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Differentiating Human Bone from Animal Bone: A Review of Histological Methods

ABSTRACT: This review brings together a complex and extensive literature to address the question of whether it is possible to distinguish human from nonhuman bone using the histological appearance of cortical bone. The mammalian species included are rat, hare, badger, racoon dog, cat, dog, pig, cow, goat, sheep, deer, horse, water buffalo, bear, nonhuman primates, and human and are therefore not exhaustive, but cover those mammals that may contribute to a North American or Eurasian forensic assemblage. The review has demonstrated that differentiation of human from certain nonhuman species is possible, including small mammals exhibiting Haversian bone tissue and large mammals exhibiting plexiform bone tissue. Pig, cow, goat, sheep, horse, and water buffalo exhibit both plexiform and Haversian bone tissue and where only Haversian bone tissue exists in bone fragments, differentiation of these species from humans is not possible. Other primate Haversian bone tissue is also not distinguishable from humans. Where differentiation using Haversian bone tissue is undertaken, both the general microstructural appearance and measurements of histological structures should be applied. Haversian system diameter and Haversian canal diameter are the most optimal and diagnostic measurements to use. Haversian system density may be usefully applied to provide an upper and lower limit for humans.

KEYWORDS: forensic science, forensic anthropology, cortical bone, histology, histomorphometry, microscopy, scanning electron microscopy, human identification, species determination, mammals

Consistently, forensic anthropologists are being asked to identify fragmented skeletal remains as their involvement in forensic situations expands. These scientists are required to aid in the identification of human remains from fires and cremations (1), mass disasters (2–4), and domestic crimes (5,6) in which human skeletal remains are often presented as highly fragmented, damaged, and potentially commingled with nonhuman skeletal remains and other debris. A common question posed to these forensic anthropologists during such situations is: "Can you tell the difference between human and animal bone?" This question is very important as the answer determines whether a forensic investigation is ultimately warranted. The microscopic determination of human or nonhuman skeletal remains may be accomplished if the remains are presented as whole or partial bones, with retention of anatomically gross diagnostic features that differentiate mammalian species. However, if bone fragments are void of any species-specific morphology, additional methods of analysis are required. Histological analysis is one such approach (5–22).

Histological analysis involves the examination of thin and block sections of cortical bone tissue to assess the appearance of bone tissue as well as the quantification (termed histomorphometrics) of histological structures within this tissue. Previous histological studies of mammalian cortical bone have commonly followed two paths. The first and more earlier path was mainly concerned with providing a thorough, in-depth description of the cortical bone tissue of several mammalian species with no or limited quantitative data (16–20). For instances, Enlow and Brown (18–20) provided a thorough description on the types of bone found in different species but their text lacks quantitative data and direct comparisons. Harsányi (7), however, provided quantitative data for one histological structure (Haversian canal diameter) but with limited qualitative description.

The second path has been concerned with distinguishing human from nonhuman bone using a small and select group of mammalian species, most often dog and farm animals (10–14). For example, Mulhern and Ubelaker (10) compared femoral midshaft sections of human subadults and adults with that of subadult sheep and miniature swine in an attempt to distinguish between human and nonhuman bone. Their study revealed that osteon banding was useful in distinguishing between human and nonhuman bone. Similarly, Whitman (12) studied the presence of Haversian systems in the ribs of humans, beef cattle, and dogs. This study concluded that human Haversian systems and canals were larger in diameter than those of both beef cattle and dog, but an overlap was present for all three mammals. Other studies, although rare, have been more specific, looking to a few mammalian species in an attempt to distinguish each species from each other (8,9,11). Rajtová et al. (8) compared cortical bone histology of sheep and goats while Hidaka et al. (9) compared cortical bone histology of raccoon dogs and badgers. More recently, Benedix (11) looked specifically to mammalian species in Southeast Asia as bones from such mammals are commonly recovered alongside human remains during Joint POW/MIA Accounting Command missions to locate and identify unrecovered and missing U.S. soldiers.

The majority of these studies have been aimed at the differentiation of human from nonhuman cortical bone at a microscopic level, particularly for forensic situations in which small, nondiagnostic bone fragments are recovered. Unfortunately, the major limitation in the examination of these fragments is that the anatomical and individual origin of the bone fragment is unknown

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and utilizing "normal" descriptions of human and nonhuman bone microstructure to differentiate is problematic and may result in an erroneous differentiation. Factors such as specific bone, bone portion sampled, age, sex, and pathological conditions all affect the "normal" appearance of bone tissue, resulting in significant variation in bone tissue appearance throughout the skeleton, within a specific bone and even a portion of a single bone (22). This review focuses on the utilization of histology to differentiate human from nonhuman bone and the potential variation in cortical bone microstructure that is dependant upon its anatomical and individual origin. The review first introduces microscopic applications in biological and forensic anthropology and then describes, both qualitatively and quantitatively, the appearance of human and nonhuman mammalian cortical bone at the microscopic level. Additionally, optimal methods of differentiation are presented and species differentiation and groupings according to cortical bone microstructure are listed and described. Attention has been paid to the difficult task of assessing highly fragmented bone for identification.

Microscopic Applications and Techniques

Within the broad field of biological anthropology, the histological examination of bone is often undertaken to facilitate the understanding of bone as a tissue as well as the skeleton as a whole. Both qualitative and quantitative methods of analysis are utilized in order to understand the various aspects of bone histology, including age-related changes, pathological conditions, and the effects of the external environment on bone (23,24).

Histomorphometrical analysis of human skeletal remains has been used in several areas of biological and forensic anthropology including: paleopathology (25–29); bioarcheology, including the assessment of differences between the sexes (30–33) and populations (30,34–36); age estimation (34,37–44); and trauma analysis (45–48). The histological examination of bone, using light (26,45,49,50) and electron microscopy (51,52), have both been used as effective methods of visualizing internal bony structures. Light microscopy most commonly involves the use of bright-field observation, the simple transmission of light to view a histological section (50). Polarized light is the second most common method and this method involves the addition of an analyzer and polarizer to a light microscope (49). These additions result in the production of bright and dark areas in the sections viewed that serve to provide further differentiation of histological structures. Circularly polarized light may also be applied to overcome dark-field effects. Other methods of observation include dark-field, Nomarski phase contrast, and fluorescence light (49).

The scanning electron microscope (SEM) has been successfully applied to studies of bone and teeth sections using predominantly backscattered electron (BSE) imaging, where the microstructural arrangement may be clearly defined and in addition the density of the bone may be assessed (51,53,54). This is important, as it allows relative osteonal age and maturation to be assessed within one section. The SEM used in BSE mode has also proved to be extremely useful in the assessment of disease and also of diagenetic alteration (52,54,55).

Diagenetic, Perimortem, and Postmortem Alteration

Diagenesis causes a net change in the microstructural arrangement of bone and teeth and it is extremely important that it is identified when making any histological assessment. When diagenetic alteration is recognized, histological analysis is cautioned and alternate analytical techniques should be investigated. Diagenetic alteration is considered mediated by the transmigration of gut bacteria into the postmortem vasculature to all the major organs of the body and thus onward to the internal cortical structures of bone (51,56). This transmigration occurs within a 12–48-h period and may be extensive by 5 days when marbling is evident (51). At the point of, or near, skeletonization, external microbial flora may gain access and this again may result in a localized or extensive disruption of the microstructure (52,53,55). Importantly, marine exposure may lead to quite different changes in microstructure due to the differential nature of decomposition and the invading microorganisms involved (51,52).

Burning of bone also alters the histological appearance of cortical bone (57–60). Heating causes melting and the recrystallization of the mineral portion of bone and concomitant changes to histomorphology (58); such changes include shrinkage in Haversian system size and blurring of the individual lamellae (59). Despite these microscopic changes to bone, the use of burnt bone to differentiate between human and nonhuman bone has proven to be adequate (60). On the opposite side of the spectrum, the freezing of bone appears to have no significant effect on the histological appearance of cortical bone (61).

Consumption of skeletal remains by predators or scavengers may also result in alteration to cortical bone microstructure. Extensive research into scat assemblages (62,63) has illustrated that gut digestion results in demineralization of cortical bone. Demineralization results in the removal of considerable regions of cortical bone, extending at times to expose cancellous bone, and also causes rounding at edges, ends of splinters, and articular surfaces, simulating a "melting" of the bone tissue. This destructive alteration often renders it impossible to differentiate species of the animals consumed, particularly for immature remains and bone fragments, the elements most adversely affected by this alteration. Furthermore, because different demineralization occurs among different predator species, predator type may be determined from the pattern of demineralization present on bone. Bell et al. (51) describe a bone fragment recovered from carnivore scat potentially exhibiting microstructural alteration as a result of gut digestion; BSE examination of the tibia shaft fragment revealed focal demineralization within two Haversian systems.

Mammalian Bone Structure and Tissue Types

The gross structure of mammalian bone is composed of two types of bone: cortical and cancellous bone. Cortical bone is hard, with an average range of densities between 1.6 and 2.4 gm/cm^3 (64), and serves as a placement for muscle attachment (65,66). Cortical bone is notionally divided into three zones, which, while not distinctly marked, are potentially different in their histological appearance: the periosteal zone or outer portion; the mesosteal zone or central portion; and the endosteal or inner portion (67). Cancellous bone, also referred to as spongy or trabecular bone, is comprised of an arrangement of bony spicules called trabeculae (66). Cancellous bone is located at the interior of bone, including the ends of long bones, the interior of cuboidal bones and flat bones, and between the inner and outer layers of cortical bone in the skull.

Microscopically, mammalian cortical and cancellous bone exhibits two types of bone tissue: woven and lamellar (66,68–70). Woven bone tissue, also know as fiber or immature bone tissue, is produced during periods of immediate necessity and is typically temporary (71,72). It is produced during initial growth as a fetus and infant, during periods of tissue repair, and in response to

pathological conditions such as bone tumors (26,66). In appearance, woven bone is poorly organized, consisting of randomly oriented collagen fibers and crystal minerals surrounding bundles of blood vessels and nerves (26,65,69,70). In contrast, lamellar bone tissue is laid down at a much slower rate and results in a highly organized structure. It consists of parallel layers of bone tissue called lamellae composed of collagen fibers and their associated minerals (70,72,73). Primary osteons exist within lamellar bone and are created during the transformation process from woven to lamellar bone (72). Primary osteons are longitudinally oriented vascular canals surrounded by concentric bone layers that supply blood and nutrients to new bone.

Cortical bone can be broken down further into primary, secondary, and avascular bone tissue (the latter of which will not be discussed but refers to bone tissue without vascularization) (66,68). Primary bone is new bone laid down in layers during primary, appositional growth, and contains primary osteons that supply nutrients to and remove toxins from the bone tissue (68,74). Many forms of primary bone are exhibited within vertebrate skeletons, including longitudinal, radial, reticular, plexiform, laminar, and acellular bone tissue (18,22,68). Longitudinal, radial, and reticular primary bone tissues are named as such because of the orientation of the vascular canals they hold (68). Laminar tissue is predominantly displayed by a large number of large land mammals, including mammals, mammal-like reptiles, and amphibians that commonly undergo cyclic periods of hibernation or experience distinct seasonal changes in feeding habits (68,75). Laminar bone exhibits distinct seasonal banding and each band is referred to as a lamina of bone. Laminae may be composed of woven or lamellar bone tissue and may be vascular or nonvascular (22).

Important to this review, plexiform bone is a type of primary bone tissue of the fibrolamellar bone tissue group (Fig. 1) (18,68,70). This type of bone tissue is characteristic in cortical bone of the long bones of large, fast-growing animals such as cows and pigs, as well as dogs and other carnivores, and less frequently in the bones of primates, including humans (present rarely in fetal bone) (69). Plexiform bone is similar in structure to laminar bone, but houses a more dense system, or plexus, of vascularization. A three-dimensional, symmetrical arranged network is formed by longitudinal, radial, and circumferential primary osteons. Also apparent within this type of bone tissue is rectilinear, residual vascular spaces, which results in a "brick wall" appearance (66).

Secondary bone refers to new, lamellar bone that has been deposited where previously existing bone has been resorbed, i.e., the infilling of a cutting cone (66,68,70). This bone is also referred to as Haversian bone (Figs. 1 and 2). This type of secondary bone contains structures called Haversian systems and these systems are composed of a central Haversian canal that contains blood vessels and nerves and is surrounded by several layers of lamella (66,76). Volkmann's canals, which run perpendicular to Haversian systems, connect the Haversian canals to one another (76). Although similar in general appearance, Haversian systems can be distinguished from primary osteons through several characteristics: Haversian systems are delimited by a cement line whereas primary osteons are not as this line is created when bone resorption ceases and new bone is laid down; Haversian systems intersect circumferential lamellae, resulting in interstitial lamellae, whereas primary osteons do not; and primary osteons also tend to be smaller (72,76).

A note on terminology needs to be addressed here to avoid confusion, as differing terminology is used throughout the world to describe histological structures of bone. In this review, the term Haversian system is used in reference to the histological structures also known as secondary and tertiary osteons. Haversian system is synonymous with both secondary and tertiary osteon; secondary osteon refers to the initial osteons created within secondary bone tissue, while tertiary osteon refers to osteons that replace secondary osteons, are larger in size, and have an increased number of lamellae (76).

Haversian bone can be broken down into three groups based on the placement of Haversian systems: irregular, endosteal, and dense (18,68). Irregular Haversian bone tissue contains relatively few Haversian systems that are isolated and scattered throughout the tissue. Endosteal Haversian bone tissue contains Haversian

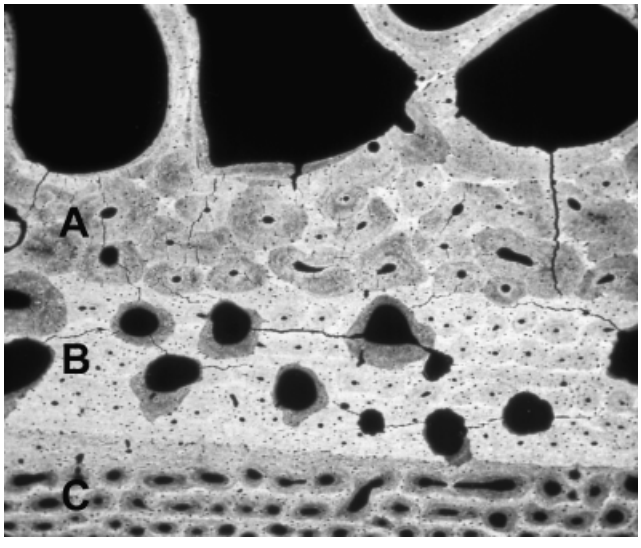

FIG. 1—*Backscattered electron image of sheep bone illustrating a range of bone tissue types in a section of compact bone. (A) Haversian bone tissue (endosteal zone), (B) transitional period between secondary Haversian bone tissue and primary plexiform bone tissue (mesosteal zone), (C) plexiform bone tissue (periosteal zone). Field width: 2.5 mm.*

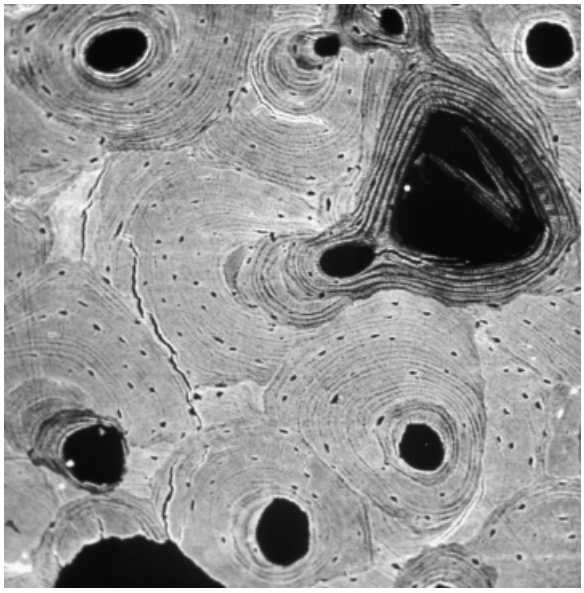

FIG. 2—*Backscattered electron image of a transverse section of modern human adult tibia. Note the presence of Haversian bone tissue, including active and complete Haversian systems. Field width: 930 μm.*

systems restricted to the endosteal margin of bone, resulting in large and incomplete Haversian systems. Dense Haversian bone tissue contains closely packed Haversian systems with reduced or absent interstitial systems or lamellae.

Human Bone Tissue Microstructure

The typical appearance of a cross-section of an adult human long bone consists of circumferential lamellae bone at the endosteal and periosteal surfaces and a middle component of dense Haversian bone (66,72,73,77). Approximately 50% of this dense Haversian bone consists of Haversian systems while the other 50% consists of interstitial lamellae, occurring at irregular angular spaces between Haversian systems (73). The Haversian systems appear as both complete and active systems. The complete systems are comprised of a central Haversian canal, often off-centered in position, surrounded by 16–20 cylindrical lamellae with an outer border consisting of a cement line (66,71). These systems are commonly oval or round in shape (7,78). The active Haversian systems, or remodeling units, differ in appearance to that of complete Haversian systems. Depending on where the cross-section intercepts the active Haversian system on its course of formation, three different appearances may be seen: (1) a resorptive bay (also referred to as a cutting cone) bordered by Howship's lacunae; (2) a forming site, with osteoblasts bordering a varied amount of freshly deposited, unmineralized bone that is contained within a cement line; or (3) a complete Haversian system (78). The circumferential lamellae appearing at the periosteal and endosteal surfaces are often times fragmentary, with the number of periosteal lamellae generally exceeding that of endosteal lamellae (26). Volkmann's canals may also be seen on a thin section of bone and run perpendicular to the Haversian canals.

While the cortical bone tissue of other shaped bones, such as flat (cranial) and short (vertebra), contain the same histological structures as long bones, their histological appearance may differ. Biomechanical forces, among other factors, influence and/or govern the shape and arrangement of bone (77–79). Accordingly, the longitudinal forces acting upon long bones that result in longitudinally oriented Haversian systems would be absent on flat and short bones, resulting in Haversian systems that are often irregular in shape (78). Most histological studies concentrate on the cortical bone of long bones and consequently, the appearance of the histological structures of the other flat and short bones, with ribs as an exception, will not be discussed in this paper as limited studies exist (80,81). Ribs have been used extensively in histological studies (42,35,82–85) and are considered within the scope of this review.

Nonhuman Mammalian Bone Tissue Microstructure

The nonhuman mammalian species in this review include: rat, hare, badger, raccoon dog, cat, dog, pig, goat, sheep, cow, deer, horse, water buffalo, bear, and nonhuman primates. These nonhuman mammalian species were chosen because both qualitative descriptions and quantitative data have been published for these species and both sets of information are required for differentiation from human bone. Bear, however, is an exception; while no quantitative data exist for this mammal, it has been deemed significant to include due to its global presence. Unfortunately, only one study includes information on bear cortical bone histology: Foote (17) describes a black bear (*Ursus americanus*, age unknown) femoral section exhibiting cortical bone composed mainly of plexiform bone, with scattered Haversian systems located near the posterior portion of the bone. It can be stated here that there is potential for differentiation of bear bone from human bone, due to the presence of plexiform bone tissue in bear bone and a virtual absence in human bone. There is also potential for misidentification of bear bone as human bone if the distribution of Haversian bone tissue in bear bone is greater than Foote (17) describes, particularly in mature bears, and if Haversian system sizes are comparable with humans.

Brown Rat—Rattus norvegicus

The histological appearance of rat long bone cortical bone is comprised mainly of primary longitudinal bone tissue. Haversian systems do appear; however, these systems are rare and scattered near the endosteal surface (17,21). Endosteal and periosteal circumferential lamellae are also present, but are poorly developed at the endosteal surface due to the presence of Haversian systems here (17). Additionally, there may be small areas of avascular and acellular bone located throughout (21).

Hare—Lepus americanus *(Snowshoe Hare);* Lepus oryctolgus *(European Hare)*

The long bone and rib cortical bone of skeletally mature hare consists primarily of dense Haversian bone tissue with small Haversian canals (7,17,20). A wide ring of periosteal circumferential lamellae and a thinner, irregular ring of endosteal lamellae surround a middle component of dense Haversian bone (17). Remnants of primary longitudinal tissue with scattered primary osteons may be present in younger individuals (20).

European Badger—Meles meles

Cortical bone tissue of badgers is very similar to that of raccoon dogs in terms of the types of bone tissue present, primarily dense Haversian bone tissue (9,17). Differences are noted between the size and shape of Haversian systems; in badgers, these systems vary in shape from round to elliptic, are present in various sizes, and contain three to eight lamellae (9). Once again, remnants of primary reticular and radial bone tissue may be present near the periosteal surface, especially in younger animals (9,20).

Raccoon Dog—Nyctereutes procyonoides

The long bone cortical bone of mature raccoon dogs consists primarily of dense Haversian bone (9). This tissue contains similar-sized and round-shaped Haversian systems with three to five lamellae. Remnants of primary reticular and radial bone tissue may be present near the periosteal surface, especially in younger animals (9,20).

Cat—Felis silvestris catus

Rib and long bone cortical bone of the common cat is composed of dense Haversian bone (20). Most Haversian canals within this secondary bone are very small (7) and Volkmann's canals are more numerous than in any other similar-sized mammal (17,20). Circumferential lamellae consist of a thin layer at the periosteal surface and a thicker, well-developed layer at the endosteal surface (17,20).

Dog—Canis lupus familiaris

The cortical bone of the ribs and long bones of mature dogs is predominantly composed of dense Haversian bone

(12,13,17,20,86,87). Periosteal and endosteal circumferential lamellae bone is well developed but often interrupted by scattered Haversian systems (17,20). Haversian systems are present in various shapes with Haversian canals classified as small (7,12). In immature dogs, remnants of osteonal banding and plexiform are present, particularly at the periosteal surface (12,20).

Pig—Sus scrofa

Femora of skeletally mature pigs consist primarily of plexiform bone with dense Haversian bone located at the posterior portion of the bone (17). Haversian canals are mostly medium in shape (7). The cortical bone of immature pig femora consists of layers of lamellar bone alternating with primary tissue containing osteonal banding (10). These bands, appearing in twos or threes, contain five to 20 primary osteons and are present near the endosteal surface along with the lamellar bone. The remainder of the femoral section consists of plexiform bone. Plexiform bone may also exist throughout an entire long bone section within immature pigs, with a complete absence of Haversian tissue or osteonal banding (11,17).

Goat—Capra aegagrus hircus

In mature goats, the long bone cortical bone consists of both plexiform and Haversian bone tissue. Plexiform bone, with scattered areas of Haversian tissue, is present near the periosteal surface; a mixture of Haversian tissue with large, sporadic Haversian systems and primary tissue is present in the mesosteal zone component and dense Haversian tissue is located near the endosteal surface (8,17,20). The layers of circumferential lamellae at the endosteal and periosteal surfaces commonly appear as narrow rings (17). Immature specimens will more likely display copious amounts of plexiform tissue as the primary tissue of growth (17,70).

Sheep—Ovis aries

The histological appearance of the long bone cortical bone of mature sheep is similar to that of goats (8,17). Ribs of mature sheep also display a mixture of secondary and primary tissue, with Haversian tissue serving as a replacement for plexiform tissue (20). The Haversian canals within the secondary tissue are classified as medium in size and irregular in shape (7). Immature sheep exhibit plexiform bone throughout entire sections of femora, with a potential for a small number of scattered Haversian systems located posteriorly (10,17).

Cow—Bos taurus

The cortical bone of immature cow rib consists of plexiform bone near the periosteal surface, Haversian bone located near the endosteal surface, and osteonal banding at the interface between both (12,17,20). Haversian canals are medium in size and irregular in shape (7). Fetal calf femora also exhibit the same pattern: plexiform bone tissue near the endosteal surface, a middle portion of laminar bone with an irregular arrangement, and a periosteal area of Haversian bone (17). No information exists for adult cow bone and this is thought to be an outcome of modern butchering practices, where subadult (13–24 months) cows are preferentially slaughtered (12).

Deer—Odocoileus virginianus

At different ages, deer long bone cortical bone consists of different quantities of plexiform and Haversian bone tissue (5,11,17,88,89). In immature individuals, plexiform bone is dominant near the periosteal surface, with Haversian bone forming near the endosteal surface. Long bone cortical bone of skeletally mature individuals consists predominantly of dense Haversian bone as it replaces the plexiform bone, especially near the endosteal surface and posterior portion of the bone (88). A thin layer of periosteal circumferential lamellae bone surrounds mature bone in all locations (89). For fetal and new-born deer, long bone cortical bone consists of primary reticular and plexiform tissue with areas of avascular and acellular bone (21).

Horse—Equus caballus

Generally, horse long bone cortical bone consists of dense Haversian tissue with remnants of the primary reticular and plexiform tissue (17,20,90,91). Large numbers of resorptive spaces exist near the endosteal surface. Circumferential lamellae at the periosteal and endosteal surfaces are often thin and fragmentary due to the spread of Haversian bone to this area (17). The cortical bone of the rib is composed of a very thin layer of periosteal circumferential lamellae surrounding an internal structure of dense Haversian bone (20). Foal cortical bone consists primarily of plexiform bone with an alternating concentric pattern of rows of "pseudo-osteons" with Haversian canal-like structures. The "pseudo-osteons" differ histologically from Haversian systems as they contain woven bone (90,91).

Water Buffalo—Bubalus arnee

Water buffalo long bone cortical bone contains both plexiform and Haversian bone tissue (11,17). Plexiform bone is located near the periosteal surface and anterior in the bone, while Haversian bone is located toward the endosteal surface and posterior in the bone (17).

Chimpanzees—Pan troglodytes

Mulhern and Ubelaker (92) report on the histology of juvenile chimpanzee lower limb long bone cortical bone and in doing so, comment on the lack of data on cortical bone microstructure for the great apes, including chimpanzees. Juvenile chimpanzees (2.0–15.3 years of age) exhibit cortical bone histology similar to juvenile humans (0–15 years of age) while differences include more secondary bone tissue in the chimpanzee cortical bone in comparison with humans, attributed to an accelerated rate of primary bone replacement. Also important to note is an increase in the number of Haversian systems in the femur as compared with the tibia and fibular of juvenile chimpanzees.

Old World Monkeys—Cercopithecidae

Included in this family of monkeys are baboons, mangabeys, mandrills, and macaques. Generally, the long bone cortical bone of skeletally mature individuals will consist of dense Haversian bone, with thin layers of endosteal and periosteal circumferential lamellae (17,21). Immature individuals, on the other hand, will display more primary longitudinal tissue, with the development of Haversian tissue beginning near the endosteal surface.

Rhesus Macaques—Macaca mulatta

Recent research on primate bone histology has centered on rhesus macaques to examine their potential as animal models for human skeletal pathology (93,94) and skeletal genetics (95). The histological appearance of the long bone cortical bone of skeletally mature macaques consists of dense Haversian bone with thin layers of circumferential lamellae near the endosteal and periosteal surfaces (17,20,21). Immature individuals may exhibit long bone cortical bone comprised solely of primary longitudinal tissue or primary tissue with areas of replacing Haversian bone (21).

New World Monkeys—Platyrrhines

Including the squirrel, spider, and capuchin monkey inhabiting Central and South America, these primates display long bone cortical bone tissue similar to that of Old World Monkeys. This includes the display of Haversian bone in skeletally mature individuals, with remnants of primary longitudinal bone in younger individuals (17,21). Thin circumferential lamellae exist near the endosteal and periosteal surfaces (17).

Quantification of Microstructure in Human and Nonhuman Species

Measurements commonly used in histological studies aimed at distinguishing between human and nonhuman mammalian species are presented quantitatively in Figs. 3, 4, and 5. These measurements include: Haversian system diameter (in microns; Fig. 3); Haversian canal diameter (in microns; Fig. 4); and Haversian system density (number of Haversian systems per square millimeter; Fig. 5). For Figs. 3, 4, and 5, the values expressed are purposely without specific reference to whole bone sampled, i.e., tibia, or bone portion sampled, i.e., mid-shaft femur; this is to illustrate the range of measurement values that may be encountered in the examination of small bone fragments of unknown anatomical (bone and bone portion) and individual (sex and age) origin. As illustrated in these figures, the ranges for certain mammals are quite large, e.g., human, dog, goat, sheep, and cow in Fig. 3, while other mammal ranges are limited, e.g., rat, hare, cat, horse, Old World Monkey, and New World Monkey in Fig. 3. For some mammals, this is reflective of their respective studies: either averages were presented without a range or the sample size studied was small, therefore limiting potential range values. For other mammals, particularly smaller-sized mammals, the ranges presented are perhaps more reflective of actual ranges in the size of Haversian systems. Hence, actual mammal size is a constraining factor on Haversian system size (97).

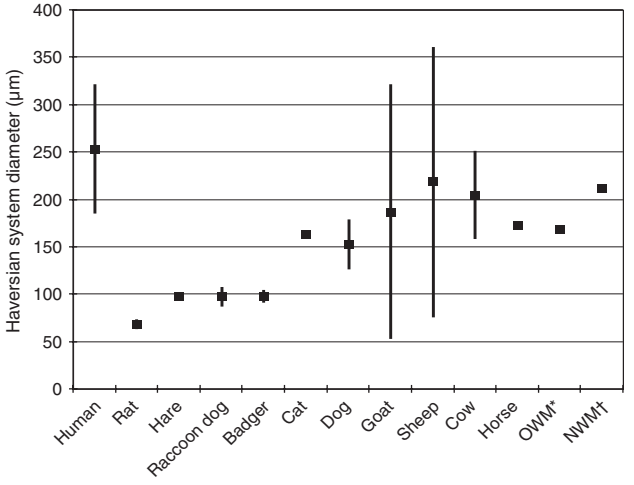

FIG. 3—*Ranges for Haversian system diameter for mammalian species. Note the overlap in values for several nonhuman mammals with humans (8,12,21,31,96–100).* *Old World Monkeys; †New World Monkeys.*

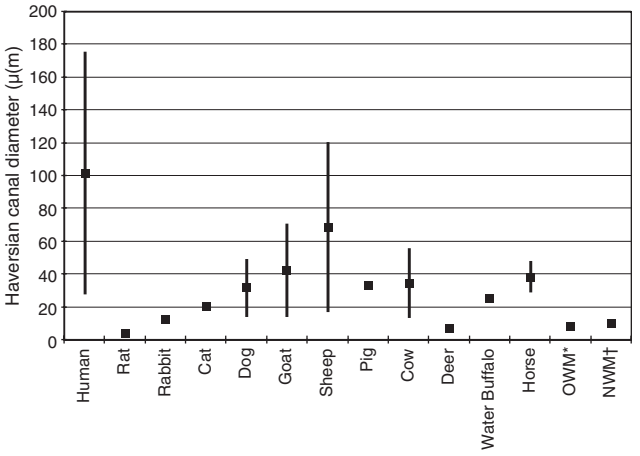

FIG. 4—*Ranges for Haversian canal diameter for several mammalian species. Note the overlap in values for several nonhuman mammals species with humans (5,7,8,11,12,21,86,96–103).* *Old World Monkeys; †New World Monkeys.*

Variation in Human and Nonhuman Mammalian Cortical Bone

In addition to the typical descriptions and values provided for mammalian cortical bone, this bone tissue has potential for variation from several influencing factors. Such factors include bone and bone portion sampled, sex, age, and pathological conditions (22). Awareness of potential variation in cortical bone microstructure is important, particularly in the examination of small and nondiagnostic bone fragments; knowledge of only the "typical" appearance of human and nonhuman bone may result in erroneous differentiation. Factors causing variation, with associated examples, are discussed below and are divided into human and nonhuman sections.

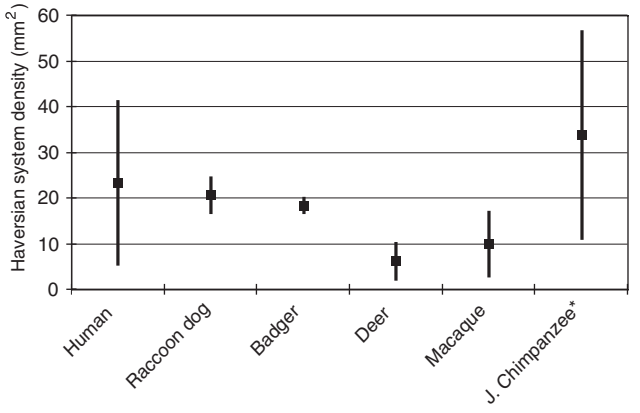

FIG. 5—*Ranges for Haversian system density for several mammalian species. Note the overlap in values for several nonhuman mammals with humans (5,9,88,92,98,101,104,105).* *Juvenile chimpanzees.*

Specific Bone

Human

It is known that histological differences exist between specific skeletal elements of humans. Pirock et al. (96), in their study on the normal parameters of microstructural elements of human bone, reported major differences between human ribs and femora. The reported value for Haversian system diameter in the ribs ($n = 63$) was 192 μm, while the reported diameter for the femora ($n = 4$) was 270 ± 35 μm. Similarly, reported values for the diameter of the Haversian canal were 46 μm for the ribs and 60 ± 34 μm for the femur. Pfeiffer (35) also reported significant differences ($p < 0.01$) for the Haversian system area between ribs and femora in her study examining skeletal populations from 18th- and 19th-century cemeteries from Canada and Great Britain and a 20th-century cadaver population from South Africa. The average Haversian system area measured 0.030 ± 0.015 mm^2 for the ribs ($n = 100$) and 0.041 ± 0.021 mm^2 for the femora ($n = 41$). While the exact cause is not known differences in Haversian area have been related to biomechanics (36), hormones and disease (106), or other factors.

Differences in the values for histological structures between human long bones have also been noted. Evans and Bang (107) reported differences for the number and size of Haversian systems between the femur and fibula. From their combined sample of 54 femora and 37 fibulae, the researchers reported femora as having a larger number (12.87/mm^2) of smaller-sized Haversian systems with little interstitial bone and the fibulae having fewer (8.69/mm^2), but larger Haversian systems and a greater amount of interstitial bone. Evans and Bang (107) emphasize medical literature that reports lower tensile strength and modulus of elasticity for the femur, due to the increase of cementing substance and therefore, an increased level of weakness, as compared with the fibula.

Nonhuman

Like the human skeleton, different bones within an individual nonhuman mammal may appear different histologically. Differing rates of remodeling experienced by various zones of the skeleton are thought to cause these differences (22). This likely holds true for humans as well. The rate of remodeling is influenced by numerous factors including the occurrence of microcracks as caused by increased mechanical loading (66,71). In a study on cortical bone organization and its relationship with antemortem microdamage in 11 Rocky Mountain mule deer (*Odocoileus hemionus hemionus*), Skedros et al. (88) reported a general proximal-to-distal increase in the number of Haversian systems per square millimeter in the forelimb: humerus 2.45 ± 1.90/mm^2; radius 8.65 ± 2.94 mm^2; metacarpal 5.93 ± 2.51 mm^2; and phalanx 10.17 ± 2.23 mm^2. The higher number of Haversian systems within the more distally located bones suggests that these bones are experiencing a higher rate of remodeling as a result of an increase in loading and contact with the ground. With both an increase in loading and ground contact, an increase in microcracks would occur and thus provide a biological stimulus for an adaptive or a preventative response in the form of remodeling (88,89).

The postural technique in which mammals move about may also be reflected in variation in the microstructural appearance of their cortical bone (108). Some bones of the skeleton may be utilized more than others, or in a different manner, resulting in the need for differential remodeling, and therefore, the development of or increase in Haversian bone. Schaffler and Burr (108) illustrate the effects that primate locomotive patterns have on bone microstructure and remodeling patterns. Significant differences ($p < 0.01$) were reported for the amount of Haversian bone in arboreal quadrupeds, terrestrial quadrupeds, suspensory animals (including chimpanzees and spider monkeys), and bipeds (humans; Table 1). Suspensory animals and bipeds utilize the femur as the dominant limb in locomotion, causing this bone to experience an increased amount of loading as compared with the femur of arboreal and terrestrial quadrupeds, who utilize all four limbs equally. Accordingly, the femora of the suspensory animals and bipeds would contain more Haversian bone, and consequently, Haversian systems, as a response to an increase in remodeling.

Bone Portion

Human

A single human bone also exhibits marked random and systematic histological variation (22,70,72,109). Histological differences are most apparent at areas where focused biomechanical forces are at work on the bone, including areas of muscle attachment and joint surfaces. At areas of muscle attachment, an increased number of Haversian systems are often present (70,110). Increased tension and compression forces with muscle movement and corresponding strain may result in microcracks and fatigue, requiring reinforcement through the remodeling process, and thus, the creation of more Haversian systems than normally required (70). Microcracks are a difficult microstructural change to ascribe as antemortem or postmortem in occurrence, as microcracks occur along microstructural planes of weakness whether wet or dry and may therefore represent a preparation artifact (111).

Within a single cross-section of human cortical bone, different regions exhibit variation in histological appearance. When considering circumferential lamellae bone, periosteal lamellae are more numerous than endosteal lamellae, particularly in younger individuals due to the process of appositional growth (112,113). With age, as remodeling occurs, the circumferential lamellae located near the periosteal surface are replaced with Haversian systems, while the lamellae at the endosteal surface are resorbed (26). With regards to Haversian systems, due to the higher rate of remodeling at the periosteal surface, these systems tend to be smaller and more numerous in size than those nearer the endosteal surface (79,97,114).

Nonhuman

Animal bone will display similar differences in specific areas of bones, particularly those undergoing additional strain such as muscle attachment sites (22,69,72). As described by Skedros et al. (89), the cranial compression cortex of the mule deer calcaneus, an area undergoing an increased level of strain, exhibits smaller and more circular Haversian systems in a smaller number as compared with the caudal tension cortex (Table 2). For both subadult and adults, these differences were significant at $p < 0.01$. The same pattern is evident in the radius from 18 skeletally mature standard-breed horses in another study (115).

Sex

Human

Several studies describe differences between cortical bone microstructure of males and females. Burr et al. (30) report significant differences ($p < 0.02$) for the Haversian system area between the females and males for the Pecos Indian population (Table 3). Mulhern and Van Gerven (31) also report similar findings for the

TABLE 1—*Histological variation in primates with different locomotive patterns.*

References	Primate	*N*	Skeletal Element	% Osteonal Bone	NH*
Schaffler and Burr (108)	Arboreal quadrupeds	9	Femur	6.79 ± 0.61	5.3
	Terrestrial quadrupeds	5		12.72 ± 2.22	5.5
	Suspensory animals	4		26.93 ± 1.15	9.85
	Bipeds (humans)	—		45.3†	12.47‡ or 12.87§

*NH represents number of Haversian systems per square millimeter.
†From Evans (105).
‡From Burr et al. (93).
§From Evans and Bang (107).

TABLE 2—*Histological variation in the calcaneus of Odocoileus hemionus hemionus.*

Reference	Age	*N*	Skeletal Element	Area	NH*
Skedros et al. (89)	Subadult	11	Calcaneus	Cranial	19.3 ± 2.2
				Caudal	16.9 ± 2.0
	Adult	10	Calcaneus	Cranial	37.7 ± 15.2
				Caudal	33.2 ± 10.0

*NH represents number of Haversian systems per square millimeter.

femora of the Nubian population (Table 3); the average Haversian system diameter and area differ significantly ($p < 0.05$) between the sexes, as do the number of Haversian systems per square millimeters ($p < 0.001$). Like other studies (116,117), females seem to have larger Haversian systems while males have more Haversian systems.

Both Burr et al. (30) and Mulhern and Van Gerven (31) state that the differences in Haversian system size are a strong indicator of sexual dimorphism in the Pecos and Nubian peoples, respectively; both an increase in the size (female) and number (male) of Haversian systems seem to enhance the structural support and reduce the fatigue properties of bone. Mulhern and Van Gerven (31) offer two potential reasons why these differences are present in the Nubian population. First, it is possible that these variations reflect similar activities performed by both males and females that placed a strain on the femur in a similar manner; however, because each sex has inherent sexual differences, there are different microstructural responses to the similar strain. Second, it is possible that different tasks completed by each sex in accordance with their sexual division of labor placed strain on the femora of males and females in different ways, resulting in the microstructural variations reported.

Nonhuman

Differences in the size of histological structures are also known to exist between sexes for nonhuman mammals. In mammals equal to or smaller than the size of a monkey, sexual dimorphism influences the size of histological structures (97). Havill (94) reports significant differences ($p = 0.045$) in the Haversian system area for male and female rhesus macaques. Male macaques ($n = 28$) had larger Haversian systems than their female counterparts ($n = 47$): 0.0254 ± 0.005 and $0.0227 \pm 0.006\,mm^2$, respectively, and this difference has been attributed to the overall larger body size of the male macaques.

Age

Human fetal

Human fetal bone deserves specific attention due to the potential for confusion with animal bone due to similarities between the size and shape of individual bones, particularly those in a fragmented state, and marked differences from that of adult cortical bone (109).

Fetal bone, depending on the stage of development, may consist of cartilaginous tissue, woven bone, and/or Haversian bone (101,102,109,118). Before 3 months *in utero*, a transverse section of fetal long bone cortical bone will consist of a central portion of cartilaginous tissue surrounded by a thin layer of periosteal bone containing primary vascular canals. Close to 4 months *in utero*, the existing periosteal bone thickens and forms a continuous layer around newly formed endochondral bone located centrally; endochondral bone is composed of woven bone organized into concentric layers. At 4 months *in utero*, the appearance is similar, with more endochondral bone and the appearance of a medullary canal. By the fifth month *in utero*, Haversian systems begin to form; these systems are round in shape with canals that are larger than average in size. At 6 months *in utero*, approximately one-quarter of the bone is comprised of Haversian systems (101). These Haversian systems have wide Haversian canals and a small number of wide lamellae. At 7–8 months *in utero*, one-sixth of the bone is comprised of Haversian systems and at the ninth-month period, one-tenth of the bone is comprised of Haversian systems. During the 7–9-month period, the bone makes the transition to a more mature organization of cortical bone, with Haversian systems that contain more numerous but smaller lamellae and Haversian canals that are narrower (101). Table 4 outlines values for

TABLE 3—*Histological variation: sex related changes in humans.*

References	Sex	*N*	Skeletal Element	NH*	DmH†	ArH‡
Burr et al. (30)	Males	28	Femur	—	—	0.034 ± 0.002
	Females	23		—	—	0.041 ± 0.002
Mulhern and Van Gerven (31)	Males	19	Femur	9.74 ± 0.39	206 ± 4.00	0.036 ± 0.002
	Females	24		6.73 ± 0.31	219 ± 3.00	0.040 ± 0.001

*NH represents number of Haversian systems per square millimeter.
†DmH represents Haversian system diameter in microns.
‡ArH represents Haversian system area per square millimeter.

TABLE 4—*Histological variation during human fetal growth.*

Reference	Age	N	Skeletal Element	NH.Ca*	DmH.Ca†
Baltadjiev (101)	Sixth month	50	Tibia	31.8 ± 0.32	62.9 ± 0.69
	Seventh to eighth month	50		35.5 ± 0.38	43.1 ± 0.70
	Ninth month	50		29.4 ± 0.44	48.5 ± 0.94

*NH Ca represents number of Haversian canals per square millimeter.
†DmH.Ca represents diameter of Haversian canals in microns.

both the number of Haversian canals and diameter of Haversian canals for the sixth, seventh to eighth, and ninth month of development.

Human Subadult and Adult

With age, general changes occur in the microstructure of Haversian bone. Subadult cortical bone changes with an increase in age, from large quantities of lamellar bone and primary osteons, to the formation of Haversian systems, and subsequent to further remodeling, Haversian system fragments (37). In adult cortical bone, with increasing age, there is an increase in the number of Haversian systems (Table 5) (37,116,117); a decrease in the size of Haversian systems (Tables 6 and 7) (97,116,117); an increase in the diameter of Haversian canals (32); and an increase in the perimeter of Haversian canals (Table 8) (97). These changes reflect both an increase in remodeling with age as well as an increase in porosity.

Qualitatively, cortical bone microstructure differs among individuals of different ages. Currey (116) describes the bone of older individuals as appearing disorganized, with an increased number of Haversian system fragments and interstitial bone, attributing the appearance to the increase in the cycles of erosion and redeposition with remodeling. The bone of younger individuals, on the other hand, appears to have complete and regularly appearing Haversian systems adjacent to one another, with limited interstitial bone.

Nonhuman

Nonhuman mammals that exhibit Haversian bone follow the same pattern of aging as humans (104,94). Accordingly, nonhuman mammalian cortical bone exhibits a decrease in Haversian system size and an increase in both Haversian canal size and Haversian system density with age (104,94). All these changes, as in human bone, reflect an increase in cortical bone porosity with age. Przybeck's (104) study on the histomorphology of the rhesus macaque rib exemplifies some of these changes (Table 9). The lower than expected value for the individual aged 31 is countered by an increase in the number of Haversian system fragments in comparison with the same values for the younger individuals, signifying an increase in remodeling. Similar findings were reported by Havill (94) for macaques, including a decrease in Haversian system size and an increase in Haversian canal size with age. In their study on juvenile chimpanzees, Mulhern and Ubelaker (92) also note, with age, an increase in the number of Haversian systems and fragments, and a decrease in both the number of non-Haversian canals and the quantity of circumferential lamellar bone for the lower limb long bones.

For nonhuman mammals exhibiting plexiform bone, an increase in age results in the replacement of this bone with Haversian bone, either partially or completely (72,119). Haversian systems begin to appear in the area of bone near the endosteal surface, gradually replacing the plexiform bone here. Occasionally, plexiform bone tissue is completely replaced with Haversian bone tissue, from the endosteal to periosteal surfaces (72,120).

TABLE 5—*Haversian system density: age related changes in humans.*

Reference	Age	Sex	*N*	Skeletal Element	NH*
Mulhern (98)	15–19	Female	7	Rib	4.72 ± 0.35
	20–29		8		10.04 ± 0.61
	30–39		12		12.87 ± 0.29
	40–49		11		13.14 ± 0.16
	50+		7		12.92 ± 0.56
	15–19	Male	3		5.71 ± 0.39
	20–29		7		9.04 ± 0.30
	30–39		10		11.55 ± 0.38
	40–49		14		11.94 ± 0.26
	50+		1		10.81

*NH represents number of Haversian systems per square millimeter.

Pathological Conditions

Human

Pathological conditions affecting the histological appearance of cortical bone of humans are numerous. They include, but are not limited to, osteoporosis, Paget's disease, diabetes mellitus, oste-

TABLE 6—*Haversian system diameter: age related changes in humans.*

Reference	Age	Sex	*N*	Skeletal Element	DmH*
Frost (99)	18.6†	Unknown	15	Rib	197 ± 56
	33		15		194 ± 34.5
	41.2		16		187 ± 57.8
	55		17		189 ± 29.6
Mulhern and	20–29	Female	6	Femur	224 ± 90
Van Gerven (31)	30–39		6		215 ± 70
	40–49		6		218 ± 70
	50+		6		220 ± 40
	20–29	Male	6		211 ± 90
	30–39		6		207 ± 90
	40–49		6		205 ± 60
	50+		1		191 ± N/A
Jowsey (97)	20–29	Unknown	26	Femur	252 ± 24
	30–39				243 ± 12
	40–49				226 ± 21
	50–59				235 ± 13
	60–69				247 ± 14
	70–79				245 ± 15
	80–90				258 ± 47
	20–29		26	Rib	213 ± 21
	30–39				217 ± 31
	40–49				222 ± 17
	50–59				214 ± 31
	60–69				164 ± 9
	70–79				167 ± 34
	80–90				—

*DmH represents Haversian system diameter in microns.
†All ages from Frost (99) represent mean age of group in years.

TABLE 7—*Haversian system area: age related changes in humans.*

Reference	Age	Sex	*N*	Skeletal Element	ArH*
Burr et al. (30)	20–29	Female	7	Femur	0.036 ± 0.008
	30–39		10		0.045 ± 0.011
	40–49		3		0.036 ± 0.003
	50+		7		0.040 ± 0.008
	20–29	Male	6		0.040 ± 0.009
	30–39		10		0.035 ± 0.011
	40–49		6		0.030 ± 0.005
	50+		6		0.031 ± 0.006
Mulhern and Van Gerven (31)	20–29	Female	6	Femur	0.040 ± 0.003
	30–39		6		0.037 ± 0.003
	40–49		6		0.038 ± 0.002
	50+		6		0.038 ± 0.001
	20–29	Male	6		0.035 ± 0.003
	30–39		6		0.034 ± 0.003
	40–49		6		0.033 ± 0.002
	50+		1		0.029
Mulhern (98)	15–19	Female	7	Rib	0.035 ± 0.0049
	20–29		8		0.033 ± 0.0007
	30–39		12		0.038 ± 0.0009
	40–49		11		0.037 ± 0.0006
	50+		7		0.032 ± 0.0008
	15–19		3		0.039 ± 0.0017
	20–29	Male	7		0.043 ± 0.0019
	30–39		10		0.036 ± 0.0013
	40–49		14		0.033 ± 0.008
	50+		1		0.032 ± N/A

*ArH represents Haversian system area per square millimeter.

omalacia, osteogenesis imperfecta, trauma, immobilization, including paralysis and disuse atrophy, primary hyperparathyroidism, acromegaly, and mastocytosis (82,36,121). Additionally, recreational and pharmacological drugs may result in changes to the histological appearance of bone (82,121). Such drugs include anticonvulsants, corticosteriods, estrogens, and alcohol. The effects of these long-standing conditions, rather than those of an acute nature, will affect the histological appearance of bone usually by increasing or decreasing the rate of remodeling within bone (81,121). For example, hyperparathyroidism results in an increase in the number of Haversian systems and their fragments due to an overall increase in bone remodeling. Diabetes mellitus, on the other hand, results in a decrease in the normal number of Haversian systems and fragments due to a depression in remodeling rates.

TABLE 8—*Haversian canal perimeter: age related changes in humans.*

Reference	Age	*N*	Skeletal Element	PmH.Ca*
Jowsey (97)	20–29	26	Femur	151 ± 35
	30–39			139 ± 18
	40–49			163 ± 15
	50–59			195 ± 11
	60–69			205 ± 14
	70–79			214 ± 29
	80–90			221 ± 7.1
	20–29	26	Rib	154 ± 18
	30–39			167 ± 30
	40–49			159 ± 5.0
	50–59			181 ± 11
	60–69			176 ± 8.5
	70–79			170 ± 15
	80–90			—

*PmH.Ca represents Haversian canal perimeter in microns.

TABLE 9—*Histological variation between Macaca mulatta of differing ages.*

Reference	Age in years	*N*	Skeletal Element	NH*	ArH†
Przybeck (104)	4	3	Rib	7.42 ± 1.63	0.028 ± 0.007
	8	3		13.41 ± 5.82	0.023 ± 0.002
	13	3		16.33 ± 2.20	0.023 ± 0.004
	24	3		22.30 ± 0.29	0.021 ± 0.002
	31	3		12.42 ± 1.46	0.020 ± 0.001

*NH represents number of Haversian system per square millimeter.
†ArH represents Haversian system area per square millimeter.

Other conditions result in the abnormal creation of new bone, such as occurs with Paget's disease. This disease, the second most common bone disease next to osteoporosis, has no known etiology; both genetic, and nongenetic factors are implicated (122,123). This disease causes abnormalities in all phases of normal bone remodeling (123): bone resorption occurs in focal areas at an increased rate and bone formation is excessively rapid and results in the deposit of bone tissue in a disorganized fashion. The end result is an increase in poor-quality cortical bone more than twice its normal value. Microscopically, this cortical bone depicts a characteristic mosaic pattern at its chronic stage (Fig. 6) (54,123).

Osteoporosis is a condition leading to the overall reduction in the amount of bone present. This disease is characterized by a rate of change in abnormal bone remodeling, resulting in low bone mass and poor microstructural arrangement of bone tissue (124,125). Osteoporosis can be primary, as is most common in the elderly, or secondary, resulting from several disorders including scurvy, diabetes mellitus, prolonged immobility, or calcium loss (124,125).

Nonhuman

Like that of humans, pathological conditions affecting the skeleton as a whole may be reflected in the appearance of nonhuman mammalian bone tissue. Such conditions may include abnormal-

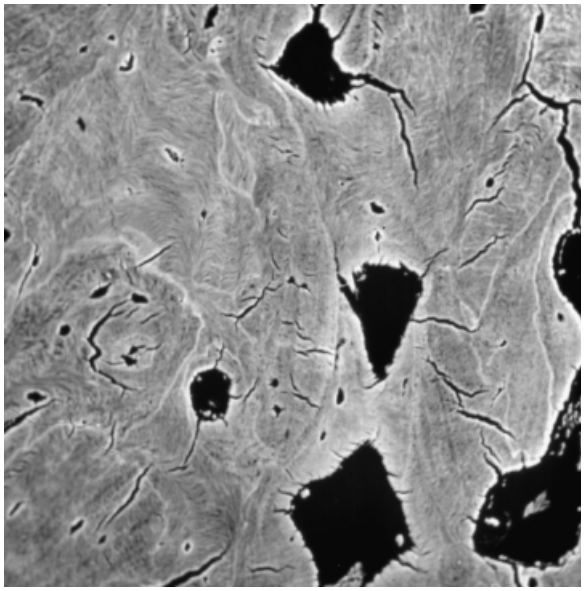

FIG. 6—*Human cortical bone that has a pathological arrangement of bone microstructure consistent with Paget's disease. Note the poor arrangement of bone packets and irregular lamellar structure. Field width: 445 μm.*

ities of development, metabolic disorders, hormonal disturbances, infections, diseases and trauma (126). For instance, age-related changes affecting the size and number of Haversian systems and canals within mammals that display Haversian bone tissue have been documented and an osteoporotic condition has been suggested (104,126). Similarly, any traumatic event resulting in immediate fracture or long-term immobility will result in the production of new, immature bone for the former and changes in normal remodeling parameters for the latter, and accordingly, differences in the size and number of Haversian systems (127).

Discussion

The histological identification of small nondiagnostic bone fragments as either human or nonhuman has been the subject of considerable research. This review has provided an overview of this body of work and illustrates the difficulty that forensic anthropologists face when trying to apply histological methods of identification. However, in certain cases, the application of microstructural analysis may positively identify bone fragments as human or nonhuman.

There are two dominant types of bone tissues present within the cortical bone of many mammalian species: Haversian bone tissue and plexiform bone tissue. Humans exhibit Haversian bone tissue, as do nonhuman primates and most small mammals. Large mammals, on the other hand, exhibit both Haversian and plexiform bone tissue. For these large mammals, Haversian and plexiform bone tissue often coexist within the cortical bone of long bones and ribs and in the same cross-section of these bones, with plexiform bone appearing near the periosteal surface and Haversian bone near the endosteal surface. For immature large mammals, plexiform bone may extend from the endosteal to the periosteal aspects of cortical bone.

Humans do not exhibit plexiform bone, except for very specific circumstances. Plexiform bone tissue may be present in humans during early fetal development and as primary osteonal formation in response to injury or inflammation, otherwise referred to as "periostitis." If a bone fragment is not fetal or pathological in appearance, the identification of plexiform bone tissue identifies it as nonhuman, a determination of extreme importance to forensic investigators.

While large mammals uniquely exhibit plexiform bone tissue, this tissue may not survive postmortem. Exfoliation of bone due to weathering (128,129) and extreme fragmentation from perimortem trauma or fire or from postmortem damage, including fire, burial, and gut digestion, may result in the removal of plexiform bone tissue from the periosteal zone, where this bone tissue is housed in mature large mammals. The remaining tissue in such bone fragments will be Haversian bone tissue and without remnants of plexiform bone to indicate a nonhuman origin, Haversian bone tissue as a shared mammalian feature requires further investigation for differentiation.

The mammalian species included in this review have been classified according to Haversian bone tissue appearance and size of histological structures (Table 10). Several nonhuman species exhibit Haversian bone tissue unique in appearance and size of histological structures as to warrant successful differentiation from humans. These mammals include rat, hare, badger, raccoon dog, cat, dog, and deer. Others exhibit Haversian bone tissue similar in microstructure and histological structure size to that of humans. These mammals include: goat, sheep, pig, cow, water buffalo, horse, and nonhuman primates. The similarity of the Haversian bone tissue between humans and goat, sheep, pig, cow,

TABLE 10—*Species grouping according to Haversian bone tissue appearance.*

Common Name	Species	Bone Tissue Appearance	Histological Structures
Brown rat	*Rattus norvegicus*	Primary longitudinal bone tissue; Haversian systems are rare; areas of avascular and acellular bone tissue	Very small Haversian system diameter and Haversian canal diameter in comparison with humans and other mammals
Hare; raccoon dog; badger	*Lepus americanus* and *oryctolgus*; *Nyctereutes procyonoldes*; *Meles meles*	Dense Haversian bone tissue	Corresponding Haversian system diameter is very small in comparison with humans and other mammals; overlapping Haversian system density for raccoon dog and badger
Cat; dog	*Felis silvestris catus; Canis lupus familiaris*	Dense Haversian bone tissue	Corresponding Haversian system diameter and Haversian canal diameter; both small in comparison with humans and most other mammals
Deer	*Odocoileus virginianus*	Dense Haversian bone tissue	Small Haversian canal diameter and low Haversian system density in comparison with humans and other mammals
Human; goat; sheep; pig; cow; water buffalo; horse	*Homo sapiens; Capra aegagrus hirucus;*, *Ovis aries; Sus scrofa; Bos taurus; Bubalus arnee; Equus caballus*	Dense Haversian bone tissue	Similar-sized Haversian system diameter and Haversian canal diameter
Human; chimpanzees; New World monkeys; Old World monkeys, including rhesus macaques	*Homo sapiens*; *Pan troglodytes*; Platyrrhines; Cercopithecidae including *Macaca mulatta*	Dense Haversian bone tissue	Corresponding values for Haversian system diameter, Haversian canal diameter and Haversian system density

water buffalo, horse, and nonhuman primates does not allow for successful differentiation, particularly when the bone under examination is fragmented, possesses no gross diagnostic features as to species or anatomical origin, and exhibits no remnants of plexiform bone tissue.

Haversian bone microstructure may be further influenced by biomechanical forces upon bone; the age and sex of the individual; and any pathological conditions affecting the individual. These factors result in differences in the rate of remodeling between bones, bone portions, and individuals; an increase or decrease in the size and/or number of Haversian systems, along with a general change of the microstructure, i.e., placement of Haversian systems or quantity of interstitial bone, are all potential manifestations of these factors. In particular, if a bone fragment appears atypical, it may be affected by a pathological condition or a diagenetic alteration and any attempt at species determination using cortical bone microstructure should be avoided.

No single methodological approach can determine species type; a combination of the overall microstructural appearance and the size of histological structures should be applied. General microstructure provides an indication as to whether the bone fragment is human or nonhuman, i.e., the presence of plexiform bone tissue indicates nonhuman origin. Measuring histological structures for which known value ranges exist provides further indication of human or nonhuman origin. Three measurements have been of the focus of this review: Haversian system diameter; Haversian canal diameter; and Haversian system density. Haversian system diameter and Haversian canal diameter have been demonstrated as being markedly different between humans and certain nonhuman species (Figs. 3 and 4) and are both advocated as measurements to be used for differentiation (7,11–13,97). Haversian system density, although not often studied for this purpose, does illustrate a range of density for humans and so, if a bone fragment exhibits a measured density outside of this range, a nonhuman origin can be assumed (Fig. 5).

Conclusion

This review has demonstrated that, in the histological examination of small nondiagnostic bone fragments, human cortical bone may be positively differentiated from certain nonhuman species. These species include the smaller mammals of rat, cat, dog, hare, badger, and racoon dog, and the larger mammal of deer. This differentiation is based on differences in the general appearance of cortical bone tissue and the size of histological microstructures, namely Haversian system diameter, Haversian canal diameter, and Haversian system density. Where plexiform bone tissue is present, differentiation of human from nonhuman cortical bone is also possible as humans do not exhibit this type of primary bone tissue (early fetal bone and periostitic bone).

Other mammals, including the larger species of goat, sheep, cow, pig, horse, and water buffalo, can be successfully differentiated from human cortical bone when plexiform bone tissue is present. However, where plexiform bone tissue is absent, due to peri- and postmortem alteration, differentiation may not be successful due to commonly shared cortical bone Haversian tissue microstructure. Hence, attention to the preservation of the bone fragment is important. Nonhuman primates share similar cortical bone histology, namely Haversian bone tissue, with humans and cannot be successfully differentiated from human bone.

The overall recommendation that can be made for the differentiation of human cortical bone from nonhuman bone is the use of bone microstructure type for primary differentiation. The presence of plexiform bone tissue positively identifies bone fragments as nonhuman and its identification would negate further forensic investigation. The examination of Haversian bone tissue for this purpose should include an assessment of the overall appearance of the tissue and an evaluation of the size of Haversian tissue microstructures. Where Haversian bone tissue is identified, while it is human in arrangement, it is not, and has not been to date, demonstrably uniquely human as it is exhibited by nonhuman mammals in a similar appearance and with similar-sized histological structures. Histomorphometry may be successfully applied and the measurements considered of most use are Haversian system diameter and Haversian canal diameter. Haversian system density, while not as comparably useful, does provide an upper and lower limit for human identification. Human identification beyond these metric parameters is therefore deemed not currently possible.

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