To the Graduate Council:

I am submitting herewith a thesis written by Mariateresa Anne Tersigni entitled "Frozen Human Bone: A Histological Investigation." I have examined the final electronic copy of this thesis for form and content and recommended that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.

Murray K Marks

We have read this thesis
and recommend its acceptance:

William M. Bass

Lyle W. Konigsberg

Accepted for the Council:

Anne Mayhew
Vice Provost and Dean of Graduate Studies

(Original signatures are on file with official student records.)

FROZEN HUMAN BONE:
A HISTOLOGICAL INVESTIGATION

A Thesis
Presented for the
Master of Arts
Degree
The University of Tennessee, Knoxville

Mariateresa Anne Tersigni
August 2002

Dedication

Vorrei dedicare quest'opera ai miei nonni: Mario e Maria Pelino e Bertha ed Andrea Tersigni ed alla mia bisnonna Angela Petrilli, i quali mi hanno insegnato che per realizzare tutti i propri sogni non occorrerà altro che lavorare sodo e con dedizione, contornata dall'amore infinito dei propri familiari.

I would also like to dedicate this work to the strongest women that I have ever met: Flora P. Tersigni, Amy R. Motyka, and Roseanne Stearns. The three of you have been a source of constant support and inspiration to me. You have taught me how to gather strength in difficult situations and to believe in myself. You may never know how much of your spirit and perseverance has become a part of me, but I hope one day I can help you half as much as you have helped me.

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Abstract

A plethora of research has been produced concerning the fate of human bone when it has been exposed to various external stressors. These include taphonomic processes such as natural weathering, decomposition and burning studies. Each study attempts to aid an investigator by trying to determine, for example, what exactly a human skeleton would look like after it had been exposed to an accelerated fire for thirty minutes. The result of this type of research may afford an investigator the opportunity to analyze the evidence at the crime scene and compare it to the research in order to draw educated conclusions about the actual evidence. This is not to imply that all of the questions of investigators have been answered. Rather, each new case brings more questions and more variables that need to be taken into consideration.

One such question asks if there is a way to decipher whether a body or body parts have been previously frozen by looking at the skeletal elements. The circumstances that surround this question suggest that the victim would have been frozen for some time and was taken out of the freezing element and was allowed to thaw and decompose to the state of skeletonization. Would it be possible at this point to determine whether or not this body had indeed been frozen? Or, at a more basic level, does freezing damage the histological integrity of bone to prevent osteon aging ability.

Few studies incorporate both the variable of low temperatures and human remains. However, two such studies focus on the decomposition of the soft tissue after a freeze thaw event, but none have focused on the bone itself. While there is no evidence that freezing can change the gross morphology, there has been no research concerning whether or not the microscopic structure of the bone would be altered as a result of the

freezing process. This research will attempt to determine whether bone that has been previously frozen would be histologically distinguishable by looking for a patterned anomaly, such as patterned cracking.

It is possible that the microscopic structure may indicate some changes due to the blood vessels, which run throughout the bone allowing for communication and nutrient flow between bone cells, undergoing the freezing process. Freezing allows for the expansion of fluids (including bodily fluids) and possibly for the forced increase in vessel diameter which may be evident, microscopically, in a section of frozen bone as small fractures in the bone microstructure.

In order to determine whether the freezing can be identified within the bone microstructure, it is necessary to freeze several human bone sections. After the sections have been frozen for twenty one days, they are allowed to thaw in accordance with the circumstances surrounding the question. The samples are thin sectioned in order to view their microstructure. This analysis affords the opportunity to look for changes in the microstructure that may be indicative of the freezing process.

Many other questions will be raised due to this research on whether or not freezing is detectable by looking at the bone microstructure. If results suggest that freezing is detectable, it may be fruitful to ask if length of freezing, temperature at which the specimen is kept before, after and during freezing or other circumstances have an effect on whether the freezing of the bone is detectable microscopically. If however, this technique is not found to be effective in the elucidation of whether or not the bone had been previously frozen, it may be prudent to investigate whether other techniques may be

more effective or if there is indeed no signature whatsoever indicating that human bone has been frozen.

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Chapter 1: Introduction

Forensic anthropology is a sub discipline of physical anthropology in which many of the techniques of physical anthropology, such as skeletal analysis, are used to aid in the identification of human remains to benefit medicolegal investigations. Forensic anthropology cannot be separated from its physical anthropological roots, for it depends on many of the techniques of physical anthropology to make a determination on the age, sex, ancestry, and stature of the individual in question. The techniques used to determine these characteristics include the analysis of the gross morphology of the skeleton (Bass, 1995; Krogman and Iscan, 1986; Schwartz, 1995; White, 2001), the histological analysis of human remains (Alqvist and Damsten, 1969; Kerley, 1965; Kerley and Ubelaker, 1978; Stout and Gehlert, 1980), cranial and post cranial metric analysis (Giles and Elliot, 1963;Trotter and Gleser, 1952).

Often, combinations of the aforementioned techniques are used to identify an individual (For Review See Byers, 2001). This is particularly the case when the skeletal remains are incomplete, due to missing parts of the skeleton, or broken or fragmented portions of bone. Many of these techniques are used to create new protocols of identification when the skeletal elements have been altered, i.e. of fire damage or dismemberment. These new protocols are the direct result of research projects that simulate real life situations that victims encounter upon traumatic death and subsequent disposal. Information of this experimental sort may prove very useful the next time investigators encounter a similar crime scene.

One ongoing avenue of research seeks to show how the skeleton reacts to being burned (Bradtmiller and Buikstra, 1984) . There has been a great deal of research done

on accidental and purposeful cremation of modern and past populations (Baby, 1954; Bennett, 1996; Binford, 1963; Gejvall, 1969; Marks et al, 1988). This research emphasizes "descriptive analysis of bone tissue when exposed to heat" (Correia, 1997: 276). To accomplish this much of the burned bone research focuses on gross characteristics that can be interpreted visually (Baby, 1954; Binford, 1963; Buikstra and Swegle, 1989; Pope et al., 2002), as well as, the histological attributes (Shipman et al., 1984). The visually assessed characteristics of burned bone include color changes on the bone (Bennett, 1996; Shipman et al., 1984), physical modification of the bone such as shrinking, fracturing and distortion of the bone (Hermann, 1977), differential survival of bones and bone fragments, and differential fracturing due to the amount of soft tissue on the bone.

Research on burned bone continues, however, the contrasting temperature extreme is almost absent from the literature. Research has not studied the changes that may occur accompanying freezing of human bone. There has been minimal work on the changes that occur in human organ tissue when it is frozen (Zugibe and Costello, 1993), and some work has been done indicating that a body that has been frozen for a period of time will decompose at an accelerated rate when it is allowed to thaw (Perper, 1993), but nothing about the changes in the bone micro-structure has been published.

This is particularly disturbing when one thinks of the vast number of specimens that are frozen and then used in the medical field on a regular basis, i.e., bone grafts (Tomford et al., 1990) and other sections of human bone for research (Fideler et al., 1994). The peculiar perspective of many of these studies is that tissue has been frozen in

order to preserve the specimen, but no where is there any reference to the freezing process and how it may affect the bone, bone cells, or bone structure.

This seems to be a grave mistake since the characteristics of the freeze-thaw process and the physical properties of ice formation could cause physical deformation including fracturing or distortion of the bone. The determination of the changes that occur in bone due to freezing could be investigated by simply analyzing the specimen's histological signature under the microscope.

Bone histology has been used in physical and forensic anthropology in association with cremation studies to determine whether the histological signature is changed by the burning process (Nelson, 1992). Bone histomorphometry has also been used to determine age of an individual and to distinguish between fragmentary human and non-human bone (Enlow and Brown, 1956 a, b, and c; Tersigni, 2001).

Early cortical bone histomorphology began with a 1936 paper by Amprino and Biarati that "provided a qualitative study that illustrated the age-associated changes in cortical bone microstructure" (in Stout, 1992: 21). Stout indicates that Amprino and Biarati provide one of the cornerstone papers for the use of bone histomorphometry to determine age at death. Stout mentions other works, i.e., Jowsley (1960), which focused on some changes that occur in cortical bone with age. Currey (1964) provided a foundation study in the quantitative analysis of how intact haversian systems and fragmentary haversian systems change with age. These three studies initiated the ongoing quest to determine the age of an individual at death by looking at the histomorphological signature. Since the aforementioned studies were published, researchers have come up with a number of techniques to try to surmise the age of an

individual from bone histology (Kerley, 1965; Kerley and Ubelaker, 1978; Thompson, 1979; Kimura, 1992; Alqvist and Damsten, 1969; Stout, 1989; Stout and Paine, 1992). These techniques differ according to what portion of the skeleton on which they focus (e.g. metacarpal, ulna, rib, femur or tibia), the portion of the bone sampled (e.g. midshaft, ends), the size of specimen needed to make the determination (e.g. small bone core or cross sections) and what is quantified (secondary osteons, haversian canals, osteon population density).

As mentioned, bone histomorphology has been used in a number of ways to glean information from the byproducts of bone metabolism within a fragment of bone. It is the hope of this research that these structures will again afford enough information to make a determination of whether the bone is compromised during the freezing process.

Chapter 2: Review of Processes

In order to understand the microscopic changes that may occur during this experiment, it is necessary to understand both the normal process that creates and modifies bone as well as the process of ice formation. This chapter begins with a review of bone histology and the use of bone histology as an indicator of an individual's age at death.

The Histology of Bone

The histological study of bone allows for the analysis of bone at the cellular level. By looking at the bone microstructure, one is able to ascertain the age of the individual using various techniques. These techniques all share the need to understand the way that the structure of bone is built and removed in order to use the techniques effectively.

The gross structure of bone is made up of two types of bone: compact bone (or cortical) and cancellous bone (also known as trabecular or spongy bone) (Figure 1). Compact bone is the outer, solid appearing portion of bone where muscles attach. Cancellous bone is the latticework of bony spicules or trabeculae that are within the shaft and the epiphyses (Ross et al., 1989). The bony spicules or trabeculae are beams of bone that form inner scaffolding. Cancellous can be converted to compact through the bone remodeling process. The remodeling process can occur in both types of bone in response to stressors such as tension, applied load, hormonal changes and the amount of activity or inactivity that a bone undergoes (Bergman et al., 1996; Eroschenko, 1993).

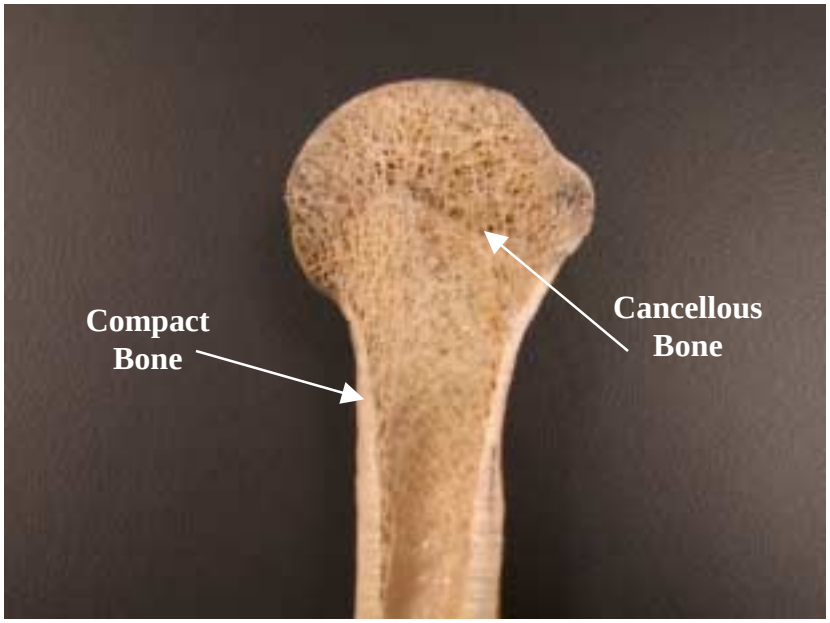


Figure 1: Compact and Cancellous Bone.
A 35mm photograph of a proximal humerus sectioned at midline.

There are two forms of bone: woven and lamellar. Woven bone is immature and is usually not found in the body after the age of five except when bone formation occurs as a result of fractures or surgery. Woven bone is found in the embryonic skeleton where it develops from calcified cartilage during the process of endochondral ossification. Woven bone is very flexible but it can be easily deformed. Thus, woven bone is eventually replaced by a more rigid lamellar bone. Lamellar bone is much stronger, and consists of haversian systems (Ross et al., 1989).

Two layers frame the microscopic structure of bone: periosteum and endosteum (Figure 2). The periosteum is an external fibrocellular sheath that contains collagen fibers and fibroblasts, which, in turn, forms an inner osteogenic layer on top of the

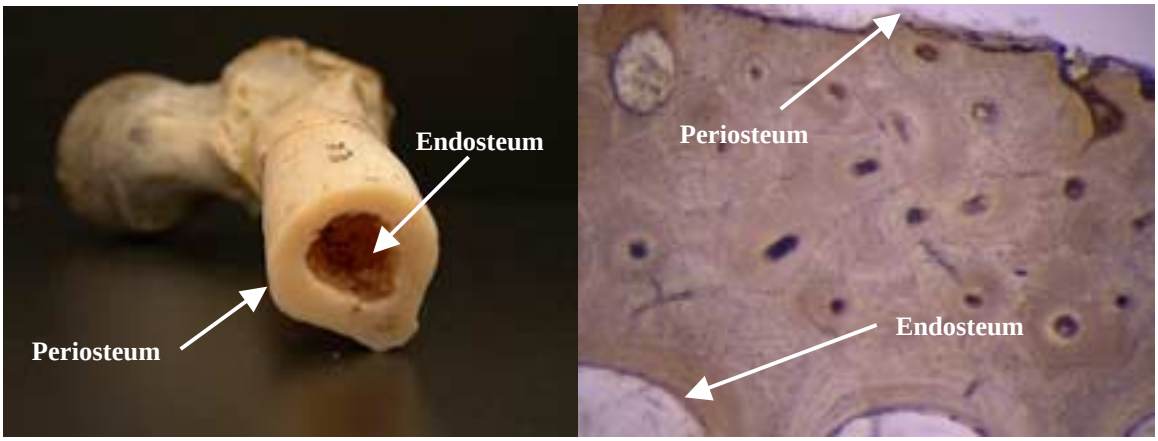


Figure 2: Human Bone Periosteum and Endosteum.
A macroscopic view of a human femur sectioned at midshaft showing the periosteum and endosteum (left). The microscopic view of human rib at 10 X magnification showing the periosteal and endosteal surfaces (right).

compact surface. The endosteum lines the inner marrow cavity surface as well as the haversian and Volkman's canals. It consists of osteogenic cells and thin reticular fibers (Bergman et al., 1996).

The micro-organization of bone consists of many small formations that allow the inside (endosteum) of the bone to communicate with the outside of the bone (periosteum) (Figure 3). One such formation is the haversian canal (Figure 4a). The haversian canal contains the neurovascular bundles that bring nutrients to the inner layers of bone. Volkman's canals connect these haversian canals to one another. These haversian canals are surrounded by osteons. Osteons are cylinders of bone that consist of concentric layers of lamellae (Figure 4b). These lamellae are made up of bone matrix and radiate outward from the central haversian canal (Bergman et al., 1996; Eroschenko, 1993).

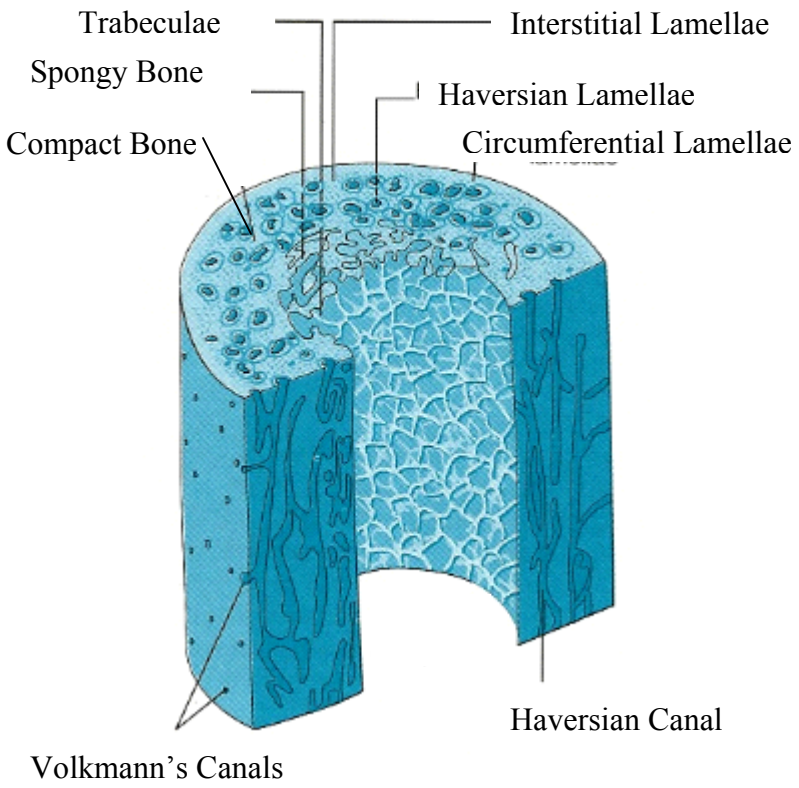


Figure 3: Schematic of Human Bone Structure.
Adapted from Bergman et al., 1996: 71.

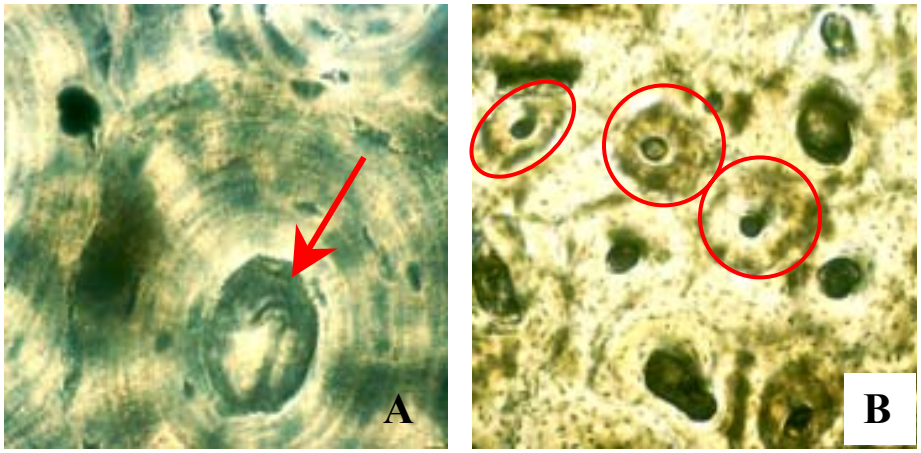
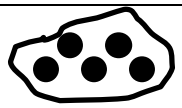
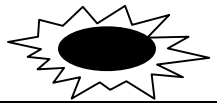


Figure 4: Bone Microstructures
The image on the left (A) shows a microscopic view of a human tibia at 40 magnification showing a circular haversian canal (indicated by red arrow).
The image on the right (B) depicts osteons (outlined in red) as seen in the same section of human tibia at 40 magnification.

Bone is laid down, resorbed and maintained by three different types of bone cells. These cells are the osteoblasts, osteoclasts and osteocytes (Table 1). Osteoblasts are responsible for the formation of bone, or osteogenesis. Osteoblasts line the bone surfaces. Their function is to package, sort and move the collagen and proteoglycans that comprise the bone matrix. Osteoblasts are responsible for forming new haversian systems around blood vessels and neurovascular bundles. As the osteoblast deposits bone, the matrix that they secrete eventually surrounds them. The cell then lives in a tiny space in the bone matrix called a lacuna. The function of an osteoblast then changes from that of laying down bone, to a function of bone maintenance. Once an osteoblast begins to maintain and monitor metabolism instead depositing bone matrix, it is termed an osteocyte (Ericksen et al., 1994). Ostocytes are responsible for the maintenance of bone. Canaliculi arte used by osteocytes to facilitate the maintenance of bone as well as the contact between osteocytes. Canaliculi are narrow channels that radiate from lacunae. The projections from one osteocyte extend to connect to other projections from other osteocytes elsewhere in the bone matrix. In this manner, the outer bone is connected to the inner bone via these projections. This allows for the transport of small molecules and ions to keep the osteocytes alive within their lacunae. Osteoclasts are responsible for the resorption or removal of bone. Osteoclasts are large multinucleated cells residing on the surface of bone inside resorptive bays known as Howship's lacunae (Bergman et al., 1996). The cell body of the osteoclast has a ruffled border that serves a very important function for the resorption of bone. The ruffles house powerful acids that are needed to dissolve bone. With the ruffled pattern around the outside of the cell, there is more

Table 1: The Cells of the Human Bone.

Bone Cell	Function	Location	Diagram
Osteoblast	Osteogenesis	Line the inside of bone surfaces	
Osteoclast	Bone resorption	In Howship's Lacunae	
Osteocyte	Bone maintenance	In the lacunae	

surface area to store these acidic ions. As a result, there is a great increase in the resorptive power of osteoclasts as compared to a non-ruffled cell (Eroschenko, 1993).

The laying down and resorption of bone is a constant process regulated by a number of systems within the body. These processes revolve around the level of calcium in the blood . If the blood calcium levels are high, hormones are released that stimulate osteoblast activity and inhibit osteoclast activity. When the blood calcium levels are low, osteoclast activity is stimulated and osteoblast activity is inhibited. This constant resorption and osteogenesis process leads to a distinct microscopic signature. When looking at a section of human bone, one can recognize haversian systems as concentric circles of bone surrounding an inner circle which would have held a blood vessel or neurovascular bundle. Osteocytes, osteoblasts and osteoclasts can often be identified in the section. Within some sections, especially those from older individuals, it is quite common to see a newer haversian system on top of another. The newer haversian system is termed a secondary osteon. A clear reversal line distinguishes

where the bone resorption ended and the bone building began (Figure 5). Secondary osteons tend to increase with age. As secondary osteons are laid down over older primary osteons, there tends to be an increase in fragmentary primary osteons (Figure 6) (Bergman et al., 1996; Stout, 1992).

These changes in the histomorphology of bone can be used to give age estimates of individuals (Kerley, 1965; Kerley and Ubelaker, 1978; Ericksen, 1991; Singh and Gunberg, 1970). The technique of aging based upon their bone histomorphology is often used on fragmentary human remains, especially in cases where no age diagnostic gross bone morphology is available. Histological age estimation can also be used to corroborate and narrow age ranges acquired using other techniques, such as sternal rib end aging, or pubic symphyseal aging.

There are many different techniques to estimate the age of an individual using bone histology (Stout and Paine, 1992; Thompson, 1979; Kerley and Ubelaker, 1978; Ahlqvist and Damsten, 1969). Many differ on what bone or combinations of bones are used to make such a determination (Ericksen, 1991; Singh and Gunberg, 1970; Thompson, 1979; Stout and Paine, 1992). Since the present study used only femur, tibia and fibula segments, this review will focus mainly on age estimation techniques using one or more of these bones.

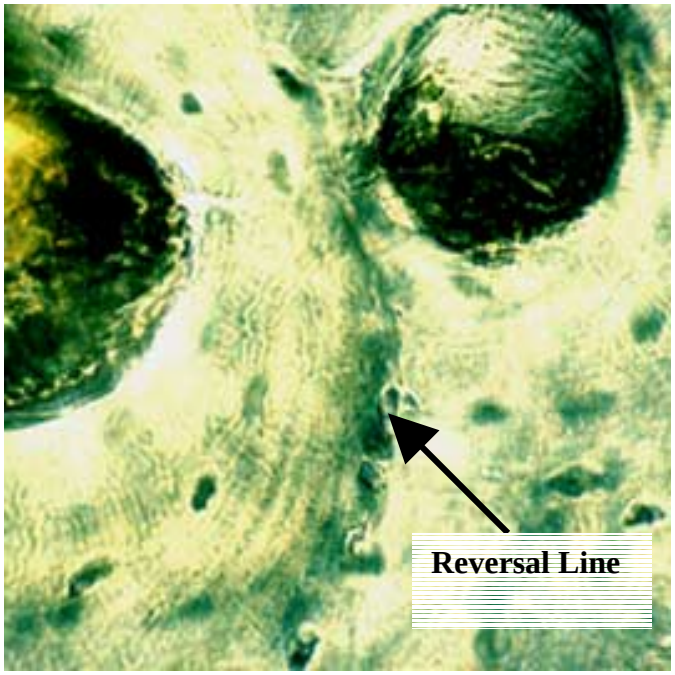


Figure 5: Reversal Line as Evidence of Bone Remodeling.
This image depicts a clear reversal line as seen in a human tibia at 40 magnification.

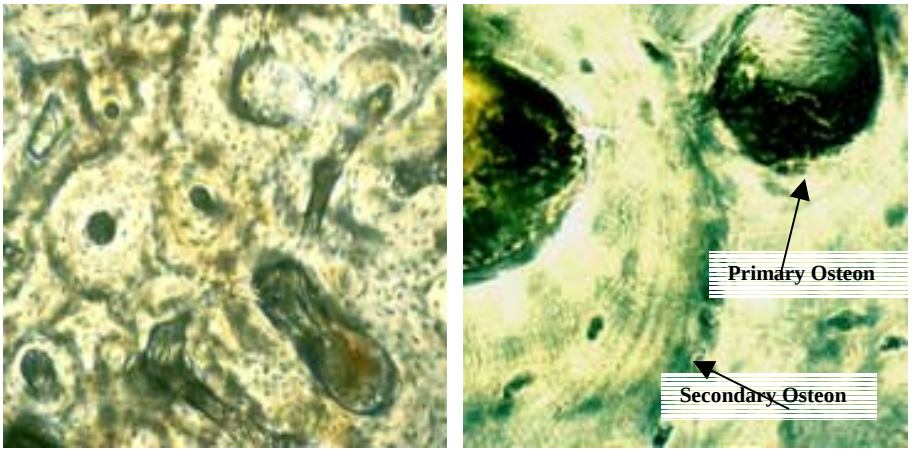


Figure 6: Microscopic View of Human Bone Remodeling.
The image on the left shows a section of bone at 10 magnification that has undergone a great deal of remodeling. This is indicated by the "cluttered" nature of the section. There are many layers of osteons in this section. On the right, a closer view of a secondary osteon laid down over a primary osteon.

One of the first techniques utilized midshaft femur, tibia and fibula sections. This technique requires the quantifications of osteons, fragmentary primary osteons, non-haversian canals, as well as, the percentage of circumferential lamellae (Figure 7). Non-haversian canals are defined by Kerley as:

> "All primary vascular channels, including those that have filled in partly with concentric lamellae to form primary osteons or pseudo-haversian systems are vascular canals that were formed by the inclusion of small, peripheral blood vessels into the bone by the rapid expansion of the cortex diameter."
> (Kerley, 1965: 151-152)

Circumferential lamellar bone is made up of "concentric bands of unremodeled primary lamellar bone that was formed during growth and through osteoblastic drift" (Stout, 1992: 25). Each of these items is quantified and all of the quantities added together at four distinct areas of the bone: midline anterior, midline posterior, medial and lateral (Figure 8a) using a circular microscopic field. The sums were placed into regression equations to get an age estimate. Kerley (1965) neglects to indicate the importance of the microscopic field size in this technique. This oversight was addressed by Kerley and Ubelaker (1978), where the authors suggest it is necessary to correct the microscopic field size if it differs from Kerley's original field size:

> "The area (πr^2) of the 100 X field of the microscope used must be calculated using a stage micrometer and that field size divided into 2.06 mm^2 (area of a field diameter of 1.62 mm) to determine the relationship between the original field size and the field size of the individual microscope being used."
> (Kerley and Ubelaker, 1978: 546)

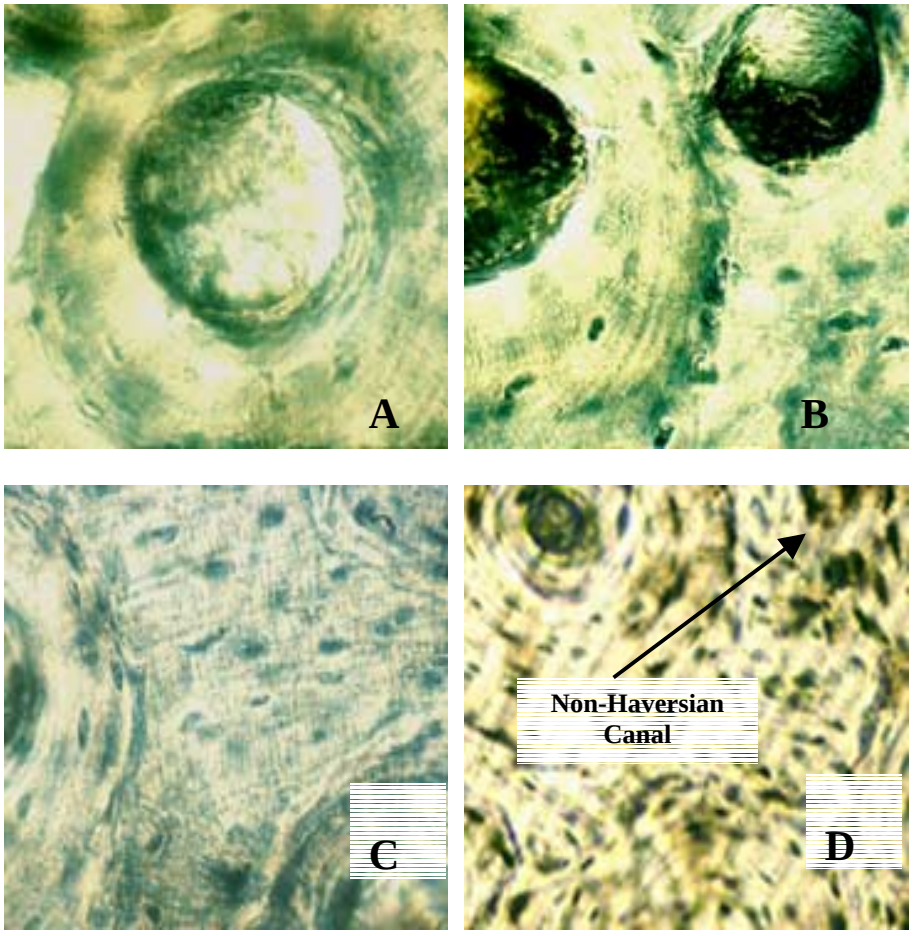


Figure 7: Features Measured in the Kerley Method of Histological Age Estimation. The osteon is illustrated in image A. Image B portrays both the fragmentary primary osteon. Circumferential lamellae are illustrated in image C. Image D shows the non-haversian canal.

Another histological age estimation technique was designed by Ahlqvist and Damsten (1969). This research actually modified the Kerley method by changing the area of measurement to half way between each of Kerley's original points (Figure 8b). This was in an attempt to avoid the linea aspera, a site of muscle attachment on the posterior femur, which they surmised would skew age estimation. Additionally, Ahlqvist and Damsten suggested that square microscopic fields should be used in an effort to include the "sub-periosteal regions of the cortex" (Stout,1992: 26). They felt that this area was the most modified region of bone with respect age-related remodeling. Their method relied solely on the percentage of haversian bone within each microscopic field. These percentages were then averaged and placed into regression equations to determine the age of the individual. Stout and Stanley (1992) showed that percentage of haversian bone was not a very accurate indicator of age, thus making this technique obsolete.

There are many other histological aging techniques utilizing the femur, tibia and/or fibula (i.e. Singh and Gunberg 1970; Thompson 1979; and Ericksen 1991) although the Kerley method (using the modifications indicated in Kerley and Ubelaker 1978) still stands as one of the best methods of histological age estimation, provided the only bone or bones available are the femur, tibia or fibula.

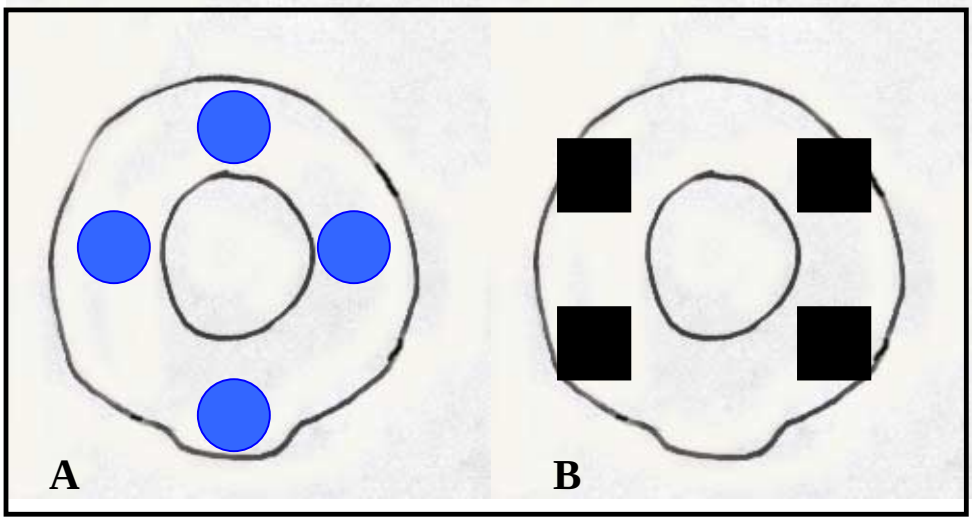


Figure 8: Methods of Age Estimation of the Femur.

The Kerley method of age estimation from the femur (a) uses circular fields and anterior, posterior, medial and lateral coordinates to make an age estimation. The Alqvist and Damsten method of age estimation of the femur uses square fields which are half way between Kerley's fields in order to avoid the linea aspera

The estimation of age at death is dependant on histomorphological change over time. As described, normal bone undergoes systematic changes which allow an investigator to quantify those changes in order to estimate age. However, not every person has normal healthy bone throughout their body, nor do all individuals have full movement of all of their limbs and muscles. Such pathological conditions and limited use of the bones and muscles (i.e. disuse atrophy) may cause differential change as part of the remodeling process (Stout 1982; and Stout 1992). Most often these changes do not occur in a systematic manner. Therefore, such changes may alter accurate age estimation. Furthermore, the changes may not be identical in each bone of the same individual. The changes may not even affect two individuals with the same pathology in the same manner, depending on other contributing disorders. It is important to look for

differential remodeling within a bone or among a set of bones in order to decide how much weight to put on the histological age estimate.

The Freezing Process

Water makes up approximately 80% of the human body. It is in every part of the body including tissues, blood and intracellular matrix. This abundance of water throughout the body makes it susceptible to undergoing freezing if the conditions are applicable. The process of freezing has a number of properties that may alter the distinct microscopic signature of bone, thus allowing a researcher to surmise that the tissue has indeed been frozen. The processes of expansion and crystal formation, in particular, are two deforming events. This can be noted in practical terms by looking at the damage caused to metal water pipes exposed to extended periods of cold weather. The water expands with enough force as it freezes to burst the pipe.

The regular form of ice, that is, when water is frozen at normal atmospheric pressure and normal freezing temperature (0˚C), is denoted as Ice Ih (Petrenko and Whitworth, 1999). The "I" stands for the first phase of ice, while the "h" indicates the hexagonal phase, which is the normal phase orientation (Petrenko and Whitworth, 1999). Ice usually forms in a crystal-lattice structure from the surface downward, covering the surface very quickly. The atoms of oxygen "are arranged on a hexagonal lattice" (Petrenko and Whitworth, 1999: 15). The oxygen atoms align with their four nearest neighbors in the shape of a tetrahedron causing the hydrogen atoms to bond covalently to the nearest oxygen atom (Petrenko and Whitworth, 1999). Figure 9 illustrates this bonding pattern.

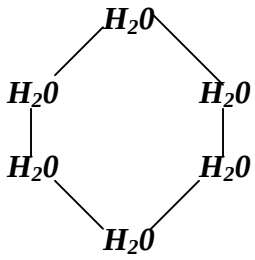


Figure 9: The Crystal-Lattice Bonding Structure of Ice.

Because of this peculiar bonding pattern, water is the only liquid that expands as it freezes. Other liquids actually contract as they cool. This expansion is due to the position of the water molecules as they move between the liquid to the crystalline state. As the water begins to freeze the molecules rearrange into a crystalline form which takes up a greater amount of space than the liquid bonding pattern. Many of the properties of ice, such as the cubic thermal expansion ($\alpha_V = - (1/V) (\delta V/\delta t)_p$) and density, depend on this unusual lattice pattern.

Routinely, human tissue is frozen in order to store it for research purposes. The freezing of tissues allows for the water component of each tissue to be transformed into ice crystals. Mellors suggests that the crystallization of water during freezing "occurs in extracellular, intracellular and intranuclear spaces and produces an artefactual distortion of the tissue section if the crystals are large" (Mellors, 1959: 11).

Mellors indicates that refreezing a previously thawed tissue can lead to even larger crystals that are even more distorting to the tissue (Mellors, 1959). Lillie (1965) concurs with Mellors and suggests that frequent freezing and thawing can damage cellular structures causing the release of various enzymes and cellular fluids. Zugibe and Costello (1993) suggest that the magnitude of cellular damage depends on how fast a

tissue is frozen and thawed and the tissue's individual properties, such as thermal conductivity, size and shape. Many of the major cellular changes only occur when a tissue is allowed to slowly freeze at normal temperatures (0˚C). This is why many histochemists freeze-dry the tissues that they need to analyze. This technique utilizes liquid nitrogen, which has a temperature of -160˚C to aid in rapid freezing. This rapid freezing eliminates the formation of crystals. The disadvantage to this is that the tissue then needs to be stored in a vacuum at -30˚C. This process drastically reduces the volume of the tissues and changes a number of the properties of the tissue including thermal conductivity (Zugibe and Costello, 1993). But what happens to human tissue when it is allowed to slowly freeze?

Zugibe and Costello (1993) suggest that freezing a body in normal freezing conditions, that is a slow freeze near 0˚C, would cause the internal microorganisms to either die or lie dormant. This, coupled with the crystalline formation of the water within vessels, organ, and tissue, effectively prevents decomposition. If it is allowed to thaw, decomposition of a previously frozen body will occur in a different manner than a body that has never been frozen. The outside of the body would theoretically thaw first while the inside of the body may remain in a frozen or nearly frozen state for a much longer takes less time for the outside to thaw than the inside. Since the outside of the body thaws more rapidly it will be susceptible to decomposition by microorganisms in the external environment that will have access to and contact with the outer thawed body surface.

In this manner, the decomposition of a previously frozen body is nearly the reverse of a previously non-frozen body (Figure 10). The normal process of

decomposition begins on the inside the body. As the normal body tissues and flora begin to break down. One of the first processes of decomposition is autolysis. This is the systematic release of enzymes to that begin to degrade organ and muscle tissues. The next process of decomposition is usually putrefaction, which is partly caused by the microflora activity within the body (Perper, 1993) If a body has been frozen, however, the normal flora will not aid in decomposition, thus, decomposition in these cases occur form the outside in. Perper agrees suggesting:

> "Exposure to cold substantially delays the decomposition process. In evaluating postmortem changes, it is, therefore, important to consider any intermittent period of exposure to cold, refrigeration or freezing. A further consideration is postmortem rewarming or thawing of the body. Experiments have shown that previously frozen and thawed animal tissues decompose significantly faster than freshly killed animals" (Perper,1993: 32).

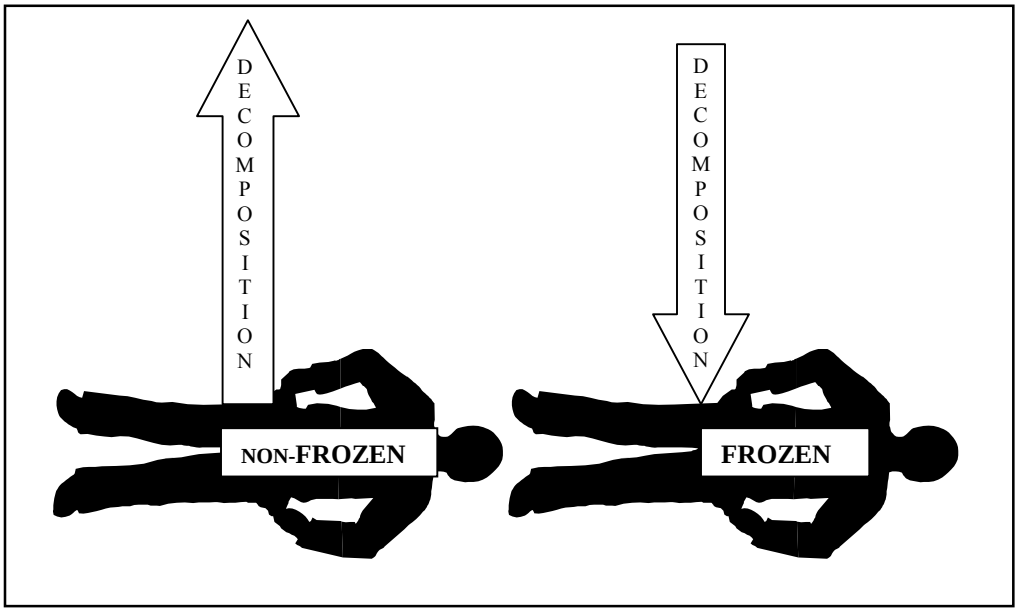


Figure 10: Differential Decomposition of Frozen and Non-Frozen Human Remains. Non-Frozen human remains decompose from the inside out, while frozen human remains decompose form the outside in.

If investigators are fortunate enough to discover a previously frozen body in the middle of this transitional state, where the external surfaces are thawed while the internal organs are still frozen or partially frozen, it would be very easy to make the determination that the body was frozen at one time. Zugibe and Costello (1993) describe one such case where the outside of the body was decomposing while the internal organs were still partially frozen and intact: "Tissue sections were evaluated for the presence of ice crystal artifacts in an attempt to confirm our impression that the body may have been frozen prior to dumping" (Zugibe and Costello, 1993: 1406). Tissues sections taken of the heart muscle in this case provided evidence of formed ice crystals within the structure of the muscle, thus confirming their suspicions that the body had indeed been frozen.

What happens when investigators are not as lucky as in the previous example and the body that was suspected of being frozen has decomposed to the point of skeletonization? The question now becomes whether the bones themselves could suggest that they had been previously frozen. This research examines the histological structure of both frozen human bone and non-frozen human bone to determine whether the bone itself can answer this question.

Chapter 3: Materials and Methods

This study was completed at the Regional Forensic Center (RFC) at The University of Tennessee Medical Center. The RFC facilities include the autopsy facilities for the Knox County Medical Examiner as well as facilities for the University of Tennessee Department of Anthropology Forensic Anthropology Laboratories. These laboratories house materials needed to clean and document individual cases and specimens such as photographic equipment, thin sectioning and embedding materials, microscopes and bone processing equipment. This study makes full use of the thin sectioning, embedding, microscopy, and bone processing materials.

This study looks at human bone to determine what effects, if any, freezing has on bone microstructure. To accomplish this, eleven human bone samples were obtained. The samples were surgical specimens obtained from the University of Tennessee Medical Center. Two of the limbs represented partial femoral and complete tibia, fibula and foot amputations. One specimen represented just the proximal end of the femur and the remaining eight specimens represented complete or nearly complete tibia, fibula and foot amputations (Table 2). From each of these samples, 3- one to two inch portions of bone were obtained from the midshaft of the tibia and fibula. In the cases where the distal femur was available, a section of bone was removed from the distal femur and split into three equal sections. The bone sections were removed with care to leave the outer muscle and skin covering intact and without removing the inner marrow content. These precautions would allow for a more realistic comparison to freezing the entire limb, since this was not possible due to space constraints.

Specimen Number	Bones Represented
1	Right Tibia, Fibula, and Femur
2	Right Tibia and Fibula
3	Left Tibia, Fibula, and Femur
4	Right Tibia and Fibula
5	Left Tibia and Fibula
6	Left Tibia and Fibula
7	Right Tibia and Fibula
8	Left Tibia and Fibula
9	Right Tibia and Fibula
10	Left Femur
11	Left Tibia and Fibula

Table 2: Description of the Specimens.

Each sample was numbered and each section of the same piece of bone was given a bone name and letter marker (1Tibia A, 5 Fibula B, or 10 Femur C) for the purpose of distinguishing between the smaller sections. Each section was placed in a separate re-sealable plastic bag. For each of the 11 samples, the section denoted A for each bone was reserved as the control section. The control sections were cleaned and analyzed in the same manner as the test groups but were not subject to any other manipulations. The other two sections of each bone (B and C) were placed in a normal freezer (0˚C) and allowed to freeze for twenty-one days.

Frozen Sections

Groups B and C were both allowed to freeze for the same amount of time and were cleaned and sectioned in the same manner. The original reason for creating two test groups was to allow one group to be frozen and thawed and then analyzed and allow the other group to be frozen and analyzed in the frozen state. Repeated attempts to keep

sample specimens frozen during processing and cutting failed. The researcher decided that it was highly unlikely that an investigator would discover a portion of bone frozen, yet still feel the need to test the bone using histology to find evidence of freezing. So this original aspect of the protocol was discarded. Instead, sample B was set aside for use in a SEM analysis of the bone, while sample set C was used in the manner it was intended: to look for evidence of scars left on the bone due to the freezing process.

Cleaning of the Sections

Each of the sections was cleaned by hand without the use of heat or detergent. This was instituted in order to prevent any distortions due to heat or chemicals. The outer layers of muscle were cut and pulled off of the bone. The inner marrow was flushed out of the cavity with a stream of cold running water. Upon completion of the cleaning process the samples were placed on absorbent paper in order to facilitate the drying of the specimen.

Microstructure Analysis

The next step was microstructure analysis. The method of analysis used required a thin section from each of the sections of bone. This thin section was then attached to a glass slide and covered with a glass cover slip. The thin sections were made using a Buehler Isomet 1000 saw with a diamond-edged oil cooled blade. The saw produced sections of bone that were approximately 0.6-0.8 mm thick. Some of the specimens were too small in diameter or too short in length to get an adequate thin-section. These specimens were embedded in an **epoxy resin** to allow the specimen to be thin sectioned.

When each slide had dried, they were viewed under a Olympus BX50 microscope and the image captured using an Olympus 35 mm camera with Fugi color slide film. The slides were digitized using a Nikon Cool Scan 4000 slide scanner. The control group was analyzed first, in order to have a base of information about that particular sample. While consecutive thin sections are not identical, there is value in using a control from the same bone to orient the observer to different nuances that may appear throughout the bone microstructure, such as color, general osteon size, shape and approximate extent of secondary osteons population (Figure 11). The control section is also a good basis of comparison for the test sections to determine if there are any structural changes or evidence of deformation of the microstructure.

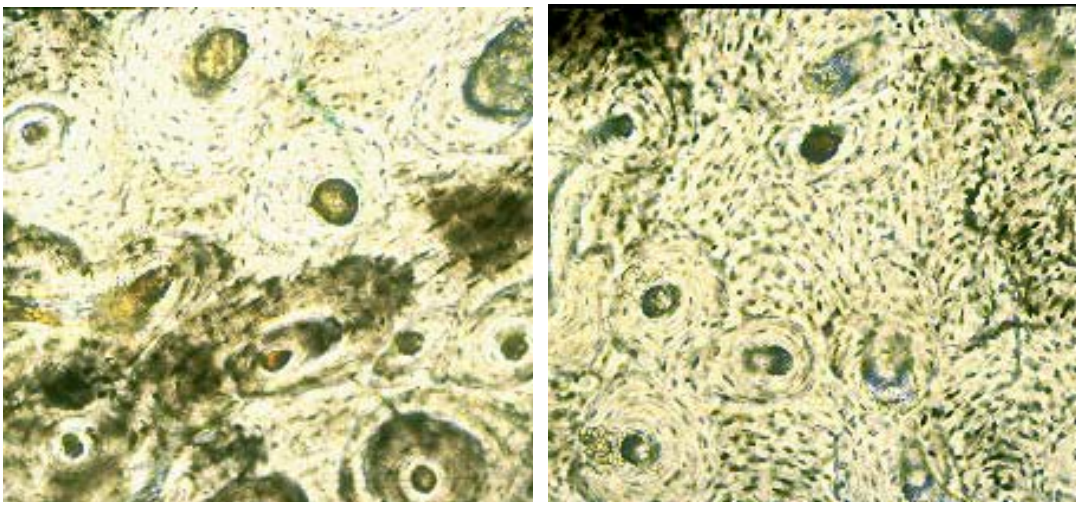


Figure 11: Non-Frozen Human Bone and Frozen Human Bone.
This figure shows thin sections taken from the non-frozen section of Tibia 1(left) and the frozen section of Tibia 1 (right) at a magnification of 10X.

Every specimen was viewed at three different magnification settings (10, 40 and 200) to determine whether any changes to the bone structure could be noted at both high and low magnification. A still image of each section was captured at a magnification of 10X, as well as a magnification 40X using the Olympus 35mm camera. These photographic slides were then digitally scanned using a Nikon Cool Scan 4000 slide scanner and imported into Adobe Photoshop 5.5 where the images were then saved to a disk. Images at 200X magnification were not clear enough to warrant photographs, since they could not be used to provide clear evidence of whether changes to the bone occurred. The images of sections A and C for all of the samples were compared. Some of these photographic comparisons are shown in Figure 12.

Statistical Analysis

In an attempt to determine if there were changes in the smaller structures of the histological section, a series of measurements were taken on each section of bone. For each specimen, thirty different haversian canals and thirty lacunae were measured. The impetus for this quantification was to see if these smaller structures had significant size changes due to the expansion of fluids that occurs during the freezing process. The measurements of the haversian canals and lacunae were compared for the non-frozen control specimen and the frozen section of each specimen. To facilitate this comparison, a series of statistical formulae were utilized in order to understand the significance of the quantification comparison.

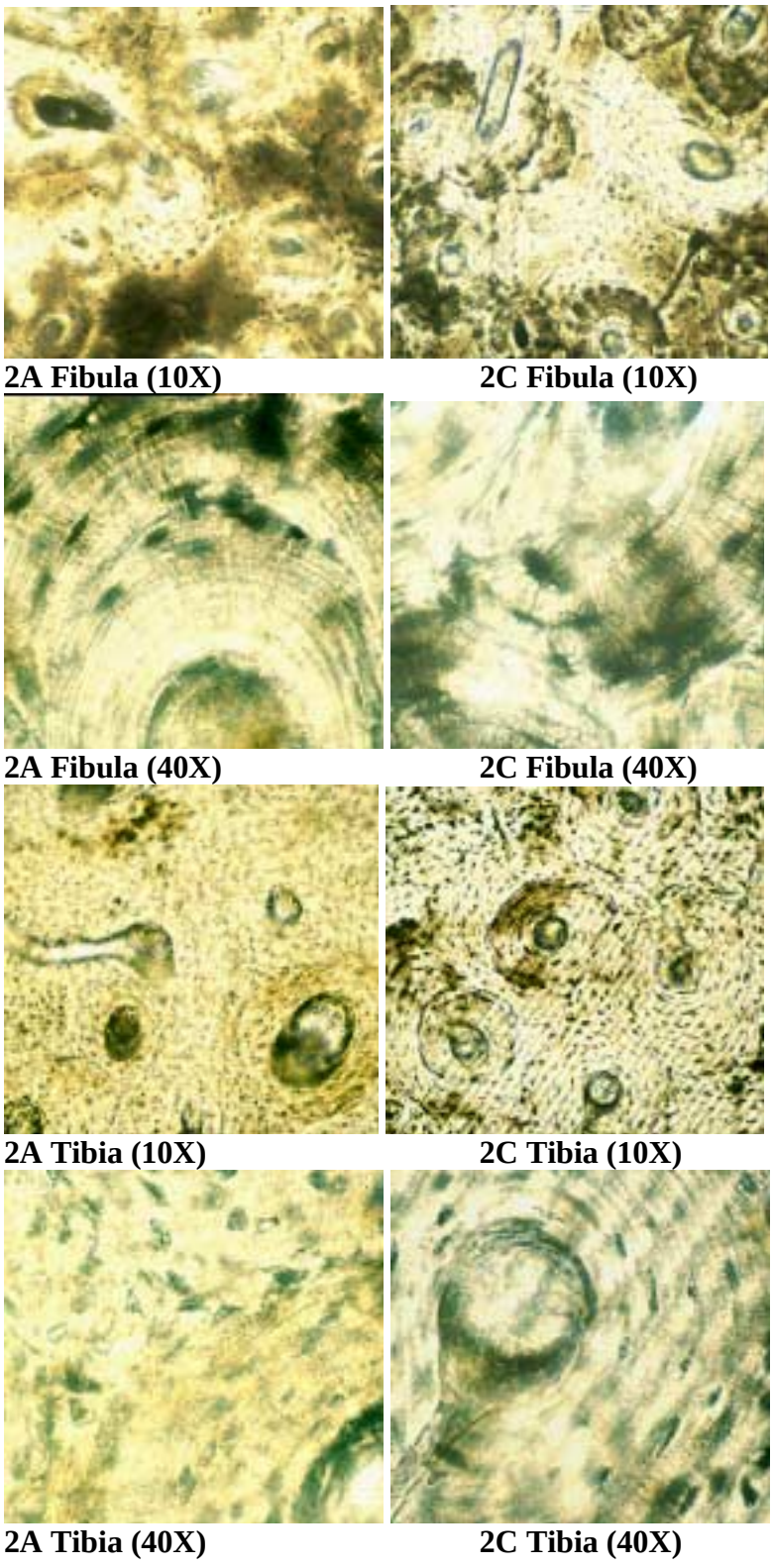


Figure12: Photographic Comparison of Several Specimens at Various Microscopic Magnifications.

The first statistical analysis used was a paired t-test in the SAS statistical program. This test examined whether or not the difference in the average size of the haversian canals in non-frozen bone and frozen bone was significantly different from zero. This test was also performed using the average sizes of lacunae in the frozen and non-frozen sample. The results are represented in Table 3.

Next, an analysis of variance was performed on the entire data set for haversian canals. Additionally, an analysis of variance was used to evaluate the entire lacunae data set. The ANOVA procedure in the SAS statistical program was used to determine if there was a significant correlation between any of the variables (individual, bone, frozen or unfrozen, and average size) in these two data sets. The results of the ANOVA are displayed in Table 4.

Finally, a discriminant function analysis using the SAS statistical program was performed to determine whether a specific bone can be identified based upon the size of the haversian canal and/or the lacunae. Table 5-6 illustrates the results for this statistical analysis. As was noted by the analysis, bone has no bearing on the size of haversian canals and lacunae. This is in direct contrast with the earlier ANOVA findings. It was noted that there were only three femoral measurements while there were ten measurements for the tibia and fibula. With this in mind, a second ANOVA was performed omitting the femur values. When the femur values were omitted, the ANOVA indicated that the type of bone was not a significant factor in the size of haversian canals and lacunae (Table 7).

Chapter 4: Results

Each of the thin sections were analyzed at 10, 40 and 200 magnification. As stated in the previous chapter, the 10 and 40 magnifications proved to be the most beneficial for the discovery of changes due to the freezing process between the sections A and C of the same specimen. No patterned histological change could be found on every specimen. A few of the frozen sections, however, did exhibit some cracking (Figure 13) around the center of the haversian canal which was not evident on their non-frozen counterpart (Stout, 2002; Personal Communication).

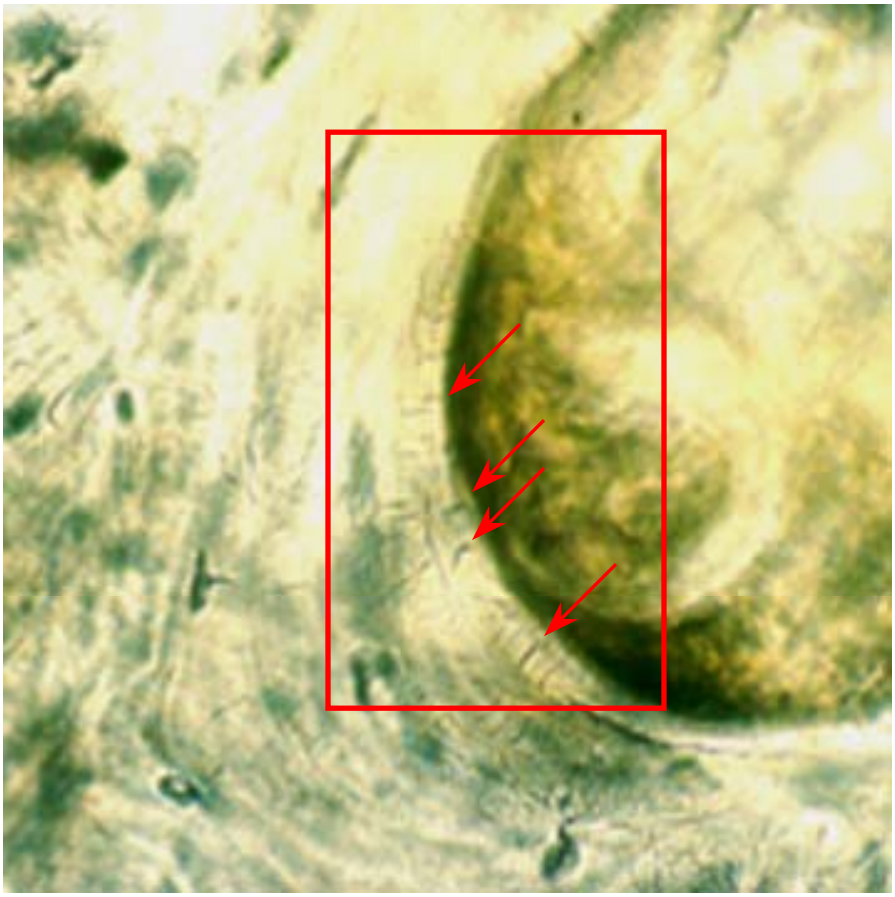


Figure 13: Specimen 3C Fibula (Frozen) that Displays Cracks Radiating from a Haversian Canal.

The numerical results of the statistical analyses performed in the SAS statistical program are presented in Tables 3-7.

Table 3: Results of Paired T-Test.

Student's T –Test	Statistical Value	Probability
Tibia- Haversian Canal	-0.16011	0.8763
Fibula-Haversian Canal	0.645957	0.5344
Femur-Haversian Canal	0.564927	0.6290
Tibia- Lacunae	-0.36365	0.7245
Fibula- Lacunae	0.223959	0.8278
Femur-Lacunae	-1.25109	0.3374

Table 4: Initial ANOVA Results.

Variable	Mean Square	Probability
Haversian Canal-BONE	0.00014116	**0.0077**
Haversian Canal-BONE(INDIVIDUAL)	0.00004400	**0.0721**
Haversian Canal-FROZEN/UNFROZEN	0.00000155	**0.7980**
Lacunae-BONE	0.00003703	**<0.0001**
Lacunae-BONE(INDIVIDUAL)	0.00000527	**0.0060**
Lacunae-FROZEN/UNFROZEN	0.00000227	**0.2619**

Table 5: Classification Results of Discriminant Function Analysis.

Number of Observations and Percent Classified into Bone

From Bone	FEMUR	FIBULA	TIBIA	TOTAL
FEMUR (percentage)	3 100%	0 0%	0 0%	3 100%
FIBULA (percentage)	2 20%	2 20%	6 60%	10 100%
TIBIA (percentage)	4 40%	0 0%	6 60%	10 100%
TOTAL (percentage)	9 39.13%	2 8.70%	12 52.17%	23 100%

Table 6: Squared Distance Results of Discriminant Function Analysis.

Generalized Squared Distance to Bone

From Bone	FEMUR	FIBULA	TIBIA
FEMUR	0	2.67760	2.90707
FIBULA	2.67760	0	0.00685
TIBIA	2.90707	0.00685	0

Table 7: Secondary ANOVA

Variable	Mean Square	Probability
Haversian Canal-BONE	0.00000514	**0.6604**
Haversian Canal-BONE(INDIVIDUAL)	0.00004152	**0.1556**
Haversian Canal-FROZEN/UNFROZEN	0.00000188	**0.7902**
Lacunae-BONE	0.00000001	**0.9221**
Lacunae-BONE(INDIVIDUAL)	0.00000568	**0.0003**
Lacunae-FROZEN/UNFROZEN	0.00000002	**0.8818**

Chapter 5: Discussion

Statistically, the results of the microscopic analysis did not demonstrate significant differences in the histology of frozen and non-frozen human bone. The changes that were noted in the test samples (C samples) do indicate that some fracturing may occur due to the freezing process, although this fracturing may not be patterned nor may it occur systematically throughout the frozen bone. While this evidence of physical changes caused by liquid expansion is encouraging to note, the cracking was not consistently present throughout the sample set, nor, when found in a section of bone, was the cracking present around each of the haversian canals within the section.

This data indicates that when an investigator is confronted with a bone that he or she believes was frozen at one time, there is no current method to determine whether or not the bone has been frozen. However, the analysis of frozen bone is not complete. Further analysis in this area should include scanning electron microscopic (SEM) analysis of bone, since this study used solely light microscopy. It is also important that additional studies are performed using whole bones. Theoretically, if a complete bone is frozen, it may react in a different manner than a portion of a bone, since the internal marrow will have nowhere to expand. The pieces used in this experiment were just that, pieces. The bone was sectioned to facilitate freezing a number of specimens at the same time. Due to this sectioning, both the proximal and distal potions of the bone were open and exposed, thus allowing the marrow to expand out of the bone section.

The statistical analyses of the haversian canal and lacunae measurements provided a wealth of information on the lack of significance that freezing human bone had upon

the microscopic morphology. The paired t-test performed on this set of data showed that the difference between the frozen and non frozen haversian canals or lacunae was not significantly different from zero. This test looked at each bone separately and took the difference of the average frozen size of the haversian canal or lacunae and the average non-frozen size of haversian canals or lacunae. Since each probability value is greater that 0.05, then the null hypotheses (the differences are not significantly different from zero) are accepted.

The ANOVA indicated that there are certain variables that are highly correlated with average haversian canal size and average lacunae size, however, frozen or non-frozen is not one of those variables. Since the probability was so much higher than 0.05 for the variable "Frozen/Un-Frozen" for both the haversian canal and the lacunae analysis, the null hypothesis (the variable is not significantly correlated with average size of haversian canals or lacunae) is accepted. The other two variables bone and bone nested within individual are lower than or very near the 0.05 value, suggesting that the null hypothesis (the variable is not significantly correlated with average size of haversian canals or lacunae) should be rejected. This indicates that both bone and bone nested within individual have a significant correlation with the size of the haversian canals and lacunae.

To test this correlation a discriminant function analysis was performed. This analysis showed that there was no significant correlation between bone and the size of the haversian canals or lacunae. This was indicated by the classification factor which did not correctly classify most of the tibiae and fibulae. The generalized squared distance has the greatest distance between the femur and the tibiae and fibulae. With this information,

another ANOVA analysis was performed without the data from the femora. This analysis shows P values of greater than 0.05 for five of the six variables. This indicates that the null hypothesis should be accepted for these five variables, indicating that these variables do not significantly correlate with average size of haversian canals or lacunae. The variable that is under 0.05 (Lacunae – Bone (Individual)), would then have a significant correlation between bone nested within individual and the average size of the lacunae.

Chapter 6: Conclusion

Even though there is not a consistent pattern to the fractures noted in the sample set, this should not rule out some histologically identifiable changes associated with freezing. It is clear that it is quite possible that bone sections may undergo noticeable changes due to the freezing process, although this experiment was unable to ascertain such information.

A more effective method of experimentation may be to freeze fully fleshed entire limbs instead of small pieces. It is possible that these small pieces of bone did not realistically reflect the processes that an intact bone incased in muscle may undergo in freezing since both the proximal and distal surfaces of the bone fragment, as well as, the marrow cavity were exposed. It is possible that the entire bone may be more likely to show differential levels of physical change throughout the length of the bone. It would be beneficial, in this instance, to sample many different parts of the bone, looking specifically for different types and proportions of cracking.

Another avenue of investigation may be to look at the sections of bone or the intact bone using a scanning electron microscope (SEM) as was intended for this research. Due to circumstances beyond the researcher's control, this technique was not yet undertaken at the completion of this manuscript. The SEM analysis of the sections of bone may uncover a pattern of change within the frozen sections that is found in each specimen that cannot be gleaned using light microscopy.

Statistically, whether the sample is frozen or unfrozen has no significance in the size of the haversian canals or lacunae. This indicates that through this experiment, there are no significant changes in the size of haversian canals and lacunae due to the freezing

process. Additionally, bone and bone nested within individual have been found to not be significant to the size of haversian canals and lacunae.

Further exploration of frozen human bone should be undertaken. Although this pilot study did not afford any significant results with respect to changes due to the freezing process this should not deter further study using other techniques (SEM analysis) and other types of samples (intact bones or entire limbs). The study of this subject has not been exhausted by any means.

References Cited

References Cited

Alqvist J and Damsten O (1969) A modification of Kerley's method for the microscopic determination of age in bone. J. Forensic Sci. *14:*205-212

Amprino R and Biarati G (1936) Processi di reconstruzione e di riassorbimento nella sostansa compatta dele osa dell nomo. Richerche see cento soggetti della nascita sino a tarda eta. Z. Zellforsch Mikorosk Anat. *24*:439-511.

Baby RS (1954) Hopewell Cremation Practices. Papers in Archaeology 1:1-7. Ohio Historical Society, Columbus.

Bass (1995) *Human Osteology: A Laboratory and Field Manual*. Fourth Edition. Missouri Archaeological Society.

Bennett, JL (1996) Thermal Alteration of Bone: Experiments in Post Burial Modification. Master's Thesis-The University Of Tennessee-Knoxville.

Bergman RA, Afifi AK, and Heidger Jr. PM (1996) *Histology*. Philadelphia, W.B. Saunders Company, pp.64-75.

Binford LR (1963) An Analysis of Cremations from Three Michigan Sites. Wisconsin Archaeology. *44(2)*:98-110.

Bradtmiller B and Buikstra JE (1984) Effects of Burning on Human Bone Microstructure-A preliminary study. J. Forensic Sci. *29(2):535-540.*

Buikstra JE and Swegle M (1989) Bone Modification Due to Burning: Experimental Evidence. In Bonnichsen R and Sorg MH (eds): *Bone Modification*, pp.247-258. Center for the Study of the First Americans, University of Maine, Orono, ME.

Byers, SN (2001) *Introduction to Forensic Anthropology: A textbook.* Boston, Allyn Bacon Inc.

Correia PMM (1997) Fire Modification of Bone: A Review of the Literature. In Haglund WD and Sorg MH (eds): *Forensic Taphonomy: The Postmortem Fate of Human Remains.* Boston, CRC Press, pp. 275-293.

Currey JD (1964) Some effects of aging in human Haversian systems. J. Anat. *98*:69-75.

Enlow DH and Brown SO (1956a) A comparative histological study of fossil and recent bone tissue. Part I. Texas Journal of Science. *8:*405-443.

Enlow DH and Brown SO (1956b) A comparative histological study of fossil and recent bone tissue. Part II. Texas Journal of Science. *9:*186-214.

Enlow DH and Brown SO (1956c) A comparative histological study of fossil and recent bone tissue. Part III. Texas Journal of Science. *10:*187-230.

Ericksen MF (1991) Histological estimation of age at death using the anterior cortex of the femur. Am. J. Phys. Anthropol. *84*:171-179

Eriksen EF, Axelrod DW and Melsen F (1994) *Bone Histomorphometry*. New York, Raven Press Ltd.

Eroschenko VP (1993) *di Fiore's Atlas of Histology with functional correlations*. Philadelphia, Lea and Febinger, pp.37-49.

Fideler BM, Vangsness Jr. CT, Moore T, Li Z, Rasheed S (1994) Effects of Gamma Irradiation on the Human Immunodeficiency Virus. Journal of Bone and Joint Surgery. *76A(7)*: 1032-1035.

Gejvall NG (1969) Cremations. In Brothwell D and Higgs E (eds.): *Science in Archaeology.* 2nd Edition. London, Thames and Hudson, pp.468-479.

Giles E and Elliot O (1963) Sex determination by discriminant function analysis of crania. Am. J. Phys. Anthropol. *21*:53-68.

Hermann B (1977) On histological investigations of cremated human remains. Journal of Human Evolution. *6*: 101-103.

Jowsley J (1960) Age changes in human bone. Clin.Orthop. *17:*210-218.

Kerley ER (1965) The microscopic determination of age in human bone. Am. J. Phys. Anthropol. *23*:149-164

Kerley ER and Ubelaker DH (1978) Revisions in the microscopic method of estimating age at death in human cortical bone. Am. J. Phys. Anthropol. *49*: 545-546

Kimura K (1992) Estimation of age at death from second metacarpals. Z. morph. Anthropol. *79*:169-181.

Krogman WM and Işcan MY (1986) T*he Human Skeleton in Forensic Medicine*. Second Edition. Springfield, Charles C. Thomas.

Lillie RD (1965) *Histopathologic Technique and Practical Histochemistry*. New York, McGraw Hill Book Company.

Marks M, Rose JC, Buie EL (1988) Bioarchaeology of Seminole Sink. Plains Anthropologist *33(122):75-118.*

Mellors RC (1959) *Analytical Cytology*. New York, McGraw Hill Book Company.

Nelson R (1992) A microscopic comparison of fresh and burned bone. J, Forensic Sci. *37*: 1055-1060.

Perper (1993) Time of death and changes after death: Part 1 Anatomical considerations In Spitz WU (ed.): *Spitz and Fisher's Medicolegal Investigation of Death: Guidelines for the application of pathology to crime investigation*. Third Edition Springfield, Charles C. Thomas, pp. 14-49.

Petrenko VF, Whitworth RW (1999) *Physics of Ice*. Oxford, Oxford University Press.

Pope EJ, Symes SA, Smith OC (2002) Burning observations of the head: An experimental model. Proceedings of the American Academy of Forensic Sciences *8:*225-226.

Ross MH, Reith EJ, and Romwell LJ (1989) *Histology: A Text and Atlas*. Baltimore, Williams and Wilkins, pp. 141-160.

Schwartz JH (1995) *Skeleton Keys: An Introduction to Human Skeletal Morphology, Development and Analysis*. Oxford, Oxford University Press.

Shipman P, Foster G, and Schoeninger M (1984) Burnt Bones and Teeth: An Experimental Study of Color, Morphology, Crystal Structure and Shrinkage. Journal of Archaeological Sciences *2*:307-325.

Singh IJ and Gunberg DL (1970): Estimation of age at death in human males from quantitative histology of bone fragments. Am. J. Phys. Anthropol. *33*:375-382

Stout SD (1982) The effects of long-term immobilization on the histomorphology of human cortical bone. Calcif. Tissue Int. *34*:337-342.

Stout SD (1989) Histomorphometric analysis of human skeletal remains. In İşcan MY and Kennedy KAR (eds): *Reconstruction of Life from the Skeleton*. New York: Wiley-Liss, pp. 41-52.

Stout SD (1992) Methods of determining age at death using bone microstructure. In Saunders SR and Katzenberg (eds): Skeletal *Biology of Past Peoples: Research Methods.* New York, Wiley-Liss, 1992, 21-25.

Stout SD (2002) Personal Communication. 17 April 2002

Stout SD and Gehlert SJ (1980) Effects of field size when using Kerley's histological method for determining age at death. Am. J. Phys. Anthropol. *58*:123-125.

Stout SD and Paine RR (1992) Brief communication: Histological age estimation using rib and clavicle. Am. J Phys. Anthropol. *87*:111-115.

Stout SD and Stanley SC (1992) Percent osteonal bone VS osteon counts: The variable of choice for estimating age at death. Am. J. Phys. Anthropol.*86*:515-519.

Tersigni, MA (2001) Microscopes and spectrometry: Identifying fragmentary human and canid bone. Proceedings of the American Academy of Forensic Sciences. *7*: 250-251.

Thompson DD (1979): The core technique in the determination of age at death in skeletons. J. Forensic Sci. *24*:902-915

Tomford WW, Thongphasuk J, Mankin HJ, Ferraro MJ (1990) Frozen Musculoskeletal Allografts. Journal of Bone and Joint Surgery. *72A(8)*: 1137-1143.

Trotter and Glesser (1952) Estimation of Stature from Long Bones of American Whites and Negroes. Am. J. Phys. Anthropol. *10*: 463-514.

White TD (2000) *Human Osteology*. Second Edition. San Diego, Academic Press.

Zugibe FT and Costello JT (1993) The Iceman Murders. J. Forensic Sci.*38*:1404-1408.

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