofluorescent technique utilizes an antibody which has been "labelled" with a chemical, which when excited by light of certain wavelengths gives off light of a different wavelength. A fluorescence microscope provides the excitatory light and registers the "excitement." Other immuno-histologic techniques are available but are limited in applicability to paleontologic applications by the background "coloration" of the specimen. One choses a technique with minimal background interference. If a background fluorescence is present at a given wavelength, an alternative immunofluorescent label is utilized (which fluoresces at a different wavelength, allowing it to be distinguished). While most immunofluorescence microscopes utilize transmitted light, a reflected (epifluorescence) optical system can also be utilized. This is a surface technique requiring that only one surface of the specimen be available for analysis, rather than a thin ground section. If the appropriate tissue site is analyzed, if the antigen has survived, and if the approriate antisera is utilized, presence of the target antigen can be documented [e.g., antisera specific for treponemal disease (e.g., the non-venereal treponemal disease, yaws) detected its presence in an 11,500 YBP *Arctodus* (short-faced bear) (23)].

Scanning electron microscopes (SEM) are currently available with ports which will accept coffee cup-size specimens, minimizing specimen sacrifice. Specimens are typically coated (e.g., with gold) to enhance resolution and reduce charging (osseous structures are fairly non-conductive). Alacrity in the SEM procedure will sometimes permit avoidance of coating, essential if x-ray diffraction (electron probe) techniques (which allow identification of contained elements and their relative concentrations) are to be applied. In contrast to extraction techniques, electron probe allows microscopic chemical localization. Ability to assess focal sites allows verification of a cystic structure as isolated from the external environment. Confidence that separation from the outside environment is complete was obtainable by noting a uniquely high sulfur content in a mosasaur spine wherein small abscesses were noted. Proof that the material was isolated eliminated the possibility of postmortum contamination and confidence that organisms seen were pertinent to the infection.

## *DNA techniques*

Low concentrations of DNA, in variable states of preservation, have been extracted from paleontologic specimens (1% of that extractable from fresh muscle) (60,87). DNA was recovered from a 40,000 year old frozen Siberian mammoth (85) and 25 million year old, amber-preserved insects (86). As yield of the extraction procedure is low and the DNA has not escaped modification, repair and replication are required for analysis. The extracted DNA is denatured, most efficiently by heating to 95-l00 degrees Centigrade (89, 90), purified and replicated (by polymerase chain reaction techniques).

The *Thermus aquaticus* (Tac) heat-stable DNA polymerase chain reaction produces strands complementary to a defined DNA segment (91). Three to five minute thermal cycles are repeated 20-40

times (90,92). Heating separates the DNA strands, while cooling allows the polymerase to again utilize the separated strands to produce additional complementary sequences. As the number of DNA strands doubles with each cycle, a million-fold amplification may be obtained in as little as 4 hours (93).

Once sufficient DNA is replicated, it can be sequenced. Separated strands can be tested for homology with test DNA by assessing their ability to anneal. The more homologous the strands, the stronger their attraction and the greater the thermal stability of the resultant DNA hybrid. The degree of homology necessary for two strands of DNA to anneal is a condition of the temperature of the reaction (91, 94).

Contamination during excavation or by reagents/glassware must be considered. As paleontologic materials are not amplifiable beyond 150 base pair size, longer segments should be considered contaminants (85,95). The antiquity of DNA is also verifiable because of resistant to enzymatic digestion (secondary to cross-linking), compared with modern contaminating materials (96). Amplification efficiency for antiquity DNA is inversely related to size, in contrast to similar sized contemporary DNA (98).

While Cytochrome oxidase I and NADH dehydrogenase I genes from Quagga mitochondrial DNA appeared to have two replacement nucleotide substitutions (compared to zebra) by the cloning technique (87,99), the substitutions did not make sense. As these sites are conserved in all other tested vertebrates (97), common sense suggested and polymerase amplification verified it as artifact (97). The source of the artifact was the repair mechanisms of cellular DNA replication, introducing alternative nucleotides (95). Robbins et al. (89) found only 12 of 229 base pair mitochondrial sequences at variance with that of the contemporary mountain zebra. Alternative DNA sequences, coding for the same amino acid, accounted for 10 of the substitutions. This suggests that the two species were derived from a common ancester 3-4 million years ago. Fossil record implications are thus confirmed (100). Marx (101) reports that mammoth muscle mitochondrial DNA is "very similar" to that of the modern elephant. DNA, extracted from 17,000 ybp bison was identified as belonging to the *Mycobacterium tuberculosis* complex. Specific segment comparison revealed the organism to actually be *Mycobacterium tuberculosis* and not *Mycobacterium bovis* (21).

## Radiology

Application of radiologic technical developments to the study of paleontology have opened new horizons for non-destructive analysis (101,1 02). Paper-wrapped film, requiring high x-ray exposures (often beyond that of routine museum equipment) has been replaced by a film screen technique. The latter utilizes phosphorescent screens (e.g., calcium tungstate) which give off packets of photons when exposed to a single x-ray photon, thus substantially reducing the amount of radiation required for an exposure. Kodak Kodabrome IIRC F2M (normal contrast) black and white paper provides the desired contrast (103).

Scatter radiation, which reduces image contrast and resolution (104) can be reduced by use of grids (3, 6, 105), airgaps (105, 106), or moving, tightly collimated photon beams (107-110). Grids are packs of parallel thin radiation-blocking materials placed perpendicular to the x-ray beam, thus blocking registry of scatter radiation. Technology developed for enhancement (e.g., Fourier transformations) of satellite photographs can now be applied to laser-scanned radiologically images, producing digital enhancement (111-114). An inexpensive radiologic technique, tomography, can even "cut" the specimen, facilitating recognition of internal structures. Computerized axial tomograms (CAT scans) offer an eloquent approach to the same question, although the power output of this expensive equipment often must be "boosted" and the technique is limited in resolution.

Conroy and Vannier (115) utilized CAT in assessment of endocranial volume and intracranial relationships. The technique overcomes the problems of calcified matrix and taphonomy, reduces specimen preparation requirements and may even facilitate preparation by localization of critical components (116).

Resolution of a "clinical" CAT scan is only l mm at best (117), whereas x-ray techniques can offer 0.l mm resolution. Microscopic CAT scanning, utilizing a 5 micrometer x-ray beam source (FeinFocus, Wunstorf, FRG), is currently available on a limited basis (118). Mounting the specimen on a rotational stage even allows three-dimensional reconstruction of the images.

Magnetic resonance imaging or MRI produces cross sectional images of hydrated materials. Application requires access to the million dollar magnets and absence of magnetizable metals in the sample of interest. While applicability to paleontology may seem limited, frozen hydrated samples,

such as Coelocanths have yielded new insights (Hans Peter Schultz, personal communication). Miocene whale vertebrae were also visualized by MRI, after protonation by placement in aqueous solution (119). The technique, however, cannot be blindly applied to assessment of relationships. Paleontologic specimens can be expected to induce non-uniform fields, a phenomena which which can significantly shift image position, distorting relationships (120).

## THREE-DIMENSIONAL IMAGING

Three-dimensional representations, though providing novel insights, must be interpreted in light of their potential compromise by distortion and discontinuity artifact. Time intensive stacking techniques (117, 121, 122) can now be pursued by direct conversion of two dimensional information (by Fourier transformation) to three dimensional images (120, 123), thus eliminating much of the artifact (120). However, the software is expensive and significant computer time is required for data acquistion, reconstruction and image manipulation. Areas of abrupt density change (e.g., bone interfaces) are not always accurately represented, because of averaging artifacts (120, 124). These imaging techniques do allow removal of portions of the structure, allowing visualization of selected interi-

or structures from almost any angular perspective (112, 120, 125-130) or display as a graded transparency manner (120, 131). Thus, specimens can be examined and internal relationships determined without matrix removal or specimen destruction/damage (120, 132-134). Microscopy, utilizing laser scanning techniques, is also amenable to tomography and three-dimensional reconstruction (135). Three dimensional images of CAT scans and MRI images and even fossil cephalopods can be recorded on holographs with sufficient accuracy for measurement of object relationships (136-138). Physical models, accurate in three-dimensions, can even be computer-generated (139).

## PERSPECTIVE

The future of paleorheumatology lies in concerted coordinated efforts to expand speculation to form testable hypotheses and then to identify valid techniques for testing them. If a testable hypothesis cannot be generated, perhaps the problem should be placed on hold or an interdisciplinary council developed for input as to application of new technologies to their assessment. Epidemiologic approaches are essential. While isolated finds are occasionally of value, placing them in perspective usually requires an understanding of the population they represent.

### SUMMARY

As paleopathology has evolved from observational speculation to analysis of testable hypotheses, so too has recognition of its contribution to vertebrate paleontology.  In the presence of significant structural and density variation (between matrix and osseous structures), x-rays provide an additional perspective of osseous response to stress and disease.  As film techniques are time and cost expensive, fluoroscopy has proven a valuable alternative.  Radiologic techniques also allow non-invasive "sectioning" of specimens, illustrating significant internal  detail.  The object can be "split" on a plane and the two portions rotated to "open" the image.  This three-dimensional approach now can be applied to other forms of sequential data to their facilitate 3-dimensional representation graphically or with solid representations.

Antigen and microstructure may be well preserved in fossils.  Molecular preservation with retention of helical structure and sensitivity to collagenase has been demonstrated in 10,000 year old collagen.  Antigen has been extracted from 100 million year old bone and documented, in situ, in 11,000 year old bone.  If the appropriate site in the tissue is assessed, if antigen is still present, and if the appropriate antisera is utilized,  fixation of the antibody to the specimen can be detected.  Minute amounts of DNA can be amplified and analyzed.  Recovery of DNA from a 40,000 year old mammoth, 17,000 year old bison and from 25 million year old insects provides opportunity for cloning and independent assessment of relationships.  Implications of available technology focuses direction for development of collaborative approaches.

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