# CHAPTER 13 A Knowledge of Bone at the Cellular (Histological) Level is Essential to Paleopathology

*Bruce D. Ragsdale and Larisa M. Lehmer*

## Introduction

An anthropologist deduces a species from a bone, a face from a skull, and life habits from a skeleton; the paleopathologist deduces disease from dry bone abnormalities by reference to medical museum specimens, pathology texts, and roentgenologic literature. But these "knowns" are classic, extreme and rare. In most instances determining the etiology of a bone lesion is extremely difficult. Bone, like all tissues, has few ways of reacting, so different causes may produce similar results. However, recognizing successive gradual pathologic stages from normal states may reveal the mechanisms of disease. Cells are the effectors of change, and an understanding of them is a necessary foundation for diagnosis. So, knowledge of skeletal dynamics in growth and adaptation is fundamental (Johnson 1966).

This chapter will review the influences that drive the cellular activities that modify mineralized tissue. A dry bone from antiquity is like a fossilized footprint left in mud. Just as the foot is obviously of more interest than the print, so, too, in paleopathology the disease is more interesting than the resulting lesion. One's frame of mind must be to mentally "put the soft tissue that abutted solid substance back on the specimen," to find clues to the host response that modified bone, and then to deduce the

*A Companion to Paleopathology*, First Edition. Edited by Anne L. Grauer.

mechanisms that produced the change. Through this process one might then determine the category of disease provoking the skeletal change, and only then, take a stab at a specific diagnosis.

## Basic Principles

Disease is the alteration of living tissue that jeopardizes "ease" and survival. The causes of skeletal disease are aberrations of normal growth, development, and maintenance, rather than the interjection of something totally new, which limits the number of ways in which bone can react. Consequently, "morphologic residua" of different diseases significantly overlap. Even the Basic Categories of disease (discussed below, and see Table 13.2) overlap to some extent, since inflammation includes repair, trauma is associated with circulatory changes, and metabolic diseases may call forth mechanical compensation.

The sequence from normal, through adaptation, to sickness and death is a continuum. However, most of the skeletal alterations that paleopathologists encounter are the result of long-standing disease, which may or may not have led to the death of the individual. Hence, using modern diseases, which are intercepted by medical professionals comparatively early, as reference standards for diagnosing diseases of the past, is problematic as they bear little resemblance to the untreated end stage of the process (Ragsdale 1997). While autopsy specimens are more likely to represent advanced expressions of disease, the morphologic expression is generally modified by therapeutic intervention. Ortner and Putschar's (1981) work is of greater relevance for paleopathological diagnosis, since many of its illustrations are cases collected in the 19th century or come from Dr. Putschar's experiences in underdeveloped countries (Ragsdale 1996). Unfortunately, further complicating the diagnostic process is the fact that well-defined structural change may appear in many diseases, and a particular disease may produce more than one morphologic expression. Only in the presence of a few extreme abnormalities can relatively specific diagnoses be made. Obviously, this casts doubt on the diagnostic specificity of a small slice of dry bone studied histologically.

Further complicating our ability to diagnose the presence of specific disease from dry bone specimens is the fact that pathologic morphology is linked to only three aberrations: circulation, metabolic factors, and mechanical stress. These three influences act on the bone fabric of collagen bundles impregnated with mineral crystals through three cell types. Only osteoblasts produce significant osteoid matrix (bone fabric). Only osteoclasts can significantly resorb (remove) bone (and do so much faster than osteoblasts create bone, so osteopenia is the usual initial expression of a bone reaction). Osteocytes can both resorb and form bone, but only to a limited degree along the walls of their lacunae where they maintain bone, serve as mechanical sensors, and control calcium flux. These three cells are normally active in the daily remodeling and rejuvenation of the living skeleton. No other cell types directly modify bone structure. Pathologic anatomy results when local (pathological) conditions stimulate abnormal activity of these cells. An understanding of these conditions is a necessary background to diagnose skeletal disease, and is facilitated by keeping a few basic principles in mind (Table 13.1).

**Table 13.1** The twelve basic principles of orthopedic pathology

1. There are seven Basic Categories of disease.
2. There are only three influences which govern cell activities: circulatory dynamics, metabolic factors, and mechanical forces.
3. Only osteoblasts make bone, are favored by elevated pH, passive hyperemia (venous congestion and edema), negative bioelectric charge, and growth hormone. Only osteoclasts delete bone, favored by low pH, active hyperemia (increased arterial input), positive bioelectric charge, and parathormone and work 100 times faster than osteoblasts.
4. Bone formation proceeds on surfaces; what is lost is gone forever, but persisting surfaces can be added to.
5. The "endosteum," if the term must be retained in adults, consists of the mature fat cells that abut internal bone surfaces and are capable of modulating into osteoblasts.
6. The interface between periosteum and cortex along growing bones can be a plane of bone formation (as in additions to diaphyseal diameter) or resorption (as in metaphyseal cutback); these same activities can be reawakened in disease as periosteal new bone or cortical lysis.
7. Turnover and remodeling proceed, even in the presence of disease. In face of disease, the mechanically most important (i.e., the stressed) bone components are preserved the longest and may indeed be strengthened.
8. Fully differentiated mesenchymal cells, e.g., "fibroblasts" and adipocytes of soft tissue and of the marrow, are not irrevocably differentiated cells, but retain a capacity to modulate into other functional cell types as conditions dictate. Fat, fascia, and muscle contribute to "periosteal reactions."
9. All cartilage formed, except for articular, ear/nasal/airway and sarcomatous cartilage, is doomed to resorption (e.g., epiphyseal and fracture callus cartilage).
10. Bone is an enclosed space bound by cortex and internally baffled by cancellous modules. This shields vessels from the mechanical compressive forces operative in soft tissue but makes bone's blood supply vulnerable, especially to compressive obliteration when anything is added to the marrow space.
11. Age epochs modify likely sites of disease, reactive potential and therefore morphologic expression.
12. Benign tumors may stabilize in size, but even so, by strategic position, can incapacitate or kill; malignant tumors inexorably grow and by definition, can metastasize.

## Categories of Disease

Factors that influence the speed with which bone lesions evolve leave clues that distinguish chronic from acute skeletal disease. The tempo of the process is a potentially important factor in diagnosis and can be discerned by "morphologic analysis," i.e., attention to the character of margins, periosteal reactions, and presence or absence of regional remodeling effects (Ragsdale 1993a). The location of solitary lesions is also helpful in diagnosis, and the character and distribution of multifocal lesions are similarly important in narrowing a differential diagnosis.

In 1996, Ragsdale and co-workers proposed using seven basic disease categories as part of the diagnosis of paleopathological conditions. These can be easily remembered by use of the acronym VITAMIN (Table 13.2) (Ragsdale and Miller 1996). Approaching differential diagnosis by looking for clues as to which of the Seven Basic

**Table 13.2** The seven basic categories of disease

| | | |
|---|---|---|
| I | V | Vascular |
| II | I | Innervation/Mechanical |
| III | T | Trauma/Repair |
| IV | A | Anomaly |
| V | M | Metabolic |
| VI | I | Inflammatory/Immune |
| VII | N | Neoplastic |

Categories of Disease is represented helps minimize the overwhelming bewilderment that can result from attempting to choose from several hundred individual entities that can afflict bone. Thus, paleopathologists should redirect their enthusiasm for diagnosing specific diseases in human skeletal remains, to deducing the less ambitious (but more often correct) classification by disease category. This will enhance the comparability of data within paleopathology.

## Vascular disturbances

There are two basic vascular mechanisms which can impact bone. The first is active hyperemia (increased arterial blood flow), which increases tissue oxygen tension, fueling mitochondria-rich osteoclasts that engage in bone resorption. The second is passive hyperemia (slow flow in patulous veins) which creates back pressure and increases permeability, leaking the edematous protein substrate that osteoblasts use to produce matrix. Due to the physics of streaming potentials, these contrasting vascular flows are characterized by opposite local electrical field effects (MacGinitie et al. 1993). Charged solutes in blood moving past fixed charges in vessel walls create energy fields similar to an electromagnet. Active hyperemia engenders a positive electrical environment, favoring osteoclasts, which work best in a slightly acidic pH. Passive hyperemia evokes a net negative field and a basic environment favoring osteoblasts.

Examples of hyperemic conditions include:

**Active hyperemia**: Enlarged cortical penetration ports of vessels near any inflammation; lytic phase of early acute osteomyelitis; juxta-articular osteolysis in rheumatoid arthritis; pencil deformity (concentric atrophy) of ainhum; loss of neuroregulation in dysvascular responses (Sudeck's atrophy, post-traumatic osteolysis) and leprosy; increased blood flow demanded by metastatic carcinoma.

**Passive hyperemia**: Dense medullary edema around a Brodie's abscess supplies protein substrates for marginal sclerosis; back pressure in varicose veins or distal to an atriovenous fistula evokes an edematous periosteal reaction; calvarial hyperostosis due to invasive meningioma clogs venous outflow (Huggins et al. 1981); and pachydermohyperostosis (Uehlinger disease) is its systemic manifestation.

**Biphasic hyperemias**: Circulation is initially active and later passive: acute osteomyelitis transitioning into chronic; diabetic osteopathy; Charcot joint; melorheostosis; hypertrophic pulmonary osteoarthropathy; Paget's disease.

Importantly, the mechanical, hematopoietic, and fat reserve functions of bone are dependent on an adequate blood supply, i.e., viability. Injury or disease elicits a vascular response. There are three noteworthy peculiarities of the vascular supply to bone which directly impact pathological processes in bone.

The first is that bone is a completely rigid compartment. The blood vessels in bone are totally protected from the mechanical compressive forces operative in soft tissue and have, therefore, thin walls. This confers a susceptibility to hydrostatic compression by anything that is added to the marrow space such as edema, pus, fat cell swelling, histiocytes filled with abnormal lipid or mucopolysaccharide, tumor, etc. Cessation of flow leads to ischemia and necrosis. At the end of a bone and in cuboidal bones this is "avascular (aseptic) necrosis." Conversely, ischemic necrosis in metaphyses or diaphyses is a "bone infarct." This can be seen with storage diseases (Gaucher's disease), vasculitis, and sickle cell disease. Bone infarcts are uncommon in the modern era and likely less so in antiquity.

Ischemic aseptic necrosis is most commonly found today in the femoral head, a precariously supplied spheroid with narrower inlet (the neck) than equatorial girth. Anything that swells fat cells (e.g., alcoholism, steroid therapy, genetically caused storage diseases like Gaucher's) will compress the only thing that can yield in the closed space defined by the cortex-marrow vessels: blood vessels. Hip dislocation and femoral neck fracture simply transect vessels, vasculitis occludes them with thrombus, and sickle cells clog them. Avascular necrosis of the femoral head in a child (Legg-Calvé-Perthes) follows a growth spurt that is unaccompanied by sufficient vascular remodeling. Whatever the cause, the attempt at repair of a dead femoral head segment begins with removal of dead cancellous bone along a reactive interface which cuts away the necrotic segment, followed by collapse and eventual severe osteoarthritis due to the resultant shape change and head fragmentation.

The second peculiarity of the vascular supply to bone is that only one-third to one-half of the vascular network in bone is open at any given time. Blood is shunted from regions of lesser to greater need by chemo-sensors (the glomoid system) that act over neural pathways to modify arterial caliber (Johnson 1971). The periosteal, osseous and medullary vascular "fields" constantly shift (take turns) from moment to moment. This is regulated by the angioglomic system that is commonly upset in disease. Therefore, circulatory disturbances are regional, affecting bone segments or bones served by the same vascular trunk.

Thirdly, the capacity of the venous system in bone is 6–8 times that of the arterial system. The shared vascular connections between muscle and bone play an important part in blood movement. The loss of bone density that accompanies disuse and muscle paralysis is in part due to the loss of the muscular "pump's" assistance to venous return. This results in progressive distention of intraosseous sinusoids caused by osteoclastic removal of bone. A further effect of disuse is the removal of the mechanical stimulus for bone formation while the lysis of physiologic turnover proceeds.

## Innervation and mechanical disease

Mechanical factors operate through bioelectric signals proportional to use or disuse. Bone is a composite, crystalline substance. Hydroxyapatite hexagonal prisms solidify the periodic organic crystal fabric of collagen. Electric charge generated by pressure

on crystals is known as piezoelectricity. Whether affecting a solitary trabeculum or an entire longbone, deformation (i.e., slight bending), results in a charge separation in the crystalline fabric. When thus loaded, concavities become sites of negative charge favoring osteoblasts that bolster the site with new bone. Convexities are the site of unburdening, positive bioelectric charge, and osteoclasia. In this way, bone is optimally "repositioned" under the load.

Bone adapts to its mechanical environment during life. The proposition that differences in morphology can be used to investigate differences in past mechanical environments is widely accepted among paleoanthropologists and bioarchaeologists (Ruff 2005), but has important limitations (see Chapter 29 by Jurmain et al., this volume, and Chapter 28 by Waldron, this volume, for further discussion). Examples should come readily to mind: hypertrophy of bone and/or alterations in shape due to specific occupations, sports activities or other specialized activities; "flowing" hyperostosis along vertebral bodies on the opposite side of handedness; relative loss of bone on the convex side of a scoliotic curve; loss of bone following denervation, and inactivity of senescence.

A negative feedback control mechanism involving bioelectricity has been proposed as the basis for changes in bone structure. Here, extrinsic force is translated by the osseous transducer (bone fabric) into a proportional electrical command signal, eliciting a cellular response that creates a structural change to resist the extrinsic force. In the feedback model presented by Lanyon et al. (1982), strain (not stress) on bone is the stimulus for the feedback loop causing bone formation. The new bone reduces strain in the area of formation, and the loop ends at what Lanyon et al. termed the "optimum customary strain level," or equilibrium. Decreased strain on an area would, by this same hypothesis, lead to bone loss, thereby increasing the strain on any single area of bone and ultimately leading back to equilibrium. The impact of strain will differ with location within a single bone and within the skeleton (Carter 1984), due to age (Currey et al. 2002), disease state, hormonal status (Lanyon and Skerry 2001) and genetics. The type and tempo of strain may also play an important role.

## Trauma and repair

This category includes both accidental and intentionally caused changes to the skeletal system, both of which provide important information about the culture and environment in which the individual lived. Trauma includes fractures, dislocations, posttraumatic deformity, cuts, borings and scrapes to bone (e.g. incomplete trephination or scalping, mutilation), amputations, decapitations or other dismemberments of living individuals, cranial deformation, cauterization, thermal injuries, and others (Ortner 2003, and see Chapter 20 by Judd and Redfern, this volume).

Repair is an accelerated recapitulation of normal growth. The stages and rate of repair are constant (though attenuated in calvaria and exaggerated in ribs) so that the elapsed time since the fracture can be determined from roentgenographic and histologic details. The three phases of fracture healing are circulatory (initial active hyperemia supporting osteolysis of injured matrix), metabolic (passive hyperemia supporting osteoblastic synthesis of internal and external callus) and mechanical (prolonged remodeling under mechano-piezoelectric guidance). Most fractures heal with good function in the absence of medical care. Healing with a false joint

(pseudoarthrosis or neoarthrosis) may mimic a tumor or infection. Surviving cells in injured muscle may modulate to bone, ossifying to contribute to an exuberant callus that eventually becomes a bony prominence (exostosis).

Pathologic fracture caused by an underlying pathological condition is relegated to the category containing the responsible condition (tumor = Neoplastic; osteoporosis = Metabolic; osteogenesis imperfecta = Anomaly; etc.) rather than in the Trauma category.

## Anomalies (misgrowths)

Anomalies, or "misgrowths," are local distortions of shape and size that result from skewed growth due to malfunction of cells or organelles (for more discussion of these conditions see Chapter 21 by Barnes, this volume) Generalized developmental failure produces dwarfism; the several types of epiphyseal and metaphyseal dwarfism (whose deformed bone ends resemble osteoarthritis) must be separated from achondroplastic dwarfism. Localized failures produce cleft palate, clubfoot, and congenital dislocation of the hip. Abnormal organelles affecting mucopolysaccharide function skew growth-plate and articular cartilage development to produce distorted dwarfism. Osteoblasts that lack a normal genetic recipe for collagen produce a thin-boned skeleton (osteogenesis imperfecta) that suffers multiple fractures and has excessive osteocyte lacunae per unit area of bone appreciable histologically. Incompetent osteoclasts in Albers-Schonberg disease (osteoporosis; marble bone disease) leave most bone that is produced, resulting in lack of metaphyseal cutback and retention of enchondral bone leaving scant space for hematopoietic marrow, which takes up residence in an enlarged liver and spleen.

## The metabolic category

Metabolic disease has to do with abnormal production, mineralization, or maintenance of the matrix of bone, i.e. the collagen fabric impregnated with mucopolysaccharide and crystals (see Chapter 22 by Kozlowski and Witas, this volume). So metabolic abnormalities are generalized throughout the skeleton with bilaterally symmetrical zones of maximal intensity, and where normal growth and remodeling are most rapid at the time of the disease. They result from an activation or suppression of cell activity and include nutritional inadequacy, vitamin deficiency, hormonal variations, metabolic errors, and poisons.

Important concepts concerning metabolic disorders include:

1. Bone is a composite material (like steel-reinforced concrete), comprised of synthesized matrix (osteoid) and mineral.
2. The simple rules, reactions, rates and investigative methods of inorganic chemistry do not apply in complex living biological systems and will not provide all-inclusive explanations.
3. Matrix made in abnormal genetic, metabolic nutritional, drug therapy, etc., or circulatory environments, fails to mineralize properly because it is *inherently* abnormal, not because of the popularly held notion that calcium is less available. (If the heart is beating, the serum calcium is sufficient to mineralize properly formed osteoid.)

4. Phosphate has more to do with the normal process of matrix building and mineralization than calcium. Understanding this is particularly important to understanding rickets, the various types of osteomalacia, and renal osteodystrophy.
5. Bones cannot become "decalcified" in the body, as they can if interred in an acidic environment. There is always total removal of the organic component (matrix) as well as mineral. This requires cellular activity and only osteoclasts and (to a much lesser degree) osteocytes possess this capability. Thus, the term "osteopenia" is preferred over "demineralization" when describing skeletal x-rays that show rarefaction.
6. Skeletal radiographs reflect a momentary summation of a dynamic interplay between simultaneous bone formation and bone removal ("turnover"). Osteoclasia and osteoblastic repair are always coupled, but one or the other may predominate in a given disease state at a given time period, and thus dominate the radiograph.

The problem of identifying the trigger for metabolic disorders is difficult in the context of paleopathological studies where skeletal remains and their archaeological context are our only sources of evidence (Walker et al. 2009). For instance, the effects of vitamin deficiency are dependent on intensity, duration, and age of the individual. Similarly, intermittent inadequate nutrition is registered simply as growth-arrest lines, while continuous malnutrition might lead to stunted stature. Recognizing the presence of these conditions in the archaeological record does not clearly lead to a precise cause. Adding to the complexity is a tendency for conditions to mimic one another. Acute infantile scurvy masquerades as a metaphyseal fracture with most of the cancellous bone missing; during healing, much of the bone is encased in a shell of new periosteal bone around organizing hematoma which will remodel to normal in later years (hematomas themselves do not "ossify": they organize). Osteomalacia will appear on roentgenograms as an osteopenia (the unmineralized osteoid will have been lost *in situ*), associated with bilaterally symmetrical incomplete cortical fractures (infractions); prolonged osteomalacia starting at an early age results in bowing deformities. Excesses of thyroxin, insulin, and cortisone all produce osteopenia without distinguishing features, but are unlikely to be seen in Stone-Age populations with a shorter lifespan.

## Inflammation and immunity

Here the primary cause of bone change is an immune reaction to outside challenges such as bacteria, fungi, parasites, and viruses; or to changes in the immune system caused by failure of the body to recognize its own constituents. Many of these conditions are acute and leave no marks on the skeleton (e.g., pediculosis, typhus, malaria); some leave highly characteristic changes (e.g., leprosy, tuberculosis, treponemal diseases); some leave nonspecific changes related to the immune system malfunction over an extended period of time (e.g., sterile subacute polyostotic osteomyelitis).

The inflammatory process is an exaggeration of the normal disposal mechanism for everyday cell turnover. However, it is activated by the immune system rather than an inherent abnormality in circulation or vasoregulatory systems characterizing the conditions in the *Vascular* category. Cells within the human body are continually shed and renewed at varying rates. Infection damages normal cells and accelerates this

**Table 13.3** The three fundamental inflammatory patterns

| | *Septic* | *Granulomatous* | *Angiitic* |
|---|---|---|---|
| *Cell* | Polymorphonuclear | Histiocyte | Plasma cell |
| *Response* | Exudation | Migration inhibition | Perivascular proliferation & cuffing |
| *Effect* | Liquefaction | Caseation | Infarction |
| *Focus* | Abscess | Tubercle | Gumma |
| *Host response* | Great | Moderate | Low |
| *Course* | Fast | Slow | Late |

turnover; the heightened response is pathological inflammation. In bone, the character, quality, quantity, and distribution of lytic change and reactive bone are indications of the tempo and character of the inflammatory fight with a foreign invader. They are also largely determined by the predominant cell type recruited and the attendant increased blood supply.

There are three fundamental inflammatory patterns: septic, granulomatous, and angiitic (Table 13.3). Septic inflammation can be suppurative (e.g., triggered by staphylococcus) with rapid production of pus that fills the marrow cavity, strips off the periosteum and isolates a dead cortex (sequestrum) within the reparative shell of new periosteal bone (involucrum). A less violent septic reaction, i.e., a bone abscess, is walled off by a dense border of bone. Important to diagnosis is the fact that extensive local repair may mimic sequelae of trauma or a neoplasm. Granulomatous infection (e.g. tuberculosis) entails the slow accumulation of rounded aggregates (granulomas) of macrophages and lymphocytes around a necrotic center that enlarges as satellite granulomas spread out with gradual bone destruction and scant if any, repair. This result can mimic neoplasm. Angiitic (or "vasculitic") inflammation (e.g., syphilis) is mediated by antibody-producing cells (lymphocytes and plasma cells) accumulating around blood vessels (angiitis); vascular thrombosis results in dead tissue (gumma) in bone and soft tissue. Angiitis is widespread over a region and in periosteum provokes bilaterally symmetrical reactions. Different still is "toxic" inflammation (e.g., rheumatoid arthritis), beginning as a fluid effusion that then becomes a low-grade inflammation dominated by the humoral side of the immune system (antibodies). Sustained active hyperemia, which stimulates osteoclasts, results in erosion of cortex at the joint capsule attachment, and erosion of subchondral bone that shares the boosted vascular supply of synovium. "Reactive" inflammation of the soft tissues and within bone to viruses (e.g., smallpox) or worms (e.g., echinococcus) is mild, nonspecific, and usually asymptomatic.

However, not all reactions are triggered by "outside" invaders. One's microbial flora includes pathogens held in check by a variety of commensal organisms and saprophytes. Infection may result from a break in the epithelial surface by malnutrition (inadequate cell shedding), exhaustion (inadequate secretions), or cuts and bites. But even germ injection does not ensure disease, for the core of the immune system, the reticuloendothelial system, thrives on a continuous diet of germs. Disease results only when the number and vigor of the organisms permit successful competition with cells for essential nutrients, enzymes, or space. The augmented turnover of injured cells drives inflammation.

The influence of the portal of entry is illustrated by the variety of diseases that can result from the same streptococcus. For instance, streptococcus entering from the skin produces erysipelas; entering via the mouth, septic sore throat and mastoiditis; from the gut, phlegmonous enteritis; venereally, puerperal fever; from the lung, hemorrhagic pneumonia; and from inoculation as by penetrating trauma, endocarditis and osteomyelitis. Furthermore, streptococci can provoke granulomatous inflammation (the Aschoff nodules of rheumatic fever), angiitic inflammation (polyarteritis and Schonlein-Henoch purpura), and collagen vascular disease (autoimmune nephritis). The lesson is clear: the disease morphology is determined by the reaction, not the specific trigger (organism).

The relevance of this to paleopathology is obvious. Through human history, specific diseases (e.g., Lucio's leprosy (Roverano et al. 2000; Choon and Tey 2009) and "malignant" or "galloping" syphilis (Cripps and Curtis 1967; Watson et al. 2004)) have burst on the scene with aggressive, rapidly progressive, even fatal expressions, later evolving into chronic illnesses with very different skeletal effects and individual case tempo. This suggests that we are naïve when we use particular modern clinical infections to model the expected histologic structures found in ancient dry bone specimens.

## Neoplasms (Tumors)

Neoplasms are overgrowths of cells that commonly arise from antecedent accelerated cell proliferation related to normal growth and development or a sustained reactive state (Johnson 1953; and see Chapter 23 by Brothwell in this volume). Benign tumors, generally solitary, may achieve dramatic proportions or stabilize at a lesser size. They can weaken a bone to the point of fracture but remain a local problem.

Malignancy is characterized by inexorable local growth and metastases. Malignant bone neoplasms, arising there or coming to bone from a visceral organ are rare, and rarer still in antiquity with the lower turnover rate, the slower growth associated with diminished stature, and shorter lifespan. Primary bone, and joint sarcomas in particular, should be rare since the annual number diagnosed in the USA is only about 2,650 as of 2010 (American Cancer Society 2010). However, the age distribution of bone tumors derived from modern surgical pathology series is irrelevant to paleopathology since, benign or malignant, the endpoint in the paleopathological specimen was the death of the individual.

Neoplasms are named for the cells of which they are composed (e.g., lymphocytes—lymphoma, vessels—angioma) or their products (e.g., osteoid, cartilage, fibrous tissue, fat, etc.). Primary bone tumors emphasize the proliferative and functional capabilities in one or more of the three skeletogenic cell types (Johnson et al. 2000): chondroblast, osteoblast and osteoclast. Particular types of primary bone neoplasms occur in that portion of a bone where the normal cells, from which they arise, are most abundant and active (Figure 13.1). Therefore, roentgenograms signal the probability of a specific diagnosis by showing the site of involvement (e.g., osteosarcomas in metaphyses; chondroblastomas in epiphyses) (Madewell et al. 1981). A list of somatic tissues, in the order of normal cell turnover rate, is also a list of the relative likelihood of neoplasms. Since neither cells nor unmineralized matrices usually remain in paleopathological specimens, most neoplasms will be perceived as large holes, some

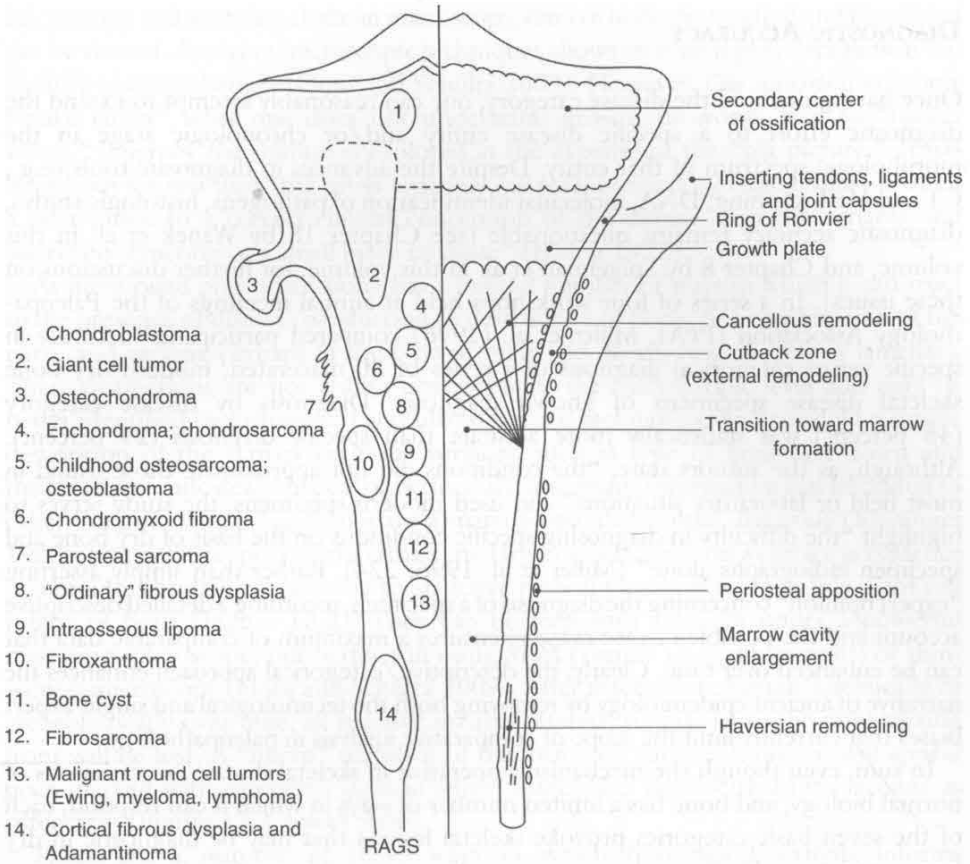


**Figure 13.1** Typical bone tumor locations as they relate to events of normal growth and development.

bordered by reactive bone. The character of periosteal reactions reflect the speed of the growth (Ragsdale 1993a). Some tumors have an internal margin of dense bone indicating slow growth.

Metastatic cancer (lytic, blastic, or mixed), frequently affects the skeleton. Due to greater blood supply of hematopoietic marrow compared to fatty marrow, more cancer cells are delivered to it, and so it is no wonder that the spine (80 percent), proximal femur (40 percent), ribs and sternum (25 percent), skull and pelvis (25 percent), humerus and shoulder girdle (7 percent) are common sites for metastasis seen in autopsy cases (Ortner and Putschar 1981). Tumors arising from the breast, prostate, thyroid, lung, and kidney possess a special propensity to spread to bone (Coleman 1997), but using modern statistics on the prevalence of various cancers to understand the past must be undertaken cautiously. For instance, since 1990 there has been a precipitous decline in gastric cancer and an exponential rise in lung cancer, both attributed to altered lifestyles. The AIDS epidemic is afflicting large numbers of individuals with new or rare expressions of "old" neoplasms such as lymphoma (e.g., primary CNS involvement). Clearly, large changes in disease incidence are possible over short time intervals and in different cultures (Ragsdale 1995).

## Diagnostic Accuracy

Once having deduced the disease category, one can reasonably attempt to extend the diagnostic effort to a specific disease entity and/or chronologic stage in the morphologic spectrum of that entity. Despite the advances in diagnostic tools (e.g., CT and MRI scanning, DNA, molecular identification of pathogens, histologic study), diagnostic accuracy remains questionable (see Chapter 18 by Wanek et al. in this volume, and Chapter 8 by Spigelman et al. in this volume, for further discussions on these issues). In a series of four workshops held at annual meetings of the Paleopathology Association (PPA), Miller et al. (1996) compared participants' accuracy in specific versus categorical diagnosis of a series of 20 macerated, modern dry bone skeletal disease specimens of known diagnosis. Diagnosis by disease category (43 percent) was statistically more accurate than specific diagnosis (29 percent). Although, as the authors state, "the conditions did not approximate those found in most field or laboratory situations" and used modern specimens, the study serves to highlight "the difficulty in diagnosing specific conditions on the basis of dry bone and specimen radiographs alone" (Miller et al. 1996: 224). Rather than simply asserting "expert opinion" concerning the diagnosis of a specimen, recording a detailed descriptive account and the possible disease *category* ensures a maximum of comparable data that can be enhanced over time. Clearly, the descriptive/categorical approach enhances the narrative of ancient epidemiology by removing both the technological and single-expert biases that currently limit the scope of comparative analysis in paleopathology.

In sum, even though the mechanisms operative in skeletal disease are variations of normal biology, and bone has a limited number of ways in which it can respond, each of the seven basic categories provoke skeletal lesions that may be diagnostic in dry bone specimens. However, because some disease conditions of antiquity may have been eliminated by evolutionary selection with no modern counterpart, paleopathologists should be cautious when diagnosing specific diseases in skeletal populations.

## Histology in Paleopathology

Various methods for preparing ground thin sections for light microscopic study, and thus eventually for paleopathological investigation, can be found in the literature (Maat et al. 2001; Schultz 2001; Beauchesne and Saunders 2006; Von Hunnis et al. 2006). Preparation involves embedding samples in plastic resin, sawing a thin slice and polishing it even thinner by hand or on a glass surface with diamond paste abrasive. Maat et al. (2001) provide a revised method which produces images of equivalent quality to samples prepared using embedding media. But only relatively dense and intact specimens hold up to the physical demands of the manual grinding procedure (Beauchesne and Saunders 2006).

### The promise

A disease unfolds as a movie; the specimen is a single frame. But bone, like a single frame, can represent a record of past events. Therefore, each specimen provides clues to earlier frames in the show. Through the use of roentgenograms, stereo- and light

microscopy and scanning electron microscopy, rates of bone destruction and formation can be viewed. Applying microscopic techniques allows an even higher "resolution" to identify osseous changes (Flohr and Schultz 2009). However, this approach may only "make larger" what one does not understand grossly, or worse, lead to "tunnel vision," whereby the minute is explored at the expense of the "big picture." It has long been known that "the gross anatomy (as corroborated by radiographs) is often a safer guide to a correct clinical conception of the disease than the variable and uncertain structure of a small piece of tissue" (Ewing 1922).

As mentioned previously, bone has a limited number of ways in which it can react to the presence of disease (production or destruction of bone, or a combination of the two), and varying perhaps in the type of bone laid down, (e.g. woven or lamellar). These distinctions are not always observable at the macroscopic level and may be better identified by histological and ultrastructural techniques. By providing a simple description of the activity on bone surfaces, such as type of bone laid down and the location (endosteal, cortical or periosteal), important information can be added to other lines of evidence and improve a macroscopic differential diagnosis (Klepinger 1983). For instance, pockmarks created by Howship's lacunae on a surface is evidence of a rapid resorptive process due to an active pathological condition as in a giant cell tumor of bone (Figure 13.2). They can be seen with sidelight under a powerful dissecting microscope, but better still using SEM (Figure 13.2, upper right) or thin-section microscopy (Flohr and Schultz 2009). After maceration though, chemically or archeologically over time, all reactive matrix (osteoid) superficial to the mineralizing front will be lost. A "micro" snapshot of the formative surface, however, will appear pock-marked with osteocyte lacunae where osteoblasts have settled into lacunae as osteocytes (Figure 13.2, lower right).

There are a number of other ways in which histological analysis informs paleopathology. The histological analysis of bone cross-sections (e.g., cortical area, cortical thickness) have been shown to be powerful tools in the assessment of nutrition, growth patterns, and adult bone loss (Martin and Armegalos 1985). Although uniform procedures for the collection and reporting of data are wanting, standardized terms for reporting are available in detail (Parfitt 1987).

The use of histology to estimate age-at-death, understand taphonomic processes, and diagnose disease in human skeletal and mummified remains is also extremely promising (Schultz 2001).

The presence of pseudopathology can be determined with a good working knowledge of the microscopic structure of bone, including the histogenesis and growth of bone and the potential and/or presence of decomposition and diagenesis. For instance, long bones of South African diamond miners who died between 1897 and 1900, were macroscopically diagnosed with periostitis. Subsequent histologic sectioning of the bones revealed no pathological changes to the structure of the bone, suggesting that these bones were normal (Van Der Merwe et al. 2010). Schultz et al. (2007), after conducting a histological examination of bones from a child buried at the Early Bronze Age cemetery, argue that the macroscopic changes initially attributed to periosteal reaction were actually caused by post-mortem taphonomic changes. These, and other findings have led Van Der Merwe et al. (2010) to declare that 'periostitis' is likely over-diagnosed in archaeologically derived samples.

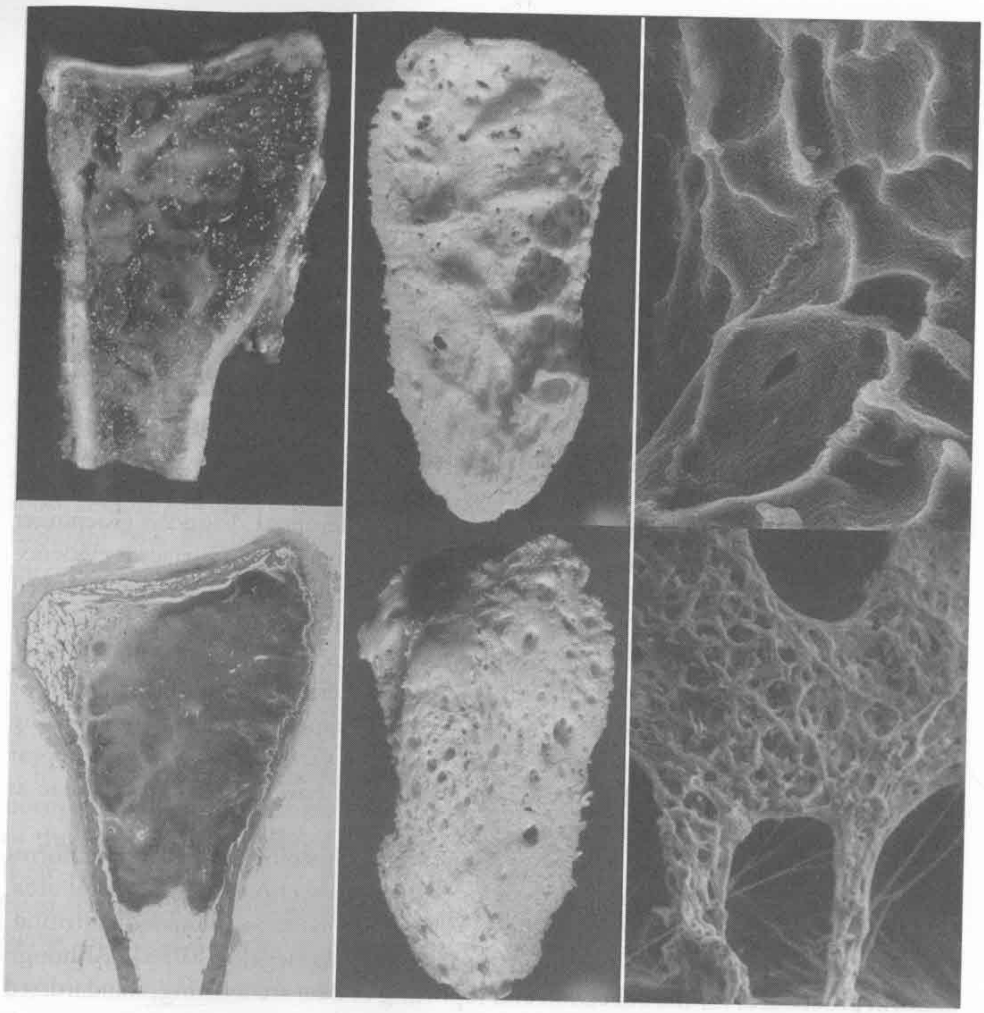

**Figure 13.2** Giant cell tumor of bone, distal radius. [Left column]: gross view of longitudinally saw-cut specimen (top) and large format histological section (bottom) with the new ridged shell-type periosteal reaction that replaced original cortex along the right side of the tumor. [Center column]: macerated specimens of the ridged shell-type periosteal reaction, endosteal surface (top) periosteal surface (bottom). [Right column]: scanning electron micrographs – Endosteal surface (top) displaying overlapping Howship's lacunae, exposing an elliptical osteocyte lacuna and above that, a somewhat erosion-resistant cement line (1300x). The periosteal surface (bottom) pockmarked by shallow depressions where osteoblasts were settling in as osteocytes beneath a layer of unmineralized osteoid, removed during maceration (520x).

Histopathology makes its most definite contributions when soft tissue is present and can be rehydrated. Sabbatani and Fiorino (2009) reviewed paleopathological studies performed in mummified tissues, with reference to infectious diseases. Through correlation of histological and biomolecular information, the authors explored dietary and hygiene conditions of ancient populations. Similarly, histological analysis of soft tissue in a 2,500-year-old Egyptian mummy enabled specific diagnosis

of a benign neurilemmoma (Strouhal and Německová 2009). However, Zweifel et al. (2009), through the use of meta-analysis, emphasize that minimum publication standards for paleopathologic studies are necessary to improve evidence-based research in paleopathology.

## The pitfalls

Despite growing awareness of diagnostic and analytical potential of histological analysis, its application within paleopathology and bioarchaeology is rare (Von Hunnis 2009). The reason for this is undoubtedly pragmatic, in part, as the cost of the equipment and materials, the preparation time, and the methodological knowledge required for the preparation of thin sections can be daunting (Beauchesne and Saunders 2006). Serving as further obstacles are the facts that the biologic basis of histomorphological features must be clearly understood in order to interpret histological data, and a comprehensive understanding of diagenetic processes that affect bone preservation is needed. Much of the biological and taphonomic knowledge required for the analysis of bone thin sections can be acquired through detailed literature reviews and experiential learning. Basic medical knowledge is necessary if histopathological evidence is to be competently interpreted (Schultz 2001). We dispute the claim that experience and familiarity with bone biology and histology can be swiftly gained (Beauchesne and Saunders 2006).

The availability of a reference collection of well-diagnosed specimens for comparison is also necessary for developing reliable histopathologic conclusions (Schultz 2001). With histology being used rarely, scant comparative paleohistopathological data is available, and much of it is presented with the enthusiasm and certitude attendant with new techniques.

Further confounding histopathological research within paleopathology is the fact that external (i.e., the macroscopic) preservation of a bone does not always correlate to internal (i.e. microscopic) preservation. A bone appearing in good condition macroscopically might have undergone substantial diagenetic change. Diagenesis operates through soil and water, plant roots, fungi, algae, bacteria, protozoa, and arthropods and their larvae, causing microscopic fungal and/or microorganism intrusion, focal destruction by microorganisms (Wedl canals) (hackett 1981), and the presence of foreign materials (e.g., soil particulates, crystals) within lacunae and/or haversian systems. Thus microscopic control is advisable as a precursor to extraction studies (Schmidt-Schultz and Schultz 2007) whose goals might be to isolate particular proteins or fragments of DNA.

Thus, a thorough differential diagnosis is essential in any paleopathological assessment (Roberts et al. 2009). The reliability of a final diagnosis increases as all diagnostics (macroscopic, radiological, endoscopic, and light and scanning electron microscopic techniques) point in the same direction. None of these is more important than the others.

## Terminology: Gross and Microscopic

Paleopathology is essentially a morphologic study. Though, fundamentally, it has many points in common with modern pathologic anatomy, it must be considered different from the latter due to the peculiar nature of the materials investigated.

Despite application of rigorous scientific criteria, sometimes no diagnosis can be made and only descriptive findings will result. This should not be interpreted as an inaccurate examination, or evidence of insufficient technical or diagnostic capacity. Indeed, the healthy trend today is towards a meticulous description rather than the stipulation of a likely but not thoroughly proven diagnosis. Descriptive criteria in pathological anatomy are well defined and can be applied with great accuracy in paleopathology. In 1981, Sweet et al. standardized descriptive terminology for the macroscopic (gross and radiologic) features of bone lesions in a three-part article series. Most of the terminology was applicable to neoplastic and inflammatory lesions. Their assertion was that attention to the three parameters of *margins*, *periosteal reactions*, and *matrix patterns*, as disclosed in plain films, permitted a diagnostic accuracy in excess of 90 percent for bone tumors. Furthermore, combinations of periosteal alterations, margins, and density changes can help refine an "Inflammatory Category" diagnosis into one of the three patterns of skeletal inflammation: septic, granulomatous, or angiitic. Post-traumatic and some metabolic (e.g., hyperparathyroid bone disease) changes are also succinctly described with these terms. The authors emphasized that for accurate description, and as a permanent record of a specimen, specimen radiographs are indispensable, since choice of descriptive terms in part relies on radiographic appearances. Though directed at today's pathologists, radiologists, and orthopedic surgeons, the terminology therein defined can be advocated without alteration for use in paleopathologic descriptions. An update of these concepts was subsequently published (Ragsdale 1993b).

Histomorphometry terminology is a much simpler matter than a standard terminology for gross and microscopic paleopathology. A review of even a dozen papers finds a host of terms used in dry bone microscopy which are unfamiliar to the modern-day orthopedic pathologist. The following is a sample:

- *Faserfilz-osteon*
- Fissural gap
- *Grenzstreifen* (= *grenzlinie*)
- Pin-like or spicular structures
- Plate-like proliferation
- Newly built bone
- Net-like bone formation
- Trabecular type of lesion
- Porotic type of lesion
- Polsters/padding
- Radiating trabeculae

How work employing these terms can be assisted by textbooks and papers describing modern-day orthopedic pathology is questionable. Schultz states that "... in the paleohistopathology of dry bones, the diagnostic criteria can be quite different from those used in recent pathology" (Schultz 2001:120), especially where soft tissue and cells are the major basis for diagnosis. Therefore, "the classification of diseases investigated by paleohistopathologists does not necessarily conform in all ways to that used by recent pathologists." (Schultz 2001:121). This contradicts the accepted paleopathological paradigm of "biomedical clinical analogy," where it is

assumed that the same pathologic changes and signs used to diagnose today's patient can be applied as diagnostic criteria in the interpretation of ancient material (Von Hunnis et al. 2006).

There is a need for greater scientific rigor, especially for data that will be compared and used by different investigators (Fulcheri et al. 1994). The approach of agreeing on a few experts to set the terms, and then agreeing on common usage in the major journals in the field, will likely help paleopathology, as it has in other fields. The first concession, of course, is that investigators must agree that their pet terms may not become the accepted terms and may need to be sacrificed in the interest of universality. With conscientious authors and reviewers working alongside diligent, strong-willed editors, rules will become habits, and manuscripts using uncanonized terminology will go unpublished.

## Tackling Histologic Specificity

Soft tissues and cells that have modified the osseous fabric during life, and which are the main basis for modern histologic diagnoses, are gone and cannot be studied in archaeological skeletal remains. All that remains is the architecture of the cortical, compact, and spongy bone, and any new bone additions. It has been declared that there is almost always a pattern in architectural elements of the cortical, compact and spongy bone substances associated with a particular disease that enable the paleopathologist to make relatively reliable diagnoses (Schultz 2001). This is contested by others (Weston 2009, and Chapter 27 this volume). Previous work has demonstrated a lack of diagnostic characteristics (qualitative or quantitative) in the macroscopic and radiographic appearance of periosteal reactions (Weston 2008). According to Hackett (1974), a diagnostic criterion of a disease is a change that by itself always indicates with confidence the presence of that disease. In the specimens examined by Weston (2008), it was shown that Schultz's (2001) characteristic traits of *grenzstreifen*, polsters, and sinuous lacunae were not characteristic of specific diseases and are extremely variable in shape and distribution, and highly dependent on the part of the section investigated (Van Der Merwe et al. 2010). It was proposed that these three traits are simply manifestations of general inflammatory processes (Weston 2008). That there are specific histomorphological features for each disease is implausible (Van Der Merwe et al. 2010). Hence, as with the macroscopic and radiographic characteristics of periosteal new bone, it appears that the microscopic characteristics are similar regardless of the disease etiology, and thus should not be relied on alone to provide disease diagnoses (Weston 2008).

Unlike the macroscopic/radiologic differences in bone changes due to suppurative, granulomatous, or angiitic inflammation, on a histological level infectious changes in bone are most likely very similar regardless of the specific condition that caused the osteomyelitis. For example, Schultz states that "Unfortunately, bone lesions caused by syphilis are frequently very similar to alterations caused by nonspecific bone diseases, particularly in long bones" (Schultz 2001:126). However, Van Der Merwe contests that it was only in conjunction with macroscopic investigation and a clear description of the distribution pattern of the lesions across a skeleton that a case of osteomyelitis could be attributed to treponematosis (Van Der Merwe et al. 2010).

Of further concern is Schultz's (2001) creation of only three groups of microscopically "proliferate patterns" bone change: hemorrhagic, inflammatory, and tumorous. While this assertion may be correct, approaching the task of diagnosis in this limited fashion may dissuade the researcher from thinking of changes caused by vascular disturbance (e.g., infarct), trauma and repair, anomalies (osteopetrosis), metabolic disease (fluorosis), and neuromechanical (osteophytosis) processes. Diagnoses become oversimplified and potentially wrong.

## The Cutting Edge

Tens of thousands of histological slides of normal bone and radiologic studies of some skeletal diseases are housed at the Anatomical Division of the National Museum of Health and Medicine (NMHM) in Washington DC. Theses slides are from the former research collection of the Orthopedic Pathology Department of the Armed Forces Institute of Pathology (AFIP). Particularly relevant to paleopathologists interested in histopathology are the stained large-format histological glass slides and ground sections of bone cut from plastic embedded, undecalcified samples. Researcher access to the collection (which is currently unavailable due to the planned relocation of the facility) is expected to resume in 2012. In addition, senior author Ragsdale's personal collection of large glass slides assembled while at the AFIP may soon be available for qualifying scholars to study.

The primary methods for creating whole-mount slides are outlined in the out-of-print 1968 AFIP Histological Staining Methods Manual (Luna 1968). Hematoxylin and eosin (H & E) stains predominate in the whole-mount collection, though Masson's trichrome and other special stains were commonly used. All processing of large-bone specimens was done by hand whereas automatic processors handled the smaller pieces (AFIP 1949).

Large glass slides of these modern-day specimens with known diagnoses have much to contribute. Soft tissue structures and cellularity responsible for the adjacent mineralized fabric changes are present in these preparations, as seen in Figure 13.2 (lower left), unlike in a dry bone sample. The disease *is* the soft tissue and cellularity; bone is merely a bystander modified by it. The goal should be to find mineralized matrix clues to specific subjacent soft tissue/cellular components. One would hope (but we doubt) that details in hard-tissue histomorphology can be reliably linked to immediately adjacent soft tissue/marrow content and then this "experience" and morphologic standards taken back to dry bone studies.

The growing number of histopathological citations in paleopathological publications indicates an interest in the regular use of histopathology and the need for a standardization of methods, descriptive terms, and reporting. More work is required in the field, particularly in characterizing the histopathological features of pathological bone from macerated specimens with known diagnoses (Weston 2009). Specimens donated to the Anthropology Department at Arizona State University in Tempe by Dr. Ragsdale were designed to meet this need. Macerates are on file with photographic documentation of what the surrounding soft tissue looked like. Figure 13.3 is an

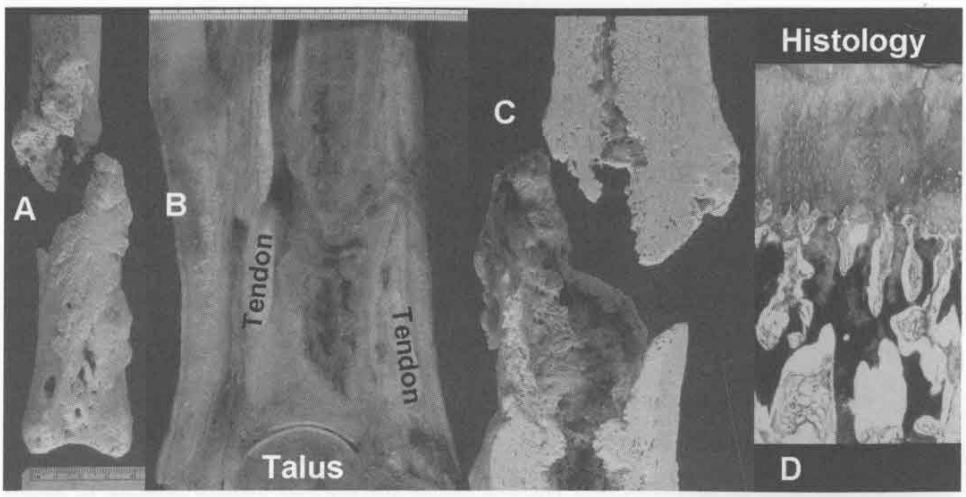


**Figure 13.3** Infected non-union of distal tibia. External (A) and internal (C) views of proximal and distal macerated tibia segments flank the saw-cut fresh specimen (B). The gap shown in "A" and "C" was occupied by a gristle-like fibrocartilage band that triggered enchondral ossification where it contacted bone as an attempt toward bony union (D). Would the histopathology of dry bone "C" predict this pathologic enchondral ossification?

example of a post-traumatic cartilagenous non-union gap in dry bone segments, flanking the longitudinally saw cut fresh specimen. The right panel is a trichrome histological view of the blue-stained fibrocartilage that filled the gap, and the enchondral ossification working to delete it from below.

A review of the literature indicates that fewer studies have used scanning electron microscopy (Weston 2009). Other techniques, such as fluorescence microscopy (e.g., for the detection of the vestiges of postmortem or intravitam fungi), phase contrast microscopy, and interferential contrast microscopy (e.g., examination of soil-living microorganisms), are also useful in paleopathology (Schultz 2001).

Confocal microscopy has also been adapted. The confocal laser scanning microscope (CLSM) is a relatively new and advanced microscopic imagining method which is rarely used in anthropological research, although numerous clinical and basic science research projects have shown its value to study bone growth, bone micro-architecture and 3D bone morphometry (Papageorgopoulou et al. 2009). The advantage of CLSM over conventional histological imaging is three-dimensional visualization, making it possible to evaluate the osteocyte distribution and the architecture of the canalicular network. CLSM users claim to differentiate pre-existing microdamage sustained *in vivo* from microdamage acquired through the processing of the sample (Papageorgopoulou et al. 2009).

Ancient bones in a good preservation state, ascertained by microscopic techniques, conserve extracellular matrix proteins and other macromolecules over thousands of years (Schmidt-Schultz and Schultz 2007). DNA analysis provides enormous potential and reliability in the identification of human remains (see Chapter 8 by Spigelman et al., this volume, for more detailed discussion on this topic). Analysis of degraded

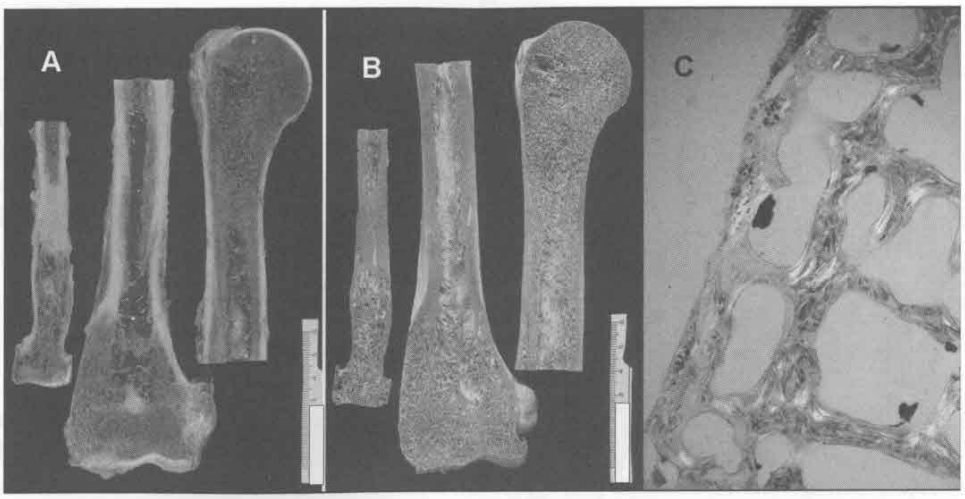


**Figure 13.4** Sickle cell disease. A: Saw cut fresh autopsy bones of the adult male who died in sickle cell crisis (Faerman et al. 2000). Proximal radius (left) and distal humerus (center) have old sclerotic residua of bone infarcts appearing white against the hyperplastic red marrow filling the bones. A recent infarct appears white in the distal diaphyseal marrow of the proximal humeral segment. B: Same specimens after chemical (papain) maceration display larger than normal cancellous modules. C: Ground section at extreme right viewed under polarized light (courtesy of Michael Schultz, MD; 40x).

DNA in forensic and archaeological specimens is still hampered by methodological difficulties and the question of the authenticity of DNA isolated from human remains. Trace amounts of highly damaged DNA in forensic and archaeological samples require the application of the extremely sensitive polymerase chain reaction (PCR) method, which is prone to contamination even deep in the specimen. However, this may be overcome, or at least predicted, by applying a set of clear precautions and controls which are enhanced by careful microscopic evaluation of the material (Cooper 1997).

Differential diagnosis of anemias in dry skeletal remains is difficult using traditional anthropological methodology. However, microscopic examination as part of preliminary screening will facilitate the molecular detection of hemoglobinopathies in archaeological specimens with suspected anemia. In anemia, cancellous-type modules are clearly carved out from antecedent compact bone to afford additional space in which to house hyperplastic marrow as in the fatal sickle cell disease case presented in Figure 13.4. Trabeculae of cancellous bone become thin and relatively long as cancellous modules of the marrow space are enlarged. In a study of a modern autopsy bone sample from a documented case of sickle cell anemia, Faerman et al. (2000) demonstrated the power of DNA analysis applied to the ß-globin gene, mtDNA sequences, Y-chromosome DNA polymorphisms, and sex identification in the creation of a genetic portrait that was corroborated by microscopic examination of bone structure, and historical and medical records (Faerman et al. 2000).

## Conclusion

Explaining the dynamic pathobiology of bone lesions must address the only factors influencing the presence of lesions: vascular, neuromechanical, and metabolic factors. These three influences operate to variable extents in the seven basic categories of disease which are capable of leaving recognizable hallmarks in dry bone specimens. Just as fossilized footprint is of interest for they tell us about the creature that made them the alteration of solid bone substance is the result of the action of ephemeral soft tissue elements acting on bone surfaces, internal and external. Understanding this dynamic interface is the essence of studying the mechanisms of disease. The analysis of a dry bone, either grossly or at the microscopic level, must be done with an eye that can "see" what is no longer there, the soft tissue that would have occupied its "holes" and covered its "bumps." We assert that only through detailed descriptions and diagnoses to general disease categories, will a stronger methodological basis for comparative research in paleopathology be reached.

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