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DIFFERENTIATING BETWEEN HUMAN AND NON-HUMAN SECONDARY OSTEONS IN HUMAN, CANINE, AND BOVINE RIB TISSUE

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DIFFERENTIATING BETWEEN HUMAN AND NON-HUMAN SECONDARY OSTEONS IN HUMAN, CANINE, AND BOVINE RIB TISSUE

By

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ABSTRACT

DIFFERENTIATING BETWEEN HUMAN AND NON-HUMAN SECONDARY OSTEONS IN HUMAN, CANINE, AND BOVINE RIB TISSUE

By

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One of the first questions a forensic anthropologist must answer is whether a bone is human or non-human. Under normal circumstances, this can be done using gross morphological features. However, this task becomes more difficult when the bone samples are extremely small or fragmentary. In this case, microscopic examination of the histology of the bone tissue may be necessary. Most adult human compact bone is composed of cylindrical structures known as Haversian systems or secondary osteons. Non-human bone typically has other distinctive arrangements of bone tissue not commonly found in humans. While Haversian bone is not common in non-humans, it has been found in the bones of a number of species. However, very little research has been conducted on the question of how to distinguish between human and non-human secondary osteons, especially in non-weight bearing bones such as ribs. For this study, rib samples from adult humans (n=11), adult dogs (n=12), and immature beef cattle (n=9) were obtained. These samples were cleaned, sectioned, examined under a microscope, and digitally measured. Non-parametric statistical testing demonstrated significant differences in the overall size of the secondary osteon and the size of the central canal between humans and the two non-human species tested. This suggests that metric analysis of secondary osteons may allow anthropologists to differentiate between human and non-human bone. More species and larger samples still need to be tested.

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Chapter 1

STATEMENT OF PROBLEM

Introduction

A number of questions must be answered whenever skeletal material is found. First of all, is the bone human or non-human? If the bone is human, is it of forensic concern? To whom do the bones belong? How did the individual to whom they belonged die? Preferably, these kinds of questions should be answered by a physical anthropologist with training in human osteology. If the bones are of forensic interest, it is even better for a physical anthropologist with training in forensic anthropology to answer them. Forensic anthropology has two main goals: assisting in the identification of human remains and figuring out what happened to them (Ubelaker 2000). It is the job of the forensic anthropologist to create a biological profile (sex, age-at-death, ancestry, stature, antemortem and perimortem trauma) to aid in the identification of an individual from their remains, since a great deal of information may be recorded within an individual's bones.

This study is concerned with addressing one of the first questions an anthropologist must answer: is the bone human or non-human? Typically, this question can be answered by examining the gross macroscopic features of the bone or bones in question. Occasionally, cases may be encountered where bone is too fragmentary to use a simple visual examination. In such cases, the microscopic features of the bone (histology) should be examined. If histological examination cannot answer this question, then molecular procedures can be used. However, the histology should always be examined initially, since molecular procedures are usually more costly and time

consuming (Ubelaker 2000). Non-human bone is usually composed of distinctive types of bone that allow it to be distinguished from human bone. Compact bone in humans is dominated by Haversian bone, which is composed of structures known as secondary osteons. However, the distinctive types of bone found in non-humans are all a type of primary bone. Over time, these types of bone may be replaced by Haversian bone, which is a secondary bone type (Martin, et al. 1998). Thus Haversian bone can also encountered in non-humans. Unfortunately, there are very few studies that address the question of how to distinguish between human and non-human Haversian bone (see (Jowsey 1966; Lackey 2001; Mulhern and Ubelaker 2001; Owsley, et al. 1985; Singh, et al. 1974).

The need for methods to distinguish between human and non-human bones at the microscopic levels may be demonstrated by two cases encountered at the Michigan State University Forensic Anthropology Lab. In the first case, burned fragments of bone were recovered from a possible crime scene. These fragments were smaller than a penny, and had no gross morphological features remaining. In a second case, two chess sets were examined from the Holocaust Memorial Center to determine if they were composed of human material. Carving of the bone-like material had destroyed the surface features of the bones (Lackey, 2001). In both of these cases, an experienced forensic anthropologist compared the fragments to known non-human samples to determine the origin.

STATEMENT OF PROBLEM

Since very few quantitative studies have been published on the characteristics of non-human Haversian bone, distinguishing between human and non-human bone

histology depends partly on the experience of the anthropologist and the reference samples available. In the case of the Holocaust material, a small pilot study had to be developed in order to compare the sizes of the secondary osteons in human and canine femora, due of the similarity of the structures found (Lackey, 2001). This research project was designed to address this growing need for microscopic methods distinguishing between human and non-human bone fragments. In addition to the scarcity of quantitative comparisons of Haversian bone, previous studies have also focused primarily on weight-bearing bones (see (Jowsey 1966; Lackey 2001; Mulhern and Ubelaker 2001; Owsley, et al. 1985; Singh, et al. 1974). Few studies have examined quantitative differences in the histological structure of non-weight-bearing bones in any species. The lack of research in this area is important, since non-weight-bearing bones such as ribs are under much different biomechanical stresses than weight-bearing bones. In the weight-bearing bones of non-humans, very distinctive primary forms of bone such as plexiform bone are commonly found. This kind of bone, which can be formed very quickly and may have a better fatigue resistance and tensile strength than osteonal bone, is typical in large, fast-growing animals such as cows and horses (Currey, 1984; Martin, et al. 1998; Stover, et al. 1992). Haversian bone is seen more often in non-humans in non-weight bearing bone like ribs, even at younger ages. Thus, I decided to focus on rib tissue for this study, since I would be more likely to find Haversian bone in all the species studied, even with sub-adult specimens. I studied samples of human, canine, and bovine rib samples and described, measured, and compared the histological structures.

Chapter 2

BACKGROUND

Bone Composition

Bone is composed of both organic and inorganic components and water. The organic component is mostly collagen fibers, which gives bone its flexibility and provides tensile strength. The inorganic component is composed mainly of hydroxyapatite crystals which give bone its strength (Martin, et al. 1998). Specialized cells carry out the formation of the bony matrix. One type of specialized cell, the osteoblast, lays down new bone by secreting a substance called osteoid. Osteoid, the organic portion of extracellular bone, contains collagen and noncollagenous proteins, proteoglycans, and water. As the water within the osteoid is replaced with minerals it forms new bone (Martin, et al. 1998). Eventually, some osteoblasts will be completely surrounded by bone and become the osteocyte. The osteocyte is now responsible for maintaining the surrounding bony tissue. Osteocytes are found in cavities known as lacunae, from which small canals (canaliculi) radiate. These canaliculi allow osteocytes to communicate with each other and receive nutrients.

Bone Classification

There are two main kinds of bone: compact bone and trabecular bone (also called cancellous or spongy bone). Compact bone is very dense, while trabecular bone is highly porous. Compact bone is found in the shafts of long bones, where it is relatively thick. It may also form a thin outer layer, or cortex, around spongy bones such as vertebral bodies, where it is also known as cortical bone (Martin, et al. 1998). At joint surfaces, compact

bone is very smooth, lies beneath articular cartilage, and is called subchondral bone (Schultz 1997). Trabecular bone is found inside compact bone, where it forms the spongy interior of bones. Inside cranial bones trabecular bone is referred to as diploë.

Both compact and trabecular bone can also contain two major types of bone tissue, classified by their arrangement of collagen fibers: woven bone and lamellar bone. Woven bone is a quickly formed, poorly organized tissue that may be found in fetal bones and repair tissue (Martin, et al. 1998; Schultz 1997). Its collagen fibers are randomly arranged in contrast to lamellar bone, which is highly organized with parallel collagen fibers. Lamellar bone is slowly formed and makes up the majority of bone tissue in humans (see Fig. 1 for an example of lamellar bone).

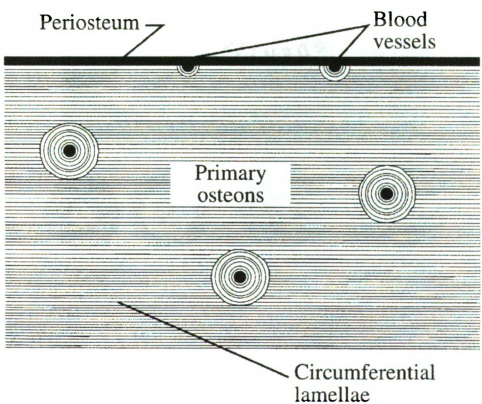


Figure 1. Schematic of primary circumferential lamellar bone with primary osteons. From Martin et al. 1998. *Skeletal Tissue Mechanics*, Springer, New York. p. 37.

Compact bone can be further characterized as primary or secondary bone (Martin, et al. 1998). Primary bone is new bone laid down on some existing calcified or bone surface. It may form circumferential lamellar bone, in which the lamellae are parallel to the bone surface, with blood vessels surrounded by several circular lamellae, forming a primary osteon (Fig. 1). Secondary bone results when the existing bone is resorbed and quickly replaced by new lamellar bone during a process known as remodeling. The process of remodeling repairs microscopic damage and prevents fatigue damage that may lead to fracturing. This process is accomplished by cells known as osteoclasts and osteoblasts working together in basic multicellular units, or BMUs (Frost 1986) (Fig. 2).

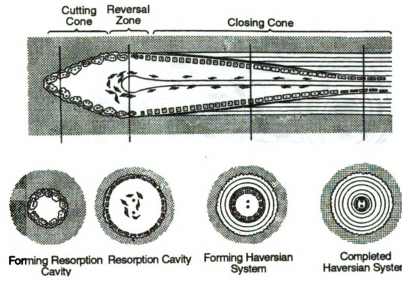


Figure 2. Diagram of a basic multicellular unit (BMU) remodeling cortical bone. Osteoclasts are found in the front cutting through the cortical bone, followed by osteoblasts filling it in. Adapted from Cowen, S.C. ed. 2001 *Bone Mechanics Handbook.* Boca Raton, FL: CRC Press

Once activated, the osteoclasts in the BMU begin to remove bone in the form of a tunnel in compact bone. This osteoclastic activity results in a temporary space called a resorption cavity that will be replaced with new bone formed by the osteoblasts (Martin, et al. 1998; Schultz 1997). The border between where osteoclastic activity stops and osteoblastic activity starts is called the cement line or reversal line. The scalloped edge of the cement line is important for distinguishing between primary osteons and from secondary osteons, since primary osteons lack a cement line. Once osteoclasts have created a tunnel, the osteoblasts follow slowly filling in the resorption cavity. This process forms new secondary tissue that consists of cylindrical structures known as secondary osteons or Haversian systems (Fig. 3).

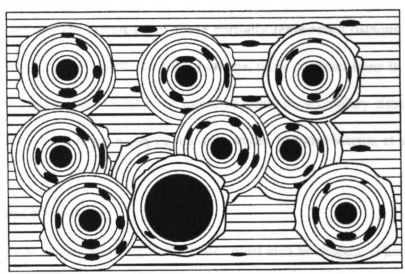


Figure 3. Schematic diagram of secondary osteons replacing primary circumferential lamellar bone. From Martin et al. 1998. *Skeletal Tissue Mechanics*, Springer, New York. p. 39.

After becoming surrounded by bone, some of the osteoblasts become osteocytes, cells responsible for transmitting nutrition and messages within the bone tissue. Thus, the tunnels created by the osteoclasts are not completely filled by the osteoblasts. A central canal is left, which contains a space for the needed capillaries and nerves. Volkman's, or transverse, canals connect the central canals to each other, and also contain blood vessels and nerves to supply the osteocytes that maintain the bone. The osteocytes, in their lacunae, are arranged in concentric layers within the lamellae surrounding the central canal. The secondary osteons are approximately aligned to the long axis of the bone (Martin, et al. 1998; Schultz 1997).

In humans, secondary osteons are about 200μm in diameter and consist of about 16 cylindrical lamellae surrounding a Haversian or central canal (Martin, et al. 1998). Resorption cavities and new osteons can cut through existing osteons, especially as an individual grows older. The remnants of these partially reabsorbed osteons are called interstitial bone (Martin, et al. 1998). In adult humans, most compact bone is composed of dense Haversian bone. Trabecular bone can also contains secondary tissue, but the trabecular structures are not large enough to contain whole osteons (Martin, et al. 1998).

Bone Modeling and Remodeling

As bones grow in size and length, the bones must also be shaped. This process is called modeling. During modeling, some bone must be removed by osteoclasts as other bone is deposited by osteoblasts. Bone must also be maintained throughout life, due to damage from fatigue, varying load conditions, and trauma (Martin, et al. 1998). Again,

osteoclasts and osteoblasts are responsible for removing and replacing bone, a process known as remodeling. The rate at which remodeling occurs depends on a number of factors, including the age of the individual, sex, metabolic diseases, and fractures (Stout 1989). Despite these variables, it is this continuous turnover of bone tissue that gives anthropologists so much information about an individual. For example, as an individual ages, secondary osteons are created that will eventually obliterate the lamella of primary bone, and then begin partially destroying other osteons. Eventually, the creation of each new osteon will result in the removal of an older osteon (Martin, et al. 1998). By measuring variables such as the number of secondary osteons, osteon fragments, and primary lamellar bone, age can be assessed (see (Ahlqvist and Damsten 1969; Kerley 1965; Kerley and Ubelaker 1978; Stout 1989).

Human v. Non-Human Bone Tissue

The previous discussion has focused primarily on types of bone tissue found in humans. Non-human animals exhibit a number of types of bone tissue not commonly encountered in humans. For example, in primary vascular bone (which contain primary osteons), humans have a laminar arrangement, with the lamellae arranged in broad circumferential sheets (Enlow and Brown 1956). Some fast growing animals, such as cows, may have plexiform bone (Fig. 4), which is formed by constructing a trabecular network then filling in the gaps with lamella, resulting in a "brick wall" appearance (Martin, et al. 1998). Still other types of primary vascular bone are characterized by the direction of their vascular canals (longitudinal, radial, or reticular) (Enlow and Brown 1956). Bone may also be non-vascular, lacking any type of osteons. One difficulty in

classifying the type of bone found within a particular animal is the diversity of bone types that can be found within a species. The types of bone may vary within the same bone, between bones in the same individual, and between individuals of the same species (Singh, et al. 1974).

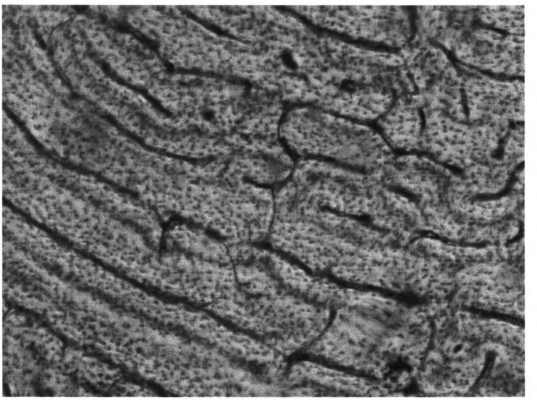


Figure 4: Example of plexiform bone found in a cow rib.

Adult humans typically display Haversian, or osteonal bone (Singh, et al. 1974). However, Enlow and Brown noted that "even in the well-described bones of the human, large areas occur in which one finds massive lamellation containing only a few, scattered, secondary osteons" (1958:193) and that extensive primary vascular areas can also be found. However, since plexiform, reticular, and non-vascular bone tissue types are so rarely found in humans, the presence of these types of bones allows an anthropologist to

easily classify the bone as non-human. A more difficult problem is that Haversian bone has been documented in a number of non-human species including non-human primates. Haversian bone is not commonly found in the weight-bearing long bones of non-humans, with the exception of dogs (Enlow and Brown 1957). However, in ribs, which are non-weight bearing, Haversian tissue has been documented in bears, cats, dogs, horses, cows, and goats (Enlow and Brown 1958). A second problem is that the distinctive non-human types of bone such as plexiform bone are all primary in nature; however, as the bone remodels they can be replaced with secondary Haversian bone. For example, the compact bone in the long bones of a dog follows a typical primary plexiform structure, but is frequently replaced by Haversian bone as the dog ages (Enlow and Brown 1958). An important question is: how do we distinguish between human Haversian bone and non-human Haversian bone in non-weight bearing bone?

Uses of Bone Histology and Microscopy in Anthropology

Microscopic examination of bone histology is not new to anthropology. Starting in 1965, a technique that allows microscopic determination of age in humans was developed (Kerley 1965). Since then the technique has been revised and refined by other researchers (Ahlqvist and Damsten 1969; Kerley and Ubelaker 1978; Stout and Paine 1994; Stout 1989). This technique is valuable since it does not require the preservation of a whole bone or specific bones, and unlike many other techniques that become unreliable after 30-40 years of age, microscopic aging is useful into the 90s. Other uses of bone histology include applications to other fields of anthropology less forensic in nature. Microscopy may be useful in paleoanthropology as a method of differentiating among

hominid species (Bartsiokas 2002). Differences in activity levels between sexes were demonstrated in another study using histology (Mulhern and Van Gerven 1997), although the usefulness of this technique in determining activity patterns is now being questioned (see Bice, 2003). Bone histology can also reveal information on generalized pathologic conditions, including metabolic disturbances, systemic diseases, osteoporoses, specific nutritional deficiencies, and more generalized dietary stresses (Martin 1981). Most recently, histological methods have been used to identify the species of bones used in bone-tempered pottery, which could yield information on regional differences and changes in native technology over time (Walter, et al. 2004). As microscopy becomes more common, anthropologists should be able to glean more information about the lives of different people, even when skeletal preservation is poor.

Species Identification Through Bone Histology

While microscopic examination of bone has become more common in bioarchaeology, little research has been done to differentiate between human and non-human Haversian systems. Enlow and Brown (1956; 1957; 1958) extensively described the bone types that may be found in different species, but presented no quantitative data. Jowsey (1966) reported differences in the sizes of secondary osteons found in the femurs of rats, rabbits, cats, dogs, cows and humans. While there were clear differences between the diameters of the osteons and the perimeters of Haversian canals, these data were not evaluated for statistical significance, and the non-human sample was quite small compared to the human sample. Singh, et al. (1974) also studied the size of secondary osteons in different species. Unlike Jowsey (1966), most of the animals in their study

lacked secondary osteons, with the exception of primates. This may be related to the samples used. Their sample size was relatively small, with 44 bone samples taken from 12 different species of mammals, with the largest number from humans, other primates, and rodents. Secondly, of the other animals in the sample, many were from newborns or very young specimens, in which one would not expect many secondary osteons. Finally, the study was a mixture of rib, tibia, and femur samples, and did not specify which bone was examined for which animal. However, when osteons were present, they found significant differences in the average number of lamellae per osteon and the average Haversian canal diameter.

More recently, Lackey (2001) compared Haversian systems in a sample of canine and human femura. She found that human bone tissue typically exhibits larger Haversian canal systems than canine tissue. The average Haversian canal diameter for humans was also almost twice that of the canine sample (Lackey 2001). Unfortunately, only an abstract of this study has been published, and no quantitative data was given. A case study that compared sections of a single deer humeri to human found human Haversian canals were typically larger than Haversian canals in the deer (Owsley, et al. 1985).

A study by Mulhern and Ubelaker (2001) examined osteon banding in human and non-human femura. Arrangement of osteons into distinct rows has been found to be common in individuals of young species that undergo rapid growth, as the compact bone replaces organized cancellous bone. Over time, these bands disappear due to further remodeling (Enlow 1963; Mulhern and Ubelaker 2001). In this study, they found that the type of bands in humans and non-humans could be distinguished. In swine, long

multiple, consecutive bands of osteons occurred frequently, while the bands that were found in two of their human samples were much shorter and isolated.

Finally, Walter, et al. (2004) used the ratio of the number of osteocytes found per secondary osteon to the area of secondary osteons for species identification. While this technique was used to identify bones fragments used in bone-tempered pottery, the technique has potential forensic as well as archaeological applications.

Most research on non-human Haversian systems has focused on weight-bearing long bones, especially femora. This may explain why so few studies have been done comparing human and non-human Haversian systems: secondary osteons are rarely seen in weight-bearing long bones due to the biomechanical stress experienced by these bones. Dogs are one exception, in that the plexiform bone in their long bones may be replaced by Haversian bone as they age. It may be more likely to find secondary osteons replacing the stronger plexiform arrangement as the animal ages in non-weight bearing bones that do not have to withstand the same kinds of biomechanical stress. This may explain why Haversian bone has been documented in the rib tissue of a number of non-human species, including large animals like horses, cows, and bears. The presence of secondary osteons in non-human non-weight bearing bone demonstrates a need for research on the characteristics of secondary osteons in non-humans. The lack of published quantitative data means that the forensic anthropologist must either be very familiar with the size and shape of Haversian canals in humans to be able to qualitatively distinguish them from non-human Haversian canals, or obtain reference sample of common mammals for comparison. This study provides a quantitative approach that can be used to distinguish human bone tissue from histologically similar canine and bovine samples.

Chapter 3

MATERIALS AND METHODS

Materials

For this study I chose to collect ribs from two non-human species to compare with human samples: cows and dogs. These two species were chosen for several reasons: 1) both are very common species that may be encountered in forensic and archaeological settings (i.e. barbeques in the case of cattle); 2) quantitative data on Haversian systems in the ribs of these two species are unavailable; and 3) ease of obtaining rib specimens.

Nine beef cattle rib specimens were obtained at local butcher shops. According to Harlan Ritchie, an animal science professor at Michigan State University, these rib samples are most likely from immature cattle, since the cuts of beef that are typically found in a meat case would be from cattle that rang in age from 13-24 months, with an average age of about 18-19 months. Beef cattle are mature at about 5 years of age (Ritchie, personal communication). The sex of the bovine samples was unknown. Twelve canine rib samples were obtained from the necropsy unit at the Michigan State University College of Veterinary Medicine. All of the rib samples were from adult dogs, from 2.5-14 years of age. Information on the sex and breed of the dogs was not obtained. Finally, a comparison sample of ten adult human 4th left ribs was obtained from a local pathologist. The ages of these samples ranged from 37 to 74 years of age, with a mean age of 55.1 years. Eight of the ten samples were male.

Procedure

Thin sections of the human and non-human ribs were prepared for this study. First, the bones were defleshed and degreased by simmering in dilute mild detergent, then sodium carbonate, and finally ammonia, using the procedure described in Fenton, et al. (2003). After the bones had fully dried, they were sectioned on a slow speed rotary saw (an Isomet 1000 Precision Saw) with a Diamond Wafering Blade. Each section was cut to a thickness of approximately 0.8mm. After sectioning, each section was air-dried and mounted using Shur/Mount™, a toluene based mounting medium. The cross-sections were photographed at 100x, using a Leica NZ12 light microscope with a Hyperhad Sony CCD-IRIS/RGB color video camera attached. Images were transmitted to a Sony Trinitron video monitor and acquired via SigmaScan Pro 5.0, an image analyzer. Three images of each bone sample showing secondary osteons were acquired.

Using SigmaScan Pro 5.0, each acquired image was examined and measured. The boundary between the osteon and the surrounding bone is known by a scalloped border known as the cement line or reversal line. However, these borders may be partially obscured by overlapping osteons, or difficult to distinguish. In particular, the bovine and canine samples lacked distinctive cement lines, so care was taken to measure only clearly defined complete Haversian systems. Three features of the Haversian systems were measured: maximum diameter (major axis), a second diameter taken 90° from the major axis (minor axis), and the maximum diameter of the central canal. Extremely atypical canals, from osteons that appeared to either be in later stages of resorption or early deposition, were not measured. Since the borders of the central canals are very clear, a larger sample of the central canal diameters was also taken, even

from partial osteons. Finally, qualitative observations about each sample, such as the presence of osteon banding, were also recorded.

Statistical Methods

Due to the small size of this study, nonparametric statistical tests were chosen to analyze the data. The Wilcoxon and Kruskall-Wallis tests replace *t* tests and one-way analysis of variance (ANOVA) since the normality assumptions of these tests were not met in these samples (Moore and McCabe 1999). The Wilcoxon Rank Sum Test was used to compare humans with dogs and humans with cows. In this test, the observations were arranged in order from smallest to largest and assigned a rank from its observation in the ordered list, starting with rank 1 for the smallest observation. The sums from each of the ranked observations are then compared. This method prevents outliers from skewing the results even with a very small sample. The Wilcoxon Two-Sample Test compares two distributions to see if one has systematically larger values than the other. The Kruskal-Wallis Test was also used to compare data sets. The Kruskal-Wallis test ranks all the responses from all the groups together and applies one-way ANOVA to the ranks rather than the original observations. Essentially, this statistic is a sum of squares groups test for ranks (Moore and McCabe 1999). These tests were performed using SAS statistical software. I choose a significance level of $\alpha = 0.01$. At this level of significance, the evidence against the null hypothesis so strong that it would appear only 1% of the time (1 time in 100) if the null hypothesis is in fact true (Moore and McCabe 1999).

Hypotheses

This study asks several questions about bone characteristics found in non-weight-bearing bone. From these questions, several hypotheses to be tested were developed.

Question 1: Are secondary osteons commonly found in the rib tissue of dogs and cows? Enlow and Brown (1956) found dense Haversian bone in the ribs of a number of species, but since the type of bone found can vary within the same bone, as well as from individual to individual, secondary osteons may not be common, even in non-weight-bearing bone. If areas of dense Haversian bone, rather than only scattered, occasional secondary osteons, are found in the rib tissue of cows and dogs, a successful argument can be made for the importance of studying the quantitative characteristics of secondary osteons of non-humans in order to distinguish them from human bone.

Question 2: Can non-human secondary osteons be quantitatively distinguished from human secondary osteons? What are the characteristics of the secondary osteons that best differentiate between human and non-human secondary osteons?

> Null Hypothesis 1 – Human/Canine Secondary Osteon Hypothesis: The quantitative data will show no statistical differences in the measurements of the a. maximum diameter; b. minimum diameter; and c. central canal of the human and canine secondary osteons.
>
> Null Hypothesis 2 – Human/Bovine Secondary Osteon Hypothesis: The quantitative data will show no statistical differences in the measurements of the a. maximum diameter; b. minimum diameter; and c. central canal of the human and bovine secondary osteons.

If any of the parts Hypotheses 1 and 2 are rejected, it may give anthropologists another tool to use to differentiate between human and non-human bone fragments. If parts of either of the hypotheses are not rejected, it will illustrate the importance of studying the characteristics of all non-human species in which secondary osteons can be found to see

which, if any, other species have secondary osteons that are similar to human and it what way. It would also demonstrate a need to develop other measures or techniques to distinguish between human and non-human secondary osteons.

Chapter 4

RESULTS

Results

The canine rib samples were predominantly composed of dense Haversian bone. The dogs younger than three years of age exhibited osteonal banding next to what appeared to be remnants of plexiform bone. However, none of the older adult dogs exhibited banding or plexiform bone in their ribs. The mean major osteon diameter for dogs was 159.6 ± 18.8 μm, the minor osteon diameter was 145.3 ± 17.1 μm, and the mean central canal diameter was 18.44 ± 3.70 μm (Tables 1-3). A comparison of the major osteon diameters and central canal diameters against the ages of the dogs from which the exact ages were known yielded a random pattern (Figs. 5 and 6), so the size of the Haversian systems was not associated with age in this sample. The breed and size of the dogs may have had an influence on the osteon size, but unfortunately this information was not collected.

The bovine rib samples exhibited both plexiform and osteonal bone. Typically, the plexiform bone was found on the periosteal surface of the rib, while the osteonal bone was found toward the endosteal surface. Osteon banding was observed at the interface of the plexiform and osteonal bone. The mean major osteon diameter for cows was 204.5 ± 25.88 μm, the mean minor osteon diameter was 181 ± 21.3 μm, and the mean central canal diameter was 19.30 ± 4.75 μm (Tables 1-3).

Due to very thin compact bone, the human rib samples contained only scattered secondary osteons. No plexiform bone or osteon banding was observed. The mean major osteon diameter in humans was 234.1 ± 20.09 μm, the mean minor osteon diameter

was 214.8 ± 19.3 μm, and the mean central canal diameter was 31.29 ± 8.34 μm (Tables 1-3). A comparison of the mean osteon diameters and central canal diameters against the age of the specimen yielded a random pattern (Figs. 7 and 8), so the size of the Haversian systems was not associated with age in this sample.

The mean values of the human osteon diameters were much larger than those of the dogs for both the major and the minor axis measurements and there was very little overlap in the ranges (Figs. 9 and 10). The difference between the osteon diameters was significant at the $\alpha = 0.01$ level ($p<0.0001$). The difference between the mean osteon diameters for both the major and minor axis was also significant at the $\alpha = 0.01$ level ($p<0.0001$). Humans also had larger central canals than dogs (31.29 ± 8.34 μm v. 18.44 ± 3.70 μm), although there was more overlap in the ranges (Fig. 11). The difference between the central canal diameters and the mean central canal diameter was significant at the $\alpha = 0.01$ level ($p<0.0001$). See Appendix 1, Tables 3-6 for the results of the non-parametric tests comparing humans and dogs.

The mean value of the human osteon diameters was also larger than those of cows but there was a great deal of overlap in the range (Fig. 9 and 10). However, the difference between the osteon diameters was still significant at the $\alpha = 0.01$ level for both the major axis ($p<0.0001$) and the minor axis ($p<0.0001$). The differences between the means of each individual were also significant ($p=0.0005$ and 0.0003 respectively). The mean value of the central canal was also larger in humans than in cows (31.29 ± 8.34 μm v. 19.30 ± 4.75 μm), but again the ranges overlapped (Fig. 11). The difference between the central canal diameters and the means were significant at the $\alpha = 0.01$ level

($p<0.0001$ and $p=0.0002$ respectively). See Appendix 1, Tables 7-10 for the results of the non-parametric tests comparing humans and cows.

When the mean osteon diameters were plotted against the mean central canal diameters, three clusters emerge (see Figs. 12 and 13). Dogs have the smallest mean osteon size and central canal size, cows are intermediate, while humans have the largest mean osteon size and central canal size. There is some overlap between the human and cow clusters.

The rib section measurements used in this study are reported in Appendices B(Human), C (Dog), and D (Cow). The average and standard deviation for each individual sample is reported in Appendix E.

Table 1: Descriptive Statistics of Human, Dog, and Cow Major Osteon Diameters

Major Osteon Diameter (μm)	*Human*	*Dog*	*Cow*
Mean	234.08	159.63	204.46
Standard Error	2.5722	1.5454	2.6556
Median	232.86	159.54	206.76
Mode	.	145.36	192.86
Standard Deviation	20.09	18.801	25.884
Sample Variance	403.60	353.462	669.97
Kurtosis	1.0783	-0.4456	-0.6456
Skewness	0.5742	0.0718	-0.1324
Range	103.48	88.72	112.47
Minimum	195.05	114.90	150.07
Maximum	298.53	203.62	262.54
Count	61	148	95

Table 2: Descriptive Statistics of Human, Dog, and Cow Minor Osteon Diameters

Minor Osteon Diameter (μm)	*Human*	*Dog*	*Cow*
Mean	214.840	145.315	180.964
Standard Error	3.13027	1.41305	2.19484
Median	214.70	145.535	179.874
Mode	.	141.206	193.032
Standard Deviation	19.2963	17.1323	21.2798
Sample Variance	372.347	293.515	452.828
Kurtosis	0.02144	0.16568	-0.345
Skewness	0.57737	0.24056	0.0886
Range	85.07	88.4563	100.381
Minimum	181.20	101.721	132.1
Maximum	266.28	190.178	232.438
Count	38	147	94

Table 3: Descriptive Statistics of Human, Dog, and Cow Central Canal Diameters

Central Canal Diameter (μm)	*Human*	*Dog*	*Cow*
Mean	31.286	18.443	19.302
Standard Error	0.9151	0.2616	0.4120
Median	29.458	17.813	19.208
Mode	22.542	16.271	16.792
Standard Deviation	8.3370	3.6996	4.7520
Sample Variance	69.506	13.687	22.581
Kurtosis	2.9628	-0.592	0.2635
Skewness	1.2789	0.2415	0.0110
Range	49.646	17.854	27.931
Minimum	16.792	9.3125	4.6104
Maximum	66.438	27.167	32.542
Count	83	200	133

Figure 5: Mean Major Osteon Diameter v. Age for Dogs

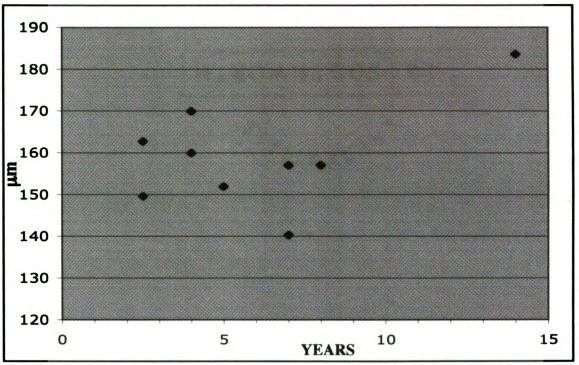


Figure 6: Mean Central Canal Diameter v. Age for Dogs

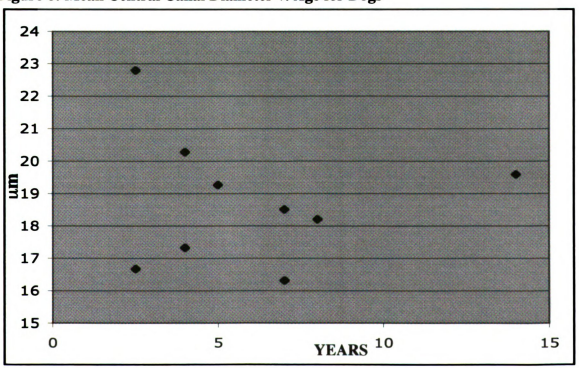


Figure 7: Mean Major Osteon Diameter v. Age for Humans

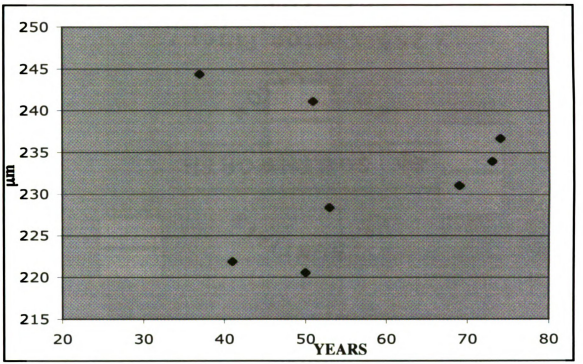


Figure 8: Mean Central Canal Diameter v. Age for Humans

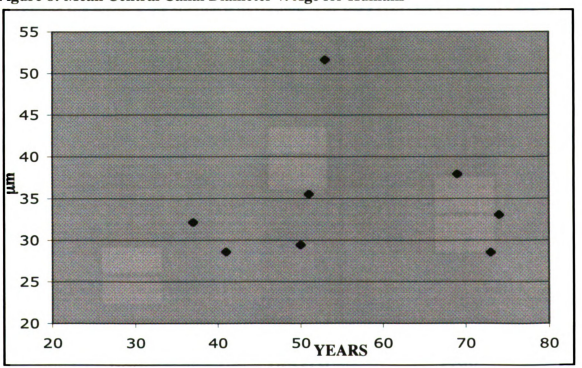


Figure 9: Comparison Human, Dog, and Cow Major Osteon Diameters. The box represents the mean ± the standard deviation. The bars represent the total range.

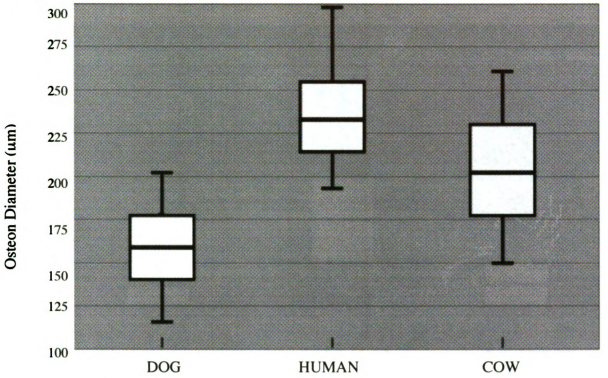


Figure 10: Comparison Human, Dog, and Cow Minor Osteon Diameters. The box represents the mean ± the standard deviation. The bars represent the total range.

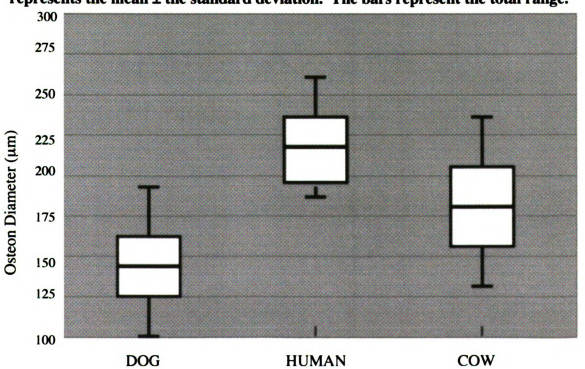


Figure 11: Comparison Human, Dog, and Cow Central Canal Diameters. The box represents the mean ± the standard deviation. The bars represent the total range.

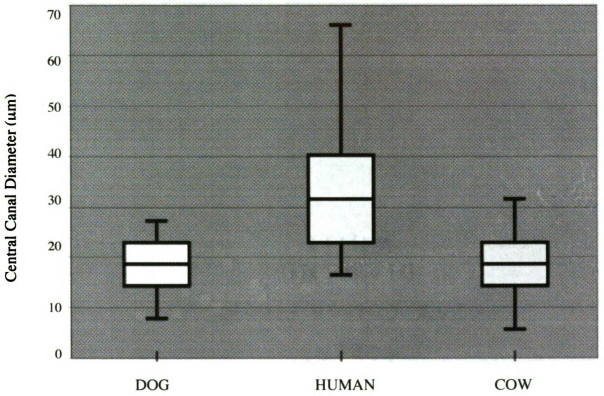


Figure 12: Comparison of Human, Dog, and Cow Major Osteon Diameters and Central Canal Diameters

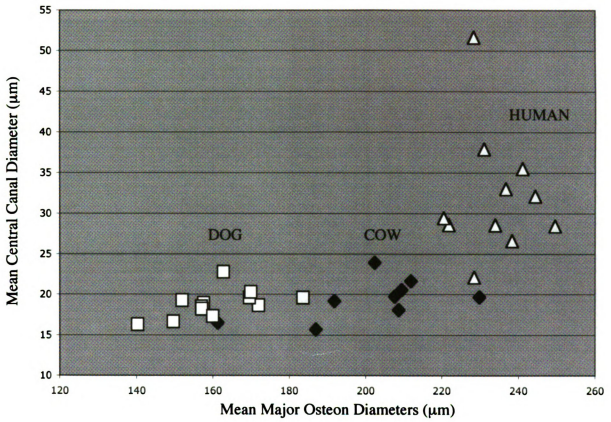
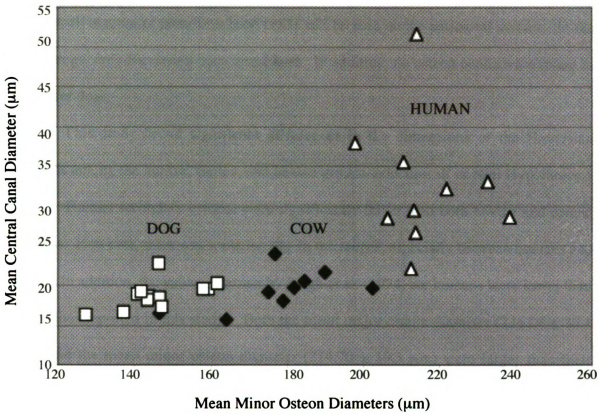


Figure 13: Comparison of Human, Dog, and Cow Minor Osteon Diameters and Central Canal Diameters



Discussion

The two non-human groups selected for this study, dogs and cows each contained dense areas of Haversian bone in their ribs. In addition to Haversian bone, plexiform bone and osteon banding was present in all of the bovine rib samples and the youngest dog ribs. The consistent presence of plexiform bone in bovine sample may have been due to the young ages of the samples and it is possible that in older cattle the plexiform bone could be entirely replaced by Haversian bone. The linear arrangement of osteons into bands is common in the young of species that have rapid areas of growth. With time this arrangement is obliterated due to cortical drift (Enlow 1963; Mulhern and Ubelaker

2001) and the plexiform bone is completely replaced by Haversian bone. This was the pattern seen with the dog ribs. In the youngest canine samples, osteon banding and a very small amount of plexiform bone could still be seen on the periosteal surface. In the older dogs, only Haversian bone was found. In addition, no osteon bands were found in the older dogs.

This study found significant differences in the dimensions of the Haversian systems among the human, canine, and bovine groups, rejecting all of Null Hypotheses 1 and 2. Human secondary osteons were significantly larger than both bovine and canine osteons. However, there was some overlap in the ranges, especially between humans and cows. In addition, the values reported by Singh et al. (1974) for humans were lower than the values reported in this study. Both the mean major osteon diameter (234.08 ± 20.1 μm) and the mean minor osteon diameter (214.70 ± 19.3 μm) were larger than their reported average osteon diameter of 203.2 ± 41.6 μm for humans. The reason for this difference is unknown, and may related to sample size, the sample location, different methods of measurement being used, or the accuracy of this study. In addition, while an osteon is roughly circular, if cut at an oblique angle, it will produce an elliptical shape. This is why two measurements were taken, a maximum diameter that would represent the major axis of the ellipse, and a measurement perpendicular to the first, the minor axis of the ellipse. The minor axis should be closest to the true value of the osteon diameter. However, even if just the minor axis was considered, or if the two measurements were averaged, the values for this study were still larger than those reported elsewhere in the literature. Another concern is that their measurements for human were very close to the values found for this study's bovine sample, which was 204.5 ± 25.9 μm. While this

study found statistically significant differences, in practice the size of the osteons overlapped in humans and in cows, and may overlap in other species as well.

The central canals of humans were significantly larger in humans than in the two non-human groups. In addition, there appeared to be much more variation in the size of the central canals in humans, which is reflected in the much larger standard deviation and range of values found for humans. Under the microscope, the central canals of the dogs and cows appeared much smaller and more regular than the central canals of the human samples. Very rarely were human central canals as small as what was regularly seen in dogs and cows. More research is needed to confirm if the presence of very small, regular central canals may be diagnostic of non-human species.

Overall, the diameter of the central canal appeared to distinguish best between the human and the non-human groups. The two non-human groups had mean central canal diameters that fell below 20μm, while the human mean fell above 30μm. More species need to be measured to see if this pattern holds true for all non-humans. A larger sampling of both humans and non-humans would also allow discriminate function analysis to be performed.

Chapter 5

CONCLUSIONS

Conclusions

Determining if a bone is human or non-human is relatively easy under normal circumstances. However, occasionally bone fragments are recovered that lack gross morphological features, which make using histological or molecular methods of identification necessary. While humans typically have Haversian bone, it is also possible that a fragment of bone may contain only Haversian bone and be non-human. In the past, the studies that have compared the histology of humans with non-humans have focused on weight-bearing bones, like femora (Jowsey 1966; Lackey 2001; Owsley, et al. 1985; Singh, et al. 1974) and with the exception of dogs have found only scattered secondary osteons. Apparently this is due to the stress placed on weight-bearing bone, since primary lamellar bone such as plexiform bone has a much higher tensile strength than remodeled Haversian bone (Currey 1984). In non-weight-bearing bones like ribs, primary forms of bone are more likely to be replaced over time with secondary osteons in a number of non-human species. Thus study demonstrates that in at least two species, cows and dogs, dense Haversian bone is found in the compact bone of ribs. The presence of dense Haversian bone in the rib tissue of non-humans means that determining if a bone fragment is human or non-human is not necessarily straightforward, and methods need to be developed to distinguish between human and non-human secondary osteons.

This study found that the diameter of the secondary osteons and their central canals are much smaller in dogs than those typically found in humans, and may be easily distinguished based on size differences. In contrast, cows have large secondary osteons

that are similar in size to human osteons. However, the central canals of cows are much smaller than those of humans, more similar in size to dogs. Not only were the diameters of the central canals significantly smaller in both dogs and cows, the size of the central canals was much more uniform in both of these species compared to humans. Humans very rarely had central canals as small as those typically seen in the non-human samples. Therefore, the range of sizes seen in the central canal may be the most diagnostic feature for determining if a secondary osteon is human or non-human in origin.

The overlap in the diameter of the osteon between humans and cows demonstrates the importance of measuring secondary osteons in more non-human species that may be encountered in a forensic setting, like deer and bear. More research involving larger samples and more non-human species may also allow statistical methods to be developed to distinguish between humans and non-humans.

The results of this study suggest a number of other issues that still need to be researched. First of all, the characteristics of Haversian bone in non-weight bearing bone in other species need to be described, especially in larger species like bear and deer. While plexiform bone may be found within non-weight bearing bones, even subadults may still have large areas of Haversian bone. There is no guarantee that an unknown bone fragment will contain the best diagnostic histological features. Walter, et al. (2004) have demonstrated that other features, like the number of osteocytes per osteon, are additional variables that can help with species identification. Additional research with a larger sample of cows, dogs, and other species is needed in order to develop standards to which unknowns could be compared. One drawback of this study was the use of human

rib tissue as a comparison to non-humans; the cortical bone in human ribs is very thin and contained very few osteons in general for comparison.

This study was designed to give anthropologists another tool with which to distinguish between human and non-human bone fragments. It demonstrates that in addition to qualitative characteristics like the presence of plexiform bone and osteonal banding, quantitative characteristics of the secondary osteons, especially the measurement of the central canal, may allow an anthropologist to distinguish between some human and non-human bone fragments.

APPENDICES

APPENDIX A

Non-Parametric Test Results

Table 4: Comparison of Dog and Human Major Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Major Osteon Diameter Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	148	11042.0	15540.0	397.478253	74.608108
Human	61	10903.0	6405.0	397.478253	178.737705
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	10903.0000
Normal Approximation	
Z	11.3151
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	128.0596
DF	1
Pr > Chi-Square	<.0001

Table 5: Comparison of Dog and Human Mean Major Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Mean Major Osteon Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	12	78.0	144.0	16.248077	6.50
Human	11	198.0	132.0	16.248077	18.00

Wilcoxon Two-Sample Test	
Statistic	198.0000
Normal Approximation	
Z	4.0312
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	0.0003
Two-Sided Pr > |Z|	0.0006

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test	
Chi-Square	16.5000
DF	1
Pr > Chi-Square	<.0001

Table 6: Comparison of Dog and Human Minor Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Minor Osteon Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	148	11036.0	13912.0	300.710970	74.567568
Human	39	6542.0	3666.0	300.710970	167.743590

Wilcoxon Two-Sample Test	
Statistic	6542.0000
Normal Approximation	
Z	9.5623
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test	
Chi-Square	91.4701
DF	1
Pr > Chi-Square	<.0001

Table 7: Comparison of Dog and Human Minor Mean Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Minor Osteon Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	12	78.0	138.0	15.165750	6.50
Human	11	175.0	115.0	15.165751	17.50

Wilcoxon Two-Sample Test	
Statistic	175.0000
Normal Approximation	
Z	3.9233
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	0.0004
Two-Sided Pr > |Z|	0.0008

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test	
Chi-Square	15.6522
DF	1
Pr > Chi-Square	<.0001

Table 8: Comparison of Dog and Human Central Canal Diameters

Wilcoxon Scores (Rank Sums) for Variable Canal Diameter Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	200	20856.50	28400.0	626.584070	104.282500
Human	83	19329.50	11786.0	626.584070	232.885542
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	19329.5000
Normal Approximation	
Z	12.0383
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	144.9396
DF	1
Pr > Chi-Square	<.0001

Table 9: Comparison of Human and Dog Mean Central Canal Diameters

Wilcoxon Scores (Rank Sums) for Variable Mean Central Canal Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Dog	12	79.0	144.0	16.248077	6.583333
Human	11	197.0	132.0	16.248077	17.909091

Wilcoxon Two-Sample Test	
Statistic	197.0000
Normal Approximation	
Z	3.9697
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	0.0003
Two-Sided Pr > |Z|	0.0006

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test	
Chi-Square	16.0038
DF	1
Pr > Chi-Square	<.0001

Table 10: Comparison of Cow and Human Major Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Major Osteon Diameter Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	95	5624.0	7457.50	275.349881	59.200000
Human	61	6622.0	4788.50	275.349881	108.557377
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	6622.0000
Normal Approximation	
Z	6.6570
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	44.3396
DF	1
Pr > Chi-Square	<.0001

Table 11: Comparison of Cow and Human Mean Major Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Mean Major Osteon Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	9	49.0	94.50	13.162447	5.444444
Human	11	161.0	115.50	13.162447	14.636364

Wilcoxon Two-Sample Test	
Statistic	49.0000
Normal Approximation	
Z	-3.4188
One-Sided Pr < Z	0.0003
Two-Sided Pr > |Z|	0.0006
t Approximation	
One-Sided Pr < Z	0.0014
Two-Sided Pr > |Z|	0.0029
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	11.9495
DF	1
Pr > Chi-Square	0.0005

Table 12: Comparison of Cow and Human Minor Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Minor Osteon Diameters Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	95	4991.50	6412.50	204.159355	52.542105
Human	39	4053.50	2632.50	204.159355	103.935897
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	4053.5000
Normal Approximation	
Z	6.9578
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	48.4451
DF	1
Pr > Chi-Square	<.0001

Table 13: Comparison of Cow and Human Mean Minor Osteon Diameters

Wilcoxon Scores (Rank Sums) for Variable Mean Minor Osteon Diameter Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	9	46.0	90.0	12.247449	5.111111
Human	11	144.0	100.0	12.247449	14.400000
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	46.0000
Normal Approximation	
Z	-3.5518
One-Sided Pr > Z	0.0002
Two-Sided Pr > |Z|	0.0004
t Approximation	
One-Sided Pr > Z	0.0011
Two-Sided Pr > |Z|	0.0023
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	12.9067
DF	1
Pr > Chi-Square	0.0003

Table 14: Comparison of Cow and Human Central Canal Diameters

Wilcoxon Scores (Rank Sums) for Variable Central Canal Classified by Variable Group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	131	9496.0	14082.50	441.292532	72.488550
Human	83	13509.0	8922.50	441.292532	162.759036
Average scores were used for ties.					

Wilcoxon Two-Sample Test	
Statistic	13509.0000
Normal Approximation	
Z	10.3922
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
Z includes a continuity correction of 0.5.	

Kruskal-Wallis Test	
Chi-Square	108.0214
DF	1
Pr > Chi-Square	<.0001

Table 15: Comparison of Cow and Human Mean Central Canal Diameters

Wilcoxon Scores (Rank Sums) for Variable Mean Central Canal Diameter Classified by Variable group					
Group	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Cow	9	46.0	94.50	13.162447	5.111111
Human	11	164.0	115.50	13.162447	14.909091

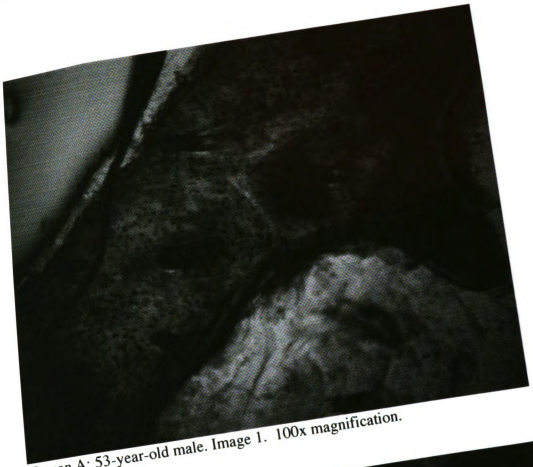
Wilcoxon Two-Sample Test	
Statistic	46.0000
Normal Approximation	
Z	-3.6467
One-Sided Pr < Z	0.0001
Two-Sided Pr > |Z|	0.0003
t Approximation	
One-Sided Pr < Z	0.0009
Two-Sided Pr > |Z|	0.0017

Z includes a continuity correction of 0.5.

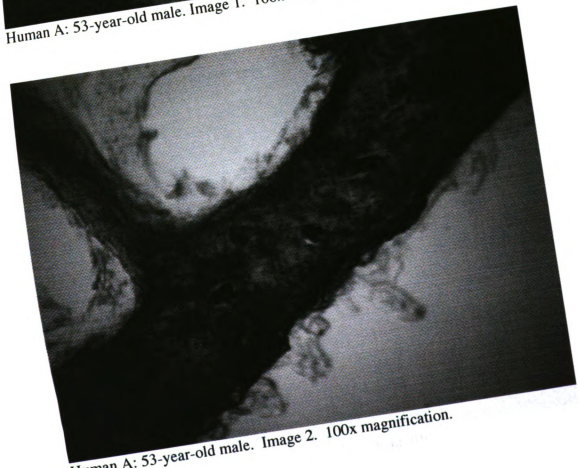
Kruskal-Wallis Test	
Chi-Square	13.5772
DF	1
Pr > Chi-Square	0.0002

APPENDIX B

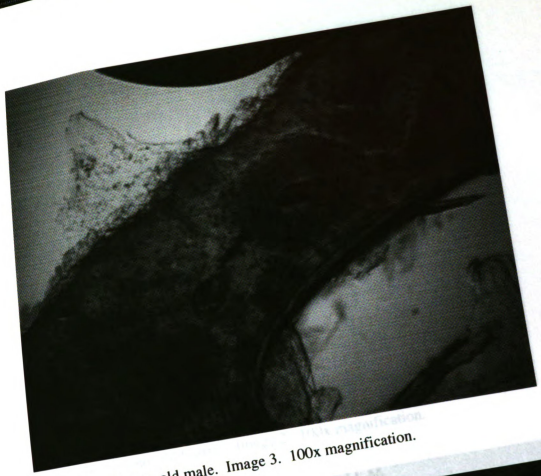
Human Rib Images



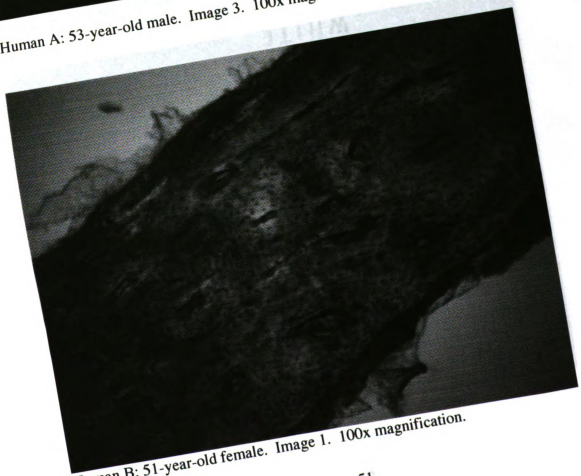
Human A: 53-year-old male. Image 1. 100x magnification.



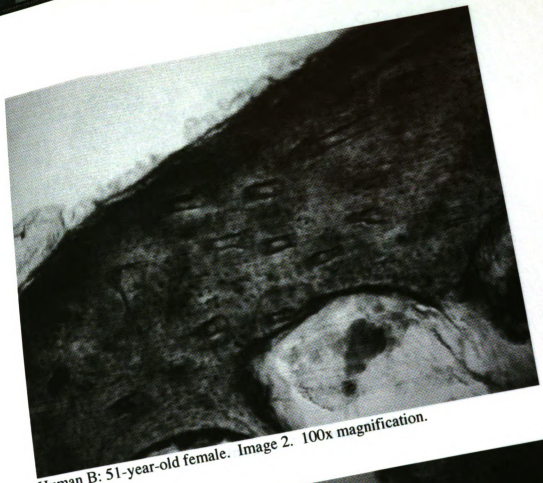
Human A: 53-year-old male. Image 2. 100x magnification.



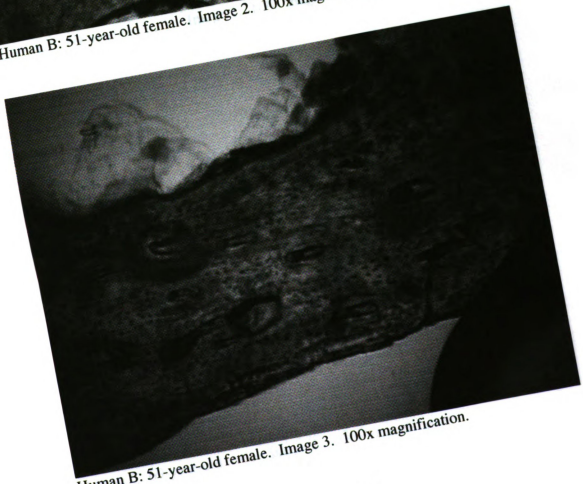
Human A: 53-year-old male. Image 3. 100x magnification.



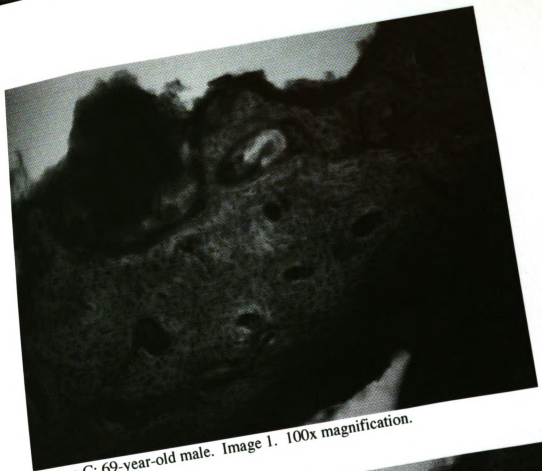
Human B: 51-year-old female. Image 1. 100x magnification.



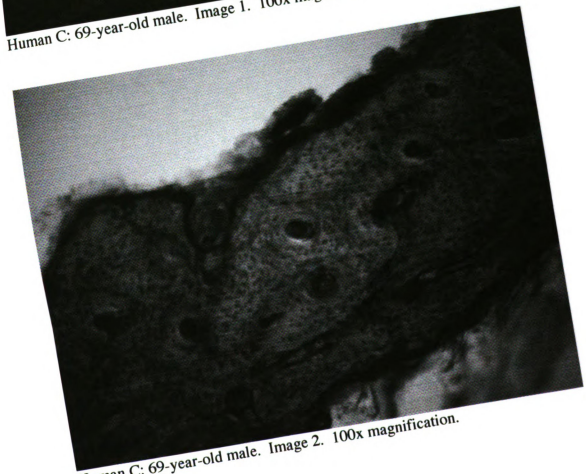
Human B: 51-year-old female. Image 2. 100x magnification.



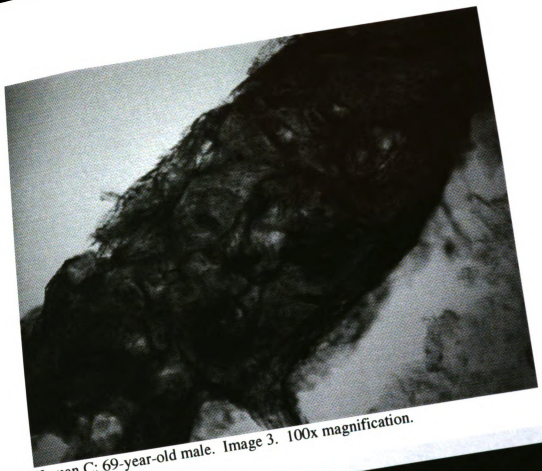
Human B: 51-year-old female. Image 3. 100x magnification.



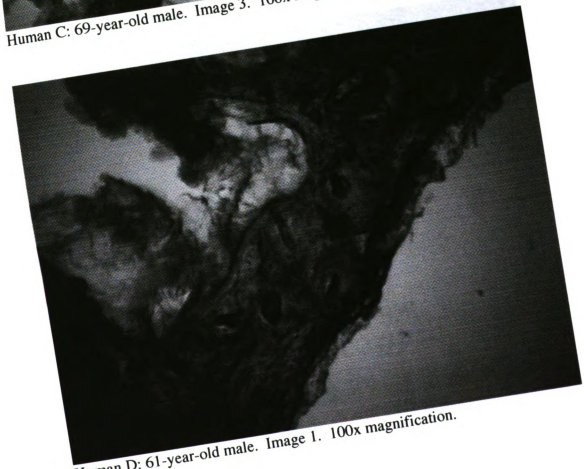
Human C: 69-year-old male. Image 1. 100x magnification.



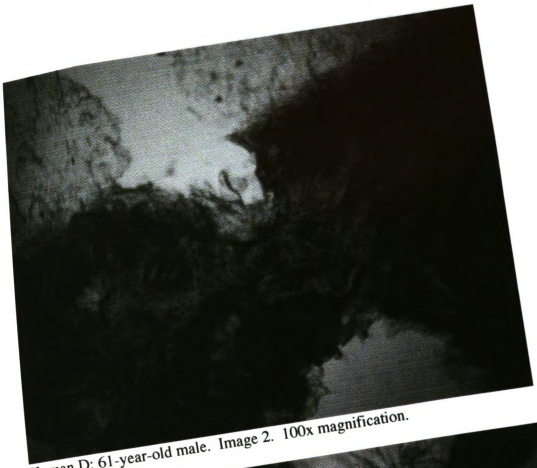
Human C: 69-year-old male. Image 2. 100x magnification.



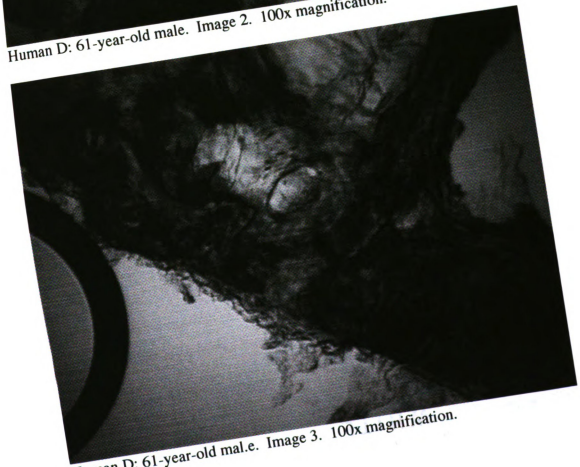
Human C: 69-year-old male. Image 3. 100x magnification.



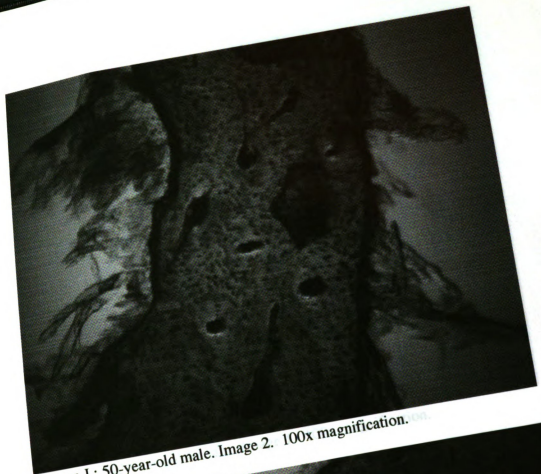
Human D: 61-year-old male. Image 1. 100x magnification.



Human D: 61-year-old male. Image 2. 100x magnification.



Human D: 61-year-old mal.e. Image 3. 100x magnification.



Human J : 50-year-old male. Image 2. 100x magnification.

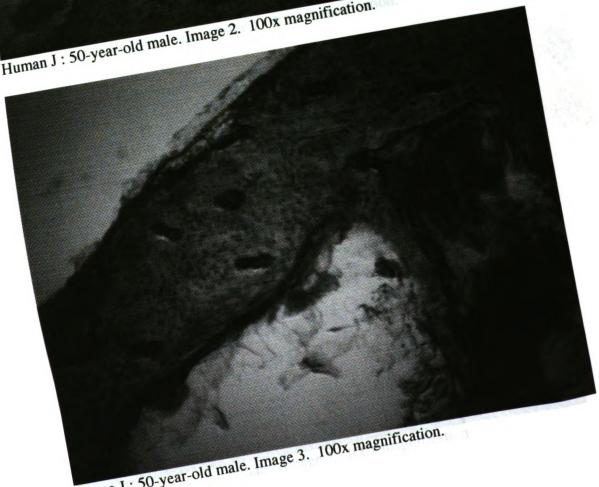
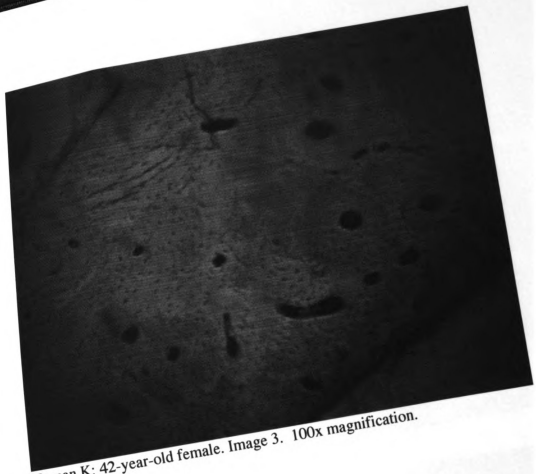


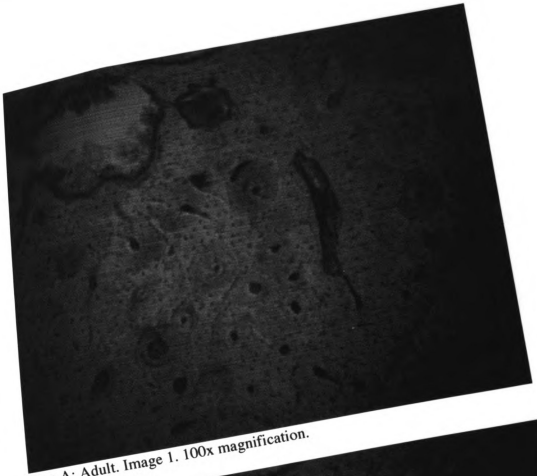
Image J : 50-year-old male. Image 3. 100x magnification.



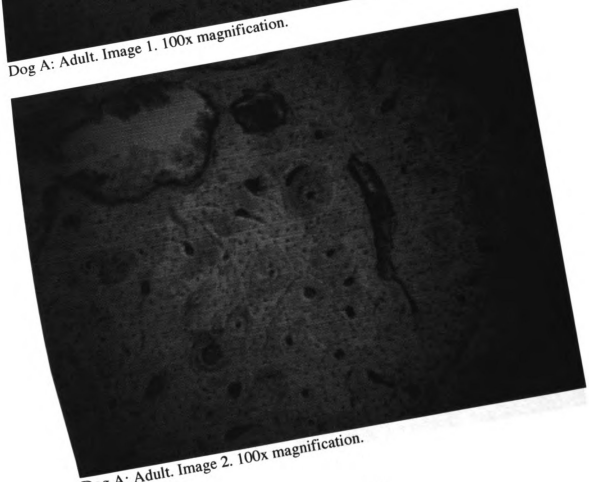
Human K: 42-year-old female. Image 3. 100x magnification.

APPENDIX C

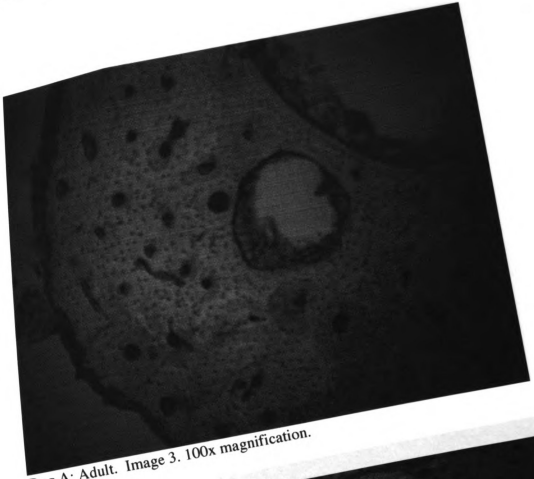
Dog Rib Images



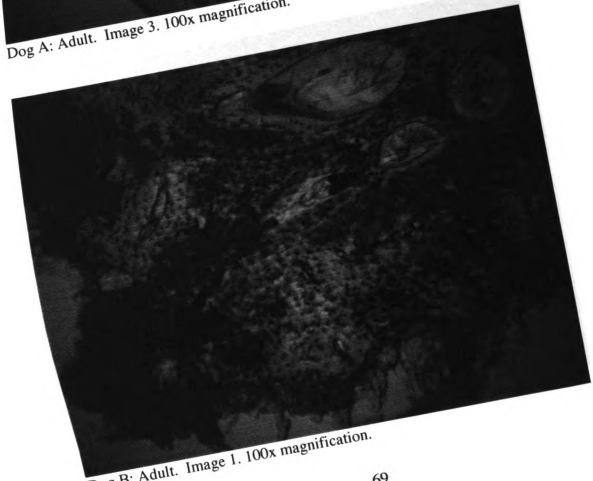
Dog A: Adult. Image 1. 100x magnification.



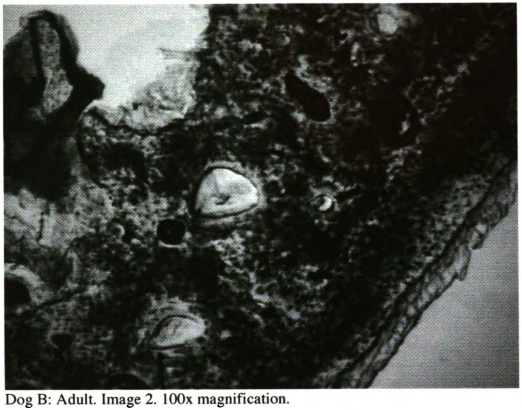
Dog A: Adult. Image 2. 100x magnification.



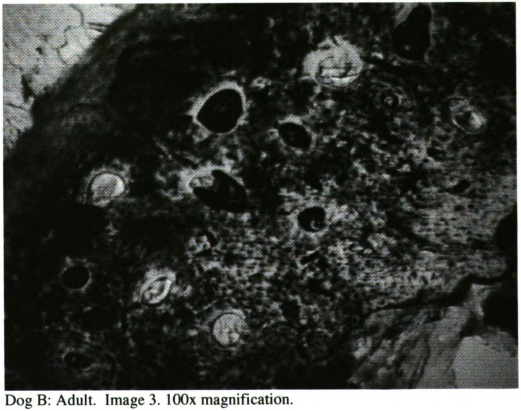
Dog A: Adult. Image 3. 100x magnification.



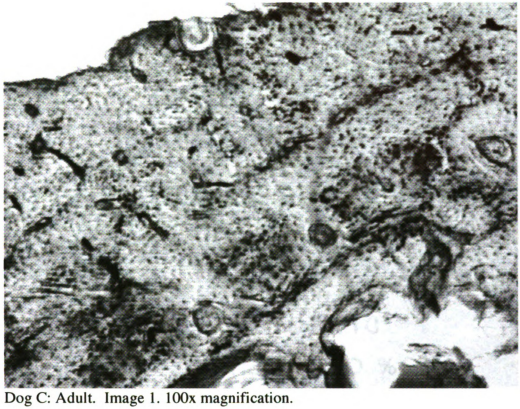
Dog B: Adult. Image 1. 100x magnification.



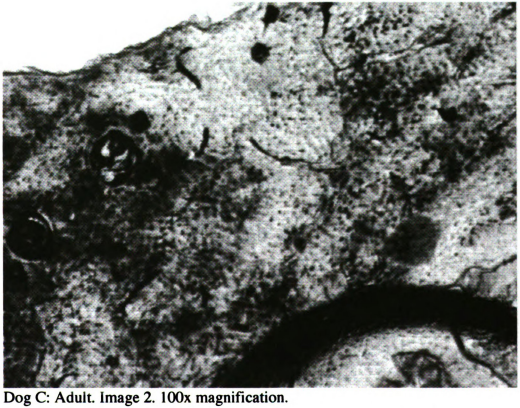
Dog B: Adult. Image 2. 100x magnification.



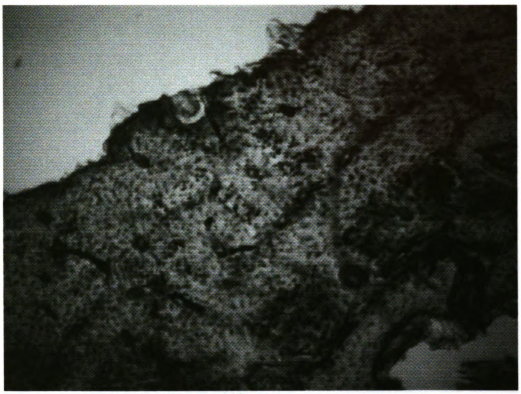
Dog B: Adult. Image 3. 100x magnification.



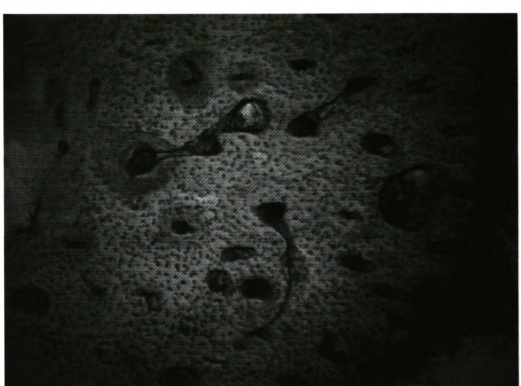
Dog C: Adult. Image 1. 100x magnification.



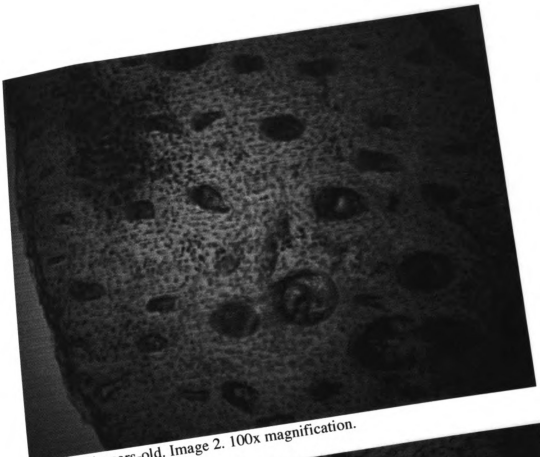
Dog C: Adult. Image 2. 100x magnification.



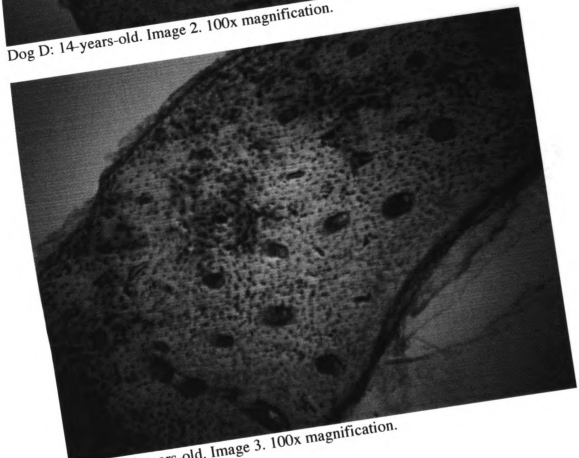
Dog C: Adult. Image 3. 100x magnification.



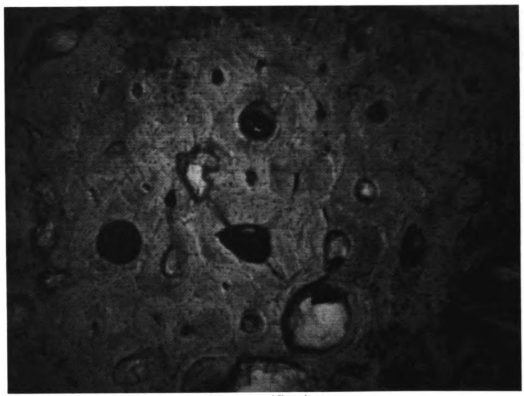
Dog D: 14-years-old. Image 1. 100x magnification.



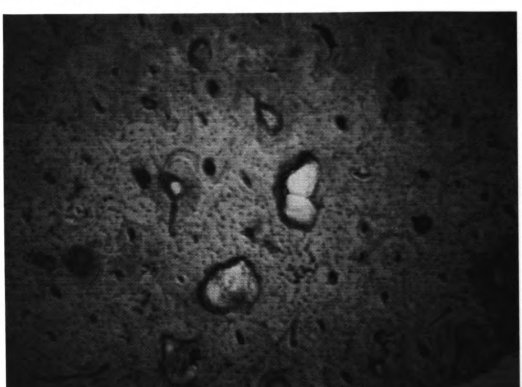
Dog D: 14-years-old. Image 2. 100x magnification.



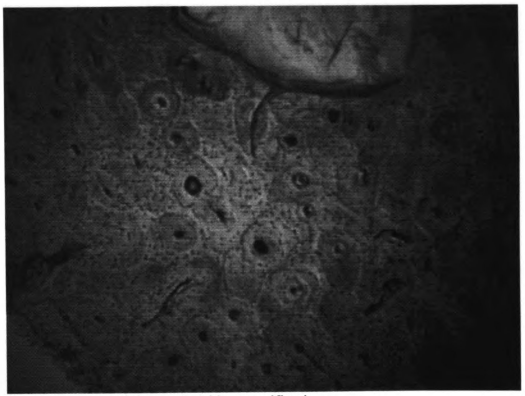
Dog D: 14-years-old. Image 3. 100x magnification.



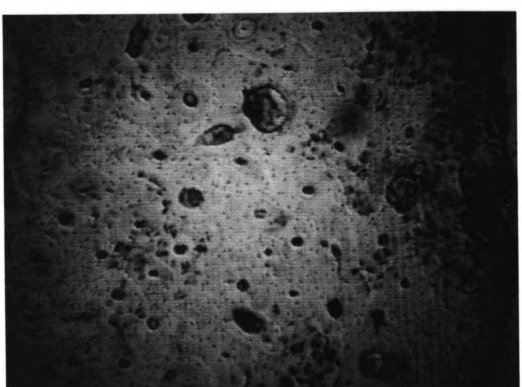
Dog E: 7-years-old. Image 1. 100x magnification.



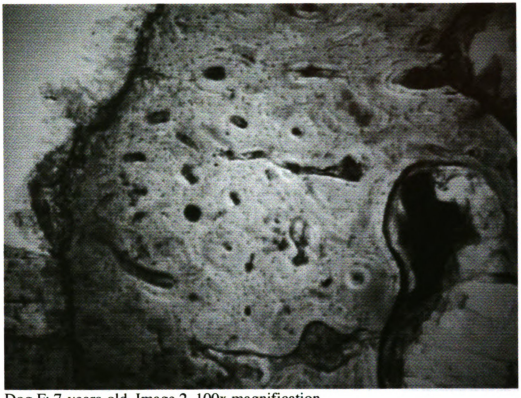
Dog E: 7-years old. Image 2. 100x magnification.



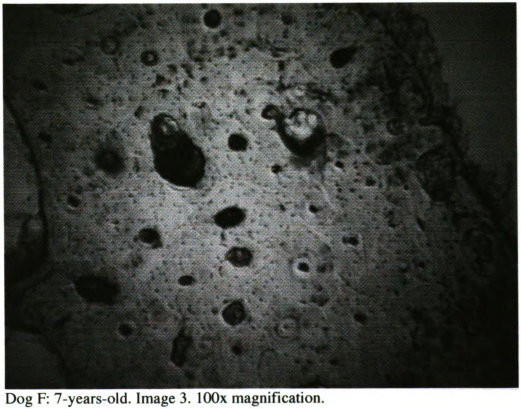
Dog E: 7-years-old. Image 3. 100x magnification.



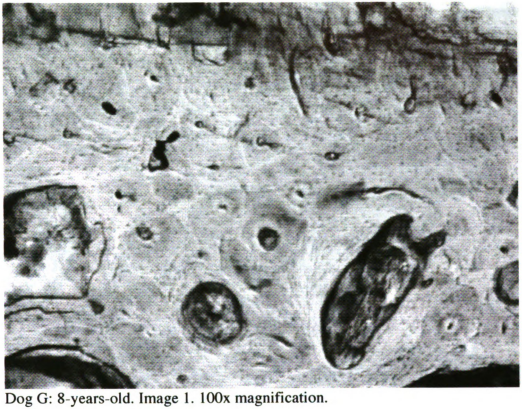
Dog F: 7-years-old. Image 1. 100x magnification.



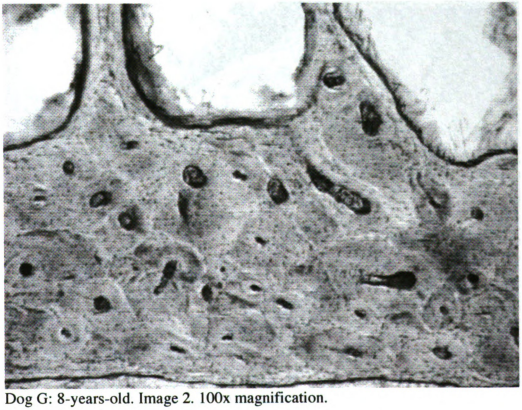
Dog F: 7-years-old. Image 2. 100x magnification.



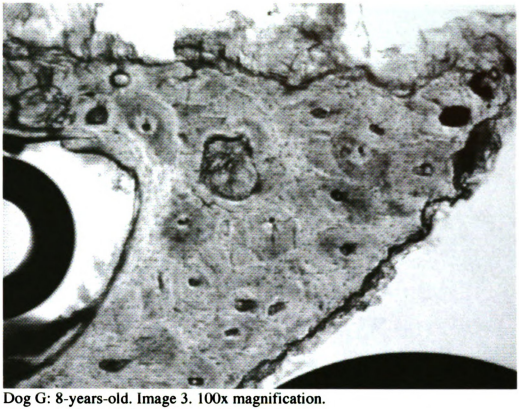
Dog F: 7-years-old. Image 3. 100x magnification.



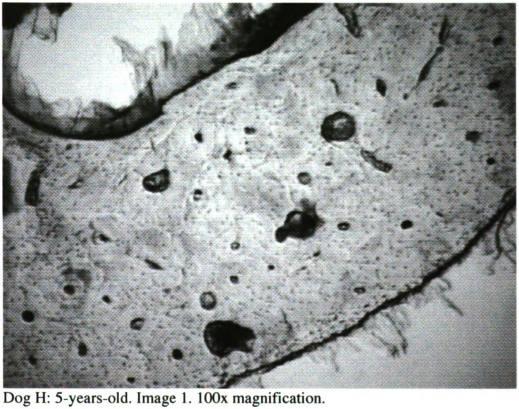
Dog G: 8-years-old. Image 1. 100x magnification.



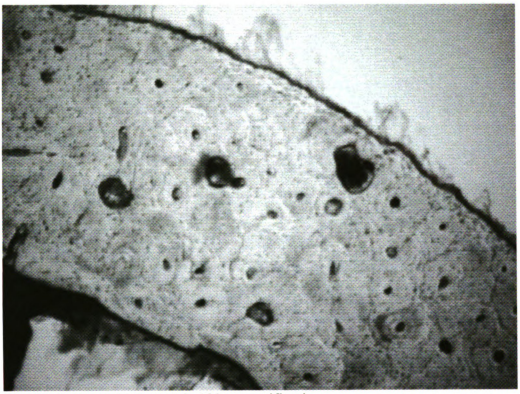
Dog G: 8-years-old. Image 2. 100x magnification.



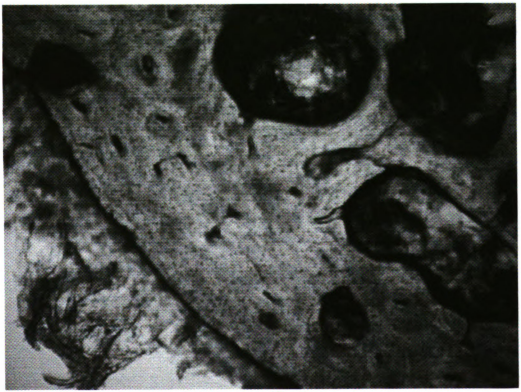
Dog G: 8-years-old. Image 3. 100x magnification.



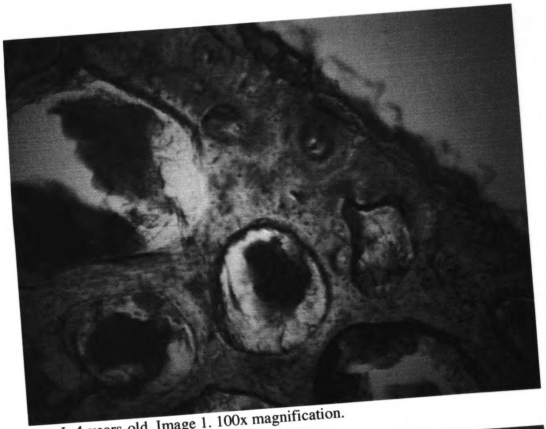
Dog H: 5-years-old. Image 1. 100x magnification.



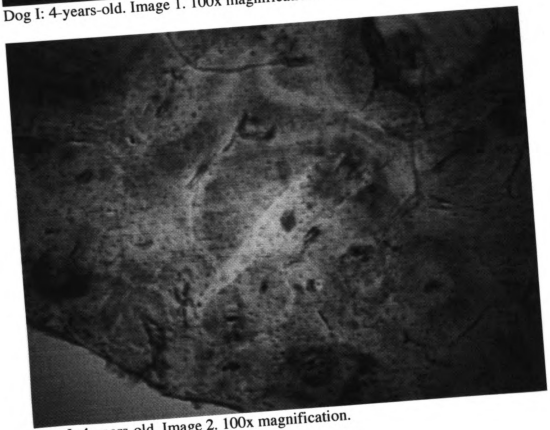
Dog H: 5-years-old. Image 2. 100x magnification.



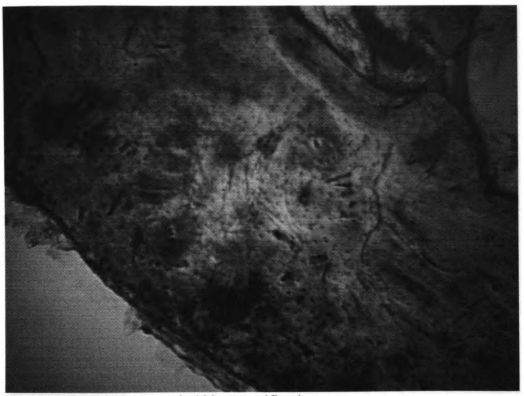
Dog H: 5-years-old. Image 3. 100x magnification.



Dog I: 4-years-old. Image 1. 100x magnification.

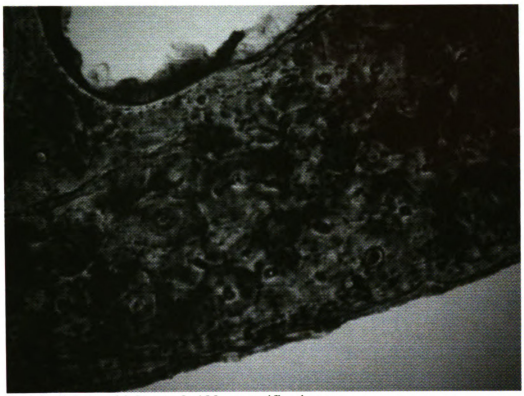


Dog I: 4-years-old. Image 2. 100x magnification.

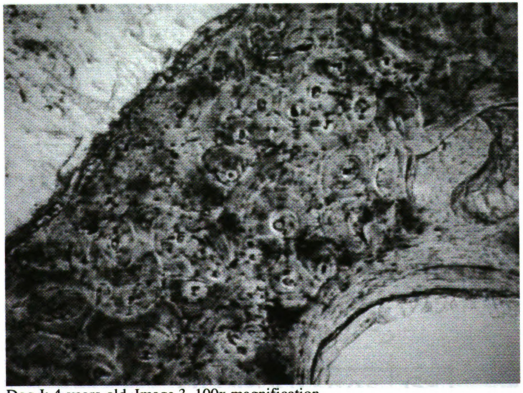


Dog I: 4-years-old. Image 3. 100x magnification.

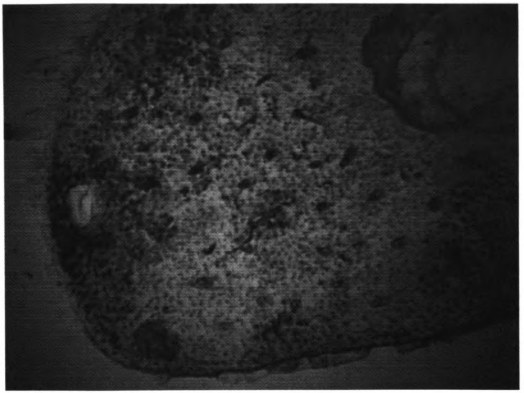
Dog J: 4-years-old. Image 1. 100x magnification.



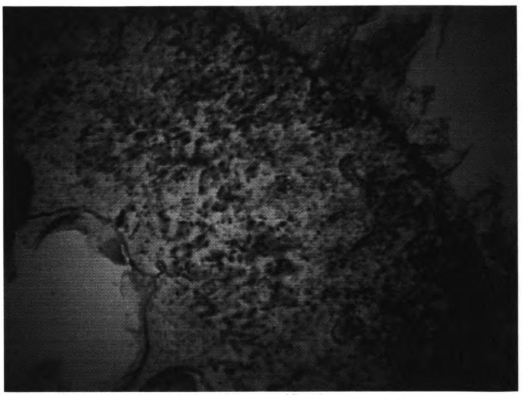
Dog J: 4-years-old. Image 2. 100x magnification.



Dog J: 4-years-old. Image 3. 100x magnification.

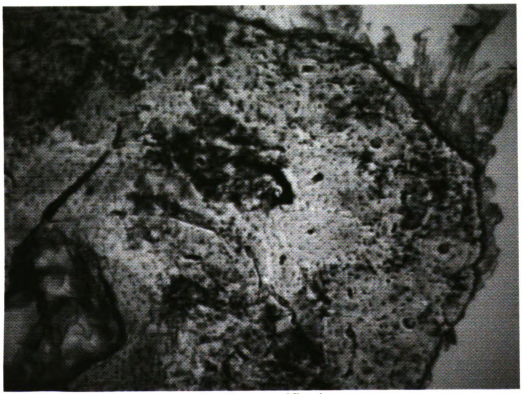


Dog K: 2.5-years-old. Image 1. 100x magnification.

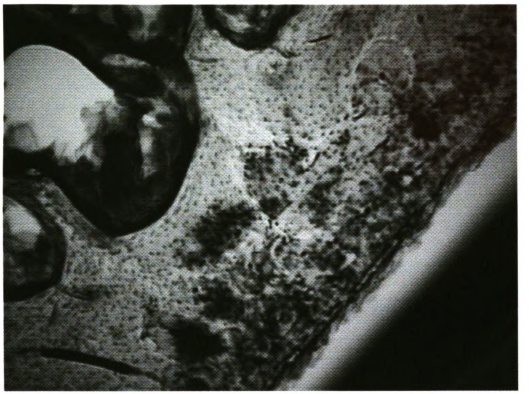


Dog K: 2.5-years-old. Image 2. 100x magnification.

Dog M: 2.5-years-old. Image 1. 100x magnification.



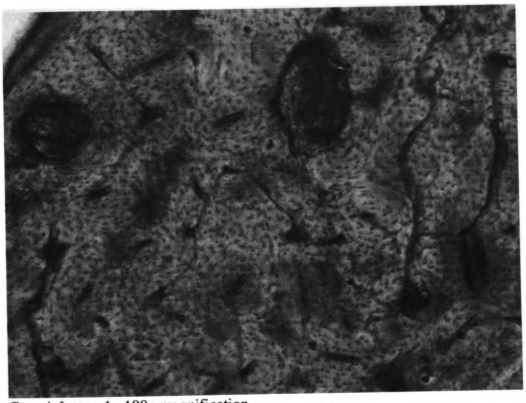
Dog M: 2.5-years-old. Image 2. 100x magnification.



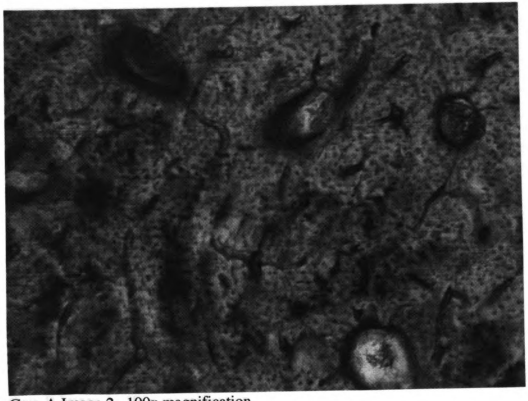
Dog M: 2.5-years-old. Image 3. 100x magnification.

APPENDIX D

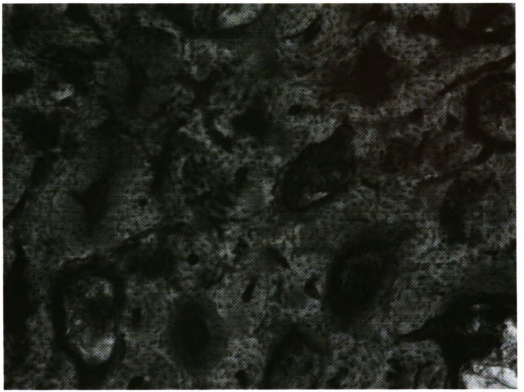
Cow Rib Images



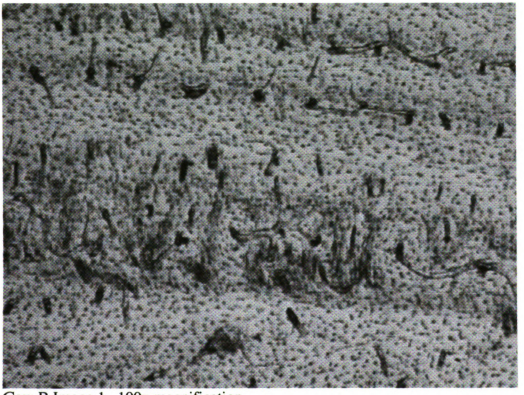
Cow A Image 1. 100x magnification.



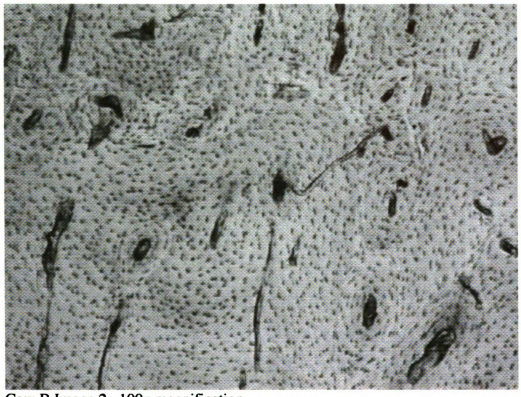
Cow A Image 2. 100x magnification.



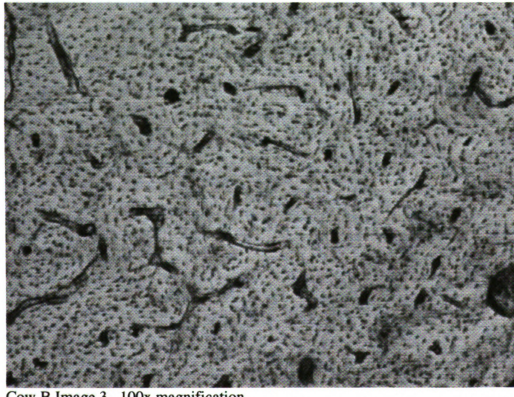
Cow A Image 3. 100x magnification.



Cow B Image 1. 100x magnification.



Cow B Image 2. 100x magnification.



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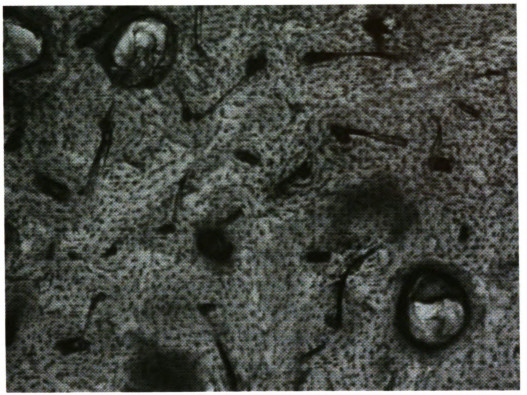

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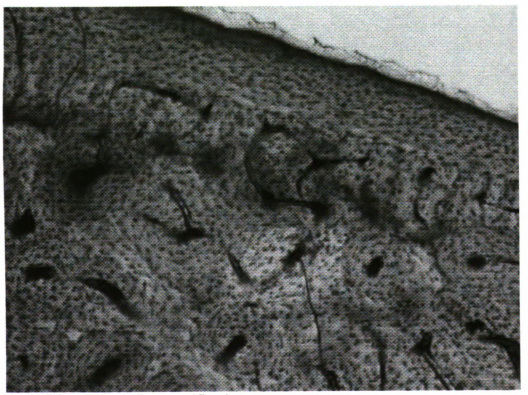

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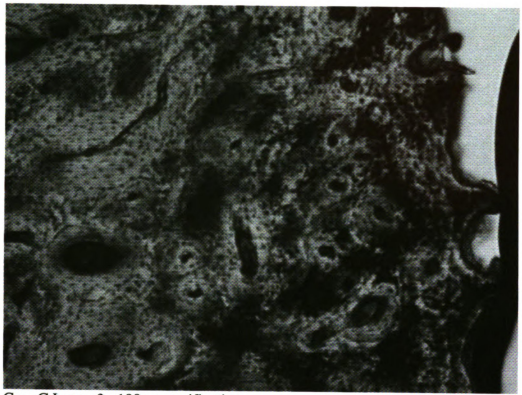

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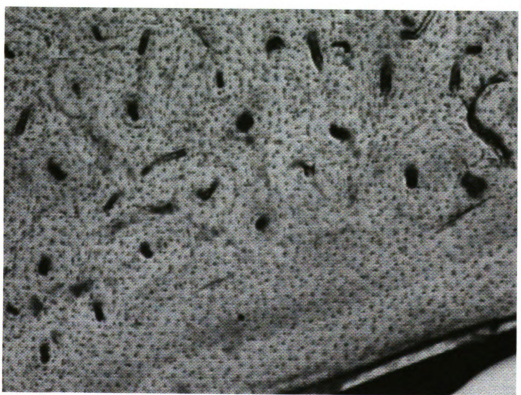

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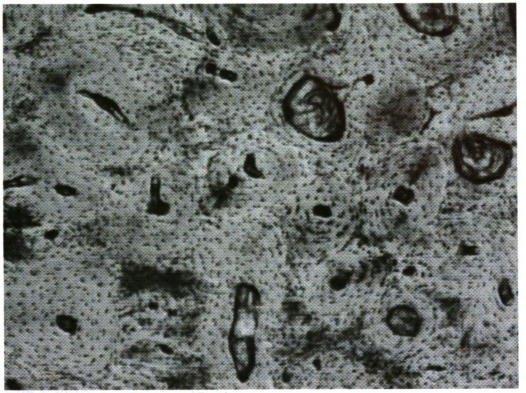

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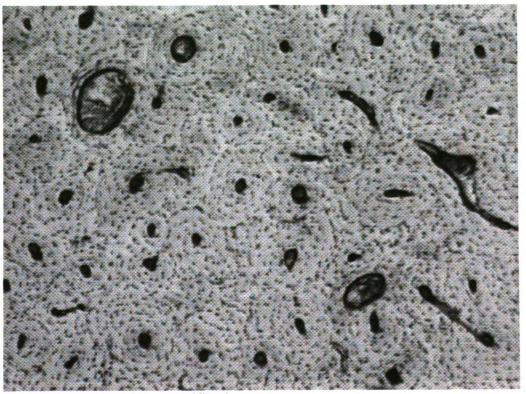

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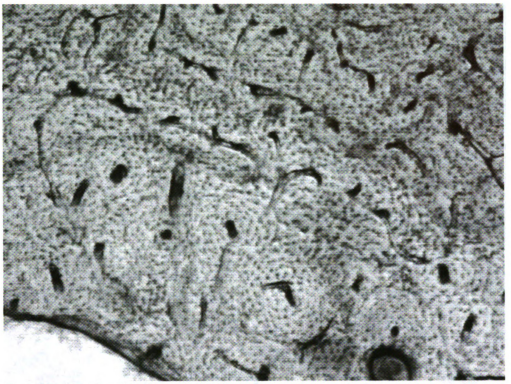

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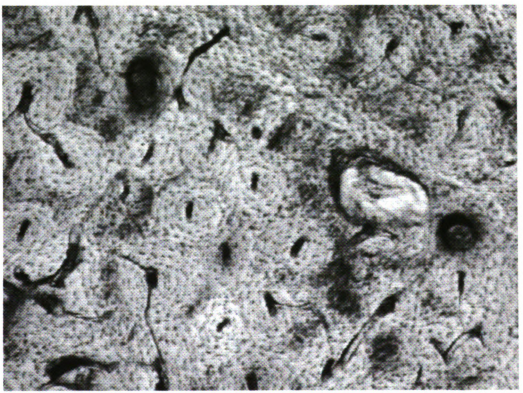

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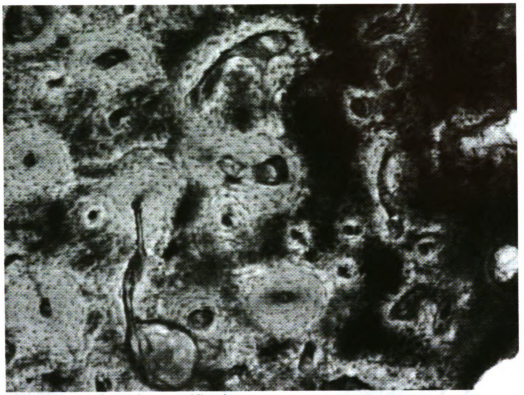

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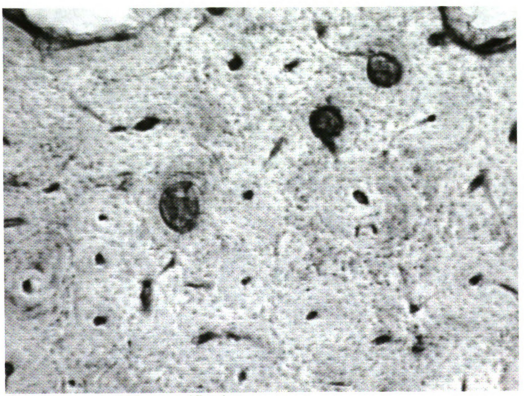

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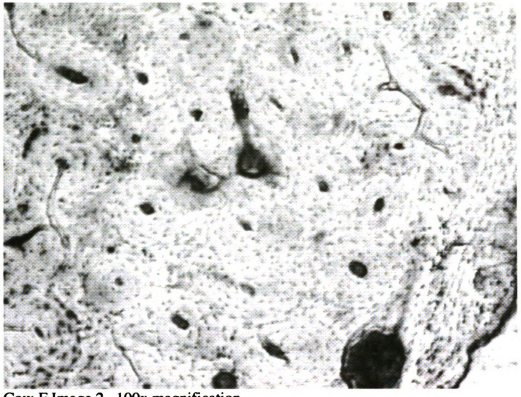

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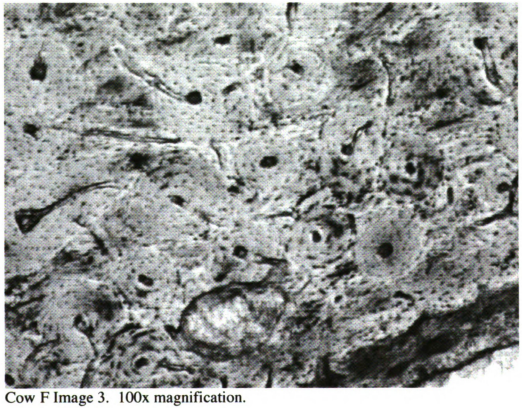

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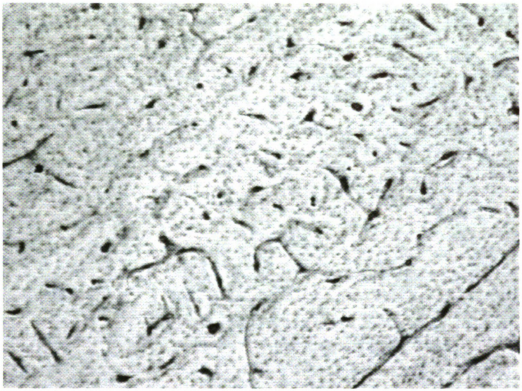

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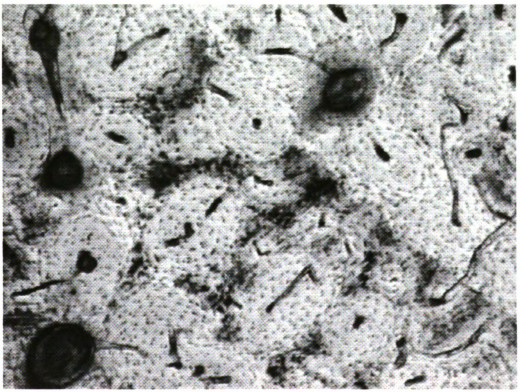

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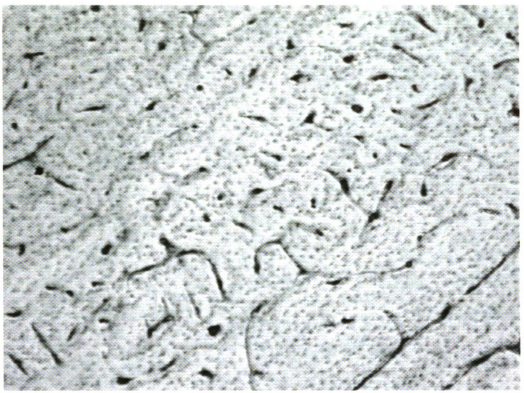

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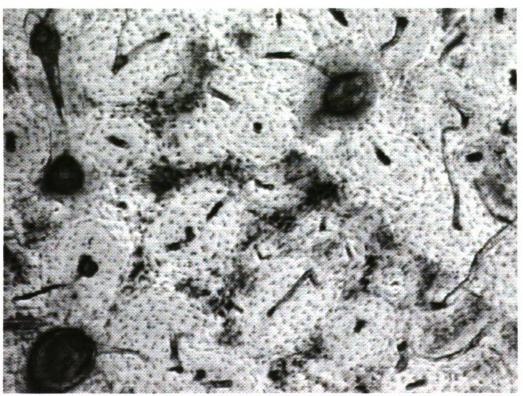

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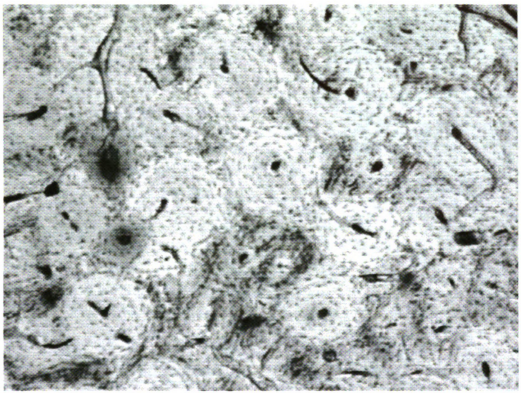

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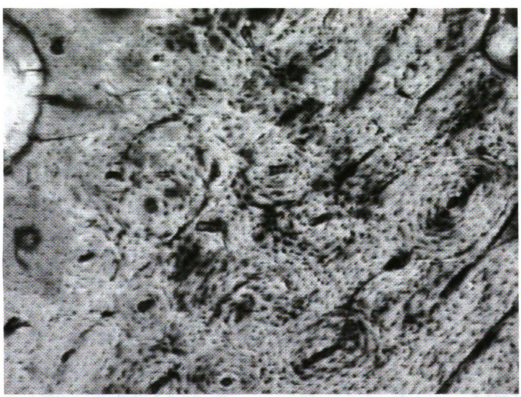

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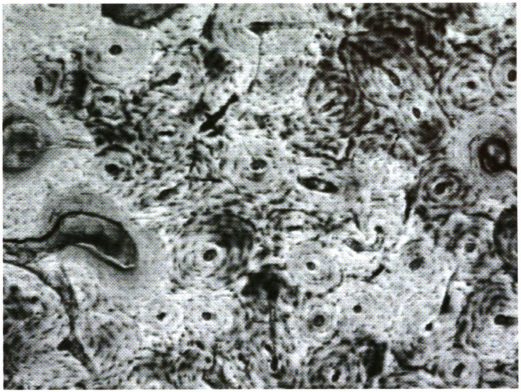

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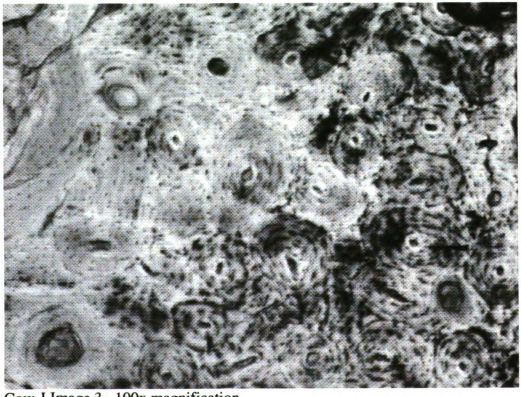

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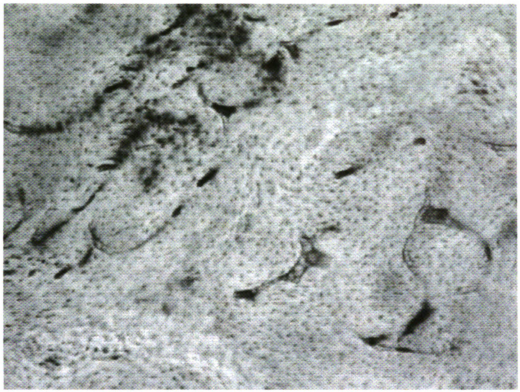

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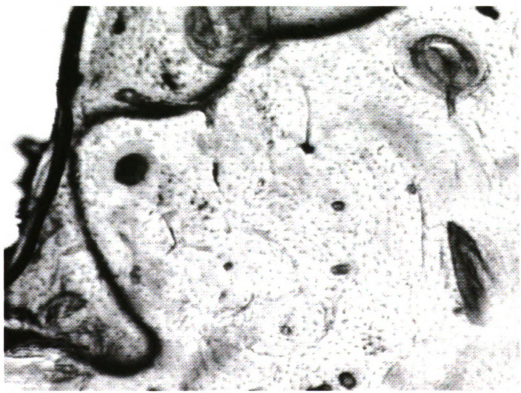

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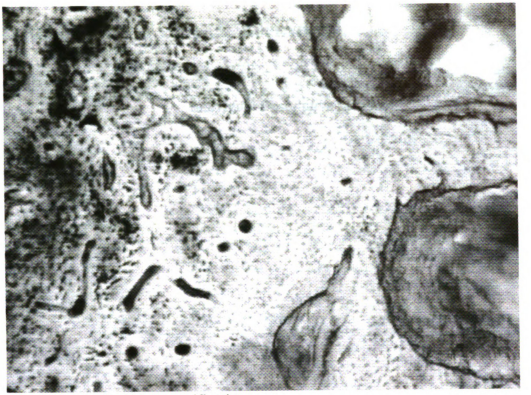

Cow K Image 3. 100x magnification.

APPENDIX E

Individual Descriptive Statistics

Table 16: Average and Standard Deviation of Each Sample.

Sample	Count	Major Axis	Minor Axis	Central Canal
Human A	5	228.4 ± 18.7	214.2 ± 12.3	51.6 ± 9.2
Human B	5	241.1 ± 12.8	210.3 ± 23.6	35.5 ± 1.4
Human C	11	231.0 ± 14.4	197.9 ± 13.9	37.9 ± 5.0
Human D	9	234.0 ± 27.4	238.4 ± 15.2	28.6 ± 3.9
Human E	5	244.4 ± 24.8	221.9 ± 21.0	32.2 ± 6.9
Human F	4	221.9 ± 13.8	214.8 ± 20.6	28.6 ± 0.8
Human G	8	236.7 ± 25.8	232.7 ± 29.1	33.0 ± 4.8
Human H	7	220.5 ± 23.8	213.4 ± 25.5	29.4 ± 4.4
Human I	10	228.5 ± 8.6	212.6 ± 12.1	22.1 ± 2.2
Human J	11	238.3 ± 10.7	213.8 ± 15.7	26.6 ± 5.1
Human K	10	249.5 ± 26.0	206.4 ± 14.6	28.4 ± 5.3
Dog A	22	157.6 ± 12.0	140.8 ± 8.09	18.9 ± 3.7
Dog B	8	172.0 ± 7.9	143.8 ± 13.5	18.7 ± 4.3
Dog C	16	169.6 ± 11.6	159.3 ± 13.3	19.6 ± 3.2
Dog D	14	183.6 ± 18.8	158.0 ± 19.1	19.6 ± 3.6
Dog E	20	140.3 ± 18.0	127.2 ± 16.3	16.3 ± 3.3
Dog F	24	157.0 ± 15.0	146.5 ± 14.3	18.5 ± 3.3
Dog G	20	157.1 ± 16.9	143.4 ± 15.3	18.2 ± 3.2
Dog H	25	151.9 ± 13.2	141.7 ±12.5	19.3 ± 4.2
Dog I	6	169.9 ± 21.6	161.7 ± 22.3	20.3 ± 4.3
Dog J	28	160.0 ± 18.1	147.1 ±16.2	17.3 ± 3.3
Dog K	7	162.8 ± 12.3	146.3 ± 16.3	22.8 ± 4.0
Dog M	15	149.6 ± 17.2	137.1 ± 16.2	16.7 ± 3.0
Cow A	16	186.9 ± 20.9	164.1 ± 15.1	15.7 ± 5.6
Cow B	6	161.3 ± 9.8	146.5 ± 16.4	16.5 ± 2.0
Cow C	17	208.7 ± 28.9	179.0 ± 19.3	18.1 ± 3.8
Cow D	22	191.8 ± 22.4	175.2 ± 15.5	19.2 ± 4.1
Cow E	15	209.5 ± 22.0	184.6 ± 20.2	20.5 ± 3.5
Cow F	26	212.0 ± 17.5	190.0 ± 12.7	21.7 ± 4.6
Cow H	13	229.8 ± 12.4	202.5 ± 17.1	19.7 ± 2.7
Cow J	12	207.7 ± 25.4	181.9 ± 23.1	19.7 ± 5.3
Cow K	5	202.4 ± 24.8	180.9 ± 15.7	23.9 ± 3.5

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