

To the Graduate Council:

I am submitting herewith a thesis written by Lindsay Hines Trammell entitled "A Histological Examination of *Odocoileus virginianus* for Forensic Application." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.

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A Histological Examination of *Odocoileus virginianus*
for Forensic Application

A Thesis
Presented for the
Master of Arts
Degree
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Lindsay Hines Trammell
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I have to also thank my family especially my mom for enduring my endless stress-filled phone calls for the past two years. Your love and unfailing patience with me have been an inspiration. I would never have made it without you.

Abstract

Few researchers have endeavored to approach the issue of bone histology in non-human specimen. In the forensic setting, it is oftentimes the norm to establish the origin of fragmentary remains as human or non-human; if the fragments are non-human then the analysis is usually said to be finished.

If the specimen does prove to be non-human, it is useful and important to learn the identity of the faunal species. The white-tailed deer (*Odocoileus virginianus*) is common to the Eastern United States and is very often found in forensic settings, commingled with or alongside of human remains. This study thoroughly examines the histological character of the white-tailed deer so that it may be identified with confidence in the forensic field or in an archaeological setting.

To assess the white-tailed deer femora and humeri were procured from five deer of varying ages -- the goal being to understand histological changes within bones from the same specimen and between bones of deer at different stages of maturity.

With the aid of a Buehler Isomet 1000 saw, two 0.08mm sections were cut at three-centimeter intervals on each diaphysis of the femur and humerus for each deer. These thin-sections were examined under a Leica DMRX light microscope at 100x magnification. The presence of all histological structures was noted for each slide and when evidence of bone remodeling was recorded, area measurements were taken on secondary osteons and Haversian canals.

The observations and measurement data from each femur and humerus of the deer samples were compared in a statistical analysis to assess inter- and intra-bone variability

among the deer. A repeated measures ANOVA examined the differences in osteon and Haversian canal area across the segments of the femoral and humeri shafts and between ages. These results indicate whether or not the histological character of the white-tailed deer differs depending on the age, the bone, or the specific location on a bone.

The results indicate no significant mean differences within shafts or across ages for osteon area in the femur. No differences exist within the shafts of the humeri. Notable variations in osteon area do exist between ages in the humerus -- the 3-year-old deer diverged from both the 1.5-year-old and the 2.5-year-old.

The results also indicated no significant mean differences intra-shaft or across ages for Haversian canal areas in the femur. A significance difference was noted between the Haversian canal areas in the nine-month deer and the 2.5-year-old deer humeri.

Based on the findings a blind test was performed on four unidentified bones samples to discern deer from other ungulates based on osteon areas. The methodology and results of this research were successfully utilized to distinguish white-tailed deer bone from pig, sheep, and goat at the histological level.

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Chapter 1: Introduction

The discipline of forensic anthropology addresses legal matters through the practice of physical anthropology. A key contribution of forensic anthropology to the criminal investigation of a homicide is the development of a biological profile from which a positive identification can be established. The forensic anthropologist develops biological-based age estimation, sex assignation, stature assessment and “race” from the human skeleton. This determination can be based on gross morphology (Bass, 1995; Schwartz, 1995; White, 2000;) or on human bone histology (Alqvist and Damsten, 1969; Kerley and Ubelaker, 1978; Kerley, 1965;). Before a biological profile is developed, the remains must be determined to be human. This step is vital for the forensic anthropologist, but it is a difficult one even for the most well-trained specialist.

Distinguishing between human and non-human remains is a necessary step to tackle due to the commonality of having both animals' remains present at an archaeological or forensic recovery site. As a result of physical and chemical processes such as weathering, degradation, or high temperatures, differentiating between the human and non-human remains is all the more difficult (Jowsey, 1966; Ubelaker, 1989). To be able to make such discernment, it is useful to understand the characteristic differences between a human and the bones most often misidentified as human.

This distinction is vital to forensic anthropology and law enforcement agencies. In such cases, microscopic analysis can be used to view the orientation of osteons and the appearance of the cortical bone. Humans have a diagnostic pattern -- osteons are larger, more scattered and are evenly space within the circumferential lamellar bone. In

nonhuman species, however, osteons are more likely to be arranged in rows or in rectanguloid structures called plexiform bone (Jowsey, 1966; Ubelaker, 1989; Woods & Ellis, 2000). Variation is important to understand, too, because even when studying the histological character of human bone, the location of interest on a long bone may differ in its microscopic features from another loci on the same long bone but further down the shaft (Horn, 2002; Pfeiffer, 2000).

As the tedious process of understanding the histological variability within human bone progresses, it is equally vital to approach the question of human or nonhuman from an angle that addresses these nonhuman species more closely. If a bone sample or fragment is indeed nonhuman, then what is it? Misidentification can occur as fragmentary human remains may be found alongside animal bones in disturbed contexts. Another source of confusion may arise when human bones and teeth are mixed with faunal remains and submitted to an osteologist or zooarchaeologist for analysis (Whyte, 2001).

The white-tailed deer, *Odocoileus virginianus*, is the most prolific mammalian species to be found alongside of human remains in the southeast United States (Whyte, 2001; personal communication, Dr. Walter Klippel). When deer and human skeletal elements are found to be commingled and incomplete or too fragmented to discern the identification of the species, forensic and physical anthropologists are beginning to turn to the field of histology for microscopic characterizations of cellular structures (George, 2003; Stout, 1992; Stout, 1989; Ubelaker, 1989). The utilization of histology in such a setting is increasing, yet is still in its initial stages and additional future research is needed to continue to prove its reliability and usefulness within the forensic discipline.

Histology is a valid method for differentiating between human and non-human bone. Microscopic examination of bone illustrates that the orientation and general arrangement of cellular byproducts such as lamellae, size of the lacunae, and the area of the osteons vary from species to species (George, 2003; Horni, 2002; Jowsey, 1966). My research utilizes histological thin-sectioning and the aid of computer-assisted analysis and a microscope to examine the cellular structures of long bones of the white-tailed deer and how they change as the deer ages. Paying close attention to and understanding the microscopic differences in deer bone as it ages is important because older, more mature deer exhibit bone remodeling and can appear histologically very similar to humans. It is useful, then, to be able to differentiate deer bone, both young and old, from human bone at the cellular level. To thoroughly assess this question, I denote any intra-bone and inter-bone variation.

The primary intention of this research is to develop a useful template that can be confidently used by forensic anthropologists as a comparative reference to discern human bone from deer bone in cases of fragmentary remains.

Chapter 2: Background

The Study of Histology

Histology has been used as a tool in anthropological research as early as 1849. Researchers used the technique to examine the microscopic structures of fossils. The science proved useful again in 1878 as an analysis was performed on preserved archaeological fauna to find evidence of histological structures still present in fossil bone and teeth (Martin, 1989). With the onset of the twentieth century, histological techniques helped to "detect abnormalities in preserved skeletons" (Martin, 1989:57).

Such findings spurred additional progress in paleopathological research and more histological studies on skeletal material from the past. Histological analysis of remains can discern pathologies within the bone (Martin, 1989; Pfeiffer, 2000; White & Fokens, 2000; Moodie, 1923). Osteoporosis, for example, causes bone thinning and results in porosity within the bone. Microscopic examination can provide an image of this condition (Pfeiffer, 2000; White & Fokens, 2000). Also, the affects of infectious diseases can be seen in bone. Bone lipping and excess bone growth resulting from infection can be viewed at the histological level (Martin, 1989; Pfeiffer, 2000; White & Fokens, 2000; Moodie, 1923).

Histological studies have led to developments in assessing age, disease, and nutritional stress in bone. Bone histomorphology, or the structure of bone tissue at the microscopic level, can help to indicate diet and interpret lifestyle (Gilbert, 1985; Harsanyi, 1993; Pfeiffer, 2000). In an archaeological context, for example, histological

examination can help to identify and characterize a faunal assemblage from a site (Gilbert, 1985; Harsanyi, 1993; Pfeiffer, 2000). The techniques of histology can be used to analyze bone fragments found at an archaeological site and infer paleodietary habits (Gilbert, 1985; Harsanyi, 1993; Pfeiffer, 2000), identify components of diet, and indicate subsistence patterns and strategies of a population (Gilbert, 1985; Harsanyi, 1993; Pfeiffer, 2000). As an innovative science, histology has led to discoveries and advancements within the field of anthropology and will provide the basic technique for examining the characteristics of animal bone.

The histological identity of the white-tailed deer has not been a predominant concern until recently and has, as a result, rarely been the subject of previous histological research and exploration. Forensic anthropologists are beginning to more often find deer and human bones alongside each other due to the rising population of white-tailed deer as well as hunting practices. Since these two species can potentially be confused, the need for the development of a microscopic chronology of the deer's cell structure as compared to humans has emerged.

Histological Thin-Sectioning

For the purpose of examining the morphological bone structures of human and deer remains, the technique of histological thin sectioning is a useful method. This means of microstructural examination is not new to forensic anthropology. Von Eber used thin sectioning in 1675 when he first recognized the remodeling processes of cortical bone (George, 2003; Lacroix, 1971). It was also utilized in 1691 by Havers to study the

microstructure of the cell -- the Haversian system (George, 2003; Lacroix, 1971; Stout, 1989).

The technique has been improved and honed over the past few centuries and is primarily used today on human bone to estimate age at death and to study skeletal pathologies and nutritional stress (Ahlquist and Damsten, 1969; George, 2003; Kerley and Ubelaker, 1978; Moodie 1923; Stout 1989; Thompson and Gunness-Hey, 1981). Thin-sectioning research has not yet fully transitioned into the field of zooarchaeology as a means of microstructural analysis, but it is indeed useful in faunal identification based on distinct bone type organization (George, 2003; Horni, 2002; Jowsey, 1966).

When examining faunal specimens via thin sectioning, numerous types of bone are visible. Lamellar bone (Figure 1) is present in three unique forms. It is "undifferentiated or homogenous" (George, 2003) with a disorganized distribution of lacunae. Lamellar bone is also present as "external circumferential lamellae" (George, 2003). In this type of bone, lacunae are parallel to the margin of the bone. And finally, lamellar bone can be seen as "internal circumferential lamellae" (George, 2003).

A second recognizable bone type is plexiform bone (Figure 2) consisting of "a series of long, thin segments of lamellar bone separated by a system of vascular canals" (George, 2003; Stout, 1989; Woods & Ellis, 2000). A third bone type is that of the Haversian system or the osteon (Figure 3). There are four distinct types of Haversian systems. These are generally associated with specific mammalian species (George, 2003; Stout, 1989; Woods & Ellis, 2000). All of these forms of bone will be discussed in more depth later in this chapter.

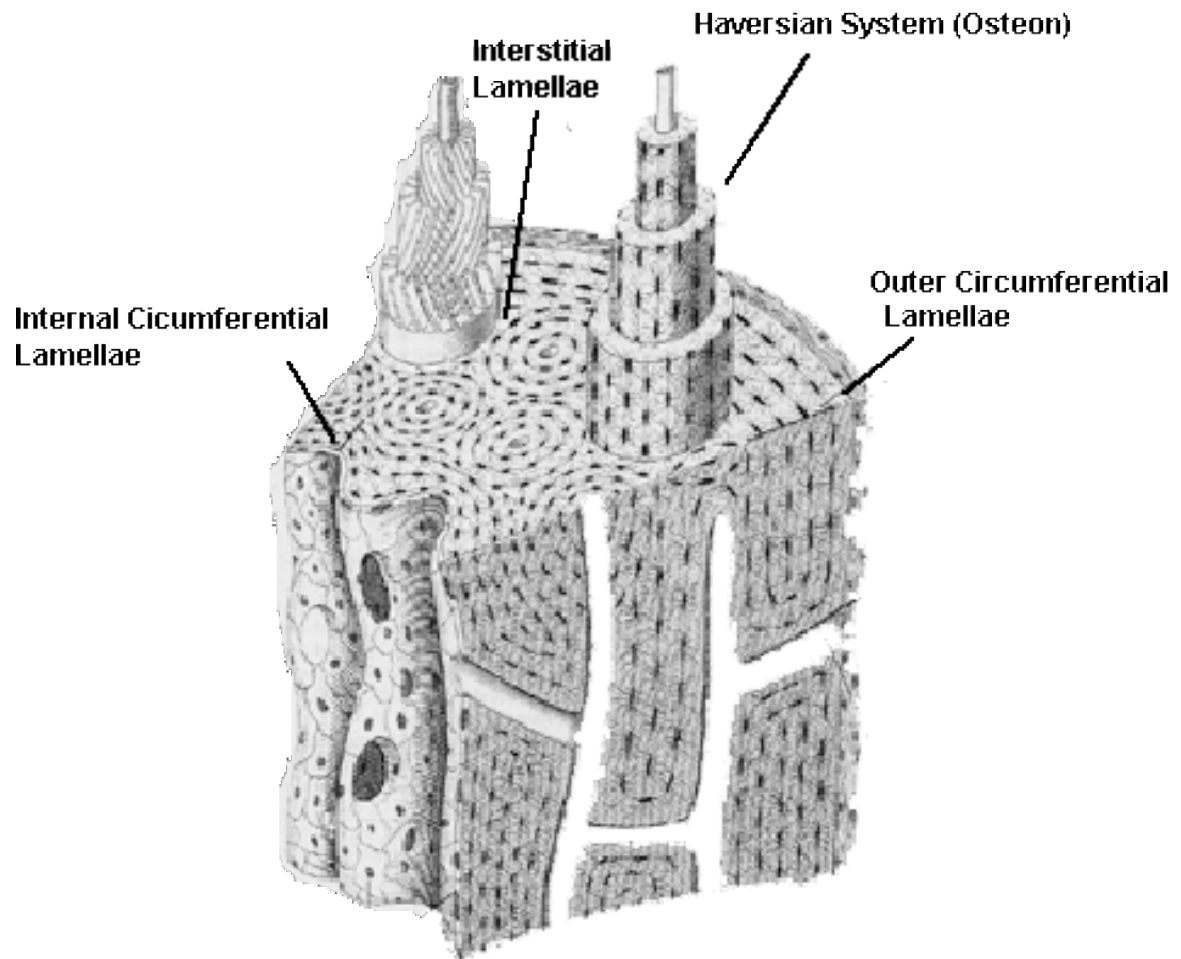


Figure 1: Lamellar bone in its three forms: Outer circumferential, internal circumferential and interstitial.

Adapted from Junqueira et al 1995.

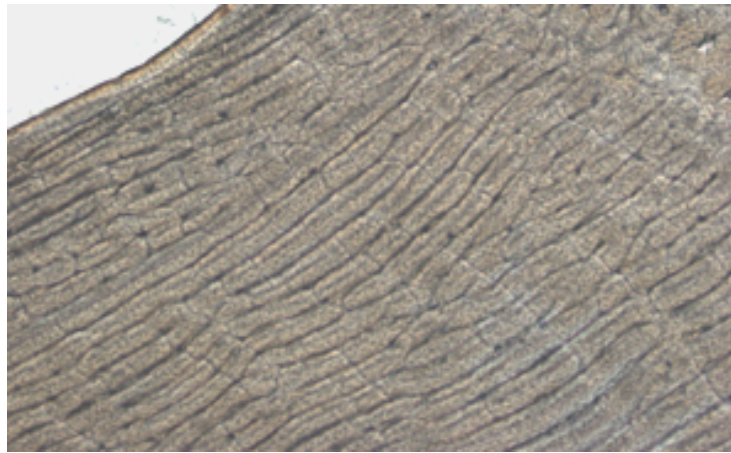


Figure 2: Brick-like structure of plexiform bone at 50x.

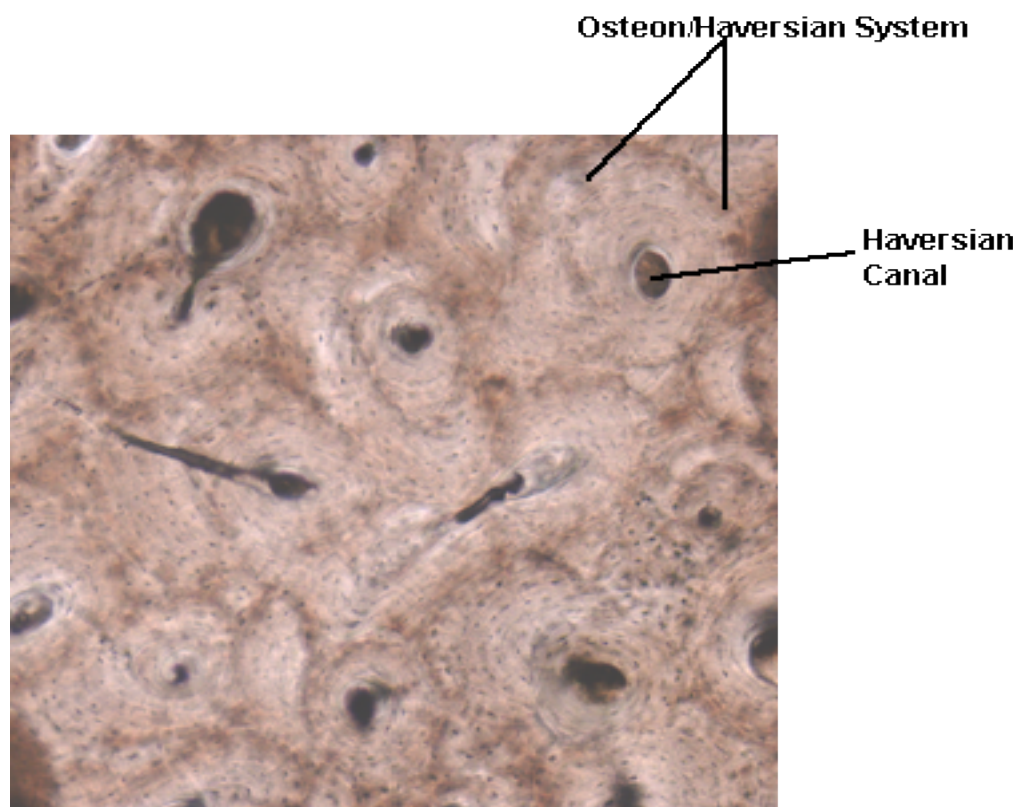


Figure 3: Illustration of the Haversian system and its Haversian canal at 100x.

George presents histological thin-sections of the white-tailed deer in his study. This specimen has "plexiform bone characterized by long, parallel segments of bone separated by a well-developed network of vascular canals" (George, 2003:1). Only the occasional Haversian system is present. These osteons appear as "circular unconformities within the plexiform tissue." George does not, however, identify or state the age of the deer nor does he characterize how the white-tailed deer changes histologically over time.

Douglas Ubelaker also conducted histological research and provided a template for preparing bone for thin-sectioning microscopic analysis. Ubelaker emphasized the importance of adequate preparation of the sample for beneficial results. He procured undecalcified, ground, thin sections for microscopic observations and first made a 5 to 10 millimeter cross-section with a saber saw. An Ingram thin-section saw was then used to remove a thinner, parallel-sided section (Ubelaker, 1989). Ubelaker's methodology for histological analysis is commonly used among current research.

Human Versus Nonhuman

Previous studies comparing human to nonhuman bone, especially deer bone, are few in number. Histological research is still a new and tedious process. Past examinations of human bone at the microscopic level were mostly to establish an accurate means of estimating age based on the size and distribution of cellular structures (Ahlquist and Damsten, 1969; Kerley, 1965; Kerley and Ubelaker, 1978). Ellis R. Kerley pioneered the microscopic determination of age in human bone. Other methods (Ahlquist & Damsten, 1969; Stout, 1992) have failed to provide better results; researchers today are still

working to test the replicability of Kerley's aging techniques (Tersigni, 2005; Stout, 1989; Stout, 1992).

In 2001, Mulhern and Ubelaker published an article differentiating between human and nonhuman bone. Their work is commonly cited and referred to in more recent studies as well. They focused on how osteons, or mature bone cells, are characteristically arranged in a banding pattern and how this pattern varies between humans and other mammalian species.

The main problem with the aforementioned research and other attempts at aging human bone via Kerley's method is the issue of destructive analysis. It is seen as unethical to destroy human bones for the purpose of histological examination (Horn, 2002). Part of this reason is that when bones are sectioned and cut for histological analysis they are fully destroyed and can no longer be used in a study collection or in other skeletal analyses such as estimations of age and stature. Currently, this taboo essentially leads to small sample sizes when research is conducted and oftentimes may result in skewed data because of such a poor representation of bones. These factors deter many anthropologists from using histology, but perhaps if the value of bone biology is adequately asserted, then it will be more readily accepted and utilized (Horn, 2002).

When examining the histology of nonhuman species, this ethical issue is not usually a factor. By utilizing the human thin section samples from prior studies, researchers can work to compare them to any number of faunal species. They will be able to gather enough data to examine the microscopic differences in human and nonhuman bone and reach significant results, though it will surely be a time-consuming process.

Only a few articles have been published pertaining to nonhuman histological studies (Enlow and Brown, 1956-58; Harsanyi, 1993; Horni, 2002; Jowsey, 1966; Owsley et al, 1985). Harald Horni has conducted one such innovative work. His Masters thesis titled "The Forensic Application of Comparative Mammalian Bone Histology," dealt directly with how accurately one can determine human versus nonhuman (Canids, Felids, Cervids, Bovids) when having only fragmentary remains. He histologically examined cellular structures characteristic to each species and whether these did or did not indicate differences in distribution or size of osteons when compared to each other and to human bone samples (Horni, 2002).

Horni considered seven species and examined numerous facets of bone at the histological level including secondary osteonal osteocyte to secondary osteon size ratio, secondary osteonal osteocyte count, secondary osteon area, and Haversian canal area. He concluded that by factoring in such data when examining fragmentary remains, an accurate distinction can be made to differentiate human from non-human (Horni, 2002).

Another noteworthy study of human versus nonhuman bone was conducted in 1985 by Doug Owsley in response to fragmentary remains linked to a homicide investigation. Bone fragments were discovered inside a man's pick-up truck who was in question for the murder of his wife. He claimed the fragments belonged to that of a deer he killed during hunting season. Owsley histologically examined these fragmentary remains and tested them against known deer bone. He was able to rule out deer as the source due to lack of plexiform bone normally seen in deer and the presence of secondary osteons consistent with those of human bone. Owsley's contribution to the case not only

indicates the value of bone histology to the forensic anthropologist, it was also essential in solving the crime (Owsley, 1985).

Bone Biology

Types of Bone

For the purpose of understanding the microscopic-level structures of faunal species it is helpful to firmly understand the histology of human bone and to first grasp the processes of bone creation, modification, and resorption as well as the basic biology of a bone cell. Collectively, these processes are known as bone remodeling.

When considering human and other mammalian bone, there are two general types of bone: compact or cortical bone and spongy or trabecular bone (Figure 4). The cortical bone is the outer layer and is more hard, dense, and porous than the inner spongy bone (Schwartz, 1995; White & Fokens, 2000). It is primarily found in the shafts, or diaphyses, of long bones, surrounding the trabeculae at the ends of joints, and in the vertebrae. Spongy bone is more isolated in the epiphyses --the proximal and distal ends of bone (Schwartz, 1995; White & Fokens, 2000).

Bone is also composed of two membranes (Figures 5, 6, and 7). The first is the periosteum, a double-layered protective membrane. This outer fibrous layer of dense cartilaginous tissue surrounds the inner osteogenic layer composed of osteoblasts and osteoclasts. It is rich in nerve fibers, blood, and lymphatic vessels and is secured to the bone via Sharpey's fibers. The endosteum is the second membranous layer, and it covers the internal surface of the bone. Within these membranes are the basic structures of bone.

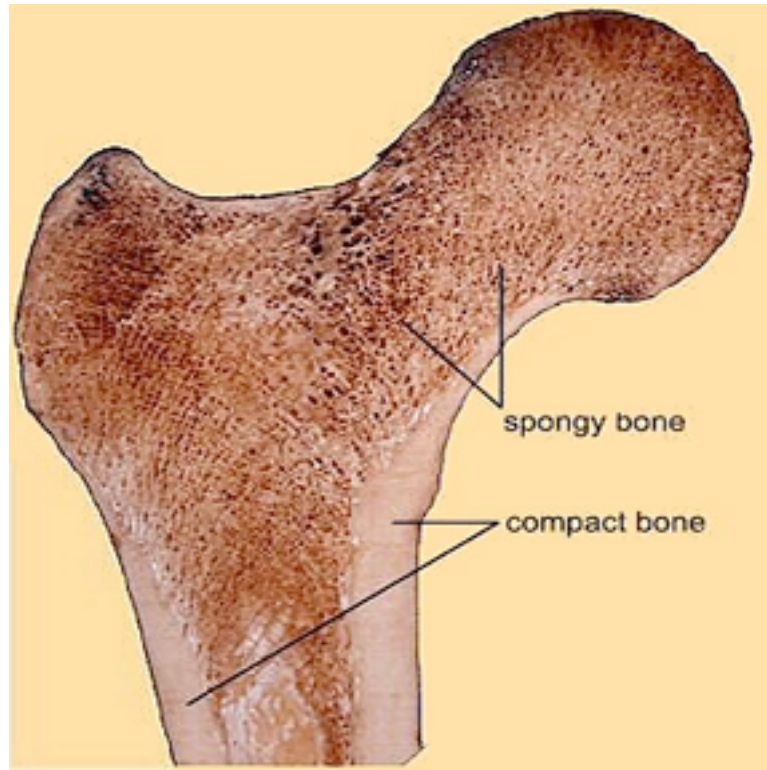


Figure 4: Two general types of bone: compact bone and spongy bone.

Adapted from <http://www.biology.eku.edu/RITCHISO/bonecompact.jpg>

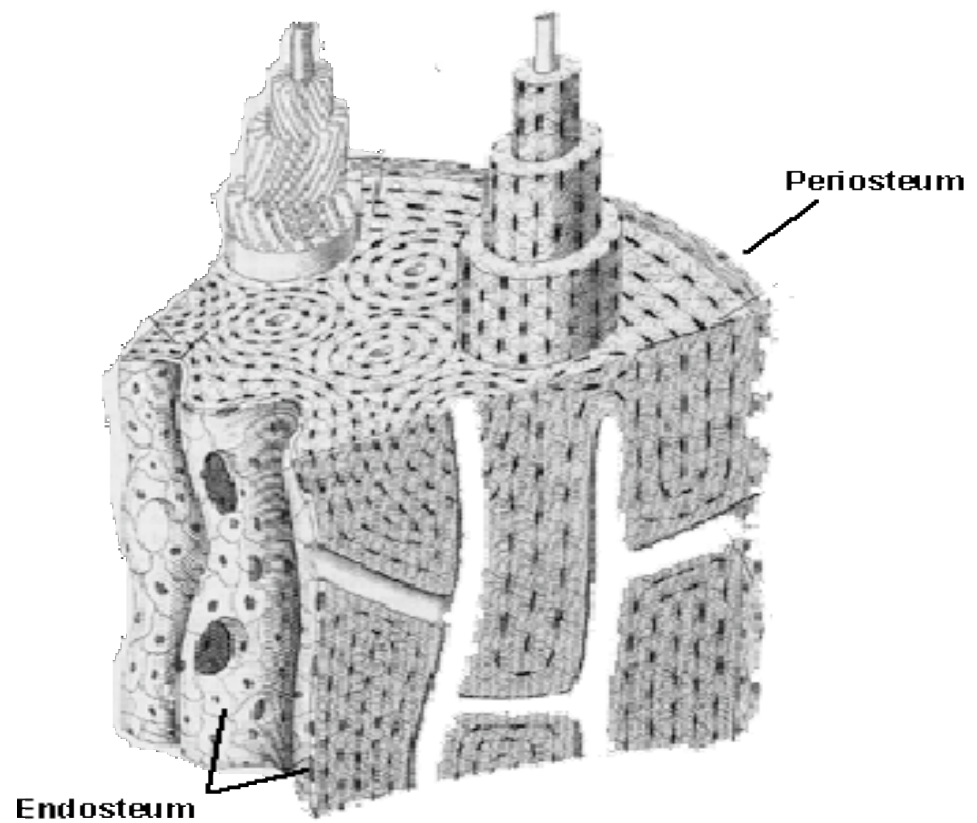


Figure 5: Periosteum and endosteum are the outer and innermost layers of bone, respectively.

Adapted from Junquerie et al 1995.

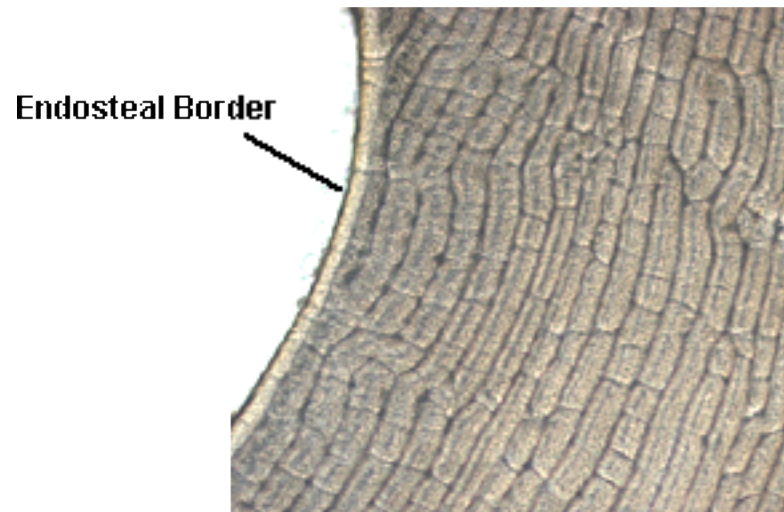


Figure 6: Endosteal layer indicated in histological photo of bone at 50x.

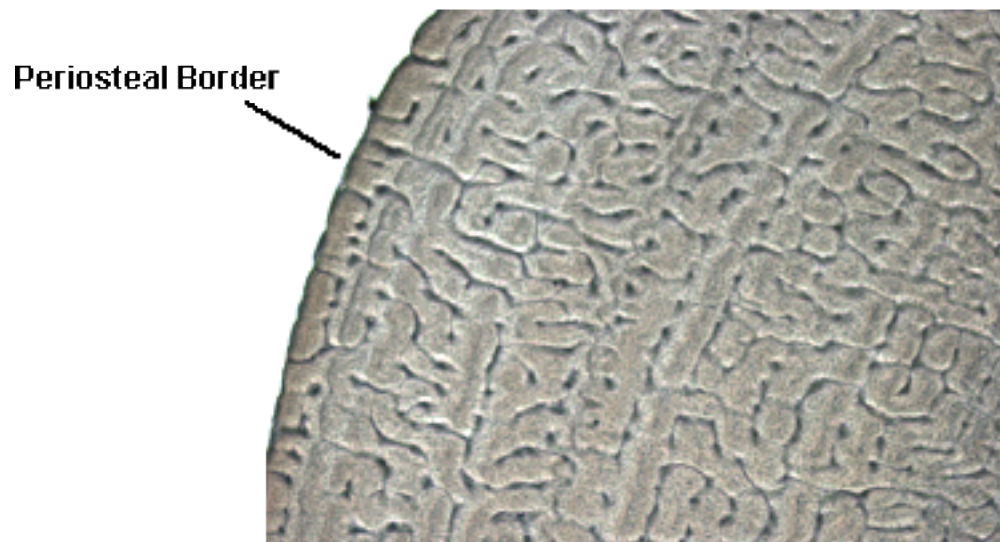


Figure 7: Periosteal layer of bone indicated in histological photo at 50x.

There are two histological types of mammalian bone -- mature or lamellar bone and immature or woven bone (Figure 8). Woven bone is known also as "coarsely bundled bone" (Ross et al., 1989; White & Fokens, 2000; Woods & Ellis, 2000) due to the presence of random cartilage bundles within the bone. It develops first in the embryonic skeleton and is gradually replaced by the growth of mature bone. Woven bone is produced more quickly than lamellar and is thus often weaker and more randomly distributed (Horn, 2002; Martin & Burr, 1989). Lamellar bone is more structured and uniform in organization. It comprises both compact and spongy bone (Ross et al, 1989; White & Fokens, 2000; Woods & Ellis, 2000). Lamellar bone is arranged in dense (collagen-rich) lamellae and loose (collagen-poor) lamellae (Ardizzoni, 2001). Lamellar bone can also be divided into primary and secondary bone with the primary lamellar bone appearing during growth and the secondary lamellar bone acting as replacement bone where old bone is resorbed (Horn, 2002).

In addition to lamellar and woven bone, a third type of bone is plexiform bone. Plexiform is essentially a combination of lamellar and woven bone and is formed very rapidly (Woods & Ellis, 2000). Since it is common for plexiform bone to develop in response to the need for increased biomechanical support, it is often seen in larger, faster growing mammals including cows, sheep, or deer (Woods & Ellis, 2000). It is characterized by rectangular, brick-like shapes and stems from mineral buds that grow first perpendicular and then parallel to the outer edge of bone surface. Plexiform bone must form on preexisting cartilage or bone surfaces (Martin & Burr, 1989; Woods & Ellis, 2000).

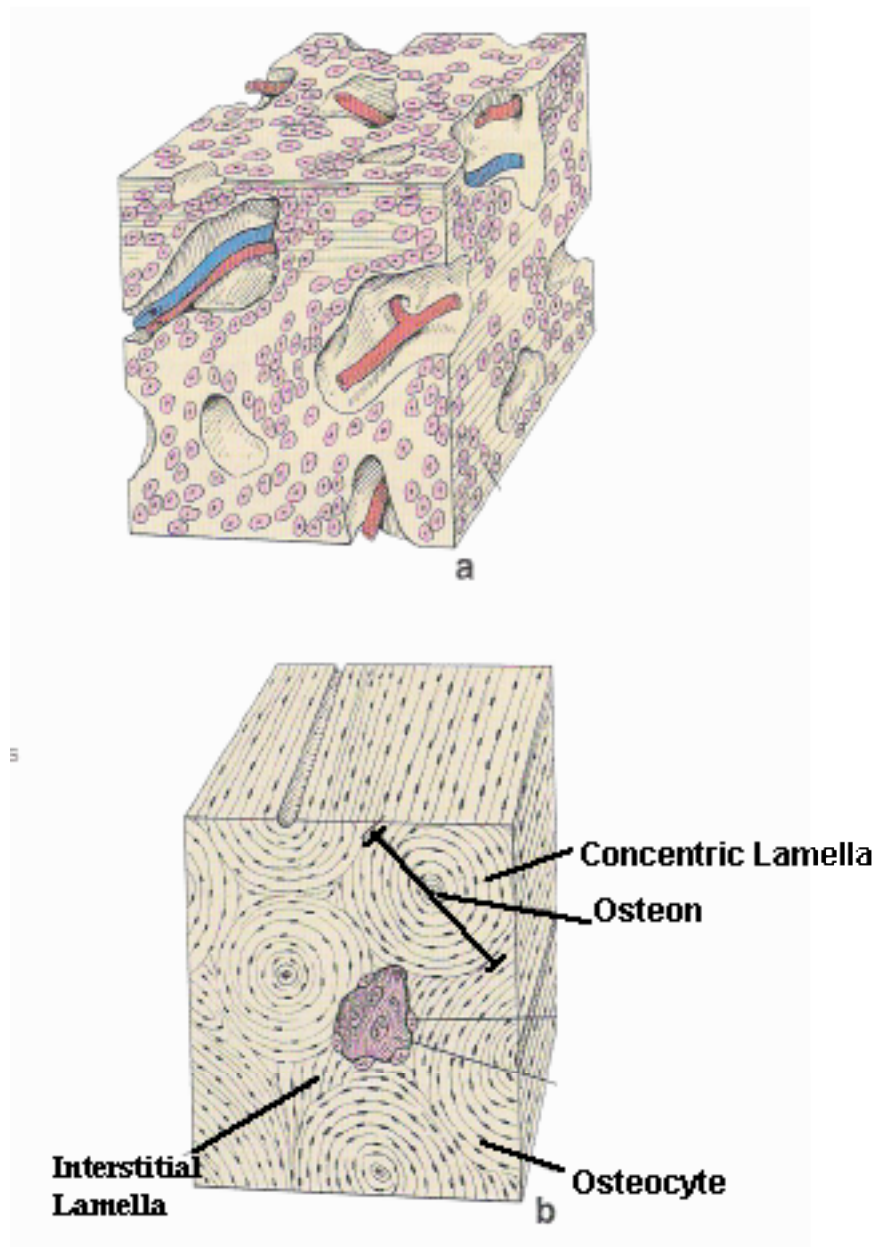


Figure 8: Picture (a) illustrates immature or woven bone. There is little to no organization. Picture (b) illustrates mature or lamellar bone and is more structured in its organization.

Adapted from Ross et al, 1989.

Bone Cells and Bone Remodeling

Compact and trabecular bone require nourishment. Microscopic structures within the bone work in communication and in movement of nutrients. An osteon is the structural unit of compact bone (Figure 9). A central Haversian canal penetrates the osteon and serves as a passage for blood cells, lymph nodes, and nerve endings. Concentric layers of lamellae composed of bone matrix surround the osteon and extend outward (White & Fokens, 2000). Also within the compact bone are small spaces called lacunae that house osteocytes during life. These lacunae reside in the loose lamellae (Ardizzoni, 2001). The osteocytes have finger-like extensions called canaliculi (Figure 10) that communicate nutrients from cell to cell. Also helping in maintaining cellular nourishment are Volkmann's canals. They transverse the cell and connect Haversian canals.

It is vital to be able to distinguish between primary and secondary osteons (Figure 11). The primary osteon is primary bone. They are small areas of bone tissue surrounded by concentric rings of more bone tissue. A central blood vessel passes through each osteon. This type of primary tissue is different from the larger secondary osteons so characteristic of human bone in its method of formation and its level of structure. It is believed that primary osteons are formed via the mineralization of cartilage while secondary osteons are the result of a remodeling process that replaces existing bone (Mulhern & Ubelaker, 2001). These methods of formation are discussed later.

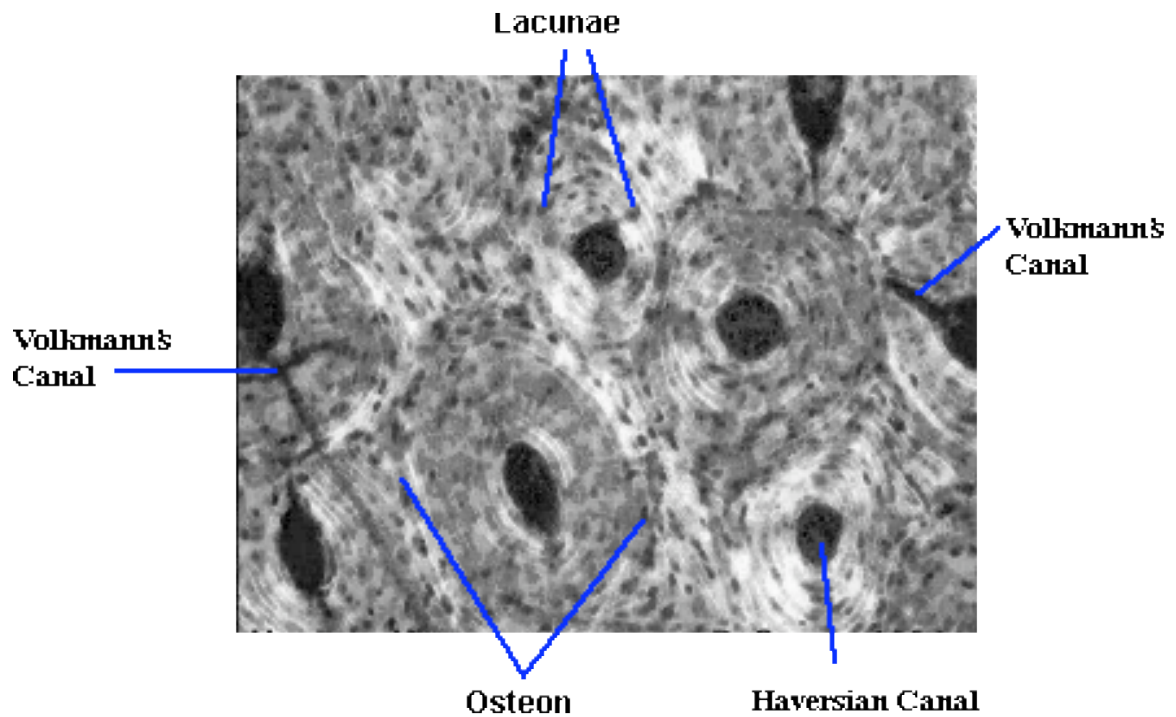


Figure 9: Depiction of an osteon in human bone including the Haversian canal, lacunae, and Volkmann's canals at 40x.

Adapted from George, 2003

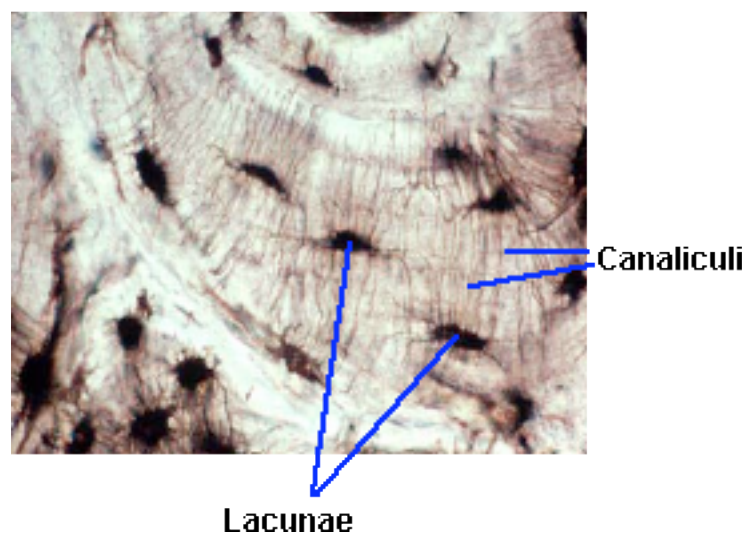
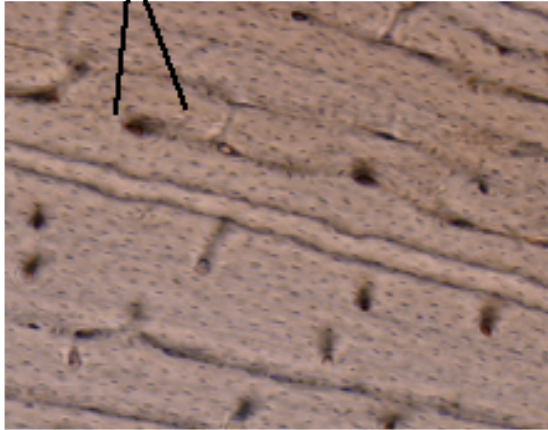


Figure 10: Spidery-like canaliculi connect lacunae and provide for a means of communication at 300x.

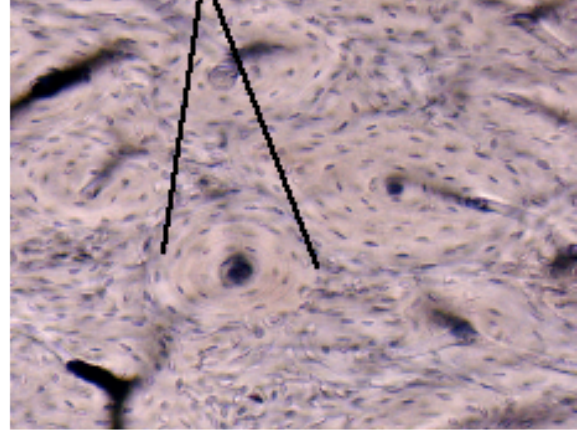
Adapted from : Ross et al, 1989.

Primary osteon



(a)

Secondary osteon



(b)

Figure 11: Primary osteons (a) are smaller and are indicative of newly formed bone while secondary osteons (b) are larger and indicative of remodeled bone. Slides at 100x.

The secondary osteon, or Haversian system, introduces a difficulty in distinguishing between human and non-human bone. It is characterized by lamellar bone surrounding a central Haversian canal with a clear cement or reversal line (Figure 12) marking its boundary. Secondary osteons replace primary bone and are oftentimes characteristic of the species. In humans, they tend to be denser, larger, and randomly scattered yet comparative studies reveal that though some secondary osteons appear to be human, they can still be confused with other mammalian species (Enlow and Brown, 1958; Mulhern & Ubelaker, 2001).

Primary and secondary bone remodeling revolves around structures at the cellular level. An osteocyte is one of the three types of bone cells that are involved in osteogenesis, the process of bone tissue formation. This ossification forms the bony skeleton of the embryo beginning at eight weeks of fetal development. It continues until early adulthood contributing to bone thickness, remodeling, and repair. There are two models of bone growth. The first, intramembranous ossification, involves bone development from a fibrous membrane. In endochondral ossification, the second model, bone forms by replacing hyaline cartilage.

In addition to the mature bone cell -- the osteocyte -- osteoblasts and osteoclasts contribute to the process of osteogenesis. Osteoblasts are small bone-forming cells with a single nucleus. Osteoclasts are multinucleated and work to resorb or break down established bone matrix.

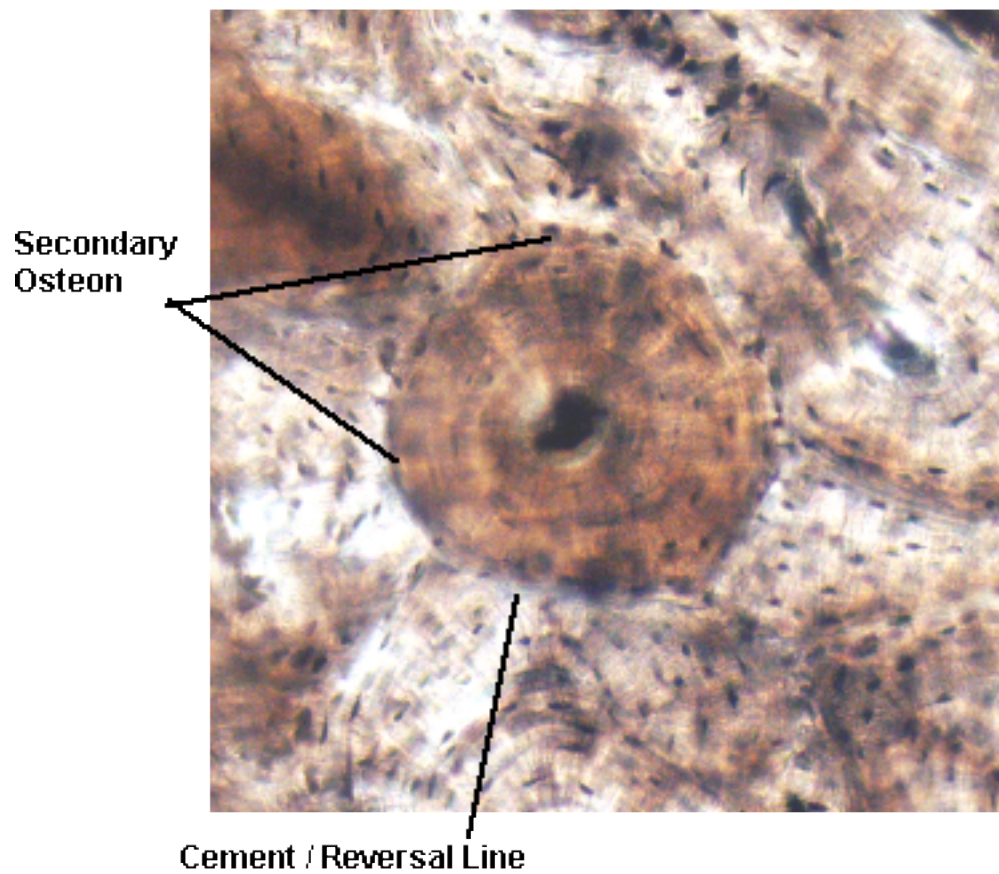


Figure 12: Secondary osteon surrounded by cement/reversal line at 100x.

These adjacent units work to deposit and resorb bone on the periosteal and endosteal surfaces. In a process known as bone remodeling, the osteoclasts carve out a space in the bone called a resorption bay (Figure 13). Lysosomal enzymes help to digest the organic matrix of bone and subsequently secrete acids to break down bone (Stout, 1992). As the matrix dissolves, osteoblastic activity is triggered by the rise of calcium in the bloodstream and begins to make bone and deposit it at the site of injury (Figure 14).

The bone remodeling process is constant and is regulated by the body's hormonal system. A feedback loop helps to maintain and control the amount of calcium ion levels in the blood -- a balance that is vital to health and bone strength. A concentration of 9-11mg/100mL is ideal. To keep such a balance, the thyroid gland releases a hormone called calcitonin when the calcium ion levels in the blood reach abnormally high levels. The calcitonin stimulates the osteoblasts to deposit calcium salts into bone. Similarly, when calcium ion levels in the blood become too low, the parathyroid gland is stimulated to release a hormone called PTH. PTH signals osteoclastic activity to break down and resorb bone matrix to release calcium ions back into the bloodstream (Stout, 1992).

These constant processes of bone deposition and resorption can be seen at the histological level. It is this pattern that characterizes human and other mammalian bone and distinguishes them from each other. One additional concept to be familiar with and aware of is the forces behind the spatial organization of bone. Wolf's Law (Hughes-Fulford, 2004; Petrtyl et al, 1996) states that bone grows or remodels in response to forces or demands placed upon it due to mechanical stress. Sites of muscle attachments on bone can pull and produce



Figure 13: Osteoclasts indicated by red circles. Resorption space is indicated by arrow at 500x.

Adapted from: http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/h_frame9.html



Figure 14: Bone-building osteoblasts indicated by red circles lining endosteal border at 500x.

Adapted from: http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/h_frame9.html

microfractures or other types of stress. This results in increased osteoblastic activity as the osteoblasts work to build more bone at sites of higher mechanical stress (Hughes-Fulford, 2004; Petrtyl et al, 1996). During bone growth, changes in physiological condition cause the bone to experience forces and moments of varying strengths and directions. Such stressors are essentially responsible for the remodeling and the growth and architecture of bone (Hughes-Fulford, 2004; Petrtyl et al, 1996).

White-tailed Deer

To understand why and how the white-tailed deer is applicable and appropriate to a histological inquiry, it is helpful to delve into a brief history of the species and its lifestyle. The white-tailed deer, *Odocoileus virginianus*, is a prolific animal in the United States. Though most commonly found in the eastern and southern states, white-tailed deer habitats are everywhere except for Hawaii (Kalbacken, 1992; Hillman et al., 1973). Since the early era of the first Native Americans, the white-tailed deer have long provided food and clothing and while the buck is generally larger than the doe, but both are used as subsistence sources (Kalbacken, 1992; Hillman et al., 1973).

Found near small fields and wooded areas where grasses and leaves are abundant, eastern Tennessee is a suitable habitat for the white-tailed deer. Deer age, sex, and related antler growth (if male) and gestation status (if female) are vital information when considering bone histology.

White-tailed deer mating season or "rut" begins in October and birthing usually occurs in late May and early June. Since deer are mammalian species, they do lactate.

Lactation can potentially pull minerals such as calcium from bone (Kalbacken, 1992; Hillman et al., 1973). A question posed by forensic anthropologists and zooarchaeologists is whether or not this action depletes bone cells and growth. A microscopic examination of female deer bone during and after pregnancy may provide information about how their bone histology does or does not change. Research has not yet to be undertaken to address this facet of the doe's life cycle and how it relates to bone architecture and osteon density.

The buck as aforementioned is usually larger than the doe and thus can be hypothesized to have more osteonal growth amid plexiform bone due to such size (Hillman et al., 1973). Also potentially contributing to such bone remodeling is antler growth. Bucks shed their antlers in the winter and grow new ones in March and April with a velvet covering (Kalbacken, 1992; Hillman et al., 1973). Peak growth occurs in June and July, and may actually exceed 1 cm in one day and can reach 120 cm or more by the time it stops in September (Kalbacken, 1992; Hillman et al., 1973). Because antlers are essentially bony projections of the frontal bone, their growth period requires ample minerals including calcium. Some of these necessary nutrients are leached from the skeleton and it would therefore be interesting to see if such osteonal resorption and remodeling affects the bones of the deer.

Cyclic bone remodeling in deer is a subject rarely addressed in research. Hillman, et al. (1973) attempted one related study concerned with antler growth and shedding and how these events can or cannot histologically be discerned. Their primary goal was to learn if bone remodeling occurs during antler growth and after cessation of growth. They histologically examined samples of ribs, metapodials, and tibia during various stages of

antler development and concluded that during the process of growth, internal bone remodeling markedly increases. Resorption spaces were observed primarily near the endosteal and periosteal borders at the following rates: 22.99% for ribs, 13.10% for metacarpals, and 10.37% for metatarsals. Little change was noted in the tibia of the deer. They believe that antler growth is indeed associated with internal bone remodeling (Hillman et al, 1973).

Chapter 3: Materials and Methods

This research was conducted at the University of Tennessee Department of Anthropology's histology laboratory. This laboratory provided access to standard equipment for thin-section preparation including a Buehler Isomet 1000 saw, a Buehler Diamond Wafering Blade Series 152 D Diamond, microscopic slides and cover slips, Fisher Scientific Permout solution, forceps, and plastic pipettes. Deer bones were acquired via the aid of Dr. Walter Klippel.

Skeletal Samples

This study thoroughly examines the histological character of *Odocoileus virginianus*, the white-tailed deer. To achieve this, 5 specimens of varying ages (Table 1) of both male and female were procured and their right femur and humerus were separated out for further processing (Figure 15). If the bone was incomplete or too fragmented for analysis it was omitted from the study.

Greatest length measurements were taken to determine midshaft. The midpoint was marked on each shaft and one-centimeter marks were placed from the midshaft to the distal end and from the midshaft to the proximal end. "M" denoted Midshaft. Distal sections were labeled as D1, D2, etc. -- D1 being the distal section taken one centimeter from midshaft (Figures 16). Proximal sections were labeled via

Table 1: Details of White-tailed Deer Specimen Used in Study				
Deer	Age	Sex	Bones represented	Stage of Antler Development
1	6.5 Years	Female	Right humerus and femur	N/A
2	2.5 years	Male	Right humerus and femur	N/A
3	9 months	Male	Right humerus and femur	Beginning of antler development
4	3 years	Male	Right humerus and femur	Antlers in velvet
5	1.5 years	Male	Right humerus and femur	Full antlers prior to shedding



Figure 15: Illustration of the femur prior to processing.



Figure 16: Depiction of one-centimeter marks from midshaft to distal end and from midshaft to proximal end of bone.

of the same procedure. Each bone specimen was given a reference number contrived by the genus and species abbreviation (OV - *Odocoileus virginianus*), followed by age, followed by the element type abbreviation (1-humerus, 2-radius, 4-femur, 5-tibia, 7-metacarpal, 8-metatarsal), and ending with the thin-section location ("m" for midshaft or "P2" for proximal 2, etc.). OV-3-4-M is representative of the midshaft femoral section of a three-year-old deer.

Preparation of Thin-sections

Once the bones were measured and marked, a hand saw was used to make a cut at the midshaft of each long bone in order for the specimen to better fit into the chuck of the saw for sectioning. These thin-sections were cut using a Buehler Isomet 1000 saw at a speed of 275 via a diamond-wafering blade (Figure 17). Samples were securely clamped onto the saw arm and a blade was used to take cross sections at each centimeter increment. The right long bones of each of the deer samples were sectioned at midshaft and from every centimeter distally and proximally from the midshaft.

Two 0.08-mm sections were prepared from each centimeter cut. Two sections were made to allow for a more thorough assessment of any variability within and between each long bone shaft and in case something were to happen to damage one of the slides. The thin-sections were oriented on pre-labeled glass slides using metal forceps, mounted with Permunt solution, and placed beneath cover slips (Figure 18). Upon the initial microscopic examination of the deer specimen and preliminary measurements,



Figure 17: Buehler 1000 Isomet saw cutting 0.08mm section from shaft of bone.

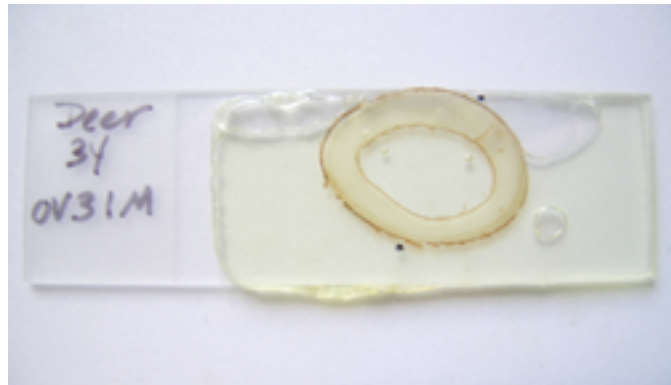


Figure 18: Prepared slide of three-year-old midshaft humerus.

it was decided that for the remaining thin-sections, measurements would be taken every three centimeters as opposed to one due to little-to-no change in histological character within such a small region of the bone's shaft. These preliminary measurements were obtained using Image Pro Express (2004) -- an image capture and enhancement computer software program designed to acquire images and take spatial measurements.

The same thin-sectioning procedure was conducted for the remaining samples. Two sections were cut at the midshaft of every long bone and at each third centimeter increment along the remainder of the shaft. These sections were also mounted on glass slides pre-labeled with their reference/catalog numbers using Permout solution and cover slips and then left to air dry.

Microstructure Analysis of Deer Bone

Once the slides were allowed time to dry they were ready to be viewed. It was at this point in the study the bone samples were narrowed down to include only the shafts of the femur and humerus. They are the more common subjects of analyses and will thus be more easily be comparable to previous similar research.

Since the goal of this current research is to assess the intra-bone and inter-bone variability slides from numerous locations along each shaft were viewed under the microscope. Table 2 and Table 3 indicate which sections on each deer were analyzed. Prior to placing each slide under the microscope they were all divided into anterior and posterior halves based on the orientation of the bone segment. This is to allow for consistency in data gathering. A circular mark was placed at the most anterior

Table 2: Analyzed Thin-section Locations on Deer Femora

OV6.5y4	OV3y4	OV2.5y4	OV1.5y4	OV9m4
Distal 4	Distal 5	Distal 4	Distal 4	Distal 6
Distal 3	Distal 3	Distal 3	Distal 2	Distal 3
Distal 2	Midshaft	Distal 2	Midshaft	Midshaft
Distal 1	Proximal 3	Distal 1	Proximal 3	Proximal 3
Midshaft	Proximal 6	Midshaft	Proximal 5	Proximal 6
Proximal 1		Proximal 1		
Proximal 2				
Proximal 3				

Table 3: Analyzed Thin-section Locations on Deer Humeri

OV6.5y4	OV3y4	OV2.5y4	OV1.5y4	OV9m4
Distal 3	Distal 5	Midshaft	Distal 4	Distal 2
Distal 2	Distal 3	Proximal 1	Distal 2	Midshaft
Distal 1	Midshaft		Midshaft	Proximal2
Midshaft	Proximal 4		Proximal 2	Proximal 6
Proximal 1			Proximal 4	

and most posterior point of the bone sections using an ultra fine permanent black Sharpie. These labels served as a guide to select the areas of bone chosen to analyze.

These sections were viewed and digital images were captured via a Leica DMRX microscope at 100x magnification and an accompanying Sony digital camera. The overall section of each slide was first examined to note prominent cellular structures, evidence of remodeling, and the distribution of said remodeling. A 2.06mm² circular field was then superimposed over the area of bone directly beside the label (Figure 19). This field is the exact size used by Kerley (1965) and other researchers (Tersigni, 2005; Horni, 2002; Cattaneo et al, 1999). This circular area was chosen to ensure this research will be comparable to previous studies and results. Since the black dot label was placed to the outside of the most anterior and most posterior facets of the section, the periosteal aspect of the bone was thus the subject of analysis for all of the thin-sections.

During the next phase of histological analysis, it is important to recall literature pertaining to the secondary osteon and its differences in human and nonhuman bone. Though usually more large and densely packed in humans, these structures can still be confused with nonhuman specimens especially when there is a lack of visible plexiform bone (Enlow & Brown, 1958; Mulhern & Ubelaker, 2001). Thus it is important to quantify and measure secondary osteonal activity in the deer samples to see whether it is comparable to human histological structures and whether the osteons are uniform in size and distribution within and between bones of each deer.



Figure 19: Example of 2.06mm² field superimposed over the midshaft of the three-year-old deer at 100x. The red circle indicated by the arrow is a secondary osteon and represents one of the osteons to be measured.

Measurements of secondary osteon area, Haversian canal area, and osteon density were taken using Image Pro Express (2004) computer software program and were recorded in squared nanometers. Only complete secondary osteons and their respective Haversian canals within the superimposed circular field were measured and counted to maintain consistency throughout the study. This process was conducted for all of the slides of the deer femora and humeri. The circular field as well as the magnification remained constant throughout the entire examination.

Overall, 98 slides were analyzed. Each of these slides was assigned a number ranging from 1-98. Fifteen slides were selected to be remeasured using a table of random numbers in the same manner as above in order to evaluate and account for observer error. These two sets of observations were compared via the student's T-test for paired samples to ensure the absence of error in data collection and measurement.

Microstructure Analysis of Human Bone

Also utilized in this research were human femora thin-sections from a seventeen-year-old male from a previous study (Tersigni, 2005). This sample provides a comparative human example to examine alongside the deer bones.

The human slides were subjected to a microstructure analysis similar to the deer. These slides were prepared prior to this research by Tersigni (2005) and thus ready to be viewed under the microscope. They were labeled in four quadrants, so each slide was oriented as to focus on the anterior and posterior quadrants. Five slides from a seventeen-year-old male femur were examined. The 2.06mm² field was superimposed

over each section, digital images were captured, and area measurements were taken for secondary osteons and their respective Haversian canals within each field.

Statistical Analysis of Deer Bones

To test for significant differences in osteonal and Haversian canals areas of the femur and the humerus, a statistical analysis by location was conducted using repeated measures ANOVA in SPSS 14 for Windows (2006) (a comprehensive statistics software program). The age variable was excluded from this analysis. Because bone remodeling and the presence of secondary osteons were not found in every segment for each femur or humerus, the proximal and distal segments were grouped together and compared against each other and against the midshaft. Kerley (1965) and Tersigni (2005) both present evidence that there is no significant difference in osteon count and percentage of non-Haversian bone within three-centimeter segments of the diaphysis of a human femur; this notion was thus applied to the white-tailed deer in this case to collapse the data for a more reliable analysis whereas these data met the assumptions for a multivariate test.

The repeated measures ANOVA specifically examined the differences in osteon and Haversian canal area across the segments of the femoral and humeri shafts. The criterion of $\alpha=0.05$ was used to test for statistically significant differences in the data. If the reported p-value is less than alpha, then the hypothesis is rejected and the means across the three areas of interest are not equal.

A repeated measures ANOVA in SPSS 14 for Windows (2006) also tested for significant inter-bone differences by age. For the data analysis of the humerus, only the proximal sections were used for the statistical comparison between ages. Since evidence

of bone remodeling was not always noted in the thin-sections, the analysis was narrowed to these sections where secondary osteons were present so that the data would be complete.

The criterion of $\alpha=0.05$ was used in this analysis to test for statistical significant differences in the data. If the reported p-value is less than alpha, then the hypothesis is rejected and the means across age groups are not equal.

Statistical Analysis of Deer and Human Bones

A T-Test was conducted in SAS 9.1.3 for Windows (2006) to compare the midshaft data (osteon and Haversian canal areas) from all of the deer femora to the midshaft data recorded for the human femur. The T-Test compares the means for two groups and looks for significant differences between these means.

The criterion of $\alpha=0.05$ was used for this analysis to indicate significant variation. If the reported p-value is less than alpha, it is decided to reject the hypothesis that the means between the deer and human femora data are equal.

Blind Test

A combination of the measurements, statistical results, and visible histological structures were utilized in a blind test. Four unidentified humeri fragments (A, B, C, and D) were subjected to histological examination. Two 0.08mm sections were cut from the shafts of the four unknown samples. These sections were mounted on slides using Permount solution, placed beneath cover slips, and allowed to air dry. The slides were oriented and labeled with anterior and posterior markings following the same methodology as the research. The samples were observed with the Leica DMRX

microscope at 100x magnification and photographed with an accompanying Sony digital camera.

The digital images were examined for plexiform structure to indicate human or nonhuman and measurements were taken of histological structures using the Image Pro Express (2004) computer software program. These data were recorded and compared with the collected data from this research in an attempt to separate out deer.

Chapter 4: Results

The right femora and humeri of five white-tailed deer were subjected for histological analysis. Every osteon area and Haversian canal area recorded for each bone section is individually listed by catalog number in Appendix A. Appendix B provides descriptive statistics including standard deviations and minimum, maximum, and mean areas for proximal, midshaft, and distal sections of the femora and humeri. In addition to the statistical analysis based measurements, the presence of plexiform bone as well as other unique structures noted during the microscopic examination are presented.

General Structures

Histological examination of the slides under the microscope revealed secondary osteons and evidence of bone remodeling for most of areas. Plexiform bone characteristic of nonhuman mammalian species was visible in all areas as were primary osteons. The plexiform bone appeared in a brick-like rectangular fashion and generally lined the periosteal and endosteal borders. If the slide only exhibited plexiform bone (Figure 20), primary canals and vascularization, and primary osteons, no measurements were taken. Osteon banding was visible (Figure 21), but secondary osteons were not present in the band, so, again, no measurements were taken of this feature but its presence was noted. Table 4 lists the sections where no secondary osteons were found. No measurements were taken from these slides and they were not included in the statistical analyses.

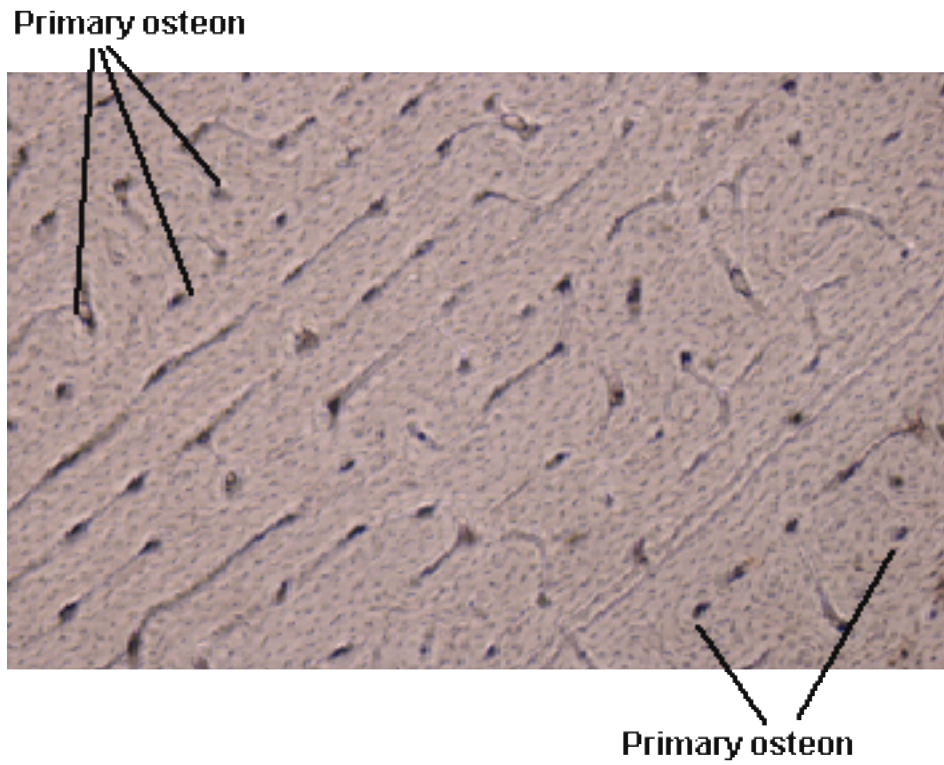


Figure 20: Distal 3rd section of three-year-old humerus at 100x. Only plexiform and primary canals are visible in this section.

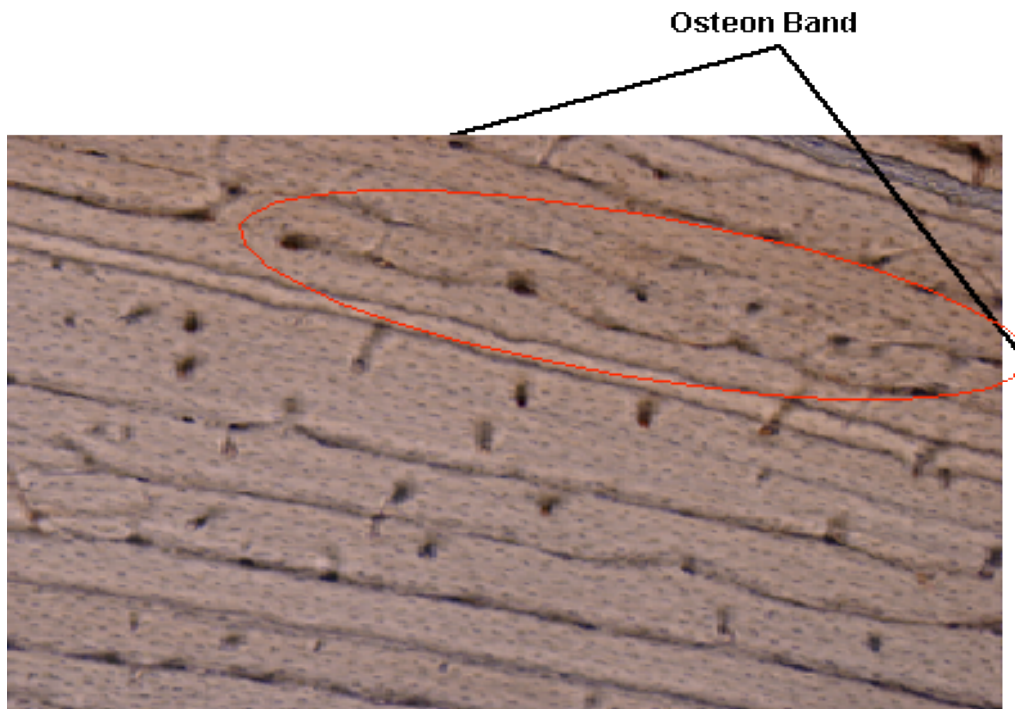


Figure 21: Midshaft of three-year-old humerus at 100x. Osteon banding comprised of primary osteons can be seen at top right of image.

Table 4: Bone Thin-sections Without Remodeling			
Catalog number	Bone	Segment	Features present
OV34P3	femur	Proximal 3	Plexiform, primary osteons, osteon banding
OV34P6	femur	Proximal 6	Plexiform, primary osteons, osteon banding
OV31D3	humerus	Distal 3	Plexiform
OV1.54P3	femur	Proximal 3	Plexiform, primary osteons
OV1.54P5	femur	Proximal 5	Plexiform, primary osteons
OV1.51M	humerus	Midshaft	Plexiform
OV1.51D2	humerus	Distal 2	Plexiform
OV1.51D4	humerus	Distal 4	Plexiform
OV9m1M	humerus	Midshaft	Plexiform
OV9m1D2	humerus	Distal 2	Plexiform
OV2.54D3	femur	Distal 3	Plexiform

Osteon and Haversian Canal Areas by Location

Since the primary objective of this research is to assess the overall histological traits of the white-tailed deer and to record osteon area and Haversian canal measurement data, it is important to understand if these secondary osteons indicative of bone remodeling are similar throughout the shaft of the bone or if they vary by location. If the osteonal and Haversian canal areas are not dependent upon their location on the shaft, it will assuage the difficulty in identifying a bone fragment as being deer.

A repeated measures ANOVA conducted in SPSS 14 for Windows (2006) tested for significant intra-bone differences in osteonal and Haversian canals areas of the femur and humerus (Table 5). This analysis specifically examined whether or not the data vary from one segment of the shaft to another.

For osteon areas of the femur, the resulting p-value of 0.103 is greater than the alpha therefore indicating that there are no significant differences between locations. For osteon areas of the humerus, the resulting p-value of 0.861 is also greater than the alpha criteria. No significant differences in location exist between the proximal, midshaft, and distal segments of the humerus.

Table 5: Repeated Measures ANOVA Summary for Femur and Humerus Osteon and Haversian Canal Areas by Location

Bone	Measurement	Locations	F-Value	P-Value
Femur	Osteon Area	Proximal/Distal/Midshaft	2.539	0.103
Femur	Haversian Canal Area	Proximal/Distal/Midshaft	1.294	0.283
Humerus	Osteon Area	Proximal/Distal/Midshaft	0.049	0.861
Humerus	Haversian Canal Area	Proximal/Distal/Midshaft	1.452	0.244

For the Haversian canal areas of the femur, the resulting p-value of 0.283 is greater than the alpha criterion; therefore, there are no significance differences in mean area between locations. For the Haversian canal areas of the humerus, the resulting p-value of 0.244 is also greater than the alpha 0.05. No significant differences in mean area exist between locations.

The implications of these statistics mean that there is no intra-bone variation for the femur or the humerus. This is important to forensic anthropology as it indicates that the location of a fragment on a long bone shaft will not affect the ability of histology to identify deer as the nonhuman source based on osteon area and Haversian canal area measurements.

Osteon and Haversian Canal Areas by Age

This research established no significant intra-bone deviations in osteonal and Haversian canal areas. The study also tested for mean differences between ages and since no locational differences were noted in prior statistical analyses, an average across locations was taken for the femora. Only the proximal sections of the humeri were compared as a repeated measures ANOVA requires data to be present for each variable and not all slides from the humeri exhibited bone remodeling.

The repeated measures ANOVA to test for inter-bone variation by age examined mean differences in osteon area and Haversian canal area (Table 6). For osteon areas of the femur, the resulting p-value of 0.487 is greater than the alpha therefore indicating that there are no significant differences between ages. For osteon areas of the humerus,

Table 6: Repeated Measures ANOVA Summary for Femur and Humerus Osteon and Haversian Canal Areas by Age

Bone	Measurement	Ages	F-Value	P-Value
Femur	Osteon Area	9 months, 1.5 yrs, 2.5 yrs, 3 yrs, 6.5 yrs	0.627	0.708
Femur	Haversian Canal Area	9 months, 1.5 yrs, 2.5 yrs, 3 yrs, 6.5 yrs	0.902	0.487
Proximal Humerus	Osteon Area	9 months, 1.5 yrs, 2.5 yrs, 3 yrs, 6.5 yrs	4.61	0.010*
Proximal Humerus	Haversian Canal Area	9 months, 1.5 yrs, 2.5 yrs, 3 yrs, 6.5 yrs	4.378	0.007*

however, the resulting p-value of 0.01 is less than alpha. Therefore, significant differences in mean area do exist between ages in the osteon areas of the humeri.

Multiple comparison Bonferroni *post hoc* statistics (Table 7) were run in SPSS 14 (2006) to determine between what ages such significant differences became evident. The 6.5-year-old deer was excluded from the *post hoc* test as only one measurement was obtained from the proximal section. The results indicate significant differences between the 3-year-old deer and both the 1.5-year-old and the 2.5-year-old deer.

For the Haversian canal areas of the femur, the resulting p-value of 0.487 is greater than the alpha criterion; therefore, there are no significance differences in mean area between ages. For the Haversian canal areas of the humerus, the resulting p-value of 0.007 is less than the alpha 0.05. Significant differences in mean area exists between ages in the Haversian canal areas of the humeri.

Post hoc statistics (multiple comparison Bonferroni test) were run in SPSS (2006) to determine where such significance differences were present. Again, the 6.5-year-old deer was excluded from the *post hoc* test as only one measurement was obtained from the

Table 7: *Post hoc* Summary for Proximal Humerus Osteon Areas by Age

Age Comparison	Mean Difference (in nanometers)	P-Value
9 months: 1.5 years	2563.74	1.0
9 months: 2.5 years	1851.33	1.0
9 months: 3 years	-3757.11	0.210
1.5 years: 9 months	-2563.75	1.0
1.5 years: 2.5 years	-712.41	1.0
1.5 years: 3 years	-6320.85	0.030*
2.5 years: 9 months	-1851.33	1.0
2.5 years: 1.5 years	712.41	1.0
2.5 years: 3 years	-5608.44	0.012*
3 years: 9 months	3757.11	0.210
3 years: 1.5 years	6320.85	0.030*
3 years: 2.5 years	5608.44	0.012*

proximal section. The results (Table 8) indicate that the only significant difference in Haversian canal areas in the humerus occurs between the 9-month-old deer and the 2.5-year-old deer.

Overall, when considering the statistical analyses, the small sample size does decrease the potential reliability of the results. Though missing data makes any statistical test more difficult, it is important to note where such gaps occur in this research because these are characteristic of the different rates of remodeling in deer. In essence, the missing data is actually a direct reflection of the amount of bone being added and resorbed.

A larger sample size may indeed strengthen the statistical results, but these preliminary tests are still useful in beginning to understand the range of variability in bone remodeling. They also suggest that when remodeling is present, the histological traits are very similar in size. This conclusion is important for understanding the microscopic character of deer.

Table 8: *Post hoc* Summary for Proximal Humerus Haversian Canal Areas by Age

Age Comparison	Mean Difference (in nanometers)	P-Value
9 months: 1.5 years	181.4	0.480
9 months: 2.5 years	289.87	0.003*
9 months: 3 years	203.57	0.217
1.5 years: 9 months	-181.4	0.480
1.5 years: 2.5 years	108.47	1.0
1.5 years: 3 years	22.17	1.0
2.5 years: 9 months	-289.87	0.003*
2.5 years: 1.5 years	-108.47	1.0
2.5 years: 3 years	-86.3	1.0
3 years: 9 months	-203.57	0.217
3 years: 1.5 years	-22.17	1.0
3 years: 2.5 years	86.3	1.0

Osteon Area Overview

Figures 22-25 illustrate the minimum, mean, and maximum osteon areas for all of the deer in this study and incorporate human osteon area measurements as well as measurements reported by Harold Horni in his 2002 study. This graphic representation serves to indicate the overall range of osteon areas recorded for white-tailed deer and will provide a template to test against in future forensic cases.

The osteon area statistical results coupled with these graphs point towards their usefulness in histological examinations. Osteon area, then, can prove helpful as a tool for the forensic anthropologist in more effectively determining the source of fragmentary remains as being white-tailed deer.

Haversian Canal Area Overview

Haversian canal areas may not be as appropriate as osteon areas in histological investigations due to the statistical results. Utilizing the Haversian canal area as a means

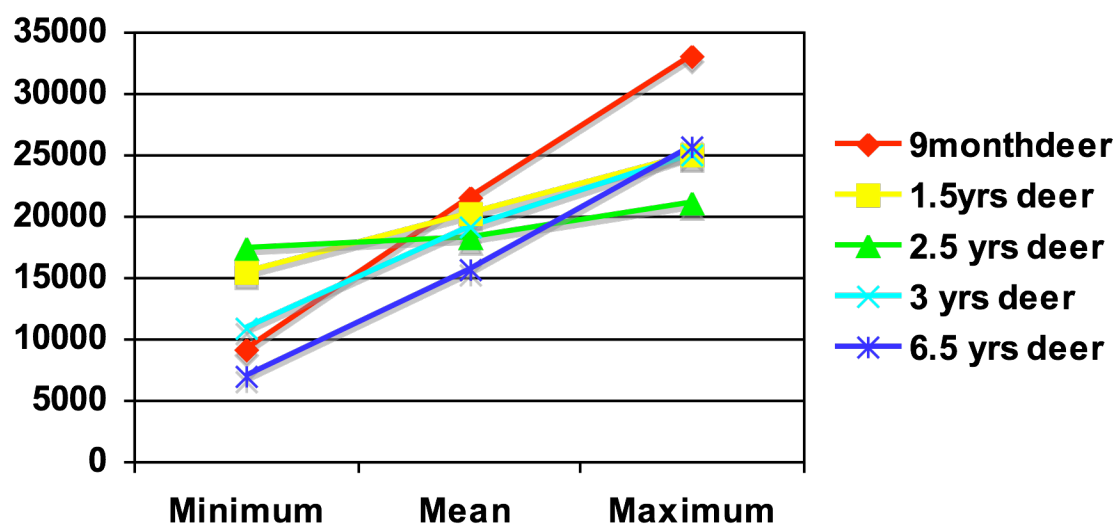


Figure 22: Comparative osteon area averages of femur midshaft.

*Y-Axis Measurements in nm^2

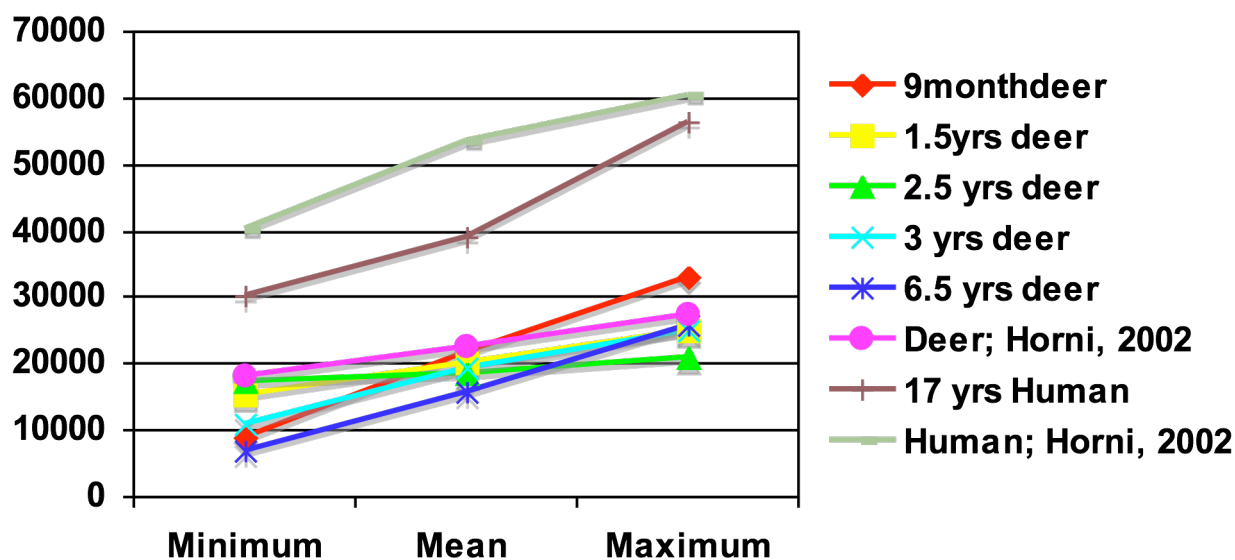


Figure 23: Comparative osteon area averages of deer femora and human midshaft femora.

*Y-Axis measurements in nm^2

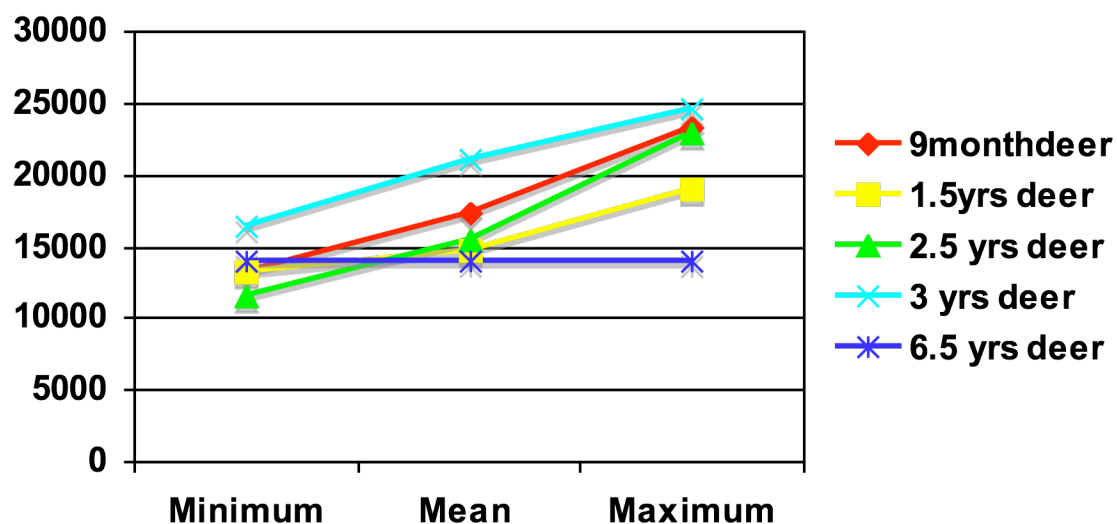


Figure 24: Comparative osteon area averages of proximal humeri.

*Y-Axis measurements in nm^2

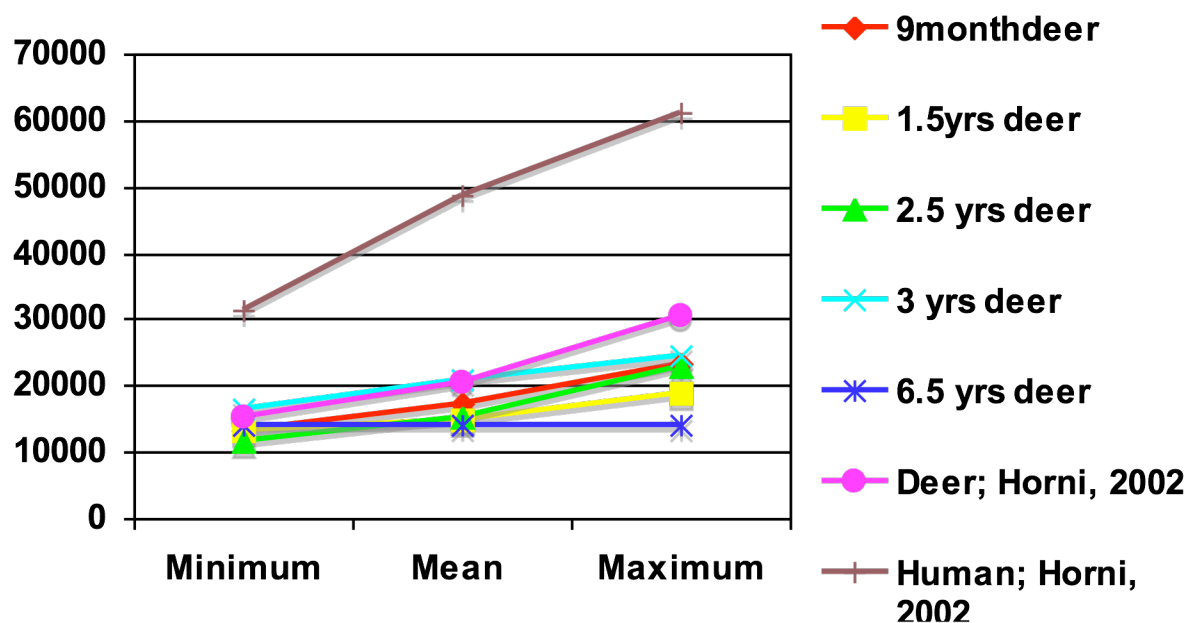


Figure 25: Comparative osteon area averages of deer and human midshaft humeri.

*Y-Axis measurements in nm^2

to separate out species has an additional source of error. Horni (2002) indicates a strong overlap in measurements between Haversian canal areas of the white-tailed deer and Haversian canal areas of humans. Figures 26-29 illustrate the minimum, mean, and maximum measurements for all deer femora and humeri Haversian canal data in this study.

Deer Versus Human

Measurements of 12 osteon areas and their respective Haversian canal areas are recorded (Table 9) for the seventeen-year-old femur. These measurements were statistically compared to those of the deer femora.

The statistical comparison of the means of the white-tailed deer femora and the human femora was examined using a T-Test. When considering osteon area averages, the resulting p-value from the T-Test was $<.0001$. This value indicates that the mean differences between the human and deer femur are indeed significant. When testing for variation in the Haversian canal areas of the human and the deer femur, the resulting p-value was $<.0001$ also indicative of significant mean differences.

These results suggest that white-tailed deer is statistically unique from human at the microscopic level. When plexiform is not histologically visible in nonhuman bone, the knowledge that the average-sized secondary osteon and Haversian canal is significantly different from deer (one nonhuman species) to the human species is indeed useful for forensic anthropologists in identifying the source of fragmentary remains.

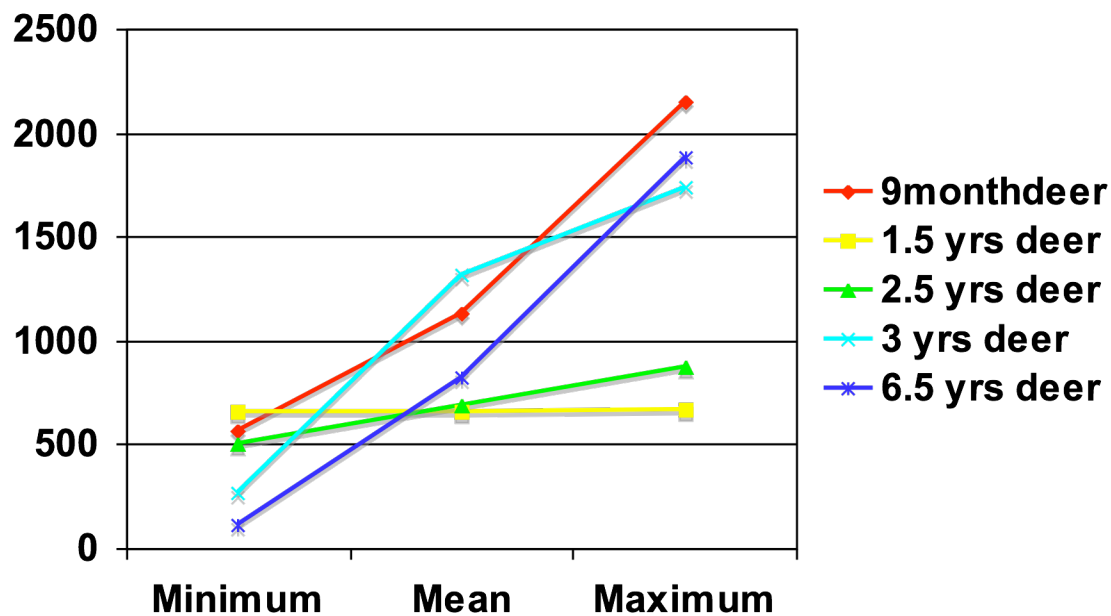


Figure 26: Comparative Haversian canal area averages of femur midshaft.

*Y-Axis measurements in nm^2

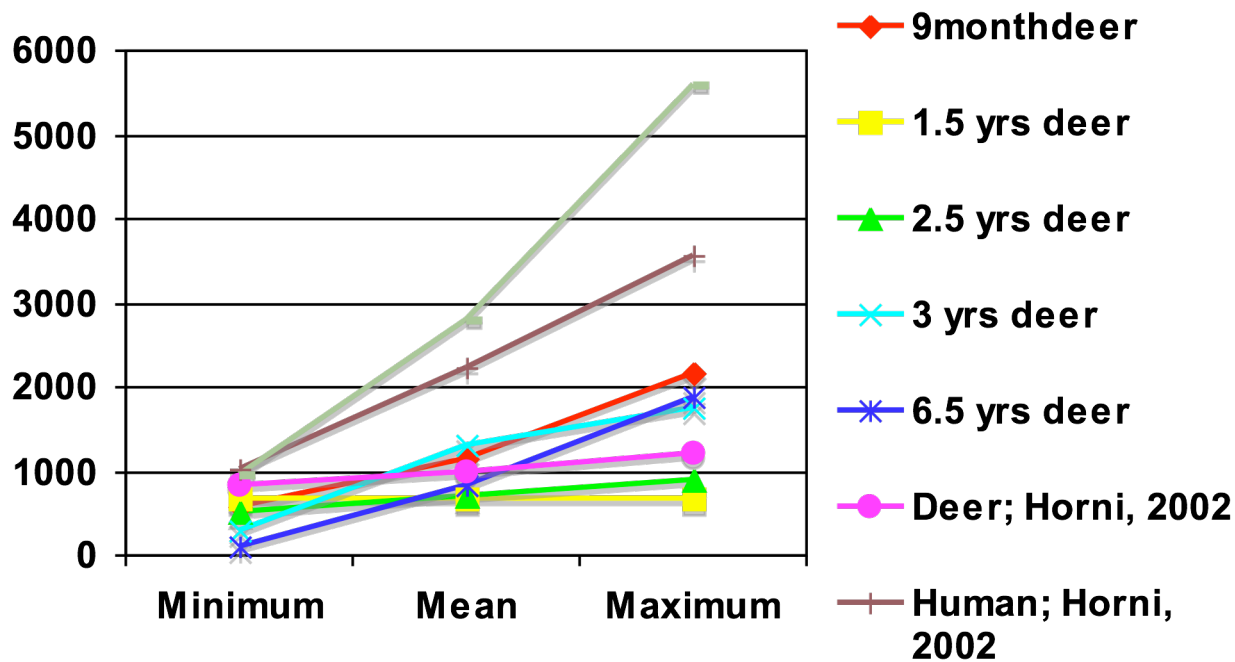


Figure 27: Comparative Haversian canal area averages of deer and human midshaft femur.

*Y-Axis measurements in nm^2

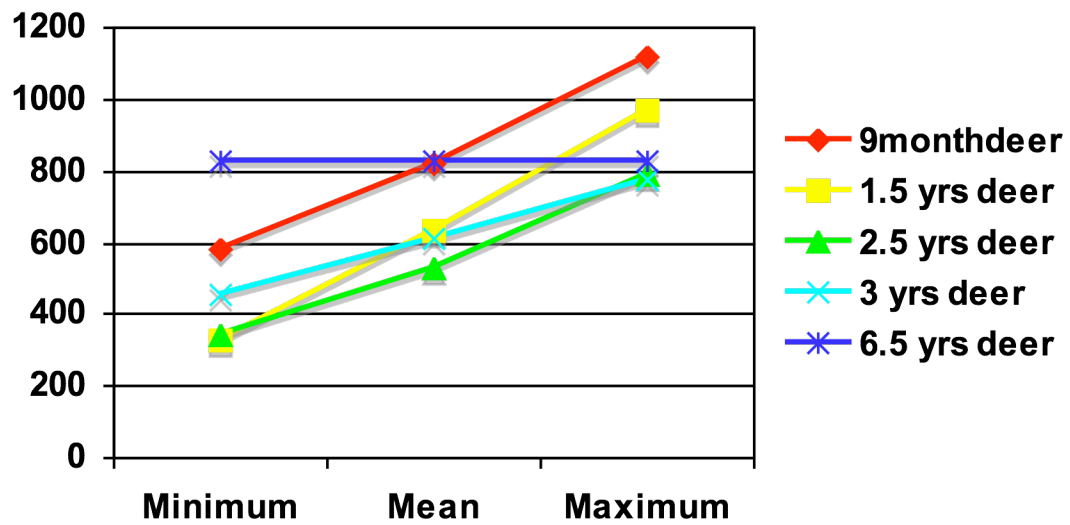


Figure 28: Comparative Haversian canal area averages of proximal humerus.
 *Y-Axis measurements in nm^2

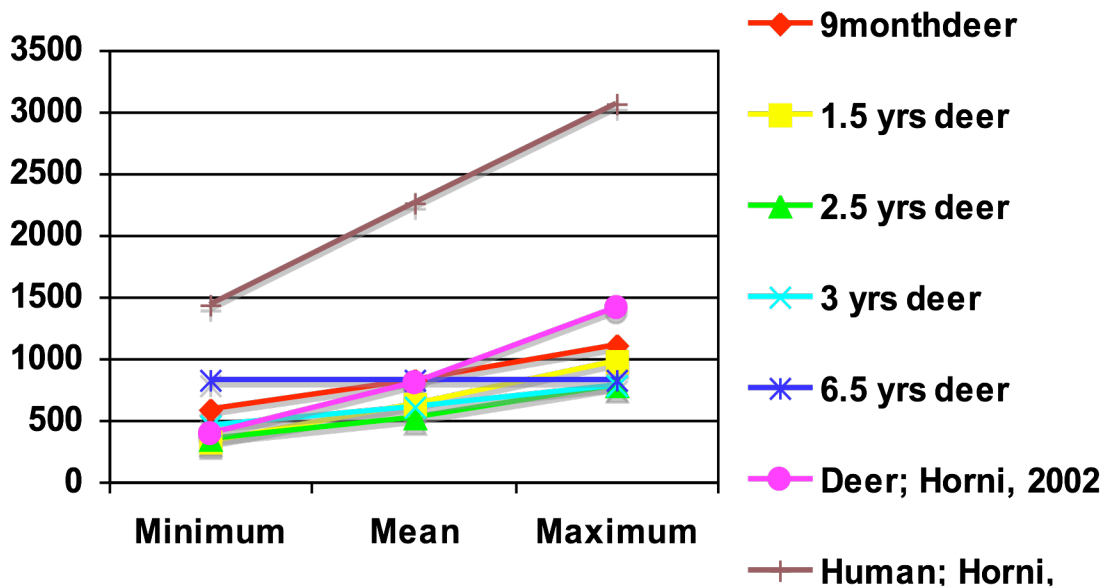


Figure 29: Comparative Haversian canal Area averages of deer proximal humerus and human midshaft humerus.
 *Y-Axis measurements in nm^2

Table 9: Osteon Area and Haversian Canal Area Measurements for Midshaft Human Femora

Catalog Number	Bone	Osteon Area (in squared nanometers)	Haversian Canal Area (in squared nanometers)
HS-B4-M	femur	34285.93	1500.1
HS-B4-M	femur	50167.43	2543.99
HS-B4-M	femur	31413.68	1224.56
HS-B4-M	femur	25183.13	1044.48
HS-B4-M	femur	34475.96	1601.42
HS-B4-M	femur	25438.76	1014.61
HS-B4-M	femur	30254.13	2759.8
HS-B4-M	femur	34158.55	1746.95
HS-B4-M	femur	42211.01	4443.5
HS-B4-M	femur	53009.22	1693.95
HS-B4-M	femur	29874.64	1816.93
HS-B4-M	femur	53191.06	1108.9
HS-B4-M	femur	48968.64	5594.85
HS-B4-M	femur	38486.38	1584.43
HS-B4-M	femur	39846.38	967.76
HS-B4-M	femur	42215.41	2828.32

Blind Test Results

Statistical results indicate the promising use of osteon area measurements in deer femora and humeri as a good method to histologically differentiate fragmentary remains. Graphic interpretations of the data also point towards similarities across loci and age groups.

Upon histological examination of the unknown specimen, plexiform bone was noted in all four samples indicating them to all be nonhuman. Three of the four unknowns actually showed a type of plexiform different than that of the white-tailed deer recorded in this research (Figures 30-31). The known deer specimen display a shorter brick-like rectangular structure of plexiform rather than the longer, uninterrupted segments seen in Samples B and D. Sample A (Figure 32) is also unusual in that it exhibits more square-shaped plexiform blocks. Since plexiform can at times be present as convoluted or rectangular and since it does vary in size and shape, a difference in general appearance is not yet enough to disregard the fragment as white-tailed deer. Table 10 lists all osteon area measurements recorded for each unknown sample and Figure 33 plots these unknowns against deer osteon area averages.

Figure 33 reveals area measurements where the unknown samples A, B, and D deviate extremely from the known deer specimen. Numerous osteon areas deviate from the range of deer and are much lower or higher than the lines indicative of white-tailed deer. These aberrant measurements are not comparable to any of those recorded for the five deer specimen in this research.

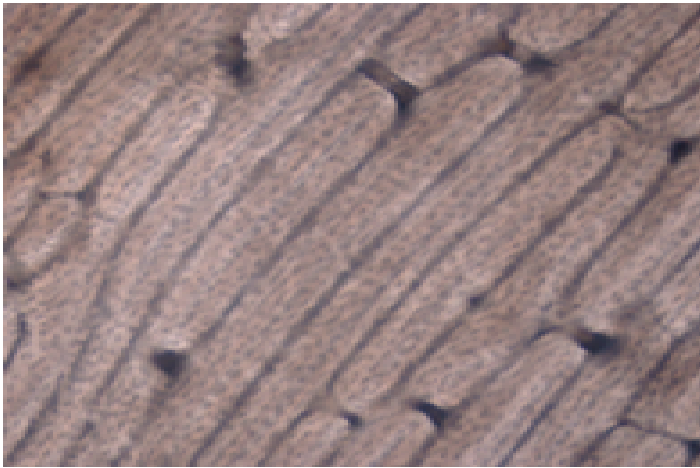


Figure 30: Plexiform bone from sample D at 100x. The plexiform bone is displayed in long, uninterrupted segments very different than known deer samples.



Figure 31: Plexiform bone from known three-year-old deer humerus at 100x. The plexiform bone is formed in shorter, rectangular segments.

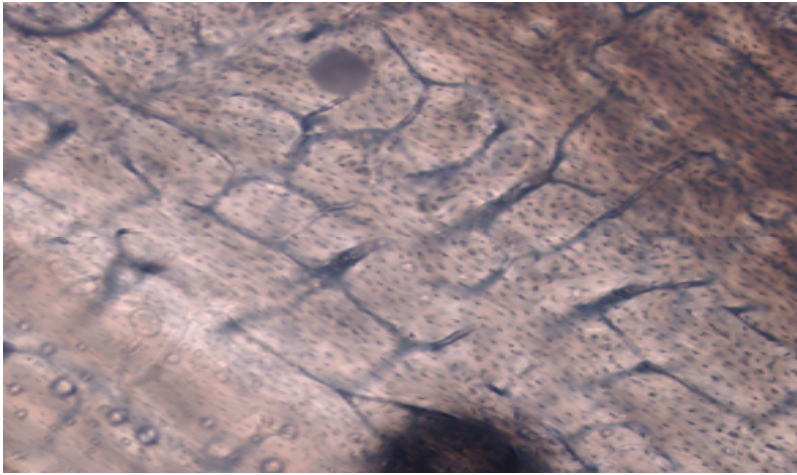


Figure 32: Plexiform bone from unknown sample A at 100x. The plexiform bone is arranged in squares unlike plexiform bone in known deer bone.

Table 10: Osteon Area Measurements for Unknown Samples				
	Sample A	Sample B	Sample C	Sample D
Measurements	15025.9	48926	23231.8	47316
in nm ²	5023.57	24714.91	16462.46	23574
	4920.49	2331.7	23595.47	5808
	18746.36	7943.24	13019.81	5881
	11274.62	8190.97	12949.53	12372
	8980	8182.18	15220.33	9678
	12933	5146.26	14836.74	72444.65
	9443	10289.58	12443.84	66384
	45043	12082.79		33966.46
				46873
Minimum	4920.49	2331.7	12443.84	5808
Mean	14598.88222	14200.84778	16469.9975	32429.711
Maximum	45043	48926	23595.47	72444.65

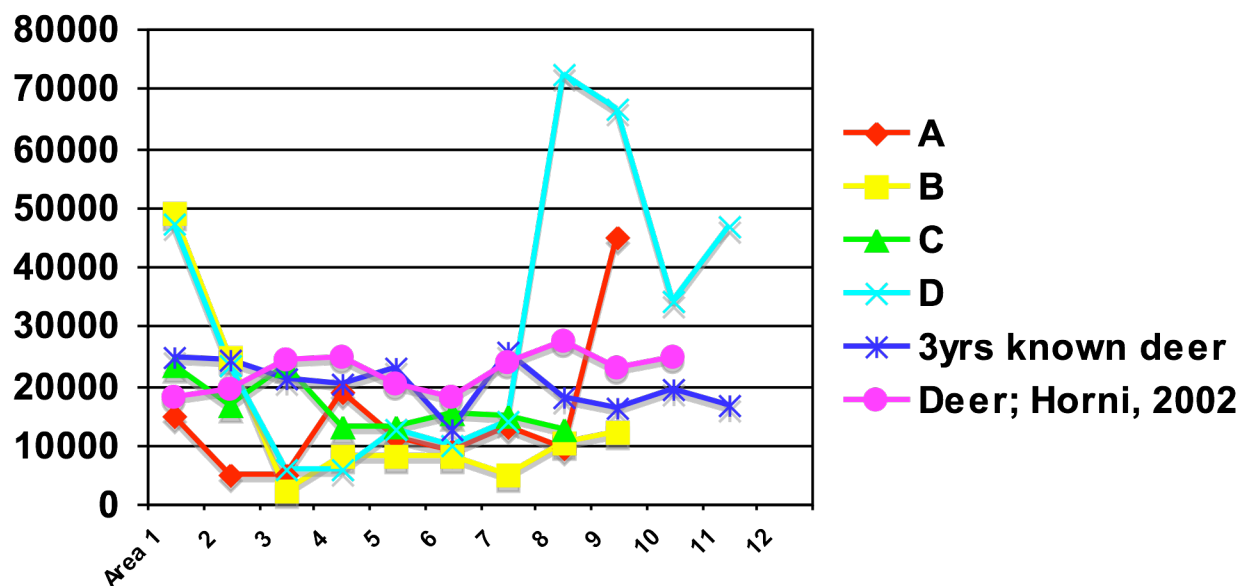


Figure 33: Unknown samples plotted against known deer humeri osteon area measurements.

*Y-Axis measurements in nm^2

Samples A, B, and D appear to be especially different from the known deer and from Sample C. This evidence coupled with the unusual plexiform bone seen in samples A, B, and D indicate these three unknowns to most likely not be deer. The data also indicate that unknown sample C is very likely to be deer as the osteon areas all fall within the observed range recorded in this research.

Figure 34 presents another graphic representation of the osteon area measurements on the unknown samples. This chart illustrates the minimum, mean, and maximum osteon areas in squared nanometers for all of the unknowns contrasted against a known three-year-old deer from this research as well as deer and goat from Horni (2002) and a cow from Jowsey (1966).

Again, unknown Sample C closely coincides to the known deer specimen indicating the source of this fragment to indeed be deer. Samples A, B, and D deviate too much to fall within the reported range for white-tailed deer and are most likely not deer based on osteon area measurements.

In this experiment, the use of osteon area measurements was successful in picking out the white-tailed deer fragmentary element. Sample C was, indeed, deer. The use of osteon area measurements was also successful in eliminating deer as the potential source of the other fragmentary elements. Sample A was *Ovis aries* (domestic sheep); Sample B was *Capra hircus* (domestic goat); Sample D was *Sus scrofa* (wild boar).

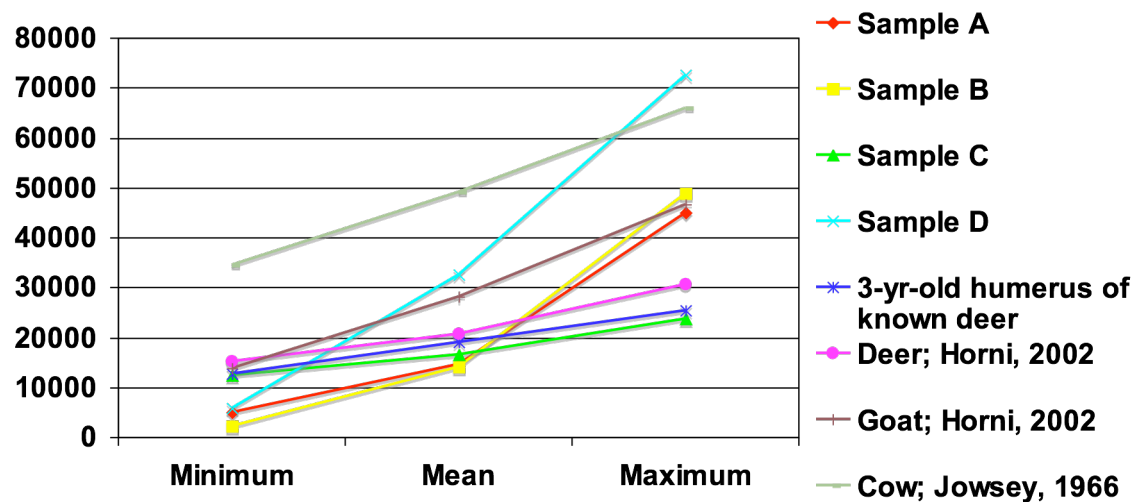


Figure 34: Osteon area ranges for unknowns, known deer, and other known mammalian species.

*Y-Axis measurements in nm²

Chapter 5: Discussion

The histological examination of *Odocoileus virginianus* revealed a unique microstructure. Plexiform bone characterized the microstructure of deer femora and humeri. Primary osteons and vascularization appeared sporadically within such plexiform. Bone remodeling and secondary osteons were also present. The arrangement and size of these secondary osteons and Haversian canals are commonly the subjects of interest as these characteristics vary among species (Jowsey, 1966; Harsanyi, 1993).

Osteon Area

With such a fact in mind, this research focused on developing a method to successfully identify and separate out the white-tailed deer species from humans and from other mammalian specimen at the histological level when gross morphological means of evaluation were unrealistic. Statistically, osteon area is not significantly different within the shaft of the femur or humerus; Locations on the proximal, midshaft, and distal segments of bone are histologically similar.

Also, mean differences in osteon area do not significantly vary depending on the age of the deer when concerned with the femur. Mean differences in osteon area do vary by age in the humerus. With a larger sample size, this test can be improved upon, but it is still suggestive of the likely trend.

Haversian Canal Area

Statistically, Haversian canal areas did not significantly differ depending on the loci of the thin-section. Mean differences in Haversian canal area are similar across

locations of the proximal, midshaft, and distal sections of the femur and humerus.

Haversian canal areas were also similar between ages for the femur.

Haversian canal areas did prove to be statistically different when considering the age variable for the humerus. A post hoc test revealed the primary variation to exist between the 9 month-old and the 2.5 year-old deer. Due to such potential disparities, Haversian canal areas may not be the most reliable variable to use in determining a species to fall within the range of white-tailed deer. Osteon area measurements, then, may be the more accurate and decisive histological characteristic to differentiate species in forensic settings.

The graphs presented in the previous chapter are very illustrative of the histological character of white-tailed deer and are telling of how osteon and Haversian canal areas are indeed very similar when examining location (proximal, midshaft, and distal) and age. It is interesting to note the parallelism between the deer data of this study compared to the osteon and Haversian canal measurements recorded by Horni (2002). The osteon areas taken from the human femur and humerus show little overlap with the white-tailed deer data. Haversian canal areas, however, do seem to overlap quite a bit when examining human versus white-tailed deer.

Another noteworthy indication seen in the graphs is that the 6.5-year-old seems to have the lowest overall osteon and Haversian canal areas while the nine-month-old appears to consistently have the highest. Though these differences are not great enough to be too inconsistent with the overall character of white-tailed deer, it coincides with the common theme pervasive in human bone histology that osteons tend to shrink with age (Kerley, 1965; Jowsey, 1966; Schultz, 1995).

This concept asserts that as an individual ages, osteoclasts continue to resorb bone and form resorption holes. In turn, bone-building osteoblasts fill in these areas with new bone atop of the broken down osteons and leaving behind these fragments. Essentially new bone is built on top of old bone and osteon density increases. This results in a greater compactness of bone and a progressive, concurrent decrease in the actual size of osteons (Kerley, 1965; Schultz, 1995). This process that has been noted and studied in humans may also serve to explain the smaller osteons noted in the older deer and the larger osteons recorded for the younger deer.

Future Implications

The results and the subsequent histological experimentation based on the graphs and the statistical findings from these results are indicative of the potential useful application of histological examination in forensic settings. A few caveats, though, should be considered for similar future endeavors.

The first is sample size. This research and the statistical results indicate that no mean differences in osteon area and Haversian canal area exist within the shafts of the bones examined. The study cannot necessarily be said to be conclusive because of the small sample size of five deer. It can be said, however, that the results provide a clear indication of potential trends. The data collected for this research did meet the assumptions for a multivariate test for repeated measures, but the conclusion reached can be strengthened by the addition of more samples from the same age groups.

An increase in deer samples will provide for more data points and thus ultimately increase the reliability and repeatability of the analysis. It will also help to more

definitively test for the importance of the age variable. Essentially, further testing will help to validate the results.

A second notion to address is the histological character of other non-human, mammalian species. As noted, the data from this investigation allowed for the identification of white-tailed deer among a series of unknown samples. The ability to do so for other specimen can greatly enhance the study of bone histology and its implications for forensic anthropology. A more in-depth examination of plexiform bone and its unique characteristics should be conducted as plexiform bone proved to be an unique variable. If plexiform bone can somehow be quantified and coupled with osteon area measurements, perhaps the results can be all the more reliable.

Chapter 6: Conclusions

To utilize the full potential of histological examination in forensic settings it is not only important to thoroughly understand the microscopic character of human bone, but it also is vital to master the defining traits of nonhuman bone. Nonhuman bone can be misinterpreted as being human in origin. White-tailed deer (*Odocoileus virginianus*) is not always morphologically distinguishable from humans, but bone histology will provide for a definitive separation between the two. Histological study can also pick out the white-tailed deer from other nonhuman species based on their unique microscopic traits.

The sample size for this research was small, but the findings are suggestive of the continuity of histological traits within long bones of a single deer and between age groups. Osteon area measurements were not significantly different depending on location or age. This is important in establishing a standard histological character for the white-tailed deer to act as a comparative norm for forensic anthropology and cases of unidentified fragmentary remains.

The implications of this study indicate the usefulness and reliability of bone histology and should draw interest for future research. A need for larger sample sizes and a focus on additional nonhuman species should not only corroborate the findings of this study but will serve to further validate the statistical results and offer a greater wealth of knowledge to the field.

Research should be expanded to include long bones other than the femur and humerus. It will also be beneficial to learn how to quantify plexiform bone to distinguish

between nonhuman species. Other histological measurements besides osteon and Haversian canal area are untapped potential sources of information. Horni (2002) suggests lacunae and osteocytes as possible differentiating variables. Mulhern and Ubelaker (2001) indicate the importance of osteon banding in separating human from nonhuman species.

To the researcher concerned primarily with human bone histology -- an understanding of nonhuman histological traits will not only allow for a more accurate identification of fragmentary skeletal remains, it will also provide for a plethora of new research. The question should not be limited to human or nonhuman; it should be expanded upon and elaborated to consider the defining characteristics of numerous species.

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Appendices

Appendix A:

**Osteon and Haversian Canal Area Differentiated by Age, Bone,
and Loci on Bone.**

Appendix A

Osteon and Haversian Canal Area differentiated by age, bone, and location on bone

*Measurements reported in nm²

A=Anterior O=Osteon

P=Posterior HC=Haversian Canal

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV9m4D6	femur	Distal 6	A	12970.03	941.41
OV9m4D6	femur	Distal 6	A	7070.65	418.44
OV9m4D6	femur	Distal 6	A	9080.54	527.95
OV9m4D6	femur	Distal 6	A	16587.78	1059.12
OV9m4D3	femur	Distal 3	A	13797.24	834.53
OV9m4D3	femur	Distal 3	A	18474.4	1482.53
OV9m4D3	femur	Distal 3	A	16822.33	1068.2
OV9m4D3	femur	Distal 3	A	13036.5	—
OV9m4D3	femur	Distal 3	A	14783.74	523.85
OV9m4D3	femur	Distal 3	A	20585.02	—
OV9m4D3	femur	Distal 3	A	35291.17	1388.83
OV9m4M	femur	Midshaft	P	33059.9	2155.13
OV9m4M	femur	Midshaft	P	21440.34	708.91
OV9m4M	femur	Midshaft	P	26993.03	1521.48
OV9m4M	femur	Midshaft	P	21543.12	874.35
OV9m4M	femur	Midshaft	P	17115.73	983.57
OV9m4M	femur	Midshaft	p	9041.31	565.14
OV9m4P3	femur	Proximal 3	P	22399.32	1410.5
OV9m4P3	femur	Proximal 3	P	18503.39	1225.44
OV9m4P6	femur	Proximal 6	P	18505.73	1761.29
OV9m4P6	femur	Proximal 6	P	18116.87	—
OV9m4P6	femur	Proximal 6	P	18713.63	1516.5
OV9m4P6	femur	Proximal 6	P	13213.65	1227.5
OV9m4P6	femur	Proximal 6	P	14911.11	756.93
OV9m4P6	femur	Proximal 6	P	16551.47	826.04
OV9mD2	humerus	Distal 2	-	-	-
OV9mM	humerus	Midshaft	-	-	-
OV9mP2	humerus	Proximal 2	P	16680.9	1044.19
OV9mP2	humerus	Proximal 2	P	19676.41	741.12
OV9mP2	humerus	Proximal 2	P	18261.52	588.27
OV9mP2	humerus	Proximal 2	P	14116.41	527.95
OV9mP2	humerus	Proximal 2	P	18797.96	741.99
OV9mP2	humerus	Proximal 2	P	15745.64	1087.23
OV9mP2	humerus	Proximal 2	P	15766.72	1028.96
OV9mP2	humerus	Proximal 2	P	23289.77	1115.34

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV9mP2	humerus	Proximal 2	P	13216.29	677.29
OV9mP5	humerus	Proximal 5	P	19300.44	755.17
OV9mP5	humerus	Proximal 5	P	13213.86	1234.81
OV9mP5	humerus	Proximal 5	P	20530.56	—
OV9mP5	humerus	Proximal 5	P	15315.79	642.15
OV9mP5	humerus	Proximal 5	P	13540.73	712.72
OV9mP5	humerus	Proximal 5	P	20624.85	903.34
OV9mP5	humerus	Proximal 5	P	14063.7	676.7
OV1.54D4	femur	Distal 4	A	13186.42	670.84
OV1.54D4	femur	Distal 4	A	12503.57	634.83
OV1.54D4	femur	Distal 4	A	15507.87	838.33
OV1.54D4	femur	Distal 4	A	12757.15	448.89
OV1.54D4	femur	Distal 4	A	12180.3	332.93
OV1.54D2	femur	Distal 2	A	25564.96	618.14
OV1.54D2	femur	Distal 2	A	18618.17	973.32
OV1.54M	femur	Midshaft	P	15381.38	654.45
OV1.54M	femur	Midshaft	P	25005.68	672.6
OV1.54P3	femur	Proximal 3	P	22399.32	1410.5
OV1.54P3	femur	Proximal 3	P	18503.39	1225.44
OV1.54P5	femur	Proximal 5	-	-	-
OV1.51D4	humerus	Distal 4	-	-	-
OV1.51D2	humerus	Distal 2	-	-	-
OV1.51M	humerus	Midshaft	-	-	-
OV1.51P2	humerus	Proximal 2	P	19193.85	791.78
OV1.51P2	humerus	Proximal 2	P	16772.55	346.4
OV1.51P2	humerus	Proximal 2	P	14796.33	550.2
OV1.51P2	humerus	Proximal 2	P	13294.76	967.76
OV1.51P4	humerus	Proximal 4	P	18819.05	517.99
OV1.51P4	humerus	Proximal 4	P	9703.66	313.02
OV1.51P4	humerus	Proximal 4	P	12234.77	623.99
OV2.54D4	femur	Distal 4	A	17363.75	330.01
OV2.54D4	femur	Distal 4	A	12305.63	626.34
OV2.54D4	femur	Distal 4	A	16109.32	509.79
OV2.54D4	femur	Distal 4	A	12350.72	226.35
OV2.54D2	femur	Distal 2	P	15823.53	511.55
OV2.54D2	femur	Distal 2	P	15905.52	507.16
OV2.54D2	femur	Distal 2	P	15903.18	454.61
OV2.54D2	femur	Distal 2	P	15920.75	922.67
OV2.54D2	femur	Distal 2	P	16450.74	469.68
OV2.54D2	femur	Distal 2	P	15889.71	433.96
OV2.54D1	femur	Distal 1	P	17347.93	640.1

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV2.54M	femur	Midshaft	P	17467.4	508.04
OV2.54M	femur	Midshaft	P	17352.91	874.06
OV2.54M	femur	Midshaft	P	21036.26	833.94
OV2.54M	femur	Midshaft	P	17419.38	526.78
OV2.54P1	femur	Proximal 1	P	11664.36	673.19
OV2.54P1	femur	Proximal 1	P	31726.41	574.51
OV2.54P1	femur	Proximal 1	P	27279.99	549.62
OV2.54P1	femur	Proximal 1	P	10517.98	713.01
OV2.54P1	femur	Proximal 1	P	18572.49	923.25
OV2.54P1	femur	Proximal 1	P	18670.59	753.42
OV2.54P1	femur	Proximal 1	P	13168.27	331.47
OV2.51M	humerus	Midshaft	P	12136.67	600.57
OV2.51M	humerus	Midshaft	P	8708.37	469.09
OV2.51M	humerus	Midshaft	P	13242.35	537.32
OV2.51M	humerus	Midshaft	P	14773.49	630.44
OV2.51M	humerus	Midshaft	P	13135.47	—
OV2.51P1	humerus	Proximal 1	A	23005.45	594.13
OV2.51P1	humerus	Proximal 1	A	18449.51	460.31
OV2.51P1	humerus	Proximal 1	A	15193.97	595.88
OV2.51P1	humerus	Proximal 1	A	16998.6	582.41
OV2.51P1	humerus	Proximal 1	A	14461.64	448.89
OV2.51P1	humerus	Proximal 1	A	12887.16	387.4
OV2.51P1	humerus	Proximal 1	A	14963.82	778.31
OV2.51P1	humerus	Proximal 1	A	12899.46	753.42
OV2.51P1	humerus	Proximal 1	P	11628.64	401.45
OV2.51P1	humerus	Proximal 1	P	18099.89	464.12
OV2.51P1	humerus	Proximal 1	P	13565.33	503.94
OV2.51P1	humerus	Proximal 1	P	13560.06	346.4
OV34D5	femur	Distal 5	A	9999.4	816.67
OV34D5	femur	Distal 5	A	15354.14	452.4
OV34D5	femur	Distal 5	A	22575.89	810.81
OV34D5	femur	Distal 5	A	23331.94	526.78
OV34D5	femur	Distal 5	A	18246.3	695.44
OV34D5	femur	Distal 5	A	21144.01	1300.69
OV34D5	femur	Distal 5	A	17174	602.32
OV34D5	femur	Distal 5	A	21218.97	1218.99
OV34D3	femur	Distal 3	A	8420.24	542.004
OV34M	femur	Midshaft	P	20269.95	1738.75
OV34M	femur	Midshaft	P	20419.58	1650.32
OV34M	femur	Midshaft	P	24911.39	1599.66
OV34M	femur	Midshaft	P	10948.71	271.44

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV31D5	humerus	Distal 5	A	22956.55	1025.45
OV31D5	humerus	Distal 5	A	12592.59	472.31
OV31D5	humerus	Distal 5	A	25423.24	1127.93
OV31D5	humerus	Distal 5	A	18047.47	511.55
OV31D3	humerus	Distal 3	-	-	-
OV31M	humerus	Midshaft	P	16147.68	765.72
OV31P4	humerus	Proximal 4	A	19292.82	635.99
OV31P4	humerus	Proximal 4	A	16435.52	578.31
OV31P4	humerus	Proximal 4	P	24664.84	618.14
OV31P4	humerus	Proximal 4	P	24094.43	773.91
OV31P4	humerus	Proximal 4	P	20935.23	457.09
OV6.54D4	femur	Distal 4	A	9345.84	416.09
OV6.54D4	femur	Distal 4	A	11532.88	350.5
OV6.54D4	femur	Distal 4	A	15486.79	568.94
OV6.54D4	femur	Distal 4	A	17195.08	493.98
OV6.54D4	femur	Distal 4	A	9089.33	-
OV6.54D4	femur	Distal 4	A	19342.02	484.61
OV6.54D4	femur	Distal 4	A	14764.12	421.36
OV6.54D4	femur	Distal 4	A	15385.77	437.18
OV6.54D4	femur	Distal 4	A	12874.28	638.63
OV6.54D3	femur	Distal 3	A	16555.57	438.93
OV6.54D3	femur	Distal 3	A	22029.78	962.49
OV6.54D3	femur	Distal 3	A	18477.33	914.17
OV6.54D3	femur	Distal 3	A	15753.25	894.85
OV6.54D2	femur	Distal 2	P	10449.17	392.08
OV6.54D2	femur	Distal 2	P	18301.35	1232.47
OV6.54D2	femur	Distal 2	P	20158.48	838.92
OV6.54D2	femur	Distal 2	P	22151.3	383
OV6.54D2	femur	Distal 2	P	12941.92	697.2
OV6.54D2	femur	Distal 2	P	11120.31	940.82
OV6.54D2	femur	Distal 2	P	10344.05	503.94
OV6.54D2	femur	Distal 2	A	13108.82	467.92
OV6.54D2	femur	Distal 2	A	10744.91	433.66
OV6.54D2	femur	Distal 2	A	11260.56	544.35
OV6.54D2	femur	Distal 2	A	5935.69	264.71
OV6.54D2	femur	Distal 2	P	13073.93	501.01
OV6.54D2	femur	Distal 2	P	17410.89	411.7
OV6.54D1	femur	Distal 1	A	18687.86	1269.07
OV6.54D1	femur	Distal 1	P	15912.55	988.84
OV6.54D1	femur	Distal 1	P	12185.28	10007.58
OV6.54D1	femur	Distal 1	P	14643.19	362.8

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV6.54D1	femur	Distal 1	P	14237.93	893.97
OV6.54D1	femur	Distal 1	P	7175.48	235.72
OV6.54D1	femur	Distal 1	P	10382.11	797.93
OV6.54M	femur	Midshaft	A	6875.05	378.32
OV6.54M	femur	Midshaft	A	13833.55	777.43
OV6.54M	femur	Midshaft	A	13138.4	789.43
OV6.54M	femur	Midshaft	A	10115.36	476.12
OV6.54M	femur	Midshaft	P	13078.96	1032.18
OV6.54M	femur	Midshaft	P	15911.37	1364.23
OV6.54M	femur	Midshaft	P	20683.41	663.23
OV6.54M	femur	Midshaft	P	22024.22	109.5
OV6.54M	femur	Midshaft	P	10880.78	667.92
OV6.54M	femur	Midshaft	P	15589.28	732.92
OV6.54M	femur	Midshaft	P	20748.12	1887.21
OV6.54M	femur	Midshaft	A	21178.56	788.56
OV6.54M	femur	Midshaft	A	19860.89	1322.65
OV6.54M	femur	Midshaft	A	25551.2	1222.51
OV6.54M	femur	Midshaft	A	16985.72	896.61
OV6.54M	femur	Midshaft	A	11889.83	705.4
OV6.54M	femur	Midshaft	P	15779.02	759.27
OV6.54M	femur	Midshaft	P	9608.49	548.45
OV6.54M	femur	Midshaft	P	12257.02	450.65
OV6.54P1	femur	Proximal 1	A	20450.62	960.15
OV6.54P1	femur	Proximal 1	A	14136.61	1011.1
OV6.54P1	femur	Proximal 1	A	11704.18	564.26
OV6.54P1	femur	Proximal 1	P	12657.59	832.48
OV6.54P1	femur	Proximal 1	P	11601.7	1115.34
OV6.54P1	femur	Proximal 1	P	13911.14	648.59
OV6.54P1	femur	Proximal 1	P	10088.42	646.25
OV6.54P1	femur	Proximal 1	P	12160.68	651.52
OV6.54P1	femur	Proximal 1	P	13242.06	525.02
OV6.54P1	femur	Proximal 1	P	9674.08	612.87
OV6.54P1	femur	Proximal 1	P	9914.49	498.59
OV6.54P1	femur	Proximal 1	P	20450.62	960.15
OV6.54P1	femur	Proximal 1	P	14136.61	1011.1
OV6.54P1	femur	Proximal 1	P	11704.18	564.26
OV6.54P1	femur	Proximal 1	P	12657.59	832.48
OV6.54P1	femur	Proximal 1	P	11601.7	1115.34
OV6.54P1	femur	Proximal 1	P	13911.14	648.59
OV6.54P1	femur	Proximal 1	P	10088.42	646.25
OV6.54P1	femur	Proximal 1	P	12160.68	651.52

Slide Number	Bone	Segment	Location on Segment	O Area	HC Area
OV6.54P1	femur	Proximal 1	P	9914.49	498.59
OV6.54P2	femur	Proximal 2	A	23638.52	526.19
OV6.54P2	femur	Proximal 2	A	12869.59	585.93
OV6.54P2	femur	Proximal 2	A	17641.34	581.24
OV6.54P2	femur	Proximal 2	A	14270.43	586.51
OV6.54P2	femur	Proximal 2	P	15628.81	1026.91
OV6.54P3	femur	Proximal 3	A	13165.92	732.33
OV6.54P3	femur	Proximal 3	A	19330.3	1018.42
OV6.54P3	femur	Proximal 3	A	15813.87	729.41
OV6.54P3	femur	Proximal 3	A	15315.2	959.85
OV6.54P3	femur	Proximal 3	P	11736.39	697.78
OV6.51D3	humerus	Distal 3	A	9604.69	761.03
OV6.51D3	humerus	Distal 3	A	8703.4	689.88
OV6.51D2	humerus	Distal 2	A	10640.67	973.62
OV6.51D2	humerus	Distal 2	P	6329.82	646.83
OV6.51D2	humerus	Distal 2	P	9790.331	501.89
OV6.51D1	humerus	Distal 1	A	11990.56	741.997
OV6.51D1	humerus	Distal 1	A	14014.21	448.6
OV6.51D1	humerus	Distal 1	A	11870.5	421.36
OV6.51D1	humerus	Distal 1	A	10876.68	446.55
OV6.51D1	humerus	Distal 1	A	10338.48	368.66
OV6.51D1	humerus	Distal 1	A	13656.1	655.32
OV6.51M	humerus	Midshaft	P	15173.18	347.57
OV6.51M	humerus	Midshaft	P	12926.4	600.57
OV6.51M	humerus	Midshaft	P	13102.09	386.81
OV6.51M	humerus	Midshaft	A	17162.87	613.45
OV6.51M	humerus	Midshaft	A	12860.52	341.72
OV6.51M	humerus	Midshaft	A	15237.31	519.46
OV6.51M	humerus	Midshaft	A	15143.02	484.03
OV6.51M	humerus	Midshaft	A	12484.54	221.96
OV6.51M	humerus	Midshaft	A	14273.06	570.11
OV6.51P1	humerus	Proximal 1	P	13902.07	823.69

Appendix B:

Descriptive Statistics for Femur and Humerus Osteon and Haversian Canal Areas

Appendix B

Descriptive Statistics for Femur and Humerus Osteon and Haversian Canal Areas

*Measurements reported in nm²

F=Femur OA=Osteon Area P=Proximal M=Midshaft
H=Humerus HCA=Haversian Canal Area D=Distal

Bone	Location	Feature	Min	Max	Mean	Std. Deviation
9 month F	P	OA	13216.29	23289.77	17327.46	2892.49
9 month F	M	OA	9041.31	33059.9	21532.24	8224.9
9 month F	D	OA	12772.53	35291.17	17797.1	8165.08
9 month F	P	HCA	756.93	1585.9	1189.72	342.35
9 month F	M	HCA	565.14	2155.13	1134.76	597.86
9 month F	D	HCA	523.85	1388.83	934.72	286.92
1.5 year F	P	OA	18503.39	22399.32	20451.36	2754.84
1.5 year F	M	OA	15381.38	25005.68	20193.53	6805.41
1.5 year F	D	OA	12180.3	19375.69	15076.38	2857.41
1.5 year F	P	HCA	1225.44	1410.5	1317.97	130.86
1.5 year F	M	HCA	654.45	672.6	663.53	12.83
1.5 year F	D	HCA	332.93	838.33	613.74	220.02
2.5 year F	P	OA	10517.98	31726.41	18800.01	8065.41
2.5 year F	M	OA	17352.91	21036.26	18318.99	1812.12
2.5 year F	D	OA	14135.74	16845.07	15862.04	976.09
2.5 year F	P	HCA	331.47	923.25	645.5	185.72
2.5 year F	M	HCA	508.04	874.06	685.71	195.17
2.5 year F	D	HCA	433.96	574.51	484.82	49.86
3 year F	P	OA	—	—	—	—
3 year F	M	OA	10948.71	24911.39	19137.41	5868.57
3 year F	D	OA	9209.82	23331.94	18531.88	4665.12
3 year F	P	HCA	—	—	—	—
3 year F	M	HCA	271.44	1738.75	1315.04	698.11
3 year F	D	HCA	452.4	1300.69	785.85	312.78
6.5 year F	P	OA	9674.08	19085.02	13240.41	2800.16
6.5 year F	M	OA	6875.05	25551.2	15578.38	4993.38
6.5 year F	D	OA	5935.69	17435.71	13362.65	3236.45
6.5 year F	P	HCA	498.59	946.68	687.16	138.22
6.5 year F	M	HCA	109.5	1887.21	819.61	408.58
6.5 year F	D	HCA	264.71	3082.4	748.69	717.56
9 month H	P	OA	13216.29	23289.77	17327.46	2892.49
9 month H	M	OA	—	—	—	—
9 month H	D	OA	—	—	—	—
9 month H	P	HCA	585.05	1115.34	816.26	182.2
9 month H	M	HCA	—	—	—	—

Bone	Location	Feature	Min	Max	Mean	Std. Deviation
9 month H	D	HCA	—	—	—	—
1.5 year H	P	OA	13238.11	19006.45	14763.72	2831.02
1.5 year H	M	OA	—	—	—	—
1.5 year H	D	OA	—	—	—	—
1.5 year H	P	HCA	329.71	967.76	634.86	262.44
1.5 year H	M	HCA	—	—	—	—
1.5 year H	D	HCA	—	—	—	—
2.5 year H	P	OA	11628.04	23005.45	15476.13	3184.8
2.5 year H	M	OA	8708.37	14773.49	12399.27	2268.34
2.5 year H	D	OA	—	—	—	—
2.5 year H	P	HCA	346.4	778.31	526.39	138.05
2.5 year H	M	HCA	469.09	630.44	559.36	71.61
2.5 year H	D	HCA	—	—	—	—
3 year H	P	OA	16435.52	24664.84	21084.57	3417.75
3 year H	M	OA	16147.68	16147.68	16147.68	—
3 year H	D	OA	12592.59	25423.24	19754.96	5674.35
3 year H	P	HCA	457.09	773.91	612.69	113.97
3 year H	M	HCA	765.72	765.72	765.72	—
3 year H	D	HCA	472.31	1127.93	784.31	340.57
6.5 year H	P	OA	13902.07	13902.07	13902.07	—
6.5 year H	M	OA	12484.54	17162.87	14262.55	1550.22
6.5 year H	D	OA	9682.48	13656.1	11021.58	1366.34
6.5 year H	P	HCA	823.69	823.69	823.69	—
6.5 year H	M	HCA	221.96	613.45	453.96	135.95
6.5 year H	D	HCA	368.66	825.55	558.8	167.32

Vita

Lindsay Hines Trammell was born November 21, 1981, in Greenville, South Carolina. She was raised in Simpsonville, South Carolina, and graduated Valedictorian from Hillcrest High School in 2000. From there, she attended the University of South Carolina Honors College in Columbia, South Carolina, and received her Bachelor of Arts degree in anthropology in 2004. She was accepted to the University of Tennessee-Knoxville anthropology program in August 2004 and earned her Masters of Arts in anthropology in August 2006. She is currently enrolled as a PhD student in anthropology at the University of Tennessee-Knoxville.