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A Study of Histological Aging of the Human Clavicle

Nikki L. Rogers
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Nikki L. Rogers entitled "A Study of Histological Aging of the Human Clavicle." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.

Lyle W. Konigsberg, Major Professor

We have read this thesis and recommend its acceptance:

William M. Bass, Richard L. Jantz, David Gerard, Esteban Walker

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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Associate Vice Chancellor and
Dean of the Graduate School

A STUDY OF HISTOLOGICAL AGING OF THE HUMAN CLAVICLE

A Thesis Presented for the

Master of Arts Degree

The University of Tennessee, Knoxville

Nikki L. Rogers

December, 1996

DEDICATION

This work is dedicated to

Dr. Robert C. Dailey

and

Dr. Rochelle A. Marrinan

For demanding nothing less than the best from their students.

I owe them my respect and sincere appreciation.

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ABSTRACT

This study attempted to augment the Stout and Paine (1992) and Stout and colleagues' (1996) methods of histological aging for the clavicle. In the 1992 study, the predictive equation was generated from a sample of only 40 individuals taken from an autopsy population (mean ages 28.6 years) with no prior sampling strategy. The 1996 expansion (Stout et al., 1996) tested the 1992 equation, added 41 males and 42 females from a Swiss cemetery sample to the original autopsy sample to generate a new predictive equation based on all 123 individuals.

In this study, an independent autopsy population was used to construct a sample of 95 individuals with a random stratified profile and an approximately equal number of individuals from each decade between the ages of 22 and 88 years. Roughly equal numbers of males (n = 50) and females (n = 45) were included in the sample. Construction of an independent sample assured comparability of all measures and excluded possibility of intraobserver error. This sample was used to achieve four research goals: 1) Test for sexual dimorphism in the OPD variable. 2) Generate predictive equations using inverse calibration, the method most commonly used in histological aging methods, achieved by regressing age upon the histological variable OPD. 3) Test the 1992, 1996, and current equations using both the Stout and Paine (1992) and the current samples. 4) Generate predictive equations for the Stout and Paine (1992) and current sample using classical calibration, which is regression of the OPD variable upon age.

The generation of predictive equations using both the inverse and classical calibration allowed comparison of the characteristic distributions associated with each, and tests for accuracy of their estimates.

While females exhibited a greater correlation between age and the OPD variable (r^2 = .5763) than males (r^2 = .2489), this difference did not result in a significant difference in the OPD variable between males and females. Based on the full sample, the relationship between age and the OPD variable was found to be highly significant ($p < .0001$).

A linear response and plateau function (lrp) was performed on the data to locate the most likely asymptote, a point where further remodeling activity cannot be detected. The lrp placed the most likely start of the plateau at the age of 73 years. Based upon this result, a subsample of those individuals between the ages of 22 and 73 years were used to generate all predictive equations (n = 74).

The inverse predictive equation reported by Stout and colleagues (1996) provided the most accurate age estimates for the current sample, followed by the equation produced from the subsample in the current study. The classical calibration equations generated for the current subsample and Stout and Paine (1992) samples both resulted in greater mean squared error values than the their inverse counterparts when applied to the full current sample.

Comparison of the mean squared error values for the classical and inverse predictive equations for the current subsample (n = 74) showed that while the inverse calibration equation shows evidence of slight bias, the error for the inverse calibration estimates is smaller than those for the classical equation across all age ranges (22 - 73

years). This suggests the inverse form of the predictive equation is the calibration method of choice for histological age estimation for the clavicle.

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PREFACE

Since Ellis Kerley introduced the first histological aging method in 1965, the use and merit of this methodology has been subject to much criticism and debate. Rather than discourage its use, the controversy has led to a proliferation of histological techniques attempting to mitigate weaknesses and utilize new sampling strategies and histological variables.

This study focuses on histological aging methods for the human clavicle. The original method was developed by Stout and Paine (1992) using a small sample (N = 40) with a relatively young mean age of 28.6 years. Stout and colleagues (1996) tested the resultant predictive equation on an independent sample from a historic Swiss cemetery (N = 83). They then tested the Swiss sample for sexual dimorphism of histological characteristics, but found none. Finally, they combined the original and Swiss samples to generate a new predictive equation. The age distribution for the combined sample (N = 123) extended through the eighth decade and had slightly older mean age of 36.6 years (Stout et al., 1996).

For this study, an independent sample was assembled according to a uniform age distribution with individuals between the ages of 22 and 88 years. A roughly equal number of males and females (N = 50 and 45, respectively) were chosen for study.

There were four goals of this study: 1) Test for sexual dimorphism in the histological variable OPD; 2) Generate predictive equations based upon the current sample using the inverse calibration, the method most commonly used in histological aging

methods. (Inverse calibration is performed by regressing age on the histological variable.); 3) Test the 1992, 1996, and current equations using both the Stout and Paine (1992) sample (N = 40) and the current sample (N = 95); 4) Generate predictive equations for the Stout and Paine (1992) sample and current sample using a two types of calibration. In classical calibration, the histological variable is regressed on age and the equation is algebraically solved for age. Generating equations using both methods allowed comparison of the characteristic patterns of age estimates for the two forms of calibration.

Each chapter in this study provides a brief introduction to a topic relevant to histological aging methods. The first chapter reviews the histological aging methods described in the anthropological literature and discusses critiques of their methodology and theory. Chapter two introduces the basics of bone remodeling and factors affecting remodeling activity, followed by a brief introduction to the histomorphometric method used in this study. The third chapter contains a discussion of the classical and inverse methods of calibration and the statistical characteristics of each method's resultant age estimates. The current sample and the technical methodology used in this study is described in chapter four, along with statistical analysis of the data collected. The results are discussed more fully with their implications in chapter five, with conclusions and suggestions for future study.

CHAPTER ONE: HISTOLOGICAL METHODS OF AGE DETERMINATION

One of the most important attributes physical anthropologists estimate is an individual's age. Actual chronological age cannot be determined from skeletal remains due to the great variability in biological aging processes among individuals. However, analysis of age-related changes of the skeleton allows determination of *skeletal age*, the best estimate of chronological age if no outside information is available to the investigator.

In archeological study, estimated ages provide demographic categories used to study cultural and environmental components of the past, and in the modern forensic setting possible identities for unknown individuals are first matched by probable age. The first reliable age indicators were introduced after World War I (Bass, 1987), each based upon age-related changes in gross skeletal morphology. The aging of children and young adults has been fully documented, and the relatively predictable sequence of tooth eruption and epiphyseal union allows small ranges for age estimates. However, after the third decade of life and the cessation of bone growth, anthropologists must rely upon more subtle changes in the skeleton. Current aging methods still rely on gross morphological patterns of change, but some anthropologists believe age estimates based on microscopic morphology may be as accurate as gross methods (e.g. Pfeiffer, 1980). This opinion has been questioned in the past, and as knowledge concerning the regulation of bone physiology increases, so does the possibility of new challenges to this idea. Examination and improvement of current methods are necessary to answer these impending challenges.

Histomorphometry, the quantitative study of histology, has been used by physical anthropologists to determine skeletal age at death, health status and degree of preservation in modern (Kerley, 1965; Thompson, 1981; Thompson and Galvin, 1983), archaeological (Stout and Teitelbaum, 1976a; Ericksen and Stix, 1991; Stout and Lueck, 1995) and fossil samples (Thompson and Trinkhaus, 1981; Trinkhaus and Thompson, 1987; Streeter et al., 1996). Amprino and Baraiti (1936) first established the correlation between age and

increasing osteon populations, but as Garland (1992:1) states, "histology simply did not fit in with the paradigms..." of early anthropology. However, one must also recognize that before the 1960's, bone histology had gaps in theory that would have limited its useful application to anthropological questions. Histomorphometry was beginning to bridge these theoretical gaps and establish itself as an independent field of study when physical anthropology began turning to the ancillary sciences for answers.

According to Burr (1985), scientists miscalculated the importance of links between cellular and organ-level processes in the early twentieth century. Coupled with a lack of technology, this underestimation resulted in studies that independently concentrated on the gross structural or cellular processes of bone physiology rather than examining the relationship between the two. In the 1960's, the work of Dr. H. M. Frost remedied this situation with the introduction of non-toxic tissue markers that allowed observation of dynamic tissue-level processes. These tissue markers led to theory describing how various bone functions were related in a hierarchical organization. This functional organization of physiological processes is called the *intermediary organization* (see chapter 2).

The discovery of the skeletal intermediary organization (IO) marked the first time cellular-level processes and organ-level changes could be traced to environmental and individual stimuli that "collectively... determine the macroscopic and microscopic anatomy of skeletons during growth and in adult life" (Frost, 1986:211). This functional concept of bone as a hierarchy of processes replaced the traditional static view of bone, and this concept of the IO was aptly dubbed the "New Bone." In the thirty years since its introduction, the IO concept has given researchers the tools to observe the mechanisms of bone modeling, remodeling, and homeostasis, and provided insight into how these processes are mediated. The IO is very complex, and many facets of bone behavior were only recently confirmed (Frost, 1985), while others still remain unknown. These basic tenets of bone physiology form the basis of histomorphometry, and while recent findings

concerning the IO have not altered the underlying concepts of quantitative histology, they *have* complicated analysis by identifying additional sources of variability in physiological mediators (see chapter 2).

BASIS FOR HISTOLOGICAL AGING METHODS

Histological aging techniques can provide more specific age estimates for elderly individuals than gross morphological methods, which often result in one large terminal category of those of 55 years and older. Histological methods also provide smaller reported error of estimate values than morphologic techniques (Stout, 1989a). However, their most attractive advantage is their proposed ability to age fragmentary remains.

All histological aging methods are based upon the fact that bone tissue is replaced or "turned over" throughout an individual's life. Living bone is a dynamic tissue that is constantly changing to meet daily demands. As Acsadi and Nemerski (1970:101) state:

> "...the skeleton...is changing throughout the course of life. It is no mere mechanical framework of the organism permanently in the same morphological state. The skeleton participates in the overall metabolism of the organism, [and] responds to exogenous and endogenous impacts (advance of age, change in environmental conditions, nutrition, working conditions, diseases, etc.) with morphological changes."

The movement and weight of body tissues affect biomechanical and physiological stresses on bone, causing microstructural damage to the mature skeleton. The productive lifespan of bone cells (osteocytes) is thought to be approximately 7 years, but may vary greatly between individuals (Enlow, 1990). The adult skeleton undergoes only slow appositional growth (Lazenby 1990a,b) and therefore populations of mature bone cells are subjected to damage from mechanical stress and strain, which would lead to functional failure of bone tissue if left unchecked. However, each area of mature bone is eventually replaced with newly deposited bone through a process called *remodeling*.

Martin and Burr (1982:137) propose that cortical remodeling is a functional "adaptation to prevent the accumulation of microdamage due to repetitive loading." This theory suggests the secondary osteons "trap" forming microfractures by forcing them to conform to the more loosely bound circumferential lamellae of the osteon. This damages the osteon, but prevents more structurally damaging linear cracks that form in primary bone tissue. The damage to the secondary osteon and its Haversian canal and the subsequent danger to regional blood supply would then stimulate the remodeling process in the area. However, the cause and effect mechanisms of baseline remodeling have not been definitively agreed upon by the scientific community. Other theories of remodeling stimuli exist based upon piezoelectric forces, streaming potentials and a whole host of chemical actions. (See Martin and Burr, 1989:120-122 and Vaughan, 1981 for discussion).

During remodeling, new osteons are built over pre-existing ones, creating a pattern of visible osteons and older osteon fragments. These patterns of complete and fragmentary osteons are interpreted in histological aging methods. The functional theory of microstructural fatigue is illustrative of the theory of histological aging methods: as one ages, the skeleton undergoes more maintenance and therefore will have more osteons and osteon fragments present.

Processes associated with metabolic bone disease also affect remodeling, but the way they initiate or accelerate the remodeling process has not been specifically described. Some conditions, like Paget's disease (Aaron et al., 1992) and hyperparathyroidsim (Cook et al., 1988) have been identified at the histological level. However, as with the organ-level response of bone, the cellular response of bone to disease is often general and several likely diagnoses may be offered for the same set of characteristics. Histomorphometry of living bone is used to diagnose conditions such as osteoporosis, osteomalacia, and primary hyperparathyroidism, but non-mineralized bone characteristics (such as osteoclast and

osteoblast counts), blood biochemistry, and in-vivo tissue markers are used to substantiate such a diagnosis. Opportunities to identify pathological conditions in archaeological populations are more limited, but are being eagerly explored by anthropologists (Grupe and Garland, 1992).

SUMMARY OF HISTOLOGICAL AGING METHODS

Many techniques for age estimation have been introduced. All are based upon the same concepts of bone physiology, but each measures different microstructural characteristics and samples different areas of different bones. With the exception of Weinstein et al.'s (1981) study of changes related to age and disease in trabecular bone, histological methods have been based on changes in cortical bone (Stout, 1989a). Age-related changes occur in other types of bone tissue, but their complex structure makes them more difficult to study (Stout, 1989b), and it is possible that their mediators are more vulnerable to physiologic stimuli. The use of cortical bone also offers more opportunity for sampling, since it constitutes almost 80% of the human skeleton (Parfitt, 1983b) and is more likely to be present in fragmentary archaeological remains.

In 1936, Amprino and Baraiti first described the correlation between the number of Haversian systems in cortical bone and increasing age. This finding was not utilized until 1960, when Jowsey described age-related changes in human Haversian systems for "medically normal" individuals to establish patterns of individual variation.. Bone mineral density, resorption, formation, and other functional activities were measured for femoral midshaft cross-sections from 24 individuals using microradiography. However, nothing similar to the now-familiar osteon counting method was used. Currey's (1964) study represents the first attempt to quantify the relationship between age and the ratio of Haversian to non-Haversian bone. Nineteen mid-femoral cross-sections were used to determine the ratio of remodeled to unremodeled bone, and the resultant ratios and

absolute osteon counts were examined for their relation to age. Currey also assessed the effects of age on the size of the both the whole Haversian system and the Haversian canal, as well as age-related thinning of the femoral cortex. After these studies found the relationship between osteon counts and age to be significant, histology became an obvious choice for age estimation techniques in physical anthropology.

The landmark method developed by Ellis Kerley (1965) measures complete and fragmentary secondary osteons, primary canals, and the percentage of lamellar bone in four areas of the femur, tibia, and fibula. Definitions of microscopic structures are clearly outlined, but some suggest (Ahlqvist and Damsten, 1969) differences in interpretation of the definitions may lead to increased interobserver error. The observed measures of the structures are used with prediction equations to estimate an individual's age, or with sample profiles to find the approximate age range in which a particular histomorphometric count would be expected (Kerley, 1969). Problems encountered due to the round shape and size of the original field were addressed by Kerley and Ubelaker (1978), who presented revised prediction equations and field size correction factors to be used with the original method. However, Stout and Gehlert (1982b) remind us that correction factors cannot transform a non-representative sample into a representative one. They claim the method is most reliable when field size and magnification are kept as close to Kerley's original study as possible. Stout (1989a) further advises averaging the ages estimated by the regression equations for each bone for greater accuracy. Despite thirty years of critique and testing, field size problems, and competition from newer methods, this technique is widely accepted as the most accurate (Bouvier and Ubelaker, 1977; Stout and Gehlert, 1982a). This may be due in large part to the characteristics of the sample from which the equations were derived. All ages were represented (0 to 95 years), and a large number of individuals were studied (N=126). The sample was mostly comprised of white males, but the inclusion of 29 females and 11 black individuals allowed possible (albeit

non-significant) noise due to racial or sexual variation to be included. This should in theory increase the method's applicability. Such complete representation across all decades of age allows accurate estimates to be made for most individuals.

Recent work on sampling location (Burr, 1985; Pfeiffer, 1992; Pfeiffer et al., 1995) indicates there may be valid concerns about the consistency of remodeling activity between areas of bone and sites near the same location. Anterior sampling sites, a common factor in the Kerley method and several that followed (Thompson, 1978,1979; Ericksen, 1973,1991) appear to have greater variability than other sampling sites due to their relationship to anterior anatomical and geometric mechanical axes. However, avoiding these areas would require computer-aided assessment of the axes, which is possible only with a complete cross-section of the bone, requiring bone invasion of the highest degree (Pfeiffer et al., 1995).

The technique proposed by Ahlqvist and Damsten (1969) was meant to lessen the confusion created by Kerley's definitions of complete and fragmentary osteons and to utilize fields not affected by the strong biomechanical factors. The modified method uses square fields and measures only the percentage of osteonal bone and fragments, making it less confusing than Kerley's method. However, the authors readily admit the ease-of-use is bought at the cost of accuracy and increased standard error, which is greater in their study than in Kerley's. The regressions presented are based on a small test sample (N=20), but Stout and Gehlert (1982a) claim they generate satisfactory age estimates above the sixth decade, possibly because more than half of Ahlqvist and Damsten's sample specimens were above the age of 55 years.

The variable representing the percentage of cortical area invested by Haversian bone (percentage Haversian bone) used by Kerley and Ahlqvist and Damsten has an "inconsistent" relationship with age, dependent on the bone and the area sampled (Stout and Stanley, 1991). Measuring the Haversian bone percentage for the entire cross section

of the radius was found to provide a statistically significant relationship between age and percent Haversian bone, while reading the 4 fields suggested by Ahlqvist and Damsten did not illustrate a significant relationship between the two variables. Some doubt may be cast on the percent Haversian variable in age determination when few fields are sampled, and subsequently on the estimates generated by the Ahlqvist and Damsten procedure. This issue is of lesser importance in methods where other variables are used in conjunction with the percent Haversian variable (Kerley, 1965: Ericksen, 1991).

Singh and Gunberg (1970) developed a method for aging from the anterior midshaft of the femur and tibia and the posterior portion of the mandibular ramus. Their small sample (long bone N=33: mandible N=52) was skewed toward older ages, with most individuals between the ages of 50 and 75 years. This allows older individuals to be aged more specifically than is possible with morphological estimators, which often end with one large category for all those older than fifty-five years. The methodology described for the mandible is different than that described for the long bones, which can cause practical problems and confusion for those trying to apply this method (Stout, 1989a).

Another concern associated with many of the histological techniques is that sampling error and histological complexity may affect the accuracy of methods that require sampling only a few specific fields. The fewer fields sampled, the greater the chance of reading a non-representative field as the basis for an age estimate. This criticism holds for the Kerley and Ahlqvist and Damsten methods, but moreso for many of the more recent methods since each attempts to lessen the sample size necessary for age prediction (e.g. Thompson, 1979; Ericksen, 1991). For example, while the Kerley and Ahlqvist and Damsten methods both sample only four fields, the method by Singh and Gunberg uses only two. The possibility for sampling error increases with older individuals, where areas of significant periosteal apposition may fall into the sample field and provide a younger mean tissue age than is representative of the remodeled cortical bone. However,

Kerley (1965) suggested the four field method as a way to insure that age estimates would not be based on a single, possibly non-representative field, while Stout and Paine (1992) claim reading the entire cross section of a small bone (i.e. clavicle or rib) provides the most representative counts. This illustrates that opinions vary as to which sampling plan is adequate to provide a representative sample.

By the late 1970's, histological age estimation was fairly well accepted and much discussed, but not widely practiced. The invasive nature of the practice was a major drawback first addressed by Thompson (1978,1979), who developed a method that requires only a small round core from the anterior midshaft of the long bones. Using a sample of 64 males and 52 females, both non-histological measures (cortical thickness, bone mineral density) and histological measurements were tested for age-related changes. A comparison of upper and lower limbs showed that upper limbs exhibit similar age-related histological changes as the lower limbs. The non-histological measures were found to be inferior to histological ones for age prediction for all long bones. Thompson's final regressions utilized 19 different variables. While the methodology is very comprehensive, it requires a great deal of histomorphological expertise on the part of the observer as well as an impractical investment of time if multiple individuals are to be aged.

Thompson and Galvin (1983) published a follow-up study using the same methodology to better estimate age from the tibia in individuals under 55 years of age since earlier work showed equations for that bone to be poor estimators of age in that particular age group.

Stout (1989b) maintains the small section utilized in this technique presents greater possibilities for sampling error (i.e., choosing a non-representative sample). However, tests on 8 forensic cases not used in the original generative sample yielded results within $\pm$5 years of actual age (Thompson, 1979). Application of this method to fossil Neandertal specimens (Shanidar 3, 4, 5, and 6) provided age estimates similar to those estimated from

their gross morphology (Thompson and Trinkhaus, 1981; Trinkhaus and Thompson, 1987). Recent work (Streeter et al., 1996) proposes that remodeling and activation rates in hominid (Shanidar 3 and Skhul 7) ribs and clavicles may be similar to those of North American native populations and modern samples, suggesting these estimated ages for these human ancestors are on sound theoretical ground.

Samson and Branigan (1987) developed a technique primarily for archaeological bone that can be applied to specimens that have less than perfect preservation of histological structures. Their method studies only Haversian canals, which were present in even the most poorly preserved specimens observed. The method samples the femur and measures mean cortical thickness (MCORTK), mean Haversian canal diameter (MHCD), and the number of Haversian canals in each square unit of area (NHC). The product of the latter two variables (MHCD and NHC) is used to calculate an additional variable, morphologic character (MC). This variable had the best statistical relationship with age of those measured in their study.

Samson and Branigan (1987) were able to generate estimates for the male test sample that were within $\pm$6 years of actual age, but ages for the female sample could not be predicted within $\pm$16 years. They suggest this might be due to hormonal regulation differences between the sexes, and recommend that sex be determined for each specimen before age estimation is attempted with this method.

Ericksen (1973,1991) also attempted to lessen the invasiveness needed for histological age estimation. Her method requires reading only a small wedge taken from the anterior cortex of the femur. Addressing sampling deficiencies of previous methods, Ericksen constructed a very large sample (N=328) that included individuals with varying diseases that affect bone remodeling, and which represented individuals from three different geographic areas (USA, the Dominican Republic, and Chile). A dimorphic pattern of remodeling for complete osteons was found between sexes: among females, the

number of complete osteons peaked in the fifth decade and did not increase thereafter, while in males the number of complete osteons continued to increase throughout all decades. The number of osteon fragments increased through all decades for both sexes. While application of this method to a historic cemetery population comprised of free blacks (Ericksen and Stix, 1991) produced ages that were significantly correlated with those determined by gross morphology, some discrepancies were found between individual estimates produced by the two types of methods.

Yoshino et al. (1994) described a microradiographic method for estimating age from the humerus taken from a sample of Japanese males. The best correlation was found between age and the number of osteon fragments, but eight variables are used in their regression, which they claim results in a standard error of estimate of only $\pm$ 5.1 years. The method remains to be tested and fully evaluated, but presents an interesting opportunity to further evaluate inter-population applicability of histological aging methods and questions of practical usage.

While most methods use bones of the appendicular skeleton, a few studies have concentrated on the axial skeleton. Boivin et al. (1981) documented age-related changes in the cortex of the iliac crest, calculating the mean number of intact and fragmentary osteons per square millimeter with a method derived from Kerley (1965). The authors suggest the method may be useful in identifying disease-related histological changes since this sampling site is quite common in clinical study. However, since Parfitt (1983b) claims that cortical bone from the ilium is unlike that found in the long bones of the skeleton, this method might require more histological sophistication on the part of the observer than the other more comparable cortical methods.

Stout and Paine (1992) described a method for estimating skeletal age from histology of the rib and clavicle, which may be more available for thin-sectioning since they are not typically used for standard anthropological estimations such as stature, sex, or

population affinity. The small cross-sectional size of these bones allows a strategy that samples the entire cortical area, decreasing the influence of non-representative fields. The authors recommended averaging ages predicted for both the rib and clavicle if possible, since sampling more sites should provide a more representative age estimate. The rib method was recently expanded to work with sternal end rib phase assessment to increase its accuracy and to further utilize multifactorial aging (Stout et al., 1994). Subsequent testing of the clavicle method on a known-age Swiss cemetery population produced ages with mean error of estimate values of 5.5 years (minimum absolute difference 0.04 years; maximum absolute difference 22.2 years) . The Swiss sample was then incorporated into the sample and a new prediction equation was generated on the expanded sample (Stout et al., 1996). (This method is discussed more fully in chapter 4.)

PROBLEMS IN HISTOMORPHOMETRY

After thirty years and the introduction of numerous methods, histological aging techniques have been viewed with criticism, suspicion, and even contempt by some anthropologists (see Lazenby, 1984). There are many precautions to observe when using these methods, but methodological limitations and biases are clearly outlined for each technique. One must be familiar with the sample characteristics and methodology used to create each procedure and assess their applicability to an individual case. However, this necessity is not limited to the use of these methods, since a competent anthropologist should explore the limitations and biases for *every* aging technique: histological or morphological.

The size of the field and its position on the bone from which the sample is taken is a very important aspect of each method (Stout and Paine, 1992; Lazenby, 1984). Recent work by Pfeiffer and colleagues (Pfeiffer, 1992; Pfeiffer et al., 1995) found significant differences in the degree of remodeling between regions of the femur, posing questions for

methods that sample anterior areas (Kerley, 1965; Thompson 1979; Ericksen, 1991), since anterior sampling sites were found to be less representative of skeletal aging processes than other sites studied. This also raises questions about applying histologic aging methods to fragmentary remains. Iwaniec and colleagues (1996) have quantified variation within the anterior midshaft of pre-and post-contact Inuit individuals and Pueblo agriculturists, and concluded that sampling of at least 17% of the anterior femoral midshaft in a specific topographical pattern provides a sample representative of the entire region. More studies of this nature are needed to help settle the question of representative sampling.

If entire thin sections of bone are read, Stout (1986; Stout and Paine, 1992) suggests a very conservative approach can be followed that allows one to "skip" areas with extremely complex or questionable histology without sacrificing statistical integrity. The Kerley and Ahlqvist and Damsten methods, which dictate the reading of *specific* fields challenge the skill of the osteon counter as well as the accuracy of the estimate if those specific fields have been affected by drift or contain structures that appear ambiguous. As Kerley (1965) stated, an obvious and most *important* factor affecting the accuracy of age estimates is the knowledge of the observer, especially in determining age in older individuals and interpreting complex patterns. However, the influence of inconsistent reading due to inexperience can be lessened by less specific and more thorough sampling. Also, methods that sample more sites and more bones increase accuracy of resultant age estimates (Lovejoy et al., 1985).

Stout and Gehlert (1982b) claim that field size affects the age estimates, even when used with correction factors like those suggested by Kerley and Ubelaker (1978) for the Kerley method. Since osteons are distributed "topographically rather than statistically" (Knese et al., 1954), correction factors for size cannot transform a non-representative sample into one truly representative of an area. Lazenby (1984) also criticizes the field

correction method, suggesting it is a superficial solution to a very severe methodological problem.

Definitions of what constitutes "complete" or "fragmentary" osteons varies by author (Stout, 1989a). To illustrate this point, Stout and Gehlert (1982a) cite four competing descriptions among the histological aging methods to date. Ortner (1970) proposed photos be taken of the observed field for more complete documentation and future study. Hard copies of each field allow further analysis with new definitions, refined methods, or in the case of questions concerning structure identification and inter-observer error. However, this may not be a reasonable option in cases when sampling strategy includes a large number of fields. Also, differences in chemical processing produced sections in my study that showed more histological detail than those processed by Stout and Paine (1992). Photos of these structures would have produced different counts than were produced by viewing the structures under different lighting and magnifications.

Pfeiffer (1980) compared ages determined macroscopically by cranial suture closure (Krogman, 1962) and pubic symphysis aging (Todd, 1920; McKern and Stewart, 1957; Gilbert and McKern, 1973) with histological ages (Kerley, 1965; Ahlqvist and Damsten, 1969) determined for a Late Archaic archaeological population from Ontario. The ages determined by the pubic symphysis and histological methods were "very similar," but the cranial suture ages did not correlate well with the other two methods. Acknowledging the intrusive nature of microscopic methods as well as the extensive equipment and knowledge needed to undertake histomorphometry, Pfeiffer recommends morphological methods be used except in cases where skeletal elements used in analysis are not available. This comparison might be questioned since the test sample consists of only six individuals and represents the only non-cremated burials from the native population. It is possible that burial practices resulted in a biased sample. Additionally, different remodeling rates (attributed to genetics and environmental influences) may cause

histological differences between archaeological and contemporary populations. Such differences have been proposed to affect the application of histological aging methods to populations from different time periods and cultures (Ubelaker, 1977: Aiello and Molleson, 1993), and may better account for the discrepancy between the estimates than inadequacy of histological methods.

Martin et al.'s (1981) critique of Pfeiffer's (1980) work stated that the histological ages were less accurate, especially among the older age ranges. They maintain that the longer an individual's life, the greater the possibility an individual will experience physiological stresses affecting remodeling. Consequently, there is greater potential for disparity of histological characteristics between individuals of the same advanced age. While conceding that histological aging techniques were useful, they claim gross morphology is less affected by these situations, and therefore provides more "stable" indicators of skeletal age. Citing work by Ubelaker (1974, 1978), Pfeiffer (1981) claimed the reverse had been previously accepted, and suggested it was presumptuous to assume microstructural ages were to blame for the discrepancy between the age estimates. She recommended further study into the accuracy of *both* types of aging techniques.

Lazenby's (1984) assessment of four histological aging methods led him to conclude :

> "Histological age estimation techniques utilizing bone do not seem able to provide estimates in which a researcher can invest a great deal of confidence. Reports of qualified success notwithstanding...the combination of limitations deriving from the nature of the bones to be aged and those inherent in the techniques themselves should be sufficient enough argument...for discontinuing use of these methods." (p.101)

He cites the normal variation between populations, environmental influences and disease processes as problems that plague the methodology of histomorphometry that can be

overcome. However, he highlights questions of sample size, constitution and definition, measurement of variables, field size problems, and the need for extensive knowledge of bone processes and microscopy as the basis for an end to histological age estimation.

Citing the lack of standardization and adequate testing of methods, Martin et al. (1981) claim that histological methods would be best used for paleodietary, stress, and paleopathological investigation rather than age determination. Work by Richman et al. (1979) did illustrate a relationship between the presence of Type II (embedded) osteons and dietary differences between historically known tribes. However, based on a suid model, Iwaniec and colleagues (1995) found no basis for the proposed relationship between high protein diet, acidosis and the differences in bone microstructure found between pre- and post-contact Inuit. They suggest the differences may be attributed to genetics, differences between mechanical usage, and calcium intake. Further studies are needed to document the relationship between dietary factors and microstructural characteristics.

Aside from the diagnostic pattern associated with Paget's disease, one wonders if diagnoses of pathology based on generalized bone responses at the microscopic level would be viewed with the same skepticism as those based on generalized macroscopic responses in archaeological bone. As Blumberg and Kerley (1966:154) state in their discussion of micromorphological analysis of paleopathology, "no one pattern is diagnostic." Interestingly, they point to aging as the more promising application of histomorphometry to anthropological and archaeological bone (p.164) contra Martin et al. (1981). Recent work by Grupe and Garland (1992) supports the idea that if the challenges presented by taphonomic and diagenetic processes can be overcome, histology may contribute valuable insight into archaeological disease patterns and processes.

Simmons (1985) proposed that selection of sampling sites presents a significant problem in histomorphometry, and suggests investigation of secular changes to assess

their influence. More recently, Aiello and Molleson (1993) cite different remodeling rates to explain the underestimation of the Spitalfields cemetery population using the Kerley method, claiming that the British (17-19th c.) population had a slower remodeling rate within its older ages than Kerley's modern sample, and therefore had fewer osteons per field than the Kerley method predicted. These results mirror Ubelaker's (1977) study of individuals from the Dominican Republic who were overaged in the older age ranges by an average of 19 years. These differences underscore the possibility of interpopulational differences that may hinder application of histological aging methods to all populations. However, most studies have found no populational differences in histological variables.

Uytterschaut (1992) blames the narrow use of histological aging methods on the "problems" of thin section techniques, field size problems, and distinguishing the structures defined by the various methods. In his original paper, Kerley (1965:149) suggested histological aging represented "an excellent *supplement* to conventional methods of age determination" (italics added), illustrating that from the beginning it was recognized that histological aging methods were not a replacement for morphological techniques. Morphological age estimates from multiple sources may provide small age ranges comparable to histologic estimates. In cases of fragmentary remains, histological aging may provide the only age estimate possible. No matter the case, histological aging methods must be explored and their strengths and weaknesses assessed before a judgment can be served.

CHAPTER TWO: BONE DYNAMICS AND HISTOMORPHOMETRY

The fast rate of primary growth in human long bones is initially accommodated by cartilage models that shape the skeleton prior to bony growth. Cartilage grows by interstitial cellular division, resulting in faster growth than osseous tissues, which grow by the slower process of cell addition. In the process of long bone growth, *primary* bone tissue is first deposited following the incipient cartilage models by a process called *modeling* (bone growth and shaping). During bone modeling, growth is directed by mechanical forces and for the most part follows Wolff's law (Wolff, 1892), which states that "form follows function."

After the second decade of life, growth ceases and maintenance of the mature skeleton begins. This maintenance process is known as skeletal *remodeling*. This process has been thoroughly described, but the causative stimuli are still in question. One theory of remodeling initiation (Martin and Burr, 1982) claims repetitive mechanical forces cause localized areas of matrix fatigue and damage. This theory suggests the remodeling process is stimulated in the damaged areas in order to maintain the structural integrity of the bone. It is possible that remodeling is an adaptation related to organismal activity: the bone tissue of slow-moving, non-dynamic animals like cows remodel very little, while smaller active animals such as dogs and larger fast-moving animals like humans and pigs undergo bone remodeling processes (Martin and Burr, 1989). While the initial activation stimuli of remodeling are not fully known, Martin and Burr (1982) posit microfractures and/or electric streaming potentials of bone provide the activation signal. Frost (1986) stated that a systemic baseline level of remodeling activity exists and is further influenced by multiple stimuli from various sources. (See Burr, 1985: Martin and Burr, 1989:120-122: Vaughan, 1981 for more on this topic).

Remodeling is carried out by osteoblasts and osteoclasts, bone cells that make a functional unit Frost (1986) termed the *Basic Multicellular Unit* or *BMU*. The cells of the BMU carry out linked functions that occur in a predictable 3-stage sequence: *Activation* of cell activity, *Resorption* of matrix, and *Formation* of new bone. This sequence is often referred to as *A-R-F*. The period of time needed to complete the A-R-F sequence, known as *sigma* (σ), has been measured by in-vivo tissue time markers and is of 3-4 months duration in adult humans. However, sigma can be highly variable, even among "medically normal" individuals (Recker, 1983) and there may be lags in time between the steps or lengthening of the time required for any stage in the processes due to age, state of nutrition, steady-state application of pharmaceuticals, or disease processes.

One of the most important discoveries in bone histology occurred in the 1960's, when modeling and remodeling were first recognized as two discrete processes (Frost 1986:191). There are simple but important basic differences between the two processes, summarized here from Frost (1986). Modeling is a function that turns over bone in the juvenile skeleton; remodeling is the function of bone turnover in the adult skeleton. Modeling changes the shape and size of a bone, while remodeling only replaces a specific volume of bone with new bone of the same type. In modeling, resorption (R) and formation (F) can occur in different areas and are not always linked functions, but in remodeling the two *always* occur together in sequence, even if the two processes are separated by a lengthened period of time. The resorption and formation functions are each carried out by cells originating from separate capillaries in modeling, while the two linked functions are carried out by the two cell types that differentiate from a single capillary in the remodeling process.

The process of cortical bone remodeling takes place only in the presence of a vascular system, since the cells of the BMU must be transported via such systems. Remodeled areas of bone are said to be distributed topographically rather than

statistically (Knese et al., 1954) because they are distributed around existing blood vessels. In cortical bone the A-R-F sequence begins with the differentiation of mononuclear haemopoietic cells into osteoclasts (Vaughan, 1981). These osteoclasts create a characteristic tunnel-like resorption space averaging 5 microns in length. This resorption area is called a *cutting cone.* Osteoblasts differentiate from mesenchymal cells and follow behind the osteoclasts filling in the resorption space with *osteoid*, a non-mineralized matrix consisting of collagen fibers and ground substance (Enlow, 1990). This process of "filling in" begins along the outside circumference of the resorption space and continues inward. Within 10-14 days, the osteoblasts begin mineralization of the osteoid, and within the first 24 hours more than half of the mineralization process is achieved. The remaining process of mineralization may proceed by fits and starts, taking as long as 6 months to conclude in the clinically "normal" individual.

Remodeled bone is defined by the presence of secondary osteons and fragmentary secondary osteons (figure 2.1), which are separated from the surrounding structures by a marked *cement line.* The fragmentary osteons are older structures that have been partially erased by subsequent remodeling processes. Complete osteons have not been remodeled, and most of their cross structure is intact and visible. Secondary (remodeled) bone is differentiated from primary (unremodeled) bone by the presence of cement lines circumscribing the Haversian canals.

As many as 10% of the working osteoblasts become trapped in the advancing mineralizing front (Martin and Burr, 1989) and become resident *osteocytes* residing in spaces in the bone called *lacunae* (figure 2.1). As mature bone cells, these osteocytes remain metabolically active and serve purposes of tissue-level communication via their canaliculi, and retain their osteogenic ability that can be utilized in cases of fracture repair and modeling.

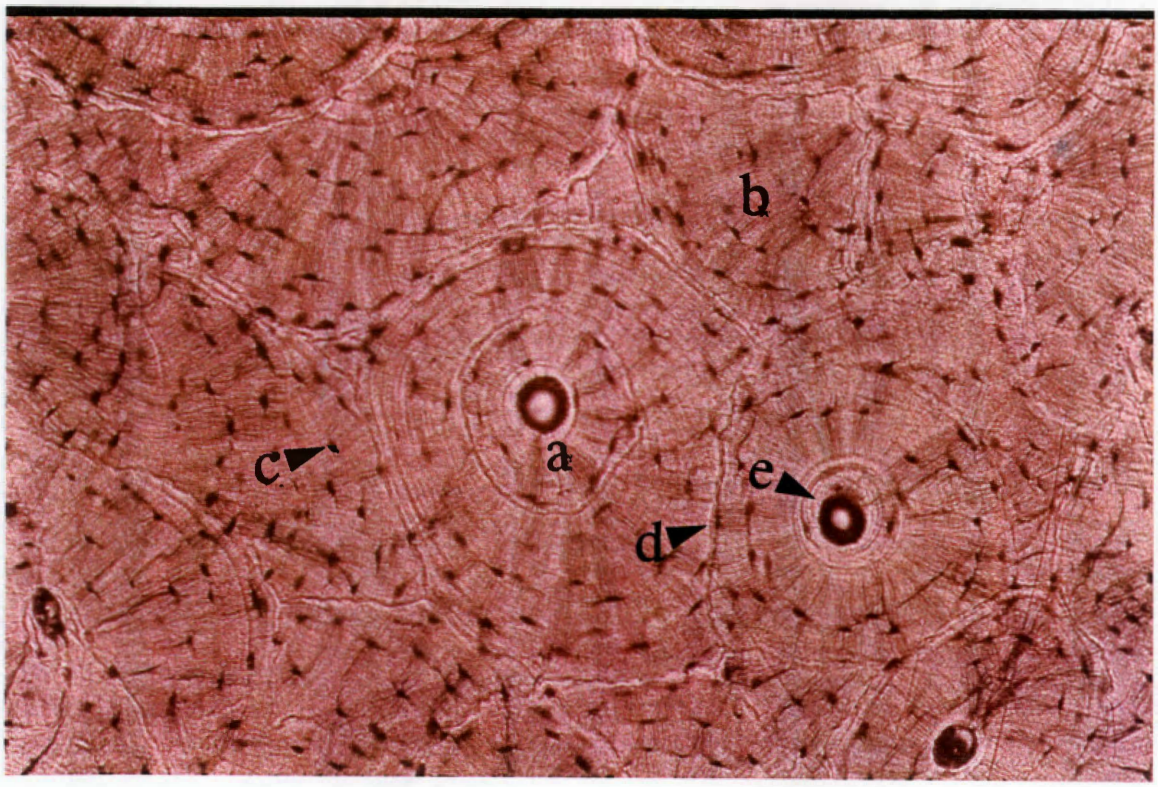

Figure 2.1: Photograph of a Clavicular Thin Section with Labeled Structures

A) Complete osteon

B) Fragmentary osteon

C) Lacuna with resident osteocyte

D) Cement Line

E) Haversian canal

Bone remodeling is a process that affects a consistent volume of tissue. Each BMU remodels approximately .05 mm^3 of bone. In trabecular bone, the formation (F) process does not completely replace the volume of resorbed bone. As a result, a small amount of bone is lost during each remodeling event. The difference between the areas resorbed and formed has been termed delta BMU (Δ BMU) and is estimated to be roughly .003 mm^3 in cortical-endosteal and trabecular bone. The annual net loss of trabecular bone in adults has been estimated to be .75% of the total bone bank, but ΔBMU values can increase due to lags in the A-R-F sequence or by conditions that further decrease the efficiency of the formation process and/or increase the number of active resorption areas, such as in the case of decreased mechanical usage (summarized from Frost, 1986). However, this loss of bone volume due to the lesser formation process does not occur in cortical bone.

THE SKELETAL INTERMEDIARY ORGANIZATION

All organs are thought to have mid-level management systems that mediate the various physiological processes and allow cellular level processes to accomplish organ level changes. However, such a system was not defined for bone until the 1960's. Frost (1986) proposed that the skeletal intermediary organization (IO) serves these functions in bone. To illustrate the functional significance of the skeletal IO, Frost often compares the IO in bone to the nephron level of processes found in the kidney. Both the nephron IO and skeletal IO occupy the tissue level of organization in the biological scheme of things, bridging the functional gap between the cellular and whole organ level:

cell -----→ tissue -----→ organ

Although the tissue level structure of bone was recognized by researchers by the mid-1800's (Martin and Burr, 1989), researchers of the early and middle twentieth century believed observed cell level processes and phenomena could be directly

extrapolated to organ-level changes. These beliefs were grounded in the tenets of what Frost (1986) calls atomistic theory, where small structural units are theorized to have direct organ level effects. This concept of organization has been summarized by Frost (1986) as:

cell -------→ organ

While the ideas underlying IO theory were described between 1960 and 1964, Frost claims that prior to the 1980's the clinical medical community paid little attention to its concepts. However, the consistent failure of hypotheses utilizing atomistic theory lead to the realization that such a direct cell ---→ organ connection was not the case. This led to the gradual acceptance of the IO theory.

The IO controls 6 functions that have influences at the supracellular level: growth, modeling, remodeling, repair, and homeostasis (Frost, 1986). However, even with 30 years of study, knowledge of the IO is still incomplete. The knowledge gained up through the mid-1980's was compiled by Dr. Frost into the two volumes of *The Skeletal Intermediary Organization* (1986) where an exhaustive description of the IO and its associated theory can be found.

Acknowledging that one could justify splitting the IO into more divisions, Frost (1986) recognizes three levels (see Table 2.1): the lower IO (L_1), the middle IO (L_2), and the upper IO (L_3), each governing different functions and processes. The L_1 level is where the cells are created (histogenesis) and controlled. The functions of this level provide basic materials that provide stiffness, rigidity, and hardness of bone structures (lamellar and woven bone, hyaline and elastic cartilage, etc.). The L_2 level organizes the basic cells and materials into functional units and allows them to interact with their local stimuli. Compact bone, trabeculae, and the spongiosa found in the epiphyseal plate are organized by the processes of the second level of the IO, and it is this level that regulates BMU-based remodeling. More is known about L_2 bone remodeling than any other IO process.

Table 2.1: Functions of the Skeletal IO by Level

LOWER IO (L_1): By organizing cells, makes the basic materials for bone processes for growth, resorption, and micromodeling, and provides the ability to heal tissue.
MIDDLE IO (L_2): Organizes processes to carry out functions of macromodeling, BMU-based remodeling, microdamage detection and repair, and drift due to mechanical effects.
UPPER IO (L_3): Provides the organized response for homeostasis, repair, RAP, joint unit loading regulation, and makes bone envelopes. Uses tissues organized by the L_2 level to make ligaments, fascia, epiphyses, and tendons.

Summarized from Frost (1986:75, 94)

The third level of the IO, L_3, regulates processes of intact epiphyses, whole joints, bone envelopes, and other complete structure functions. This level of organization is also responsible for the remodeling of the woven callus associated with fracture repair into more organized lamellar tissue and also regulates bone's homeostatic biochemistry (Frost, 1986:125).

The three levels of the IO are intricately linked, and small problems within a level of organization can spell larger ones in others. Some aspects of the IO have been identified due to failures within a single functional level of growth. Osteomalacia and osteoporosis are of the most interest to us here since they both arise from dysfunction of the L_2 remodeling process. However, in many conditions, a multiplicity of functions and processes lead to unanswerable questions of causation.

FACTORS AFFECTING REMODELING

Remodeling rates are site-specific and bone-specific (Simmons, 1985). Remodeling has a baseline level and is affected by age, microdamage, disease, drugs, sexual dimorphism, diet, and mechanical usage. Further, Frost (1986:118) stated "Neurologic, ... physical, diverse endocrinologic, biochemical, and multifactorial factors appear able to affect BMU-based remodeling, although most of the details remain unknown." For example, certain drugs may induce osteopenic conditions by increasing the minimum effective signal strength (MES)[1] for remodeling. This multifaceted influence complicates the issue of causality. The degree of response and the parallel action of different stimuli on remodeling activity are also unknown. The effects of various

1: MES is a concept associated with the skeletal IO that explains how varying levels of a signal can activate a biological process through an "on/off" type mechanism. Increasing the level of the MES would cause a previous healthy level of activity become inadequate to keep remodeling activity turned "off."

combinations of stimuli may prove to be indecipherable. However, while the order or magnitude of effects remain to be defined, many of the stimuli themselves have been identified.

AGE-RELATED MICROSTRUCTURAL CHANGES

Martin and Burr (1989) claim that aging alone creates a fairly consistent change in cortical bone geometry, size, and number of secondary osteons. This explains the relative success of histological aging methods. The cortical thickness of the clavicle has also been found to have a consistent relationship to age (Walker and Lovejoy, 1985). As with many long bones, with increasing age the medullary cavity of the clavicle expands and the periosteal surface increases. This loss of cortical area may result in less cortical area to sample for aging methods, and increases the possibility of sampling error among older individuals. The cross-sectional thickness of a clavicle can suggest a general age category of "young" or "old" for many individuals.

Aging affects the size of secondary osteons, with increasingly smaller osteons occurring with age. This supports the findings of Martin and Burr (1989:31-32), who suggested that osteocytes are arranged geometrically to be close to their nutrient source, i.e., the Haversian canal. On average, they claimed osteocytes are located within a diameter of 100-150 microns of the canal. However, with the general systematic decrease in efficiency that accompanies advanced age, it is logical that osteocytes would not be distributed as far from the canal as those in younger individuals, to compensate for age-related decreases in efficiency. Osteon diameter was not measured in my study, so no comments concerning this effect in the clavicle can be offered here.

As age increases, osteoblast activity slows, and lags between the resorption and formation processes may occur. Due to decreased osteoblast efficiency, mineralization

often becomes less efficient as age increases (Ortner, 1975). This may introduce a problem with osteon aging methods due to unresolved A-R-F sequences. This is especially true for women, who are more at risk for poor mineralization due to hormonal changes after menopause. These lags in formation and mineralization result in bone that is more porous than usual and therefore much weaker than normal. This also decreases the observable cortical area and osteons in older (female) individuals, again increasing the possibility of sampling error. However, in the current study, females exhibited a stronger statistical relationship between age and the observed histological structures, suggesting this may not be a significant problem (see chapter 4).

HOMEOSTASIS

During the first three decades of life, the growing body creates a store of calcium in the bones that is often referred to as an individual's "*bone bank.*" These nutrient reserves contain 99% of human calcium, with the remaining 1% located in the blood system. Biological problems like poor nutrition can cause serious imbalances in serum calcium levels, but the body can activate bone resorption to restore the balance (Junquiera et al., 1986). Hormones initiate osteoclast activity within bone to restore blood mineral level homeostasis. Processes associated with homeostasis require as little as 5 minutes or as long as 3 months to carry out (Frost, 1986:71). Most of the body's calcium stores (bone bank) are found in cortical bone (Martin and Burr, 1989), but trabecular bone has greater contact with body fluids contained in marrow space. This close contact allows the chemical and mineral resources found in trabecular bone to be more easily exchanged with the fluids (i.e., it is more labile than cortical bone). Therefore, trabecular bone is first affected by metabolic insults. This is well illustrated by the seasonal loss of trabecular bone seen in deer and birds when metabolic needs exceed serum levels of calcium and the bone is excavated to restore the balance. A parallel condition has been observed in cases

of human osteopenia, where trabecular bone is affected by osteoclast activity before cortical bone (Martin and Burr, 1989).

The fact that bone near blood vessels is more labile (Ortner and von Endt, 1971) also suggests that the concentric lamellae surrounding Haversian canals of a secondary osteon are subject to excavation and utilization when homeostasis of serum calcium levels is in jeopardy. This is illustrated by the incidence of Type II (embedded) osteons and their relationship to conditions like heart disease, poor nutrition, and diabetes described by Stout (1989a). However, Type II osteons may be found only in chronic disease states, since the ionic transmission available through the trabeculae, canaliculi and lacunae also presents numerous possibilities for homeostatic processes. The large number of surfaces available for transmission within these structures suggests they would be more efficient sites for calcium exchange than Haversian systems.

EFFECTS OF DISEASE / DRUGS

Chronic metabolic bone diseases and medications have been shown to affect bone processes. Transient effects, like short-term dietary stress or programs of medication that last less than three months, have limited effect on bone (Frost 1986:71). Physiological effects are only seen in histologic structures after one sigma has passed (3-4 months). However, sigma may be affected by disease, poor nutritional conditions, and age. In some cases of osteoporosis and osteomalacias, sigma can be increased to 2-10 years (Frost 1986:112). There is a definite "on-off" system in bone processes, and lags may exist between one function and the next in the series. A common example of this is the lag time often observed between the resorption (R) and formation (F) functions in remodeling associated with disease (like osteoporosis) or advanced age.

Pathological conditions tend to affect remodeling rates in focused areas, while non-pathological remodeling (like that associated with increasing age) affects areas more

equally (Stout 1989a). This focal nature of pathology is illustrated by a condition known as Regional Acceleratory Phenomenon (*RAP*), which is caused by phlebitis and metastases (Stout, 1989a). RAP results in a localized increase in the remodeling rate and therefore a higher mean tissue age for that area relative to others. Other conditions that affect remodeling rates include secondary hyperthyroidism, which increases remodeling rates, senile osteopenia, and diabetes mellitus, which both decrease the osteon densities per unit of area and reduce the mean tissue age (Stout 1989a).

The effects of many drugs on remodeling activity associated with homeostatic regulation are not completely known, since imperfect models used for research have provided information concerning the effects on isolated components of remodeling units (osteoclasts), rather than complete BMU's. However, it has been established that immunosuppressants, fluoride, excess doses of diphosphates, and radiation damage can result in decreased remodeling efficiency and affect age estimates for individuals exposed to them (Frost, 1986:120).

MECHANICAL INFLUENCE

The case supporting the effects of disease on BMU-based remodeling is sound. However, Frost (1985) suggested that the changes in remodeling as well as those associated with drug use, hormone anomalies, and nutrition are usually due to changes in mechanical usage rather than the condition or its treatment. This statement was based upon the similarity between bone losses due to adult osteoporosis and those due to decreased mechanical usage. This suggests that mechanical influence is the main stimulus of BMU-based remodeling. Indeed, biomechanical influence is thought to be the greatest determining factor of osteon distributions. Sampling bones with lesser mechanical usage or choosing long bone sampling sites away from the cross-sectional geometric axes may be the most efficient way to maximize the effectiveness of histological aging methods.

Microdamage caused by mechanical forces is known to affect L_2 remodeling. Incidence of microdamage (Frost's MDx, 1986:193) increases with the age of the bone tissue, time, strain magnitude and repetition of movement. If an area of bone has been subjected to frequent repetitive strain, its older areas will show evidence of increased remodeling activity. This strain theory of remodeling is supported by the fact that areas of muscle attachments show higher numbers of Haversian systems (Enlow 1966). Study of the IO has illustrated the influence of local versus global effects of mechanical usage (see Frost, 1986:193). Local effects are regional increased remodeling due to localized strain, such as at the site of muscle and tendon attachments or the geometric axes of long bones. The result of such local effects on osteon distribution has been recently shown by Pfeiffer and colleagues (1995), who found statistically significant differences between sampling regions along the femur.

Martin and Burr (1982,1989:208-209) suggested that the osteonal nature of cortical bone serves to distribute microfractures so as to "trap" them. They use the example of horse hooves to illustrate the concept. The keratin of a horse hoof is formed into small circles with distributions similar to that of secondary osteons. These keratin structures are oriented horizontally to keep fractures from traveling upward into the sensitive area of keratin growth. A trapped fracture is forced to travel horizontally and ultimately detaches the disposable lower hoof from the unfractured area above it. The authors proposed that fractures in cortical bone become trapped in the concentric lamellae and affect only a limited area or cortex. Fractures would ultimately be remodeled by initiation of remodeling in the affected area.

SEXUAL DIMORPHISM

With the intricate response systems found in living bone, it seems improbable that differences in hormone levels and physiology between males and females would not

affect BMU-based remodeling. While the first histological aging methods were developed when the skeletal IO concept was in its infancy, researchers did assess the influence of sex and "race" on histological characteristics. Neither Kerley (1965) nor Enlow (1966) found significant sexual dimorphism in osteon counts. Based upon Kerley's report, Ahlqvist and Damsten (1969) report that "no attention was paid ..." to differences between males and females in their study. Singh and Gunberg (1970) tested the male-derived formula on a sample of 7 females, but did not find evidence of sexual dimorphism. However, they noted that future studies with larger sample sizes should further explore this possibility. Thompson and Galvin (1983) found no sexual differences in histology in their modern autopsy sample, but only 5 of the 53 individuals studied were female. Stout and Paine (1992) did not examine sexual differences in the OPD (osteon population density) variable for the clavicle or rib, but Stout and colleagues' (1996) follow-up study of the clavicle failed to find sexual dimorphism in a historic cemetery sample with similar numbers of males and females.

Ericksen (1991) generated sex-specific equations to provide more accurate age estimates due to the sexual differences she found in the ratio of complete and fragmentary osteons. This could be related to the sexual dimorphism in cortical bone area that exists, with females having less cortical area than males. There is greater probability that a remodeling event will encroach upon a previously formed Haversian osteon in females. Ericksen found the sex-specific equations performed better than the general one or the one generated for the opposite sex. However, when the sex-specific equations were used by Ericksen and Stix (1991) on the First African Baptist Church population, the female population was aged less accurately than the male population.

Samson and Branigan (1987) found dimorphism in their histologic variable MC (morphologic character), defined as the product of the number of Haversian canals per area times the average minimum Haversian canal diameter. This MC variable was shown

to have the best mathematical relationship with age among the variables studied. MC and age were highly correlated for Samson and Branigan's male population, but it was not significantly related to age for their female population. This difference could be related to the definition of the variable and the influence of the sexually dimorphic remodeling patterns found by Ericksen (1991:177). The earlier asymptote of complete osteons in females should result in higher numbers of osteon fragments in females, and therefore fewer observable Haversian canals in the females relative to the males. Due to this lack of a relationship between MC and age, Samson and Branigan could not age adult females more accurately than ± 16 years of actual age. This led them to state that sex determination must be made before accurate age estimates can be calculated. However, they recognized this limited the applicability of their method for specimens for which histological aging methods are most helpful (i.e., fragmentary remains). They suggested a fairly successful method of sexing fragmentary remains based upon cortical thickness and pointed to citrate analysis as a possible aid in sex assessment (Samson and Branigan, 1987:106)

Ross (1992) found dimorphic remodeling evidenced by 7 variables for specific sites in the human rib. These findings suggest that remodeling may vary between the sexes in some sites and not in others. It is perhaps more probable that different variables provide different results for the same sampling site. Those techniques that measure many factors with subtle dimorphism may find dimorphism due to a cumulative effect of these multi-factors, while those that measure few variables or those that are physiologically less susceptible to hormonal influence will not illustrate any dimorphism. While sexual dimorphism in histological characteristics is a possibility, it appears that the effect may be very complex, very slight, or at least very elusive. More definitive answers may be generated in the future, but the variation between sampling sites and populations may confuse the issue for a great time to come.

BONE HISTOLOGY AND TERMINOLOGY

When viewed longitudinally one can easily discern the dynamic structures of remodeling, but these characteristics are most often viewed in transverse cross-section by researchers. Constituents of bone run longitudinally in the long bones, but a cutting cone may be oriented in any direction, as can the resulting secondary osteon. However, the majority appear to follow an approximately longitudinal path. In cross section, resorption spaces created by the cutting cones are seen as circular empty areas (figure 2.2), and the observed size of the resorption space depends on the location of the sectioning plane along the cutting cone. The resorbed area is separated from the surrounding bone tissue by a prominent cement line, a distinguishing characteristic of secondary (remodeled) bone (see figure 2.1). The osteoblasts move inward from the surface of the cement line, making their way toward the center as they fill the resorption space with *osteoid* (figure 2.2) that later mineralizes. A space for the Haversian canal remains at the center.

In histological aging methods and paleopathological analysis, differentiating types of osteons is very important. A *primary osteon* is the result of modeling processes associated with growth. They consist of a blood vessel surrounded by concentric lamellae and osteocytes laid down about a central blood vessel (but not necessarily a Haversian canal). However, primary osteons have no cement line separating them from the surrounding bone (figure 2.3). These osteons result from the modeling and mineralization of growing bony tissue around an existing blood vessel and are characteristic of modeled bone. Primary bone tissue is quite common in very young individuals, but becomes more rare as age increases. A *secondary osteon* is the result of resorption of older bone and subsequent formation of new bone (remodeling), and consists of a blood vessel (Haversian canal) surrounded by concentric lamellae and osteocytes. As previously stated, they are separated from the surrounding interstitial bone by a marked cement line. Basic Structural Units (BSU), a sub-unit of a secondary osteon, are recognized by some

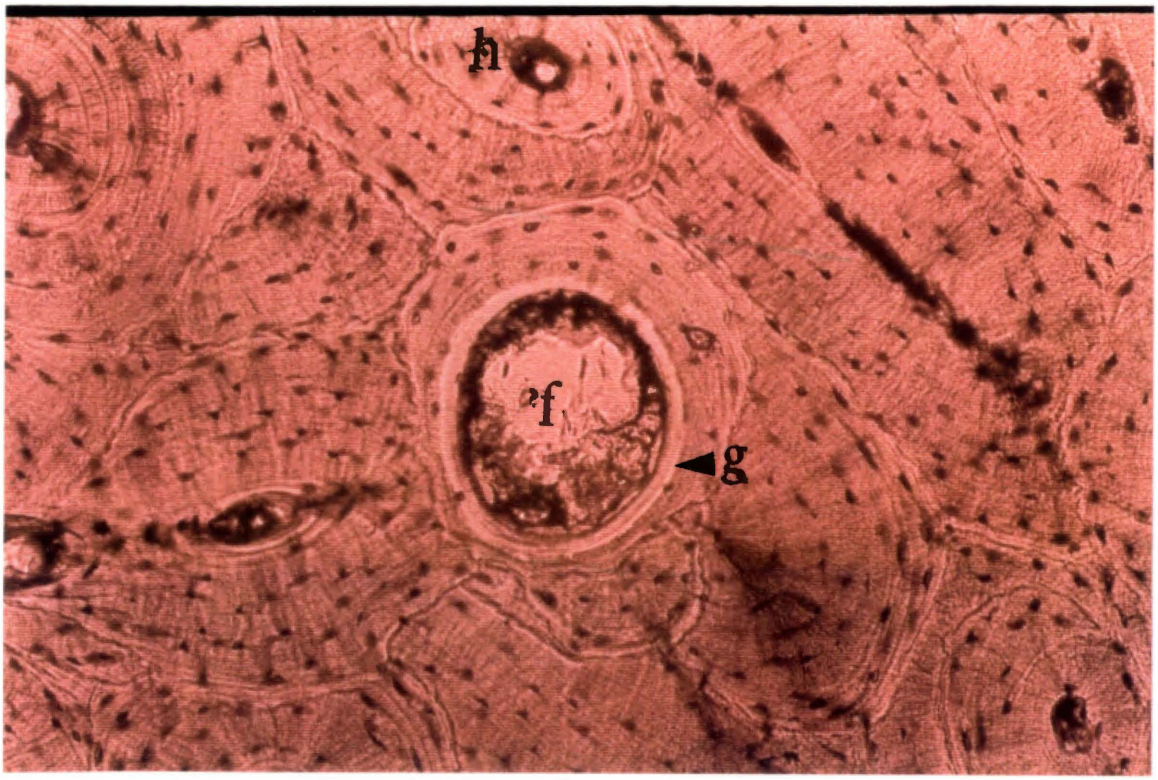

Figure 2.2: Histological Structures Associated with the Remodeling Process

F) Resorption space

G) Osteoid (non-mineralized)

H) Embedded (Type II) osteon

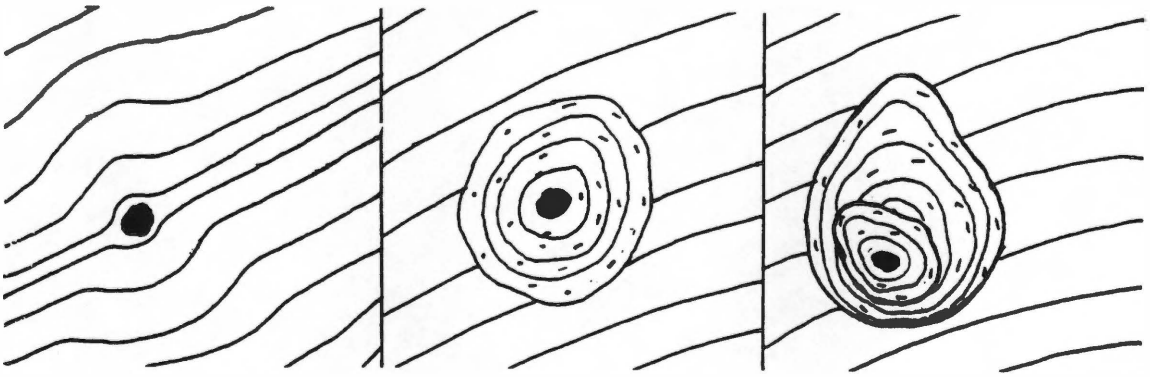

Figure 2.3 : Three Types of Osteons

1) Primary osteon

2) Secondary (Remodeled) osteon

3) Embedded (Type II) osteon

authors (Jaworski, 1976; Parfitt, 1983b) and are related by definition to their parent BMU's. However, these BSU's define a three dimensional volume of bone that lies within the area circumscribed by the cement line and the Haversian canal (Martin and Burr, 1989:105). *Type II* or *embedded osteons* develop inside pre-existing secondary osteons, and are thought to be the result of less intensive remodeling processes within existing secondary osteons. These small osteons are identifiable from their off-center location inside larger osteons (figure 2.2, 2.3). They have been associated with some forms of chronic heart disease, poor nutrition, and diabetes (Stout,1989b). Junquiera et al. (1986) define interstitial lamellae as the remains of earlier osteons that have been partially remodeled, but in my study these partially obscured osteons are defined as secondary osteon *fragments* (see figure 2.1). Since both complete and fragmentary secondary osteons represent past remodeling activity, both are counted as evidence of remodeling activity. Following Stout and Paine (1992), a structure is defined as an osteon fragment if an estimated 90% of the Haversian canal perimeter is visible. Complete and fragmentary osteons are counted separately in the initial analysis for future reference, but the categories are added together to represent the osteon population density (OPD) variable. Use of this variable allows greater latitude in determining a fragment or complete osteon, and allows for interobserver differences in identifying structures.

The distribution of osteons between consecutive cross-sections may result in variable observations. Koltze (1951) and Cohen and Harris (1958) suggested osteons occur as groups of tangled resorption tunnels, rather than the single straight tunnel often presented. Martin and Burr (1989) claimed these tangles of osteons become more common toward the midshaft of bones than toward the metaphyses, suggesting that sampling in the midshaft will result in a different picture of remodeling activity than sampling further toward the metaphysis. However, Stout and Paine (1992) proposed a

comprehensive sampling plan, such as the reading of more than one section per specimen, results in a more accurate representation of the true osteon distribution.

A secondary Haversian osteon averages a lifespan of 2-15 years before it is replaced. In this context, the concept of *mean tissue age* (MTA) becomes very important. Due to modeling and constant remodeling processes, each region of bone has a different history and is of a different age. The evidence of remodeling activity due to mechanical usage, hormonal effects, and metabolic influences in each area will be different (Martin and Burr, 1989), and therefore the estimated age from each area may be different. This variability in tissue age demands comprehensive sampling plans to counteract its effects. It is important to note that the *tissue* age for one area is not necessarily representative of the skeletal age due to localized variation in MTA. A mean age value from many sampling sites provides the best estimate of the true representative values for each individual. This is why rigorous sampling plans are recommended.

There may be a point at which further remodeling activity cannot be discerned from static histomorphometry. This point defines the *skeletal asymptote.* It is a circumstance in which every available area of bone has been previously remodeled and further remodeling activity is carried out over existing osteons and osteon fragments, replacing existing structures instead of adding additional evidence of activity. In other words, new remodeling maintains the number of osteons and fragments rather than increasing that number. This would result in a plateau of visible osteons and an end to the ability to accurately age the bone from direct observation. The asymptote should be stated as a range, and will differ for each bone due to biomechanical forces and individual variation. Each bone has a different estimated decade of asymptote, based on the amount of modeling drift incurred throughout the lifespan. In the rib, Stout (personal communication) estimates an asymptote in the sixth decade. Stout and colleagues (1996)

did not find a clear asymptote for the OPD variable in the clavicle, but my study found an asymptote beyond the age of 73 years. (See chapter 4).

CHAPTER THREE: CLASSICAL AND INVERSE CALIBRATION METHODS

Although there are many variations upon the theme of histological aging methods in anthropology, most described to date use the same statistical method as Kerley's study (1965). However, none has yet directly recognized that the method used is not simply linear regression, but a special case of regression called *calibration.* Calibration carries with it many theoretical and technical considerations and has been the subject of controversy in the field of statistics. Examination of this controversy and of calibration itself makes up the next chapter and illustrates the consequences of the statistical method that has been uniformly applied in the field of histological aging.

In least squares regression, related variables x and y are fitted to the linear model

$$y = \beta_0 + \beta_1 x + e_i$$

where $i = 1,2, ...n,$ the β's are unknown parameters, and the e_i's are normally and independently distributed (Koopmans, 1987:456). Minimizing the sum of squares values of the residuals allows calculation of the least squares estimates for the parameters β_0 and β_1. Under the Gauss-Markov theorem, these parameter estimates are unbiased and have minimum variance (Neter et al, 1990:43). The fitted line allows the estimation of a value of y given a value of x.

When given a data set with a variable x, the independent (predictor) variable and a variable y, the dependent (response) variable, one has two choices: (1) regress x on y or (2) regress y on x. Only when the two variables sets are perfectly correlated (i.e., $r^2 = 1$ or -1) do these regression formulae predict a line of the same slope (Berkson, 1950; Brown,

1993). If the correlation between the two variables is not perfect ($r^2 < 1$) , two different lines are fitted for the two regressions. If one wishes to predict *y*, the minimum sum of the square residuals for *y* are calculated to fit the line, and conversely, the minimum sum of square residuals for *x* are calculated if one wishes to predict *x* (Berkson, 1950).

In statistics, the accepted method for linear regression uses fixed values of *x* (the predictor variable) and random values of *y* that are dependent upon *x*. This is often attributed to the suggestion by Eisenhart (1939), that states error should be retained in the direction is was observed. More simply, if values of *x* (the predictor) variable are chosen in an experiment, they are accurate and not subject to error, while the observed *y* (response variable) values are subject to error. Reversing this relationship of fixed and random variables places the error (of the estimate) in the wrong variable.

A useful example of calibration is described by Brown (1993:3-4). He describes experiments by nineteenth-century physicist J.D. Forbes that related altitude above sea level measured by a barometer (*x*) to the boiling point of water (*y*). Measurements of altitude and the boiling point of water were both recorded for many locations throughout the Alps and Scotland. Once the scale of boiling points and altitudes was calibrated, Forbes was able to simply measure the boiling point of water (y) in a new location and estimate the altitude of the location above sea level (x) without use of a barometer.

The regression of *y* on *x* is often called *classical calibration*[1] since it is the

[1] Many statistical texts identify the classical regression as the method of *inverse prediction*. In this paper, I follow the precedent set by Krutchkoff (1967, 1969) in defining the inverse method and contrasting it with the so-called classical method.

original form proposed by Sir Francis Galton in the late 19th century to relate the stature of fathers and sons (Neter et al., 1990:26). The work of Eisenhart (1939) stated the classical form of regression in relation to problems in biological and industrial research. These problems often deal with calibration. Rosenblatt and Spiegelman (1981) described absolute calibration (versus comparative calibration) as calibrating a "quick or nonstandard" measurement against a "standard or defined" one.

The regression of x on y is often called *inverse regression* or *inverse calibration* since it is the reverse form of the classical calibration. In many cases, the fixed value of x is expensive or difficult to obtain by standard methods and the variable y is not (Scheffe, 1973). In these situations, a "training set" of observations of the hard-to-obtain x values may be used to establish the linear relationship between two variables and values of x (the predictor variable) may be predicted from future observed values of y (the response variable). The error is then introduced to the x variable and y is fixed. Simple linear regression is used to establish the relationship between the variables, but the prediction of x from y uses the special case of linear regression called calibration.

While the inverse regression was previously known, its application to the calibration problem was highlighted by a controversial paper by Krutchkoff (1967). The inverse regression uses an easily obtained response (y) variable to predict an associated value of the predictor variable (x). Since this method went against the established statistical conventions concerning calibration, there were many critiques of Krutchkoff's methods (Osborne, 1991). The harshest criticisms addressed his conclusion that inverse calibration was superior to the classical due to the inverse's smaller mean squared error

values. Critics (Berkson, 1969; Halperin, 1970: Martinelle, 1970: Shukla, 1972) showed that the smaller mean squared errors for the inverse are only generated in a restricted range of values around the mean of the x values used to generate the calibration line. Outside that range, classical regression produces smaller values for mean squared error.

Williams (1969) also criticized Krutchkoff's support of the inverse regression, claiming that choosing a calibration method on the basis of minimum mean square deviation cannot be justified. Berkson (1969) submitted that the inverse calibration method was "inconsistent" (in the sense of probabilities) due to its minimization of the x residuals. Further, he stated that the resulting estimates of x are dependent upon the original chosen values of x used to generate the calibration line.

Krutchkoff (1969) conceded that situations did exist in which the classical estimator was superior to inverse calibration in terms of smaller mean square error values. In cases of extrapolation beyond the plotted calibration line and in designs using 5 or more observations at each design point, the mean square error values are smaller for classical methods than inverse. However, this concession did not end the debate over the merits of the inverse estimator.

Using Pitman's (1937) measure of "closeness," Halperin (1970) confirmed that Krutchkoff's inverse calibration was superior to the classical regression in a restricted range of x values situated around the calibration sample's mean x values. He stated (1970:727) that the size of the range of the inverse method's relative superiority varies with the standard deviation of the model's independent variable. Further, he claimed that in many practical applications "..the interval where the Krutchkoff estimate is superior

will be trivially small." He seconded Berkson's (1969) claim that the inverse regression is "inconsistent."

The Pitman measure was again used to compare the inverse and classical least squares regression methods by Keating and colleagues (1991) using the estimators for the slope parameter. They found that the slope was affected by the distribution of the predictor variable and by the "signal-to-noise ratio." They arrived at the conclusion that the inverse estimator is "PN-inadmissable with respect to the classical estimator" but added that neither estimator is favored in all situations.

Hoadley (1970) introduced the Bayesian perspective to the debate of the inverse regression. Bayes Rule is a way to calculate the posterior probabilities of an event (or events) given the occurrence of another event (ie, calculate posterior probabilities based upon conditional probabilities)(Koopmans, 1987:148). A Bayesian perspective assumes knowledge of prior distributions of data sets and uses this information in the form of probabilities to increase the power of predictive equations. As Brown (1993:2) states, "The most flexible techniques arise out of a fully Bayesian analysis and incorporate realistic prior assumptions which supplement the data inadequacies." If the probability of a new data set meeting the prior assumptions concerning distribution are realistic and correctly assessed, the resultant predictions are more accurate. However, when these probabilities are unrealistic or incorrectly assessed, the resulting estimates are inaccurate.

Hoadley (1970:356) concluded that the inverse estimator "is Bayes with respect to a particular informative prior" that is for the distribution of x and squared error loss. (The

effects of the Bayesian perspective of the inverse estimator is discussed more fully below.)

STATISTICAL CHARACTERISTICS OF CLASSICAL CALIBRATION

When applied to large samples, the classical regression is an unbiased estimator. This means that characteristics of the calibration sample are not used to augment the calibration by the application of *a priori* assumptions. Such prior assumptions are used in Bayesian forms of statistical analysis. The classical estimator generates smaller mean squared error values in cases of extrapolation and in ranges outside that surrounding the mean of the calibration sample. However, for very small samples the construction of confidence intervals may be complex: they may be non-symmetrical about the regression line, or in some cases they may remain unresolved (see Fisch and Strehlau, 1991). A very small bias may result from the classical method when it is applied to very small samples, but this bias is negligible compared to that for the inverse estimator.

The classical estimator can be expressed as:

$$Y = \beta_0 + \beta_1 (X)$$

where Y is the response variable, X is the predictor variable, and β_0 and β_1 are the parameter estimates. When using this form as a calibration equation, the response variable is the more easily observed variable. In order to predict the accompanying value for the predictor value, one must use basic algebra to solve the equation for the predictor variable as follows:

$$(Y - \beta_0) / \beta_1 = X$$

Shukla (1972:548) described the classical estimator as the maximum likelihood estimator when its error terms are distributed normally with mean and variance σ^2. He further explained that the classical estimator's analysis of variance is easily generated. These attractive characteristics are not necessarily also true for inverse regression. This discrepancy of characteristics formed the basis of Eisenhart's (1939) rejection of the inverse method of regression in favor of the classical (Shukla, 1972).

STATISTICAL CHARACTERISTICS OF INVERSE CALIBRATION

Krutchkoff (1967) stated that the inverse regression method results in smaller standard errors than the classical method. However, this only occurs in a limited range of values surrounding the mean of the calibration sample. If large samples are utilized in the generation of the predictive equation, 95% confidence intervals can be constructed using two times the error of estimate. The use of small samples to generate the inverse calibration equation results in the larger confidence intervals at both ends of the values of x (i.e., away from the sample mean). If the slope of the regression line is near zero, a "nonsensical" confidence interval can result (Montgomery and Peck 1992:404).

Brown (1993:31) describes inverse calibration as "naive" and claims that it has a bias "that persists even as the number of replicates goes to infinity."

Konigsberg and Frankenberg (1992:96) stated that :

> "...regressing age on an indicator to estimate ages in future forensic cases makes the critical, often dangerous assumption that future cases come from the same age-at-death distribution as does the original reference material of known age."

This assumption may in fact be an appropriate one if the forensic cases in an area are of very defined and restricted characteristics. For example, if most of the autopsy population in an area can be consistently defined as a population of young males between the ages of 15 and 25, then the assumption of similar age-at-death distributions for future cases may be valid. However, what if an individual does not come from a population of similar age distribution? For example, what if an elderly individual of unknown age was autopsied at the location of the consistently young autopsy population suggested above? In this case, the actual age of the unknown individual is outside the range of the calibration sample and the assumption of similar age distributions is not valid. Applying the inverse regression to estimate the individual's age is not appropriate.

In an archaeological context, prior assumptions concerning the age-at-death distribution from which an individual comes may be mistaken. Inverse calibration should be cautiously applied to archaeological samples due to the biasing behavior of many of the inverse age estimation techniques used in anthropology.

APPLICATION TO HISTOLOGICAL AGING METHODS

While Neter et al.(1990:28) warn that there is not necessarily a cause/effect between related variables, in some cases a causal relationship can in fact be established. Konigsberg and Frankenberg (1992) suggest that these causal relationships are to be modeled as they exist in nature, such as is done by biologists modeling growth in the fisheries literature. For example, in histological aging methods, the observed histological variables are the result of the aging process. This suggests that AGE is the true

independent variable, and the histological variable (in this study OPD) is the dependent variable. The resulting linear model would be of the form:

$$\underset{(Y)}{OPD} = b_0 + b_1 \underset{(X)}{(AGE)}$$

where b_0 and b_1 are the parameter estimates of the intercept β_0 and slope β_1, respectively.

One may argue that reversing this relationship is simply an application of statistical calibration, as defined earlier. This is true, but one must heed the warning Brown (1993:4) issued in discussing Forbes's boiling point/altitude data: there are "counter-considerations" concerning the calibration relationship when "the explanatory variable is subject to appreciable error." Added to this is the wisdom of Karl Popper (1959) who emphasized the need of data "to be theory laden" (Brown, 1993:13). This suggests that modeling AGE as if it is dependent upon the histological variable OPD is not the model of choice.

Currey (1964) regressed the histological indicators on age in years in her study of aging effects on Haversian systems. For example, to assess the relationship of Haversian canal diameter with age (which was non-significant), she fit the line

$$y = 58.0 + 0.34\,x$$

where y is the estimated minor diameter of the canal in microns and x is age in years. Since age is independent of histological characteristics, it is correctly modeled as the independent variable x. The canal diameter, which she hypothesized might be dependent upon age, was modeled as the dependent variable. A survey of the literature concerning

histological aging methods found Currey (1964) and Lazenby et al. (1989) to be the only investigators utilizing classical regression.

The landmark aging method described by Kerley (1965) used regression formulae to predict age from histological variables, and modeled the relationship between age and the histological variables as an inverse calibration problem. The function of the equation suggests this form is the most straight-forward and appropriate, since he wanted to "plug in" the observed value for the histological character(s) and generate an estimate for age. However, there is no indication in his report that he was aware of the statistical trappings of the inverse method. This makes sense, since the Krutchkoff controversy did not erupt until 1967 and was entirely contained in the statistical literature.

The histological aging methods described by Ahlqvist and Damsten (1969), Singh and Gunberg (1970), Samson and Branigan (1987), Thompson (1979), Thompson and Galvin (1983), Ericksen (1991), Ericksen and Stix (1991), and Yoshino et al. (1994) all utilize the inverse method of regression. It is quite probable that the investigators simply followed the form of regression used by earlier investigators without question since Kerley (1965) set the precedent, not realizing they were using a calibration model. The inverse calibration is in fact the most simple and straight-forward way to model the relationship, since it involves simple math and no algebra to solve for the estimate.

The discussion of inverse and classical calibration methods and their statistical implications are recent additions to the anthropological literature. Konigsberg and Frankenberg (1992) describe the biasing effects of methods using Bayesian assumptions in anthropology and illustrate the biased age structures produced by applying age

estimation methods to populations of differing age distributions. In another article, (Konigsberg and Frankenberg, 1994) they suggest that the lack of older individuals in archaeological age distributions is more likely the result of our biased methodologies rather than a reflection of the truth. Using models of relationships taken from biological growth studies of fish, they show the method of least squares regression most commonly used in anthropology is inverse calibration and warn that currently used methods may produce biased age estimates.

Lucy and colleagues (1996) introduced a Bayesian perspective to ordinal and categorical data used in morphological dental aging methods. Using prior probabilities and likelihoods with a number of accompanying assumptions, they proposed a non-parametric method to assess the posterior probability of an individual with a particular set of dental maturation scores belonging to a particular age group. In their test, the Bayesian method provided slightly smaller absolute standard errors than multivariate regression (7.0 v/s 7.8 years difference, respectively). The constructed confidence intervals for the Bayesian method were almost half the size of those for the multivariate regression technique (19.4 v/s 37.9 years in the range, respectively). However, the small confidence intervals resulting from their Bayesian method of age prediction contained the true age of the individual for only 79% of the test cases, leaving 21% of test cases for which the true age was not placed within a 95% confidence interval. The larger multivariate regression confidence intervals included the true age of the individual in 99% of the test cases run by the authors. This illustrates the potential problems of augmenting data with Bayesian assumptions: when these assumptions are not met, the resulting estimates are not

accurate. For samples of unknown characteristics, there is no way to test the accuracy of the estimate.

In this study, the classical and inverse methods of calibration are examined in the context of histological aging methods for the human clavicle. The distribution and accuracy of the estimates are examined for patterns characteristic of the statistical method applied to the data (see chapter 4).

CHAPTER 4: MATERIALS, METHODS, AND RESULTS

The histological aging methods Stout and Paine (1992) and Stout et al. (1996) described for the clavicle have the advantages of using the entire cross-section and generating OPD values from two cross-sections for each individual. Both practices decrease the probability of non-representative sampling. The clavicle may be less affected by biomechanically induced remodeling processes than human long bones, the more traditional subjects of histological aging, since it does not bear any significant weight. The clavicle's limited anthropological use for determining personal characteristics, such as stature, make it more easily obtained for invasive techniques. These benefits make age estimation from the clavicle a useful technique.

The original method described by Stout and Paine (1992) was based upon a small sample of 40 individuals with a relatively young mean age of 28.6 years. The high male to female ratio in the sample did not allow for testing of sexual dimorphism in the histological variables (see Table 4.1). The resultant predictive equation was tested and the sample deficiencies were corrected in a letter by Stout and colleagues (1996) using a historic Swiss cemetery population (n=83, mean age 36.6 years). The original equation performed very well on the Swiss clavicles, providing a mean absolute difference between actual and estimated age of only five years (minimum absolute difference = 0.04 years; maximum absolute difference = 22.2 years). However, this equation consistently underaged those over 40 years of age.

The roughly equal number of males and females in the Swiss sample allowed for

Table 4.1: Sample Size and Constituents of Previous Studies

	Males	Females	Unknown	Total
1992	32	7	1	40
1996: Swiss	41	42	0	83
1996: Combined	73	49	1	123

1992: Stout and Paine (1992) ; 1996: Stout et al. (1996)

tests of sexual dimorphism in histological variables. None was found in the Swiss cemetery sample. The Swiss sample was combined with the original autopsy sample (1992) to generate a new predictive equation (Stout et al., 1996) in hopes of providing more accurate age estimates for older individuals.

PART I: GENERATION OF A PREDICTIVE EQUATION FOR THE CURRENT STUDY SAMPLE

The independent sample assembled for the current study allows an additional test of the 1992 equation and an initial test for the combined (1996) equation.

THE CURRENT STUDY SAMPLE

The sample used in this study was chosen from the McCormick Forensic collection housed at the Forensic Center at the University of Tennessee, Knoxville. Invasive techniques were allowed on the chosen specimens by the permission of Dr. William F. McCormick, Deputy Chief Medical Examiner for the State of Tennessee and Dr. William M. Bass, Professor Emeritus of the University of Tennessee and director of the UT Forensic Center. Thorough medical histories were available in the form of autopsy records so the primary causes of death, persistent medical conditions, and (in many cases) prescribed medications were noted and available for reference. Events related to the cause of death include gunshot wounds, uncontrolled diabetes mellitus, automobile accidents and acute alcoholic intoxication, among others (see appendix 1).

The McCormick collection reflects the ethnic composition of northeast Tennessee and as a result, few black individuals were available for study. Only white males and females were of sufficient number to include in the sample. Individuals were chosen at random without regard to medical conditions to better represent normal human variation. The great number of deaths attributed to auto accidents and gunshot wounds suggests there was no bias toward any particular medical condition. Left clavicles were chosen for study, and those with evidence of fractures were deleted from the sampling pool. A 73 year old male (case 90-61) was said to have a healed fracture of the left clavicle in the autopsy report, but since it was not in the area of the midshaft sampling site, the individual was included in the sample.

Roughly equal numbers of males (n = 50) and females (n = 45) were chosen to assess possible differences between the sexes. Similar numbers of individuals were selected from each decade between the second and ninth decade of life (see Table 4.2) in order to represent individuals along the length of human lifespan. Individuals younger than 22 years were not included in the sample since methods such as dental eruption and epiphyseal closure can be used to successfully estimate age for this younger age group.

MATERIALS AND METHODS

Each specimen was measured for maximum length, midshaft sagittal diameter, and midshaft vertical diameter according to Moore-Jansen and Jantz (1989:70) so that this information would be available for future investigators after the clavicles were sectioned. Using the maximum length measurement, the exact center of each clavicle was

Table 4.2: Current Sample Description

Decade	Males (n =)	Females (n =)	Total (N =)
22-29	7	6	13
30-39	7	6	13
40-49	7	7	14
51-59	8	7	15
60-69	7	6	13
71-79	7	6	13
80-88	7	7	14

Total Sample N = 95

marked with a pencil so that the sections could be taken from this area. Midshaft sections of roughly one centimeter were removed from each clavicle using a Craftsman 16 inch direct- drive scroll saw. These midshaft portions were rehydrated, fixed, and embedded in Spurrs epoxy resin (Electron Microscopy Sciences, Ft. Washington, PA) in preparation for thin sectioning. A Leitz 1600 microtome was used to cut 300 μ sections from each clavicle within 4 mm of the exact midshaft. A Mark V Lab grinder equipped with 1500 grit waterproof hardware cloth was used to polish each thin section to the desired thickness, which varied between 60 and 90 μ. For each specimen, one side of each section was polished and super-glued to a plastic slide and allowed to dry overnight under pressure of a vice-clamp. The remaining side of the slide was then polished until the microscopic detail of the clavicle section was judged to be of high enough resolution to be read. Use of Permount mounting solution and coverslipping increased the optical properties of the slide. Two slides were prepared in this manner for each individual.

Each slide was analyzed using a Leitz Dialux 20 microscope equipped with a 16X objective and paired 10X eyepieces. Two Tiffen 52mm polarizing camera lenses were used with the microscope's light source to aid in identification and differentiation of structures. Fully polarized light can introduce artifacts into the field (Stout, personal communication), but semi-polarized light avoided this problem while maintaining the benefits of polarization.

The entire area of each clavicle was sampled in a "checkerboard" pattern (figure 4.1) to insure sampling of all topographic areas (Stout, 1986). The number of complete and fragmentary osteons were recorded for each field, and the cortical area sampled was

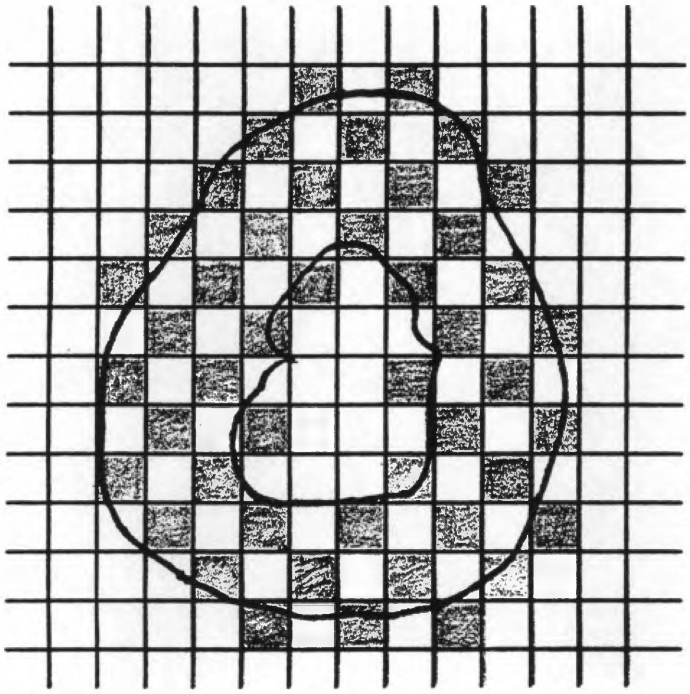

Figure 4.1: Illustration of "Checkerboard" Sampling Pattern for the Clavicle

measured using a Merz grid reticule following common histomorphometric methods (Recker, 1983). If a field contained ambiguous or unidentifiable structures, it was skipped and the next field to be sampled was counted. The observed measurements were used to calculate the osteon population density (OPD). This variable represents the number of complete and fragmentary osteons per square unit of area. The weighted mean of the OPD values for the two slides was used to calculate the final OPD value for each individual following Stout and Paine (1992).

The slides were divided into two sets of 95, with one slide from each individual in each set. The first set was ordered by decade so that the less complex slides were read first to gain experience with the histomorphometric method. The "20's" age group was read first, followed by the "30's" and so on through the "80's". Demographic information was not available during the reading of the slides to create a "blind" study situation. The second set of slides was read in random order. Again, no information concerning the age or sex of the specimens was available during the reading process.

The two OPD values for each individual were compared using a paired difference t-test to see if their values were significantly different (N = 95). The OPD data failed to meet the assumption of normality needed for the parametric paired t-test as measured by the NORMTEST macro (D'Agostino et al., 1990). The non-parametric Wilcoxin signed rank value of 87.5 ($pr > |S| = 0.747$) supports the null hypothesis that the difference for the OPD value between the first and second set of slides was not significantly different than zero.

A one-way anova with replication using CASE, OPD, and the two slides for each individual (SLIDE) was run. This tested for differences in OPD values for the two thin sections for each individual and assess the replicability of OPD values for subsequent thin sections and observer consistency in method. The ANOVA showed significant differences between individuals as represented by the CASE variable ($F = 12.12$, $p < 0.0001$). No significant differences were found between the two slides for the individuals as represented by the SLIDE variable ($F = 0.33$, $p = 0.5681$) and no variation was attributed to the interaction between the SLIDE and CASE variables ($F = 0.32$, $p = 0.5705$). This result suggests that the methodology used in reading the two sets of slides was consistent and that intraobserver error is negligible. Further, subsequent thin sections for each individual show evidence of similar remodeling rates.

STATISTICAL ANALYSIS

The data were screened for outliers and influential observations using descriptive statistics available from the SAS statistical package (University of Tennessee Computing Center, Knoxville). The variable AGE was regressed on OPD since this is the natural cause-and-effect relationship of the data. Five observations were identified as influential or outliers using criteria outlined by Freund and Littell (1991:60-67). These values were checked for accuracy and data entry errors. All flagged observations were found to have acceptable slide quality and to be accurately recorded and were not deleted from the data set, retaining the sample size of 95 individuals (See Table 4.3).

Table 4.3: Sample Summary Statistics for Full Sample (N = 95)

Sex	N =	Mean	SD	Min. Age	Max. Age
M & F	95	54.83	19.88	22	88
F	45	55.47	19.87	23	87
M	50	54.26	20.08	22	88

The method of least squares was used to test for homogeneity of linear functions for the two sexes. The OPD variable was modeled as dependent upon SEX, the treatment variable, AGE, the independent variable (n = 95) and the interaction of SEX and AGE. The overall model was highly significant ($F = 24.950$, $p < 0.0001$, $R^2 = .4513$). The variable AGE was found to be highly significant ($T = 7.012$, $p < 0.0001$) as was the interaction term ($T = -2.199$, $p = 0.0304$). The variable SEX was not significant in this model ($T = 1.223$, $p = 0.2246$). These results suggest that the value of the OPD variable is significantly affected by the aging of an individual and that males and females have different rates (i.e., different slopes) of OPD increase.

Separate classical calibrations were performed for males and females to generate the sex-specific regression lines. The resultant equation for each sex was as follows:

Females: $OPD = 11.48 + 0.15\,(AGE)$ $(n = 45)$

Males: $OPD = 13.55 + 0.08(AGE)$ $(n = 50)$

Females proved to have a stronger relationship between age and osteon number based on the R^2 values for the two models (See Table 4.4).

The graph of age and OPD values for the data set by sex (Figure 4.2) illustrates the differences between the male and female data set. The Pearson correlation coefficient values for the full sample of both sexes (n = 95) show AGE and OPD are significantly correlated ($r = .62$, $p < 0.0001$). As with the regression coefficient, the Pearson coefficient for females ($r = .76$, $p < 0.0001$) was greater than that for males ($r = .50$, $p < 0.0002$).

Table 4.4: Results of Single-Sex Regressions for Males and Females

	Model	R-Square	OPD
Males n = 50	F=15.903, P< 0.0002	.2489	P< 0.0002
Females n = 45	F=58.481 P< 0.0001	.5763	P< 0.0001

Total N = 95

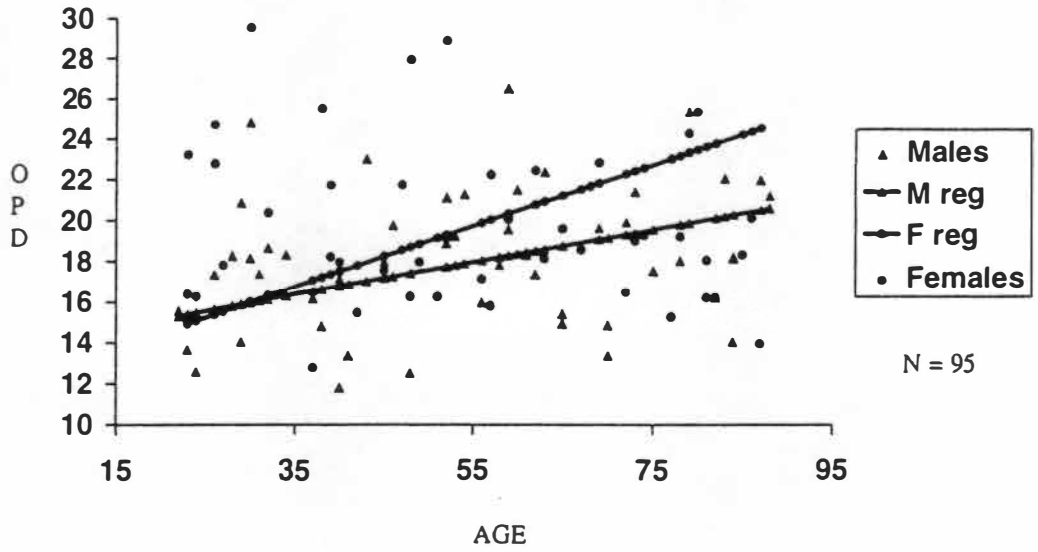

Figure 4.2: Plot of the Male and Female Subsets: AGE by OPD

The linear relationship between AGE and OPD is apparent in Figure 4.2, but the terminal end of the distribution is slightly more dispersed and the higher ages have more widely distributed values of OPD. This is not unexpected, since the cumulative effects of variation in skeletal changes due to chronological age produces more varied observations in the later decades of life for many skeletal age indicators. However, a bone has a set amount of area, and it is possible to fully remodel the entire area of a bone so that further remodeling activity does not affect unremodeled areas, but rather affects previously remodeled areas. In this case, the new remodeling activity will erase evidence of the previous activity and replace it, creating a situation where the OPD variable will not increase, but will level off. This is called an *asymptote.*

To explore this possibility, non-linear least squares regression was used to perform a linear response and plateau function (LRP) as follows:

$$y = A + \delta Bt + (1- \delta)\, Bt_c$$

The interpretation of this equation by Masters et al. (1990) has been adapted to suit this situation: OPD values begin at age A and increase in a linear function with age at rate B for the range of ages t until an increase in the OPD value can no longer be discerned beginning at a particular age t_c (age of increase cessation). The age of OPD increase cessation (t_c) was found to be 73 years. Modeling the OPD variable as dependent upon AGE, the plateau was plotted using an algorithm following Konigsberg et al. (1990). This yielded the maximum likelihood estimate of the asymptote, i.e., the most likely point where the line of OPD increase changed. The value of $\delta = 0$ when the AGE > 73 years and $\delta = 1$ when AGE $\leq$ 73 years (see Figure 4.3). The resultant LRP equations were:

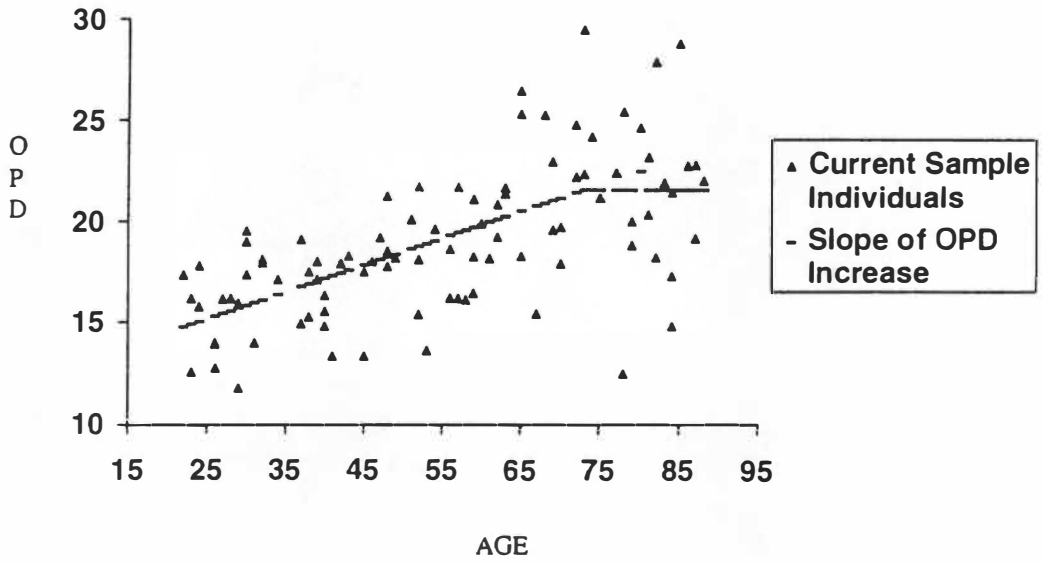

Figure 4.3: Linear Response and Plateau Function Results

$$OPD = .13375948\ (AGE) + 11.84165775 \text{ if } AGE \leq 73$$

$$OPD = 21.74 \text{ if } AGE > 73$$

since 21.74 is the OPD value at the plateau.

The Stout et al. (1996) study does not include a detailed description of the age distribution. Since the autopsy sample maximum age is 62 years, it is apparent that the Swiss cemetery sample provides the extreme ages (63-75 years) of the combined sample. The graph of the Swiss age distribution (1996:141, Figure 1) shows only 4 individuals over the age of 70 years. It is possible that the 1996 sample did not include enough individuals in the range of the asymptote to pinpoint this behavior. However, it is also possible that interobserver error and/or sample-specific characteristics are responsible for the results of the current study concerning this asymptote. Further examination of this result is necessary, since only intraobserver error has been considered here.

Based on the implication of an asymptote in the OPD variable, a new calibration was performed on a data set of those individuals between the ages of 22 and 73 years (n = 74: see Tables 4.5 & 4.6), truncating the data set at the plateau outlined by the LRP. To avoid confusion, this truncated subsample will be referred to as "the subsample." Following the common methodology of histologic aging techniques, AGE was modeled as dependent upon the histologic variable (OPD). This relationship of the dependent and independent variables is based upon logic concerning the final use for the equation as a prediction tool rather than the causal relationship between the two variables.The histologic variables are the result of the aging process, so in reality the observed OPD variable isdependent upon the AGE of the individual. Reversing this causal relationship

Table 4.5: Sample Summary Statistics for the Subsample (N = 74)

Sex	N =	Mean Age	SD Age	Minimum Age	Maximum Age
M & F	74	47.23	15.48	22	73
F	34	47.24	15.36	23	73
M	40	47.23	15.77	22	73

Table 4.6: Descriptive Statistics for the Subsample Variables (N = 74)

Mean OPD	18.18
Variance OPD	11.19
Standard Deviation OPD	3.35
Mean AGE	47.23
Variance AGE	239.66
Standard Deviation AGE	15.48
Covariance AGE & OPD	33.621
Minimum AGE	22
Maximum AGE	73
Minimum OPD	11.79
Maximum OPD	29.54

so that the final predictive equation models (estimated) AGE as dependent upon the observed OPD value is an application inverse calibration (Krutchkoff, 1967). This inverse model is the most common form used to generate predictive equations for histological aging methods.

The resultant inverse calibration for the subsample was highly significant ($F=52.426$, $p < 0.0001$) with an r-square value of .4213. The residuals were found to be normally distributed as measured by the NORMTEST macro (D'Agostino et al., 1990). The resultant predictive equation was:

$$AGE = -7.375492 + 3.003387\ (OPD).$$

The Pearson correlation coefficient value for the relationship between AGE and OPD for the subsample ($N = 74$: $r = .65$, $p < 0.0001$) is comparable to that for the full sample ($n = 95$: $r = .62$, $p < 0.0001$). The correlation coefficient for OPD and AGE increased for the subsample males ($r = .67$, $p < 0.0001$), while that for females decreased ($r = .63$, $p < 0.0001$). However, both relationships were still highly significant.

PART II: TEST OF INVERSE EQUATIONS

In order to assess the predictive accuracy of the previously published equations, (Stout and Paine, 1992; Stout et al., 1996) and the equations in this study, the complete sample of individuals ($N = 95$) was used rather than the subsample ($n = 74$). This study's best predictive equation was generated from a calibration sample with only those individuals of 73 years and below (the subsample), but since the age distribution of a sample to which an equation is applied is unknown, individuals older than 73 years of age

may be present. Use of all 95 individuals as the test sample allowed assessment of each equation to all age groups, including those beyond the estimated asymptote of 73 years.

Two statistical measures were used to assess the predictive performance of each equation. The mean absolute differences between actual ages and the estimated ages is used to allow comparison with Stout and Paine (1992) and Stout and colleagues' (1996) discussion of equation predictive performance. In addition, the root mean squared error value for each equation was calculated since it is a standard measure of predictive capability. The effects of the calibration method used in each technique were also examined.

STOUT AND PAINE (1992) EQUATION

$$AGE = 2.216 + 0.070280\ (OPD)$$

Application of the Stout and Paine (1992) equation to the test sample provided the results found in Figure 4.4, where estimated ages for the current sample are plotted relative to a line of perfect prediction. The mean absolute difference (MAD) between actual and estimated ages (table 4.7) was 19.96, much greater than that reported for first test sample (N = 7), which resulted in a MAD of only 1.1 years (Stout and Paine, 1992). The Swiss test sample (N = 83) resulted in a MAD of 5.5 years (Stout et al., 1996), again, much smaller than that for the current test. Individuals from the current test sample older than forty years were systematically underaged. The estimated ages were slightly more accurate for females than males, but this was expected given the stronger correlation between age and the OPD variable for females in this sample.

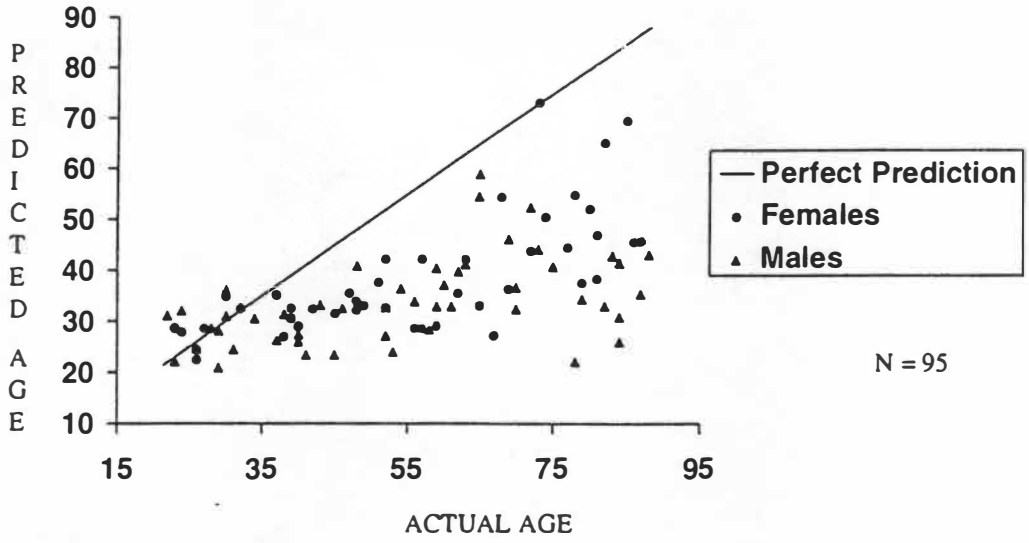

Figure 4.4: Stout and Paine (1992) Equation Applied to Current Sample

Table 4.7: Summary Statistics for Absolute Differences Between Actual and Predicted Ages

Equation Applied	Test Sample	N =	Abs. Mean	Abs. SD	Abs. SE	Abs. Minimum	Abs. Maximum
Stout and Paine (1992)	Current Test Sample	95	19.96	14.71	14.71	0.12	57.92
Stout and Paine (1992)	Swiss Sample *	83	5.5	6.548	2.05	0.04	22.2
Stout et al. (1996)	Current Test Sample	95	17.04	13.69	13.69	0.03	56.97
The subsample Equation (Current Study)	Current Test Sample	95	12.84	10.38	34.92	0.21	47.74
The subsample Equation (Current Study)	Stout and Paine (1992)	40	10.14	6.62	6.62	0.64	24.19

* = Stout et. al., 1996

The RMSE value of 24.75 (table 4.8) is at least in part a result of the differing age distributions of the two samples. Since the Stout and Paine (1992) sample extended to only 63 years of age, applying the equation derived from it to the current sample, which includes many individuals beyond the age of 63 (i.e., extrapolating) resulted in large error of estimate values (as outlined in chapter 2). This RMSE value was the highest for all the equations tested in this study, making this equation the least accurate predictor of age.

The tendency of this equation to underage individuals in the test sample over 40 years of age is also the result of the calibration method used in the 1992 study. Stout and Paine used inverse calibration, where estimated age was modeled as dependent upon the OPD variable. Inverse calibration biases age estimates toward the mean age of the calibration sample. This biasing effect is clearly illustrated by the age estimates: all are biased toward the mean age of the calibration sample (28.6 years), and the equation predicts ages most accurately in a range surrounding that age. This is illustrated by the plot of estimated ages crossing the perfect prediction line at the mean of the sample. Since many individuals in the test sample are beyond that range, many estimated ages are inaccurate. However, the standard formula for calculating confidence intervals accommodates small sample size, and calculates wider intervals at the terminal ends of the regression line. This recognizes the lesser accuracy of estimates as one moves away from the mean of the calibration sample.

Table 4.8: Root Mean Squared Error Values

Equation Tested	Test Sample	N =	Root Mean Squared Error
Stout and Paine (1992)	Current Test Sample	95	24.75
Stout et al. (1996)	Current Test Sample	95	13.69
The subsample Equation (Current Study)	Current Test Sample	95	16.48
The subsample Equation (Current Study)	Stout and Paine (1992)	40	12.07

STOUT AND COLLEAGUES (1996) EQUATION

The equation derived from the combination of the 1992 sample and the Swiss cemetery sample provided more accurate age estimates for the current sample than the 1992 equation, but the improved (1996) equation provided less accurate estimates for many older individuals. Figure 4.5 illustrates the distribution of the 1996 equation estimates for the current test sample plotted relative to a perfect prediction line. The mean absolute difference between actual and estimated ages was 17.04, slightly less than that for the 1992 equation applied to the same sample (see table 4.8). Again, the estimated ages for females are slightly more accurate than those for the males, since females in this sample exhibited a stronger correlation between age and the OPD variable.

The RMSE value of 13.69 (v/s the 1992 equation's RMSE of 24.75: see table 4.8) better illustrates the significant improvement of the 1996 equation over the 1992 equation. This is to be expected, since the age distribution of the sample used to generate the combined (1996) equation is more similar to that of the current sample. Since applying the 1996 equation is not a case of extrapolation as was applying the 1992 equation, the MSE values were expected to be much smaller.

The 1996 equation was also generated using inverse calibration, modeling estimated age as dependent on the OPD variable. While not as severe, the results are similar: age estimates biased toward the mean of the calibration sample (36.6 years), and the perfect prediction line and the plot of estimated ages cross at that mean. The range surrounding the mean calibration age of 36 years is the range of greatest accuracy. This

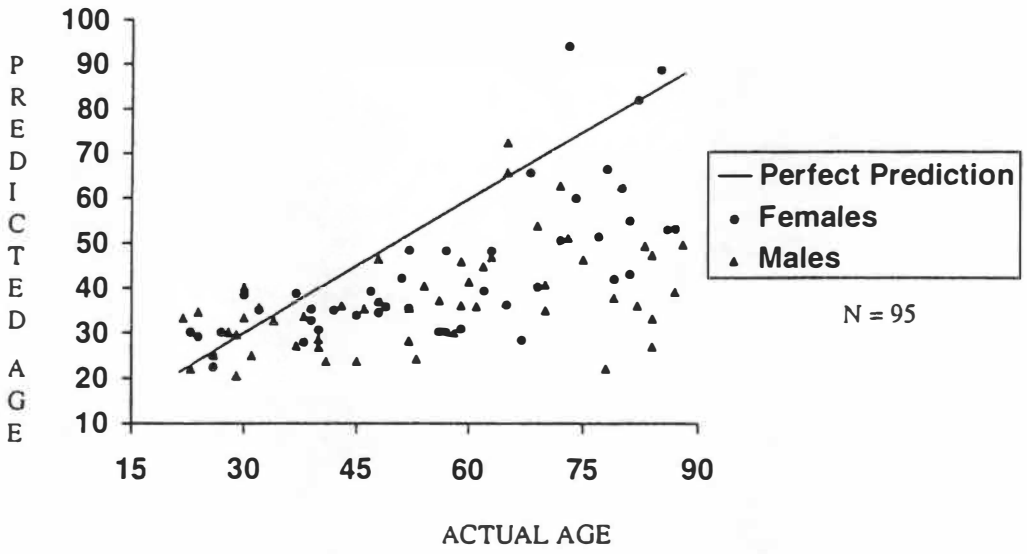

Figure 4.5: Stout and Colleagues (1996) Equation Applied to Current Sample

reduces the error in the estimates for the test sample: since more individuals fall within this range of increased accuracy, there is less cumulative error.

THE SUBSAMPLE EQUATION: DERIVED IN THE CURRENT STUDY

The subsample equation applied to the full test sample (N = 95) shows fair results, with a mean absolute difference of only 12.84 years between actual and estimated ages(See Table 4.7). The minimum absolute difference was 0.21 and the maximum absolute difference was 47.74, lower than the previous test cases in this study. However, this is not a very impressive fit of the equation to its a sample that is mostly comprised of its own data. Since 74 of the 95 individuals in the test sample were used (as the subsample) to generate this predictive equation, one would expect a better performance of the equation. The fair performance reinforces the fact that the correlation between the variable OPD and age is relatively low, and may also be partially the result of the slight extrapolation involved.

Inverse calibration was used to generate the subsample predictive equation. As Figure 4.6 illustrates, the best estimates are generated at the mean of the sample and this is where the perfect prediction line and the plot of the estimates cross. Since the equation is applied to its own generative sample, this area of best estimates is obviously in the center of the equations age distribution (22-73 years), placing it at 47.5 years.

The RMSE value of 16.48 (table 4.8) suggests a relatively good fit of the subsample equation to the full sample (n = 95). It is larger than the MSE of the subsample generated during the regression analysis (MSE = 11.86). This is an expected result, since

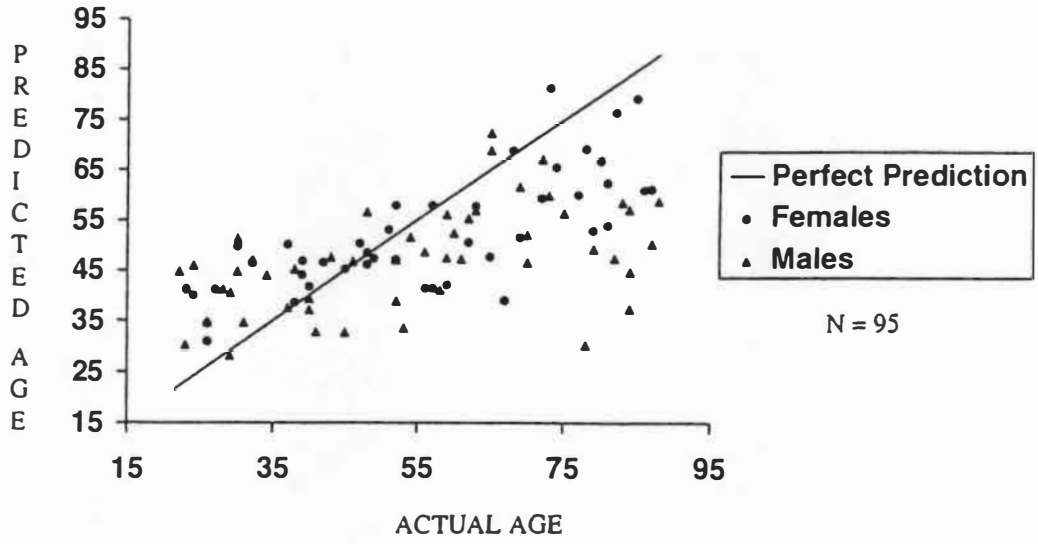

Figure 4.6: Subsample Equation Applied to Full Current Sample

applying the predictive equation (generated from the subsample of individuals 22-73 years of age) to the full sample (individuals 22 to 88 years of age) is a case of slight extrapolation.

The current study's predictive equation was tested by applying it to the Stout and Paine (1992) sample (see Fig.4.7: Table 4.7). The mean absolute difference of 10.14 years was the smallest of all tests in this study. The minimum and maximum absolute differences were among the smallest of the tests in this study (minimum = 0.64 years, maximum = 24.19 years). Predicted ages for some of the younger individuals were reasonably accurate, but most of the sample was systematically overaged.

However, this application of the subsample's inverse calibration equation did not produce the typical distribution of estimates: all ages are not biased toward the mean age of the calibration sample (47 years). Many of the estimates for the young end of the distribution are accurate or slightly *lower* than actual, when the pattern of the bias would place them all *higher* than actual age. The older individuals should be *underaged* by the inverse estimator, but those in this sample are all *overaged*. This suggests the observed OPD values for the two samples are not comparable. The most likely explanation for this would be interobserver error between the two studies or sample-specific characteristics.

The accuracy of the estimates for the very young individuals could be due to a greater correlation of OPD values between the current and 1992 study for the easier to read thin sections found in young individuals with limited evidence of remodeling. That is, OPD values for the two samples are more comparable for the younger individuals than in the older, more histologically complex individuals.

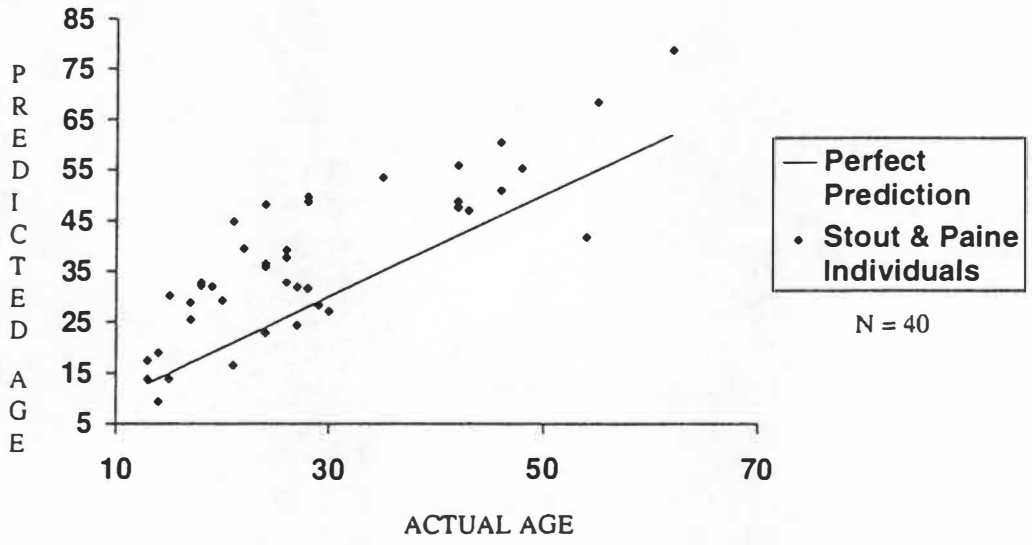

Figure 4.7: Subsample Equation Applied to Stout and Paine (1992) Sample

The RMSE value of 12.07 (table 4.8) is the lowest for all the test cases. This likely due to the fact that Stout and Paine's sample age distribution is more restricted than that

of the subsample used to generate this study's predictive equation. Since this is the case, applying the subsample equation to the Stout and Paine sample is not a case of extrapolation. Applying the equation to a truncated version of a similar distribution should decrease the standard errors (Krutchkoff, 1967, 1969).

In this case, the biasing effect of the inverse calibration is not evident. The best age estimates are not generated around the mean of the generative sample, most likely due to interobserver differences between the two studies.

PART III: CLASSICAL EQUATION RESULTS

Predictive equations were generated from the Stout and Paine (1992) sample and current subsample (n = 74) using classical calibration to contrast their results with those of the inverse prediction equations. The variable SEX was not significant when classical calibration was used to generate an equation from the subsample (N = 74). Absolute differences between actual and estimated ages and mean square error values were again calculated to compare the predictive performance of the equations.

CLASSICAL STOUT AND PAINE (1992) FORMULA

Applying the classical calibration equation generated from the Stout and Paine sample set to its own data produced excellent results. Of course, applying a predictive

equation to its own data set is expected to produce very small errors. As figure 4.8 illustrates, there is no systematic bias to the distribution of the age estimates. The mean absolute difference of 6.98 years (table 4.9) is not much larger than the mean absolute difference for the corresponding inverse equation, which produced a mean absolute difference of 1.1 years in its first test (Stout and Paine, 1992) and 5.5 years in its second test (Stout et al., 1996).The minimum absolute difference between the actual and estimated age is quite small (0.04 years), and the absolute maximum difference (20.80) compares favorably with the smallest of the absolute maximum values for the inverse equation (maximum absolute difference = 22.2 :Stout et al.'s 1996 application of the 1992 equation to the Swiss sample). The root mean squared error value of 8.38 (table 4.9) also reflects an excellent predictive performance and is the lowest MSE for any test case produced for this study.

When the Stout and Paine classical equation was applied to the current sample, the mean absolute difference between actual and estimated ages increased to 16.51 (see table 4.9). Again, the plot of the estimates shows no systematic bias (figure 4.9). The minimum absolute difference (0.10) is comparable to that of the Stout and Paine inverse equation applied to the same sample (0.12 years). The maximum absolute difference of 56.44 years is quite large, but it is slightly smaller than the maximum absolute difference for the complementary inverse equation applied to the this sample (57.92 years). This suggests that the inverse and classical equations derived from the 1992 data set are quite evenly matched in their predictive ability.

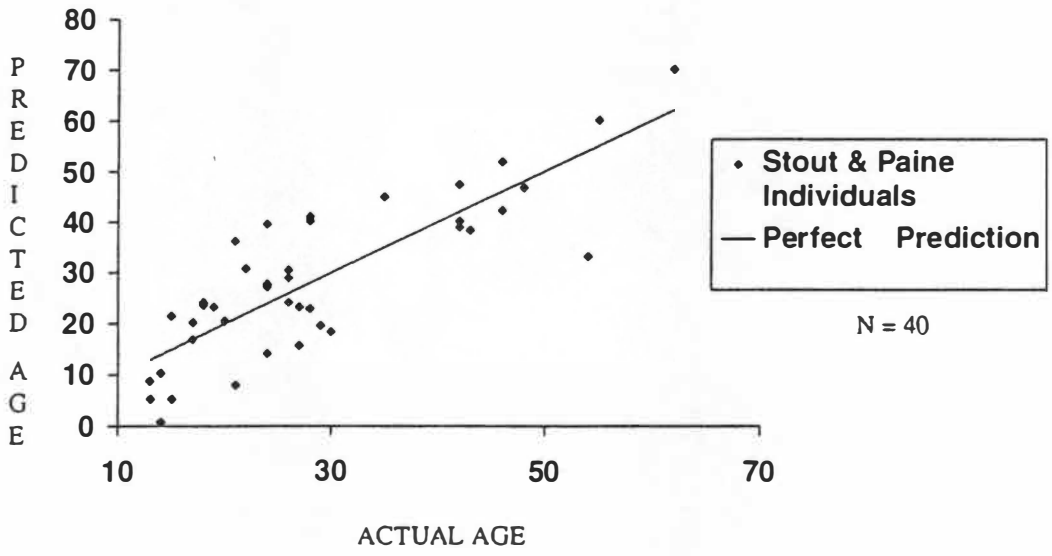

Figure 4.8: Classical Stout and Paine Equation Applied to Stout and Paine (1992) Sample

Table 4.9 : Absolute Differences Between Actual and Predicted Ages for Classical Equations.

Classical Equation Applied	Test Sample	N =	Abs. Mean	Abs. SD	Abs. SE	Abs. Minimum	Abs. Maximum
Stout and Paine (1992)	Stout and Paine (1992)	40	6.98	4.70	4.70	0.04	20.80
Stout and Paine (1992)	Current Test Sample	95	16.517	13.03	13.03	0.10	56.44
The subsample Equation (Current Study)	Stout and Paine (1992)	40	21.98	15.69	15.69	1.45	60.21
The subsample Equation (Current Study)	Current Test Sample	95	16.21	13.58	13.58	0.08	71.05

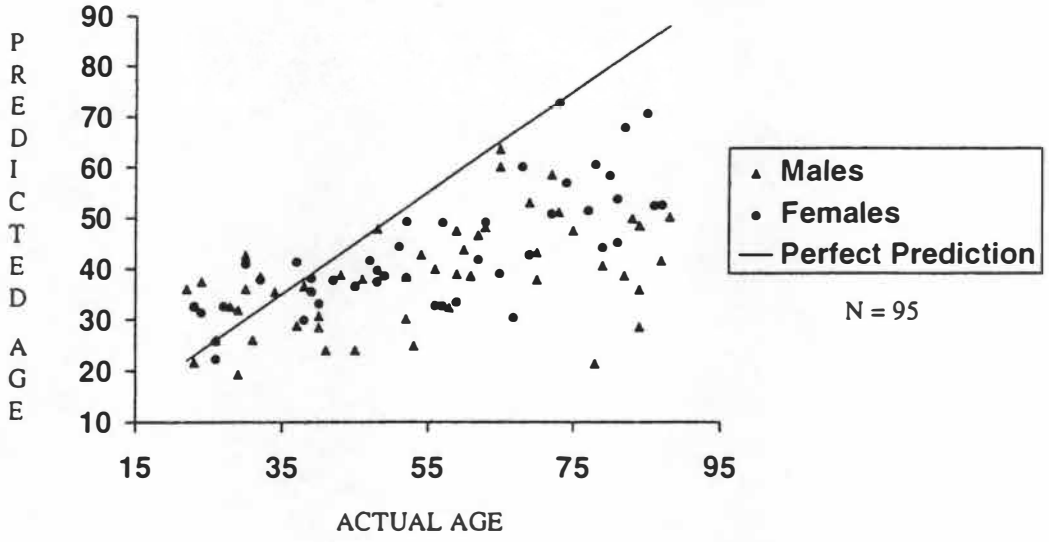

Figure 4.9: Classical Stout and Paine Equation Applied to Current Sample

However, a different conclusion is drawn when the mean square error values for the inverse and classical Stout and Paine predictive equations are compared. The classical equation applied to the current sample resulted in an RMSE value of 20.99 (table 4.10), while the inverse equation applied to the same sample resulted in an RMSE of 24.75 (see table 4.8). The greater error for the inverse method is the result of the distribution of the current sample. An inverse estimator produces a smaller standard error only in the range surrounding the mean of the generative sample. In the case of the 1992 equation, this range surrounds 28.6 years. Since the current sample extends well past this range, application of the 1992 equation to this data set is a case of extrapolation. The error of estimate becomes increasingly larger as the OPD values increase from the 1992 sample mean. The unbiased classical estimator has consistently larger errors for estimates for the entire test sample, but they result in a smaller total RMSE. This supports Krutchkoff's (1969) claim that the classical estimator is superior to the inverse in cases of extrapolation.

CLASSICAL SUBSAMPLE EQUATION: DERIVED IN THE CURRENT STUDY

The classical regression equation generated from the subsample provided less satisfactory results than the inverse when applied to the Stout and Paine (1992) sample. The mean absolute difference of 21.98 years is twice the mean absolute difference of (10.14 years) of the subsample's inverse equation. The classical equation also produced a greater maximum absolute difference (60.21 years) than the inverse equation on the same sample (24.19 years).The plot of the age estimates relative to a perfect line of prediction

Table 4.10: Root Mean Squared Error Values

Classical Equation Applied	Test Sample	N =	Root Mean Squared Error
Stout and Paine (1992)	Stout and Paine (1992)	40	8.38
Stout and Paine (1992)	Current Test Sample	95	20.99
The subsample Equation (Current Study)	Current Test Sample	95	21.10
The subsample Equation (Current Study)	Stout and Paine (1992)	40	26.89

is illustrated in figure 4.10.This plot shows no systematic pattern to the resultant age estimates, suggesting that the classical equations estimates are not significantly biased.

Comparing the RMSE for the classical and inverse equations generated from the subsample proves the inverse equation produced more accurate age estimates for the Stout and Paine data set. The RMSE value of 12.07 for the inverse equation (see table 4.8) result suggests the range of the Stout and Paine sample is close enough to the range of the current sample mean (47.23 years) to keep the standard error lower than that for the classical equation. Since this is not a case of extrapolation, the inverse estimator is expected to have smaller errors. The problem of non-comparability between the Stout and Paine and current data set is not possible in this case, since there is no expected pattern of bias to interpret.

Applying the subsample classical equation to the full test sample (N = 95) resulted in a mean absolute difference between actual and estimated of 16.21 years (table 4.9). The minimum absolute difference was very small (0.08 years), but the maximum absolute difference of 71.05 years is quite extreme. The plot of the age estimates relative to a line of perfect prediction (figure 4.11) illustrates no systematic bias to the estimates.

The RMSE of 21.10 (table 4.10) illustrates that the equation is not biased toward any particular sample distribution, since applying the same classical equation to the smaller age distribution of the Stout and Paine sample provided a very similar RMSE value (26.89). However, comparison of the RMSE value for the complementary inverse equation to the same data set (16.48, see table 4.8) shows the inverse equation produced much smaller errors than the classical equation. This is expected, since the inverse

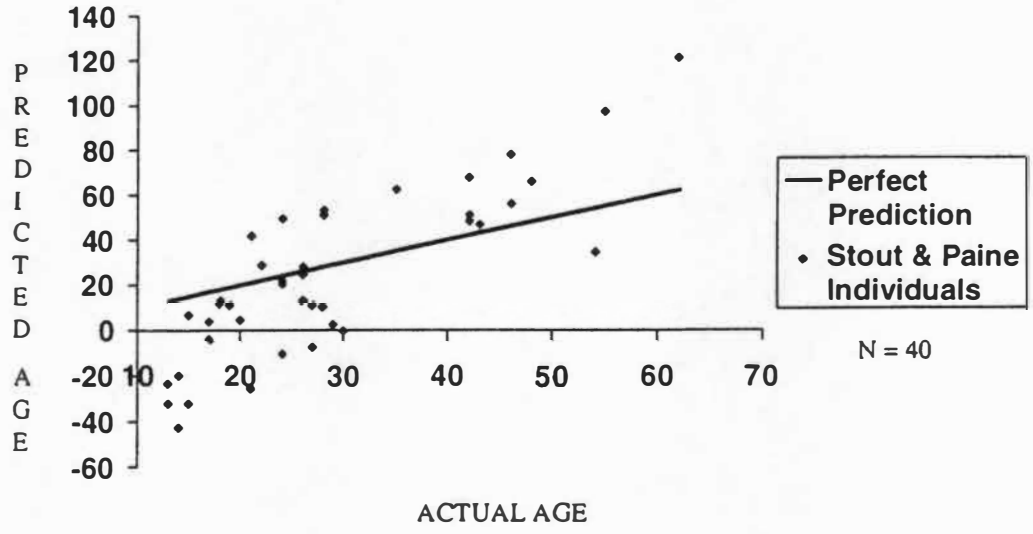

Figure 4.10: Subsample Classical Equation Applied to Stout and Paine (1992) Sample

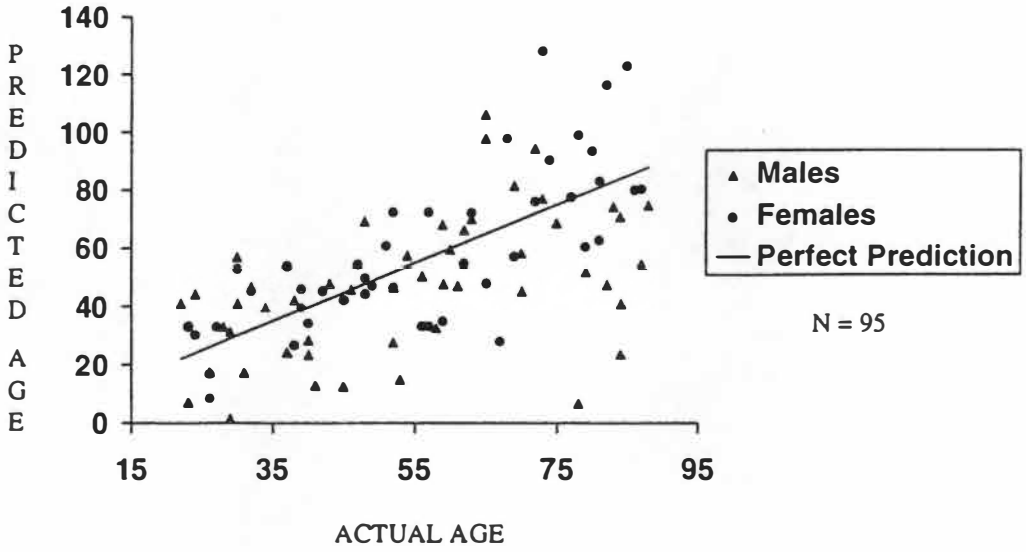

Figure 4.11: Subsample Classical Equation Applied to Current Sample

estimator is more efficient in cases where extrapolation is not involved. The Bayesian assumption of similar distributions that accompanies the inverse estimator is met since the two distributions are exactly the same. This small RMSE value illustrates that using members of generative sample as test cases for an inverse equation will provide over-optimistic expectations of prediction accuracy, since it is unlikely that future test sample distributions will so closely match that of the generative sample.

PART IV: CALCULATION OF THEORETICAL MEAN SQUARED ERRORS

Shukla (1972) describes a method for computing the bias, conditional variance, and mean squared error (MSE) for regression equations. While the MSE, bias, and variance for the classical estimator are infinite if there is no regression relationship between the variables, they are always finite for the inverse estimator. The formulae assume normal distributions of error terms, since they are less complicated than non-normally distributed terms.

Following the method described by Shukla, the mean squared error, bias, and conditional variance terms were generated for both the inverse and classical subsample equations (n = 74, age range = 22 to 73 years) generated in the current study. A plot of these values (figure 4.12) shows that the inverse estimator produces the smallest MSE throughout the entire age range of the current sample. The MSE values for the classical estimator are not lower than those of the inverse for any age in the current sample. This illustrates the high conditional variance of the classical estimator in this case. Although its estimates are subject to some bias, the comparison of estimator efficiency suggests the

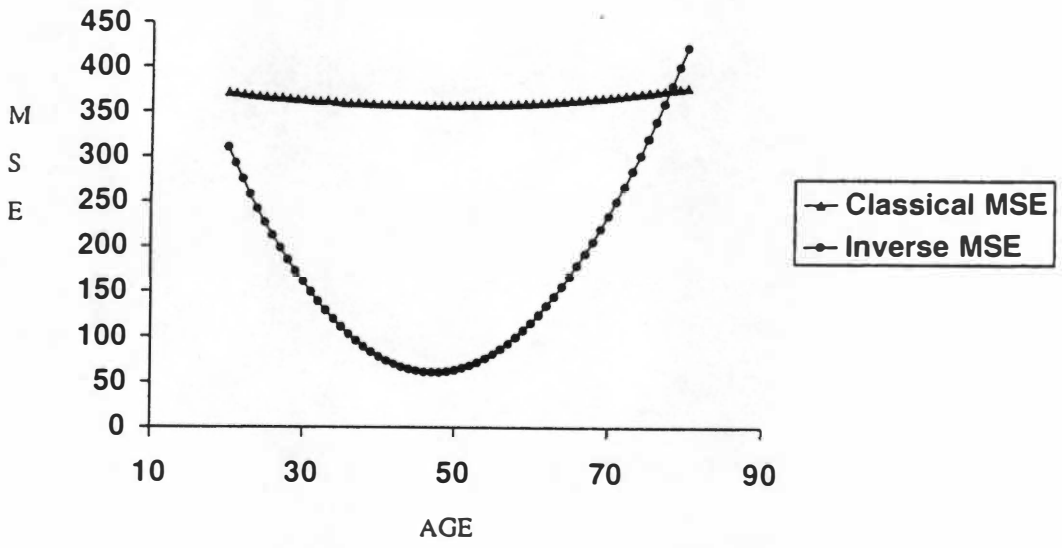

Figure 4.12: Comparison of Mean Squared Errors for the Subsample Classical and Inverse Predictive Equations

inverse calibration is the favored estimator across the age range of 22 to 88 years. The more conservative classical estimator is not necessary since the bias of the inverse equation is not substantial in this equation. Since the current sample includes the age range one expects to encounter in most cases of unknown remains, aging techniques using inverse calibration may be routinely applied. However, since the effects of the many factors on histological characteristics of humans are not yet completely known (see chapter 2), use of the more conservative classical calibration may be a viable option for archaeological applications.

CHAPTER FIVE: CONCLUSIONS

In this study, generation of single-sex classical regression lines indicated the clavicular cortical bone remodeling rates for males and females were different, evidenced by different plots of the classical regression lines for each subsample (males n = 50, females n = 45). However, this difference was not significant when the combined-sex sample was used to model classical and inverse equations, illustrated by the non-significance of the SEX variable.

The difference between the single-sex and combined-sex regression lines is likely because females of the current sample exhibited a stronger correlation between age and the OPD variable than the males, although the difference was not great enough to necessitate sex-specific predictive equations. This finding is contrary to previous studies (Samson and Branigan, 1987: Ericksen and Stix, 1991) that found females to be less consistent than males in their relationship with the histological variables observed. Samson and Branigan (1987) suggested their inability to produce accurate ages for the females in their sample was related to hormonal regulation. The stronger correlation between OPD and age for females in my study suggests the OPD variable is not affected by physiological hormonal variabilities in the same manner as the variables studied by Samson and Branigan. This result has not been previously reported, and may due to a real effect or some characteristic(s) unique to the Northeast Tennessee autopsy sample, although none have been identified.

This introduces the idea that the relationship between age and histological structures may depend upon which variable one defines. If this relationship is sample-specific or subject to population differences, this would spell more barriers for histomorphometry. The dynamics that produce the observed structures must be robust to population, temporal, and geographical differences if they are to be utilized for cross-populational investigations. In addition, the variables used in each study must be studied for their relationship to other variables and to the dynamic processes of bone physiology.

An asymptote was found for the OPD variable at the age of 73 years using a linear response and plateau function. This indicates that individuals beyond this age may be more difficult to age accurately due to an inability to detect increasing remodeling activity. However, since only intraobserver error was controlled in this study, further study of this result is warranted.

When measured by mean absolute differences or by mean squared error values, the newer inverse equation reported by Stout and colleagues (1996) provided more accurate age estimates than the original Stout and Paine (1992) equation for the independent sample gathered for this study. This is the result of the more similar age distributions between the (1996) combined sample and the current sample. As Krutchkoff (1969) described, in cases of extrapolation the inverse method of calibration results in greater standard errors than in cases where the same equation is applied to samples of similar distributions as the generative sample. Applying the 1992 equation to the current sample is a case of extrapolation, but applying the 1996

equation is less so, since the age distributions of the current and 1996 samples are quite similar. This similarity between the two samples results in lower MSE values when the current sample is used to test the 1996 equation.

Both inverse and classical predictive formulae generated from the current subsample generated less accurate age estimates than the equation by Stout and colleagues (1996), illustrated by the greater mean absolute differences and larger MSE values for the tests of subsample equations. This is due to the variation in the current sample not attributable to age or sex. The various incidental and major medical conditions related to cause of death for the current sample (appendix 1) may contribute to the unexplained variation. Two other possibilities include interobserver variation and natural variation introduced with advancing age due to individual life experiences and metabolic regulation, as suggested by Martin and colleagues (1981). The greater number of elderly individuals in this study increased the latter natural variation over that contained in the Stout and Paine (1992) and Stout and colleagues (1996) studies. In any case, the relationship between age and the OPD variable in this study was weaker than those found in previous histological studies of the clavicle.

A surprising result showed that the range of MSE's for the inverse estimator is smaller than those for the classical estimator in almost the full range of the subsample (22-88 years). In this study, the range of inverse superiority is not "trivially small" as Halperin (1970) suggested the range might be in many cases. However, the low r-squared values in this study may contribute to this result. The stronger the relationship between the regression variables, the tighter the distribution of the age

estimates, and possibly, the smaller the range of the inverse method's superiority in terms of MSE. While use of the inverse predictive equations for clavicular aging methods appears to be acceptable, each of the existing histological predictive equations for other sampling sites should be examined for the range where bias is minimized and estimate efficiency is maximized. Classical forms of each equation should be studied as a conservative option for age estimation techniques.

The possible non-comparability between the subsample and the Stout and Paine data set, suggested by the non-characteristic distribution of the subsample equation's age estimates for the Stout and Paine individuals (chapter 4, page 78), is troubling. If in fact the two are not comparable, there may be implications to consider. In this case, the error could be due to the greater amount of detail observable in my slides versus Stout and Paine's slides due to the differential processing of the samples. If this is the case, the methodology for processing samples should be standardized in order to produce consistent results.

If the alleged non-comparability is due to problems above and beyond that which can be explained by differential chemical processing, it is quite possible that histological age estimation techniques will continue to be practiced by only a small number of researchers. After study of bone dynamics, the histological method, and hands-on experience, one should expect satisfactory and replicable results. Comparable studies using the same variables must be done to establish acceptable guidelines for interobserver error and assess the comparability of interpopulational samples. The Stout and Paine (1992) study and the 1996 expansion (Stout et al.)

illustrate a unique situation that should be replicated for all histological aging techniques: the same variable was studied in geographically and temporally different populations and the results were compared for consistency. In the case of the 1992 and 1996 studies, the results were quite consistent. However, several observers should carry out this research, for if only a select number of individuals can produce dependable results, histological methods will be of lesser value to anthropology in general.

In addition to technical concerns, methodological problems exist in the form of statistical application. This is the first study to examine this problem, and other types of statistical analysis should be explored as possible aids to histological aging techniques. Anthropologists need not be statisticians, but they must be aware of the methodology they employ, especially as concerns bias and confidence bounds. It is troubling that more than a quarter of a century after the introduction of histological aging methods, the statistical method all apply has only recently been correctly identified as an application of statistical calibration (Konisberg et al, in press). Calibration is a specialized form of linear regression, and constitutes its own field of study in statistics, yet anthropologists have never used the word "calibration" in their reports of histological aging.

While this study showed that the inherent bias of the inverse calibration method was not severe enough to warrant discontinuing the method for the clavicle, its use to generate predictive equations should be further examined and regarded as a potential problem in cases of forensic and archaeological age estimation. This is

especially true for equations based upon small sample sizes and those with restricted age distributions. Until the classical and inverse forms of each histological predictive formula (and each method) are compared, there exists a hazard of producing biased age estimates for unknown individuals.

In summary, histological aging methods represent an area that has much new territory to explore, and potentially, much new insight to contribute to the study of human variation and skeletal biology. However, until the complexities of the biological, the methodological, and the statistical dynamics at work are studied and understood, histological methods will remain the domain of a small number of researchers patiently searching for the answers that are not yet available.

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APPENDICES

Appendix 1
Cause of Death and Major Medical Conditons for the Current Sample

Case	Death Related to:	Notes
87-131	Myocardial Infarction	Renal failure
87-132	Diabetes mellitus, uncontrolled	Kimmelsteil-Wilson Kidneys, Hysterectomy
87-142	Aneurysm	
87-143	Multiple trauma	Renal cortical tuberculosis
87-147	Acute alcoholic intoxication	
87-154	Marfan's syndrome	
87-164	Vehicular trauma	
88-014	Pneumococcal Pneumonia	Osteoporosis
88-021	House fire	
88-022	House fire	Complete hysterectomy
88-023	Arteriolar Sclerosis	
88-031	Cerebral atrophy	Mild-moderate osteoporosis
88-036	Gunshot wound	
88-046	Suffocation / Drug OD	Oophorectomy
88-047	Vehicular trauma	
88-058	Burns, smoke inhalation	Prescribed drug list
88-059	Multiple head trauma	
88-064	Gunshot wound	
88-065	Alcohol and drug OD	
88-067	Drowning / Drug OD	
88-068	Crushing trauma	Prescribed drug list
88-077	Gunshot wound	Prescribed drug list
88-089	Anoxia / Drowining	Epileptic / Dilantin
88-102	Vehicular trauma	
88-109	Shotgun wound	Diabetic
88-124	Asphyxia / Drowning	
88-125	Obstructive emphysema	Prescribed drug list
88-137	Gunshot wound	
88-157	Cardiomyopathy	S/O remote hysterectomy (-1 ovary)
88-161	Anoxia / Drowning	
88-162	Gunshot wound	
88-163	Vehicular trauma	Total hysterectomy - S/O
88-170	Coronary atherosclerosis	Known heavy drinker
89-015	Gunshot wound	
89-017	Atherosclerosis	
89-018	Atherosclerosis	
89-028	Atherosclerosis	High blood pressure
89-042	Gunshot wound	Mild-moderate emaciation / Prescribed drug list

89-068	Thermal burns	Type III Polycystic Disease (Kidneys and liver)
89-072	Vehicular trauma	
89-075	Vehicular trauma	
89-076	Diabetes mellitus	
89-085	Gunshot wound	
89-097	Vehicular trauma	
89-098	Hanging	Cerebal palsy
89-099	Acute heart failure	
89-120	Gunshot wound	Hysterectomy / S/O
89-121	Gunshot wound	Drug list available
89-132	Vehicular trauma	
89-147	Chronic emphysema	History of alcoholic demetia: Prescribed drug list
89-151	Stab wound	
89-163	Vehicular trauma	
89-173	Anoxia / Drug OD	
89-175	Anoxia / Hanging	
89-178	Gunshot wound	
89-182	Gunshot wound	
90-013	Gunshot wound	
90-039	Anoxia / IV Drug OD	Probable chronic hepatitis
90-049	Vehicular trauma / Heart attack	Thyroidectomy, 1 ovary missing
90-055	Cirrhosis	Moderate osteoporosis / Diabetes / Prescribed drug list
90-061	Multiple gunshot wounds	
90-068	Drug overdose	
90-081	Vehicular trauma	
90-082	Shotgun wound	
90-083	Vehicular trauma	Total hysterectomy & oophorectomy
90-092	Shotgun wound	
90-100	Artherosclerosis	Prescribed drug list
90-102	Asphyxiation / Alcohol	
90-106	Vehicular trauma	
90-125	Asphyxia	Prescribed drug list
90-126	Gunshot wound	
90-141	Severe fatty liver	
90-146	Thermal burns / Alcohol	
90-150	Vehicular trauma	
90-161	Atherosclerosis	Alcoholic steatosis & hepatitis
91-018	Anoxia / Drowning	
91-026	Drug overdose	1 ovary present
91-030	Gunshot wound	
91-051	No identifiable cause	Subtotal hysterectomy

91-059	Gunshot wound	
91-084	Chronic alcoholism	Wernicke's syndrome / Malnutrition
91-088	Vehicular trauma	
91-105	Vehicular trauma	
91-111	Thermal burns	
91-129	Gunshot wound	
91-130	Anoxia /Drug overdose	Obesity : Prescribed drug list available
91-132	Gunshot wound	
91-148	Vehicular trauma	
91-159	Bronchitis with Bronchopneumonia	
92-008	Atherosclerosis / Liver cancer	Parkinson's disease
92-025	Vehicular trauma	
92-051	Alcoholism / Acute intoxication	Severe obesity
92-066	House fire	
92-138	No cause determined	
92-165	No cause determined	
92-178	Gunshot wound	
93-043	Gunshot wound	Prescribed drug list available

Appendix 2
Demographic Information and Histological Measures for Current Sample

Case	Age	Sex	Complete Osteons Slide 1	Fragmentary Osteons Slide 1	Area Read Slide 1	Complete Osteons Slide 2	Fragmentary Osteons Slide 2	Area Read Slide 2
87-131	81	F	264	310	24.13	259	284	23.96
87-132	40	F	176	137	18.83	188	114	18.77
87-142	56	F	136	168	18.48	136	155	18.18
87-143	80	F	231	175	17.30	198	184	14.58
87-147	40	M	313	244	36.01	308	205	32.75
87-154	53	M	216	204	30.44	248	185	32.06
87-164	23	M	286	188	32.53	268	80	32.86
88-014	86	F	190	207	17.60	209	241	19.57
88-021	48	F	253	188	24.55	223	173	22.46
88-022	73	F	290	318	20.93	259	289	18.20
88-023	84	M	168	253	22.96	168	196	22.31
88-031	81	F	207	185	19.25	241	222	22.70
88-036	26	F	138	95	18.88	129	132	19.84
88-046	78	F	260	197	18.04	223	200	16.51
88-047	57	F	405	234	28.98	338	219	26.08
88-058	82	M	313	257	30.67	320	208	29.46
88-059	62	M	246	284	25.38	232	269	24.05
88-064	49	F	219	184	24.54	197	315	25.74
88-065	42	F	272	150	23.96	319	232	30.27
88-067	26	M	317	159	34.70	321	185	35.23
88-068	72	M	307	391	27.00	275	386	27.76
88-077	67	F	127	208	19.16	137	136	20.13
88-089	33	M	393	282	39.88	367	337	36.16
88-102	22	M	423	371	49.20	339	457	42.37
88-109	56	M	407	300	35.82	406	275	38.60
88-124	43	M	247	288	30.06	286	288	30.50
88-125	58	M	279	351	38.84	238	347	36.49
88-137	40	M	398	235	42.07	370	244	42.02
88-157	45	F	194	174	21.67	197	160	19.74
88-161	29	M	234	28	26.20	274	153	32.23
88-162	34	M	350	261	37.12	327	313	35.91
88-163	65	F	186	156	26.64	217	163	27.72
88-170	45	M	287	165	34.67	292	163	33.25
89-015	69	M	185	226	18.12	158	180	14.45
89-017	70	M	359	253	36.03	362	308	35.35
89-018	70	M	463	307	37.29	469	311	41.20

89-028	52	F	359	257	27.01	336	234	27.57
89-042	78	M	287	253	39.17	267	63	30.24
89-068	82	F	140	156	10.55	101	168	9.68
89-072	57	F	211	200	26.80	224	223	26.11
89-075	79	M	347	202	30.11	374	207	29.82
89-076	59	M	334	289	28.13	350	305	32.37
89-085	32	F	280	148	23.43	226	175	22.75
89-097	89	M	248	296	25.93	219	239	26.20
89-098	48	M	478	389	38.67	445	341	39.02
89-099	23	F	253	207	27.74	248	205	28.53
89-120	85	F	177	268	15.35	164	252	14.49
89-121	37	F	167	188	18.84	183	199	19.71
89-132	29	M	243	205	27.90	214	244	28.87
89-147	66	F	177	224	13.90	160	199	13.45
89-151	24	M	352	206	34.58	360	371	37.78
89-163	24	F	289	143	28.04	306	140	27.57
89-173	72	F	184	175	16.96	208	230	18.87
89-175	65	M	375	351	28.31	368	374	27.11
89-178	30	M	464	225	35.55	393	283	34.23
89-182	82	M	206	253	21.63	256	243	22.92
90-013	59	M	453	276	39.64	454	246	38.45
90-039	30	M	361	217	32.02	350	183	32.02
90-049	79	F	197	217	22.05	206	257	21.68
90-055	77	F	178	195	15.69	166	151	15.03
90-061	73	M	312	361	28.29	292	358	30.82
90-068	52	F	217	184	24.46	251	245	25.07
90-082	37	M	302	238	36.30	291	212	33.48
90-083	69	F	204	198	20.21	185	181	18.99
90-092	52	M	165	127	19.18	173	130	19.40
90-100	48	F	314	313	31.89	265	294	32.02
90-102	54	M	231	214	22.96	234	223	22.98
90-106	84	M	247	191	27.20	249	156	29.50
90-125	41	M	268	155	31.07	254	187	33.53
90-126	60	M	374	239	31.72	382	271	31.82
90-141	63	M	171	192	17.00	192	217	19.10
90-146	38	M	419	237	37.15	429	240	38.48
90-150	46	M	310	267	31.80	274	182	25.54
90-161	65	M	384	288	26.96	352	262	23.76
91-018	28	M	250	325	33.62	304	189	32.33
91-026	63	F	216	222	21.16	200	234	19.5
91-030	88	M	295	154	22.29	276	219	13.07
91-051	87	F	158	138	15.64	190	262	17.11

91-059	61	M	296	131	32.64	332	408	41.43
91-084	52	M	261	269	27.07	249	223	28.19
91-088	59	F	198	142	21.13	217	134	20.85
91-105	30	F	316	161	24.53	283	172	24.54
91-111	62	F	353	235	29.74	376	213	31.37
91-129	31	M	296	161	32.60	275	181	32.56
91-130	38	F	256	137	24.68	245	117	24.70
91-132	47	F	225	208	22.27	220	181	21.16
91-148	74	F	126	138	12.01	126	219	13.07
91-159	68	F	166	214	14.41	165	202	15.08
92-008	83	M	274	277	25.53	253	265	23.12
92-025	27	F	224	158	24.19	257	146	24.34
92-051	39	F	382	241	28.45	355	220	38.00
92-066	23	F	233	90	19.56	225	98	20.39
92-138	65	F	252	193	25.42	230	193	21.99
92-165	51	F	319	253	28.59	354	289	31.87
92-178	75	M	343	308	30.68	336	262	28.20
93-043	26	F	214	140	24.04	180	105	21.79

VITA

Nikki Lynn Rogers was born in Covington, Virginia, in 1971 and moved with her parents and older sister to Oak Hill, West Virginia, where she lived for most of her childhood. In 1981, the family relocated to Kissimmee, Florida, and she graduated from Gateway High School in 1989. After graduation, she attended Florida State University, where she earned her A.A. and B.A. in Anthropology with a minor in geology, graduating with honors at the end of 1992. In the beginning of 1993, she began her graduate studies in physical anthropology at the University of Tennessee in Knoxville. After almost four years of research, study, and marrying the man of her dreams, she received her M.A. in anthropology with a minor in statistics in December of 1996.