REVIEW OF THE HISTOLOGICAL METHOD FOR DETERMINING AGE AT DEATH IN HUMAN SKELETONS

BY

CHRISTOPHER G. NEILL
University of Toronto

ABSTRACT

The histological method for determining the age at death of adult human skeletons is critically reviewed and standardizations are suggested. The accuracy of the method varies with the technique applied but is generally more accurate than morphologic and morphometric methods. A S.E.E. of 2.55 years was obtained by Singh and Gunbewrg (1970) using the mandible. Kerley (1965) obtained a S.E.E. of 5.27 years and Thompson (1978, 1979) obtained S.E.E.'s within 6.5 years using this "core technique". Disease must be screened out and specific equations are required for different "racial" groups. Sex difference does not significantly affect the age estimates. The histologic method is applicable to archaeology, forensic medicine, demography and palaeophysiology. A manual detailing the essentials of the histological method would be useful to future investigators.

RÉVISION DE LA MÉTHODE HISTOLOGIQUE SUR LES SQUELETTES HUMAINS POUR DÉTERMINER L'ÂGE À LA MORT

RESUME

La méthode histologique appliquée aux squelettes d'adultes humains pour déterminer l'âge à la mort fait ici l'objet d'une révision critique et nous suggérons des normes d'unification. La précision de la méthode varie selon la technique employée. Toutefois, elle est plus précise que les méthodes morphologique et morphométrique. Singh et Gunberg (1970), travaillant avec la mâchoire inférieure, sont arrivés à une E.T.E. de 2,55 ans. Kerley (1965) est arrivé à une E.T.E. de 5,27 et Thompson (1978, 1979), utilisant cette "technique du noyau", a abouti à des E.T.E. dans les environs de 6,50 ans. Les maladies doivent être passées au peigne fin et des équations spécifiques sont requises pour différents groupes "raciaux". La différence des sexes n'affecte pas de façon significative les évaluations de l'âge. On peut utiliser la méthode histologique en archéologie, en médecine légale, en démographie et en paléophysiologie. Un manuel expliquant en détail la méthode histologique serait utile pour les futurs chercheurs.

INTRODUCTION

This paper is an evaluation of the histological method for determining the age at death from age-related metamorphic changes in intra-cortical structure in human cortical bone. A review of the methodologies employed by various investigators, their reliability and facility, are detailed in order to assess the appplicability of the method. Areas where further research is needed are delimited by a review and synthesis of the pertinent literature which, hopefully provides an up-to-date critique of the method.

Histological aging techniques were developed to provide a more accurate method for estimating the age at death of human skeletal remains, especially among individuals who had achieved skeletal maturity. In general, all aging methods are based on one or more of the following; 1) the amount of growth, 2) the stage of development, and 3) the amount of degeneration (Stewart 1979: 128). The subadult methods of aging (see Table 1) are based on the correlated processes of growth and development, while adult aging methods are generally based on the amount of "degenerative" change including non-pathological metamorphic, transformation, as well as continued-growth change. It is noteworthy that the histological method is based on all three types of age-related change. Further the method is applicable to subadults (from prenatal to approximately 25 yrs.) as well as adults (over 25 yrs.), but is used mostly in adult aging applications.

The first observer that quantified the histological changes that occur with age in human cortical bone was J.D. Currey (1964:69=75). Currey took transverse sections from the midshaft of the femora of 19 autopsy subjects ranging in age from 23-89. The individuals were free of bone disease and trauma, but many had been invalids for some time prior to death and this appeared to have affected cortical thickness.

Currey's main interest was to describe the factors that cause the change in the ratio of non-Havesian to Haversian bone and their effect on gross skeletal morphology. This involved observing the effect of an increase in lumen size on the amount of non-Haversian bone, a decrease in the size of the Haversian systems with age, and a decrease in corticla thickness. Other factors affecting this ratio were the same as those described by Kerley (1965, see below).

Regression equations, derived by Currey for the age-related changes in the bone microstructure, were used to quantify the microstructural changes, but were not used for estimating the age at death of the subjects. Kerley's work (1965), then, was the first study that applied the age-related changes in cortical microstructure to estimates of age at death of human skeletons.

The changes that occur in the cortex with age are the natural outcome of the process of bone remodelling and other biological reactions of bone. The essentials of bone growth and remodelling are given below. Note the general patterns of change and the appearance of the cortical microstructure at the various stages of aging.

THE PROCESS OF CORTICAL BONE FORMATION AND DEGENERATION

Bone Formation and Structure

An elementary understanding of the growth of long bones will be given since most of the experiments discussed below used long bones for their studies. The remodelling process described is generally the same for all cortical bone. (Ham and Cormack 1979: 384-387).

Growth in the length of long bones is by interstitial (between tissue) growth, and the width increases by appositional (additive) growth (Ham and Cormack, 1979 See Fig. 1). The generalized parts of the longbone are shown in Fig. 2. Looking at a sagittal section of the diaphysis, the fine structure of the cortex (compact bone) can be appreciated (see Fig. 3). Figure 4 is a photomicrograph of the cross-section showing the microstructural detail of compact bone.

Comparing figures 3 and 4, the main microfeatures of compact bone can be see: the periosteum, the external and inner circumferential lamellae, Havesian systems (secondary osteons), osteoblasts, osteocytes, and osteoclasts, interstitial lamellae, Volkman's canals, resorption cavities and endosteum.

The periosteum consists of an outer fibrous layer and a thin, osteogenic layer. The cells in the osteogenic layer are responsible for almost all of the growth and development of the skeleton (Hamm and Cormack 1979:389).

The cells responsible for the resorption of bone are the osteoclasts. The canals created by the osteoclasts are approximately 300 m in diameter and up to 5 m in length (Ortner 1976). The canals may be invaded by blood vessels, some of which run perpendicularly and connect the Haversian systems to the periosteum or endosteum. These are known as Volkman's canals; they are not surrounded by concentric lamellae as are the osteon (Hamm and Cormack 1979:438-439; Enlow 1963:60-61; Jowsey 1977:82). Osteoclasts also form resorption cavities. A resorption cavity is formed from the remodelling of a lacuna (a space of dead bone) by osteoclastic resorption of the dead bone (see Fig. 4).

A secondary osteon (Haversian system) is formed when osteogenic cells enter a resorption space or a canal that has been enlarged by osteoclasts, and differentiate into osteoblasts which secrete the unmineralized, bone-forming matrix called osteoid. The osteoid is deposited in concentric layers starting at the surface of the canal wall and building inwards to a central blood vessel which has invaded the space. After approximately two weeks, the osteoid is mineralized, thus forming bone (Eanes et al. 1967 in Bourne 1971:209).

Once formed the secondary osteon is comprised of a canal containing one or two blood vessels and innervation, and surrounding concentric layers of bone. Usually up to 6 concentric layers can be seen marked by rings of osteocytes (see Figs. 3 and 4). Osteocytes are osteoblasts that have completed their bone forming function and have become entrapped in their own boney secretions. The osteocytes then complete the

mineralization of the bone (Ortner 1976: and see Ham and Cormack 1979: 401-406; Harris and Heaney 1970:10-11, for details on the role of the osteocyte in bone biology).

Secondary osteons (Haversian systems) are the primary unit of remodelled bone. They are distinctive from primary osteons because of their cement line which interrupts the flow of the interstitial lamellae, and the several concentric lamellae circling around a central blood vessel, or vessels, and nerves (Enlow 1963), (see Fig. 3).

Primary osteons are often arranged in groups or layers (Enlow 1963:63-65). The few concentric lamellae of the primary osteon may be difficult to differentiate from the interstitial lamellae (Fig. 5), particularly in young individuals and in areas of strong muscle attachment (eg. the linea aspera) (Kerley 1965:153).

The primary osteon is formed by the encapsulation of vascularized canals within the cancellous bone matrix. They also form subperiosteally during rapid bone growth in neonatals and within endosteally deposited bone. Primary osteons are prevalent in young and rapidly growing bone and remain until they are resorbed endosteally later on in the remodelling process (Enlow 1963:63-65).

Young individuals (under 18 yrs.) tend to have avoid-shaped secondary osteons because bone deposition occurs on on behalf of the resorbed canal while resorption continues in the other half. Adults tend to have circular osteons because bone is deposited more evenly on all of the surfaces (Lacroix 1971, in Thompson 1978:15).

BONE REMODELLING

Bone is formed rapidly during the first two decades of life. There is a period of stability until about 40 years of age and then the rate of bone loss becomes greater than the rate of bone formation (Kerley 1969; Enlow 1963).

From birth, the diaphyseal cortex and medullary cavity expand rapidly. Most of the cortical bone growth is the result of the deposition of circumferential lamellar bone by osteoblasts in the inner periosteal layer. During the period after birth until about 4 years of age, most of th eosteoclastic activity occurs in the endosteal third of the mid-shaft of the diaphysis (Kerly 1969). By the fourth year of life, the osteoclastic activity occurs more evenly throughout the cortex. As the osteoclasts create tunnels in the circumferential lamellae, or expand pre-existing canal structures, they interrupt the flow of the circumferential lamellae (Enlow 1963). The unresorbed lamellar tissue remaining between the remodelled bone is called the interstitial lamellae (Harris and Heaney 1970:1-2).

From 10 to 17 years of age bone goes through a growth spurt. Most osteoclastic activity, at this time, occurs in the region under the periosteum (Kerley 1969). Continued random osteoclastic activity resorbs portions of the already-formed osteons leaving osteon fragments. In later years (40-60+) there is an increase in the rate

of bone resorption in the medullary third of the entire shaft, and the rate of bone formation is reduced (Kerley 1969). The osteocytes continue to grow in both size and number, with age, contributing to the reduction in the amount of non-Haversian (interstitial) bone (Jande and Belanger 1971:282, in Ortner 1976:39).

The outcome of the process of bone remodelling is that there is an absolute and proportionate increase in the number of secondary osteons and osteon fragments with increasing age, and a corresponding decrease in the percent of interstitial lamellar bone. There is also a decrease in the number of primary osteons as the process of new bone formation slows down. Some other changes that have been observed with increasing age include a reduction in the size of the secondary osteons, and increase in the average diameter of the Haversian canals, an increase in the number of secondary osteons that are incompletely mineralized (less than 70%), and an increase in the number of closed Haversian canals (Thompson 1978:18-19). The thickness of the cortex also decreases as well as the bone density and bone mineral content (Thompson 1978)(see below).

BACKGROUND: THE DEVELOPMENT OF THE TECHNIQUE

Initial Stage: Kerley

Kerley's initial study involved 126 cross-sections from 67 subjects ranging in age from birth to 95 years old. The sex, age at death and clinical history of each subject was known. Thin sections were prepared from the cross-sections which were taken from the approximate (±3 inches) mid-shaft of the left femur, tibia and fibula. Care was taken to screen out any pathological bone or bone that was lying in the near vicinity of a visible pathology. Pathologies were detected by gross examination, radiographic examination, and by microscopic examination.

The outer third of the cortex was chosen for observation of the ag-related changes because it was believed to be the least affected by excessive resorptive changes. It is an area that remains intact through all ages and is therefore useful for comparison.

For circular, microscopic fields, viewed at 100X, were chosen. The fields were situated on the anterior aspect of the bone (at mid-shaft), on the medial and lateral edges, and posteriorly (on the linea aspera of the femur). The field size was reported (Kerley 1965) to cover an area on the bone of 1.25mm, but Kerley and Ubelaker (1978) reconsidered this estimation and determined that the actual field size was 1.62mm.

The histological features observed for each bone specimen were the total number of osteons (including those that were partially obscured by the periphery of the field), the total number of osteon fragments, non-Haversian canals, and the percent circumferential lamellar bone.

In his observations, Kerley (1965) noted that the number of secondary osteons and osteon fragments increased with age. There was

a predominance of non-Haversian canals in childhood decreasing to almost nil be age 55, and a decrease in the percent interstitial lamellae to negligible in extreme old age (95 yrs.). These general results were later corroborated by subsequent observers (see below).

Kerley (1965) derived regression equations, linear and curvilinear, where appropriate, from the histological quantifications and then tested the accuracy of the method via deriving the standard error of the estimate. The age estimates obtained in this manner showed that the number of osteon fragments were more highly correlated with age and gave the best estimates of the actual age. The lowest S.E.E. was obtained for the osteon fragments of the fibula at ±5.27 years. The total number of secondary osteaons for the femur gave a S.E.E. of ±6.69 years., and for the fibula of ±8.83 years. (Kerley 1965:160).

Kerley was able to improve on the accuracy of the estimation by preparing an "age-profile chart" for the four variables (see Fig. 7). the age profile chart plots the range of the age estimate given by each variable and marks the limits where the estimates overlap. This gives a narrower range of error for each estimate. The accuracy of the age-profile chart was checked on 56 additional subjects ranging in age from birth to 32 years. It was found that 87.3% of the estimates were within ±5 years of the actual ages, and that 100% were within ±10 years of the chronological age. When two or more bones were compared, the accuracy was 100% within ±5 years.

Generally, the accuracy of the estimates was higher for those subjects under 30 years of age (91.7% within ±5 yrs.) than for those subjects over 30 (78.9% within ±5 yrs.). Kerley attributed this to the natural result of greater biological variability with age, and the fact that only two of the variables could be counted in very old individuals (the number of secondary osteaons and osteon fragments) (Kerley 1965).

Kerley mentions that the reliability of the age estimates increases when two standard deviations of the estimate are taken but that the age range of the estimate is then so great as to be useless, except for the number of osteon fragments of the fibula. Note that many of the studies following Kerley's work emphasize the use of the femur because it is frequently found in archaeological context and bone samples are more easily taken from the femur than the other long bones with less damage occurring as would be with a sample from the skull. The best age estimates obtained from the femur were for the number of secondary osteons.

Some problems with the method were recognized by Kerley at the initial stage. The first source of error discussed involves the problem of distinguishing osteons from osteon fragments. Kerley proposed a definition of an osteon to aid the standardization of the counting. The osteon must be "...easily distinguishabel over 80% or more of its area and (have) the canal intact" (kerley 1965:162). Osteon fragments are defined by Kerley as "...any osteons that have a discernable encroachment by subsequent generations of osteons and are

exemplified by arcs of concentric lamellae between new osteons" (Kerley 1965:162).

The problem of sex and racial differences were not strictly investigated by Kerley in his initial study (1965). His sample consisted of 88 males and 29 females, and although Kerley states that no sex differences were evident in the age estimates, he does not present a discussion of the difference in the age estimates obtained between the sexes. He does give the correlation between sex and the outer zone age factors (the extreme ends of the age estimates that fall outside of the range of the age-profile, see Fig. 7). The 'r' value for the secondary osteons of the femur was +0.022, and for the non-Haversian canals of the fibula r = +0.391 (see Bruning and Kintz 1977 for the calculation of Pearson's r). This shows an extremely weak, positive correlation meaning that there is little chance that the sex of the individual accounts for the extreme ends of the age estimates. In contrast, the r for sex and secondary osteons in the fibula (0.391) is statistically significant, at less than the .001 probability level (see Thomas 1976: Table A.11). Kerley also noted that race did not affect the results. Only 11 "Negroid" subjects were present in the sample, the remaining 56 were "Caucasian", but Kerley explains that the "Negroid" subjects were randomly distributed throughout the age graphs which he accepts as an indication that race does not affect the estimates (Kerley 1965:163). Later work (see below) has challenged Kerley's claim.

Another area of biological 'noise' is disease. Seventy-eight of the subjects used by Kerley had one or more of 24 common pathological conditions including trauma, inflammation, cardiovascular disease, atrophy of some part, neoplasm and developmental and metabolic disorders. In areas on the bone where pathology was visible, the microstructure was disturbed and the age estimates were unreliable. However, it was noted that Paget's disease causes an excessive number of osteon fragments because of the increased osteoclastic activity, and if the cause of death of the individual were not known the estimated age would have been much older than the true age. In a review of his 1965 study, Kerley (1969) recognized that not only Paget's disease, but osteomalacia and hyperparathyroidism also mimic normal age changes and produce faulty results.

SUBSEQUENT STUDIES

Ahlqvist and Damsten

Following Kerley's work, Ahlqvist and Damsten (1969) modified some of the aspects of Kerley's method to make the technique simpler. They focused on the problem of distinguishing osteons from osteon fragments, and calculating the percent circumferential lamellae. Kerley (1965) did not describe how this had been done. Ahlqvist and Damsten also standardized some parts of the method to reduce inter-observer error. In their investigations, they used 25mm thick, unstained, ground femoral cross-sections of decalcified bone (with archaeological bone they used 30-50mm thick sections because of its fragility). Their sample was small, consisting of only 20 subjects all with known age at death.

The outer third of the midshaft was observed but at different points than Kerley used (see Figure 8). Ahlqvist and Damsten chose the antero-medial, antero-lateral, postero-medial and postero-lateral regions on the bone in order to avoid the linea aspera, an area where histological changes not related to age are likely to occur (Kerley 1965).

Ahlqvist and Damsten also replace the circular field with an ocular square-ruled network divided into 100 squares. They standardized the level of magnification of the microscope so that one side of the square field would measure 1.00mm on the bone surface. the use of the square field eliminated the blurring of the features on the periphery that Kerley had to contend with when using the circular field. The standardized grid also allowed the easy calculation of the percentages of the variables present, in particular the percent circumferential lamellae. Polarized light was used to observe the features because it gave a clearer view.

A further modification involved the treatment of the variables. To make the calculations easier and to reduce inter-observer error, the number of osteons and osteon fragments were treated as one variable (ie. that increases with increasing age), and the percent circumferantial lamellae and the number of non-Haversian canals were treated as a second variable (that decreases with increasing age). The number of squares that were more than one-half filled with a feature, of either member of a variable, were counted and then the counts were turned into percentages (because there were 100 ruled-off squares the absolute number served as the percentile value). The average was taken for all four fields and the percentage values were applied to the appropriate regression formula to get an age estimate.

The results gave a S.E.E. for the femora of 6.71 years. Kerley's (1965) results gave a S.E.E. for the femur of 9.39 years, and $\pm$5 years using the age-profile chart (see above). Although Ahlqvist and Damsten's results are comparable to Kerley's, their sample size was small (N=20). Bouvier and Ubelaker (1977) state that Ahlqvist and Damsten's error would be much higher with a larger sample. The benefit of using Ahlqvist and Damsten's superimposed grid is that the variables are easier to quantify. Their new field locations exclude the linea aspera where poor age estimates are obtained, and the calibration of the field size ensures that there is no error due to the application of regression equations derived from quantifications made on fields of a different size.

Bouvier and Ubelaker

Bouvier and Ubelaker (1977) tested Kerley's (1965) method against the modifications made by Ahlqvist and Damsten (1969). They used 40 of the thin sections originally used by Kerley, recoded them, and retested them using both methods to compare the result. The percentage of remodelled bone (the number of osteons and osteon fragments) was determined after Ahlqvist & Damsten using their four fields and respective regression equations. Then, the total number of secondary osteons in the four fields used by Kerley were counted and his regression formula was applied to the resulting values. A standardized field size of 1.45mm was used. All fields were re-evaluated by the same observer and 25 of the 40 thin sections were examined by a second investigator using the same procedures.

The suspicion that the counts from the linea aspera were unreliable was corroborated by their findings. The most reliable results were obtained from observations made by the same investigator. The overall best correlation occurred using Kerley's method, r=0.992 for the same observer, and r=0.908 for different observers. The Ahlqvist and Damsten method yielded an overall correlation of r=0.939, same observer and, r=0.929, different observers. Bouvier and Ubelaker concluded that the Ahlqvist and Damsten method resulted in less inter-observer error but that the difference was not significant.

The comparison of the accuracy between the two methods showed that Kerley's method was more accurate ($\pm$8.20 yrs., versus $\pm$9.50 yrs. for Ahlqvist & Damsten). The greatest accuracy was obtained on the 45-90 year age range which is not in agreement with Kerley (1965) who found that the best results were obtained on the subjects under 30 (Table 2).

Bouvier and Ubelaker concluded that their small sample (N=40) resulted in a high average difference between the age estimates. They proposed that a sample size of 50 or more is necessary for reliable results.

Bouvier and Ubelaker discussed the error that enters into the estimate as a result of an age bias in the sample (Table 3). Ahlqvist and Damsten's ages lean to the older end ($\bar{x}$ = 55.45 yrs.), and the skewness is negative (-0.582). The fact that Ahlqvist and Damsten's estimates were more accurate for the older age group ($\pm$7.5 years for the 45-90 year old group, and $\pm$13.87 years for the 20-45 year old group; see Table 2), may be a function of the greater number of individuals in the older age range. The fibula sample used by Kerley would be more reliably applied to a younger age group since the mean age was 34.48 years, and was positively skewed at 0.652 (see Table 3). Skewness approached statistical significance (Table 3) in these two cases (the P=0.05 critical valve is $\pm$0.71 for N-25). To eliminate the effect of skewness on the age estimates, the ideal situation of an evenly distributed sample, over all ages, would be necessary. This might also produce more reliable regression equations.

Singh and Gunberg

The study done by Singh and Gunberg (1970) produced the lowest standard error of the estimate of any of the preceding or following work. Their study was also novel in that they used the mandible and obtained an age estimate within 3 years.

Forty specimens from 59 cadaver skeletons with known sex, age at death, and medical history, were chosen for having all of the mandible, femur and tibia intact. No macro or microscopic evidence of pathology was visible. There were 52 males, and 7 females. Sex Differences were tested with the 7 female specimens but the results are tentative because of the small sample size. The standard errors of the estimates, given below, are for the males which includes 33 specimens of femora, tibiae and mandibles that range in age from 39-87 years, and a sample of 19 additional mandibles (total N=52 for the mandibles), ranging in age from 40-80 years. One centimeter-square sections were removed from the anterior midshaft of the femora and tibiae, and a 1.00 cm^2 sample was taken from the posterior

border of the mandibles along the ramus at the point opposite the lingula. For the 40 individuals, with each of the three bones intact, 3 samples were taken from each bone and 4 slides prepared from each sample, giving 120 thin sections for each bone. Each of these samples was fixed and decalcified and one of every four of the slides was stained. The remaining 19 mandibles were left unstained and only one thin section was made for each one. The thin sections were ground to 30-50mm thickness and two 2mm wide visual fields were chosen at random from the periosteal third of the cortex and viewed with a 10 x 10 power wide-field ocular microscope.

Three quantifications were made. The total number of secondary osteons were counted (only 100% complete Haversian systems were counted). The average was not taken of the number of secondary osteons from the two fields, and obliquely cut osteons were counted only if the complete canal was visible. The average number of lamellae per osteon was taken over the two fields, and the average diameter of the Haversian canals was measured using a calibrated ocular micrometer of 1 cm. scale micrometer. Only the diameters of complete Haversian canals were measured and if the osteon was obliquely cut then the diameter was measured only if the canal was three times as long, or less, than it was wide (Singh and Gunberg 1970).

Multiple linear regression analysis was done and the standard error of the estimate taken for each variable. The 52 male mandibles were used to prepare a nomograph using the number of secondary osteons and the average diameter of the Haversian canals.

By comparing the values obtained for the variables between bones, an estimation of the between-bone variability was possible. The results showed that the mean diameter of the Haversian canals was significantly higher ($p=0.05$) for the mandible. The other two variables (number of secondary osteons & concentric lamellae/osteon) did not vary significantly betwen the bones. The least between-bone variability occurred with the number of secondary osteons. Based on these findings, any researcher wanting a single variable to estimate the age at death of a subject would be best to choose the number of secondary osteons.

The most important finding from the Singh and Gunberg study was that the histological estimates of age were more consistently accurate for the mandible than either the tibia or femur, for each independent variable.

Multiple regression analysis derived a standard error of the estimate for the mandible of 2.58 years, 67% of the time, and of 5.16 years, in 95% of the cases (using the nomograph). The average r-value for the mandibular age estimates was 0.978. Kerley's most accurate result was for the osteon fragments of the biula at $\pm$5.27 years. In both cases (Singh and Gunberg 1970; and Kerley 1965) the most accurate results were obtained when all variables were considered together (see Table 4). Where Kerley used an average taken from the age-profile chart, Singh and Gunberg used a statistical analogue, vis a vis multiple regression analysis.

Thompson; Thompson and Gunness-Hey

Thompson (1978, 1979) introduced the use of the high speed drill to remove small (0.4 cm) bone samples, causing little damage to the bone. He also produced the first reliable regression equations for both left and right sides of the major long bones (femur, tibia, humerus and ulna).

Cortical thickness, bone mineral contents and bone density (3 "whole-bone" variables) were used to compare age changes between male and females in the first study with a large female subsample (males=64, females=52). Thompson and Gunness-Hey (1981), and Thompson et al. (1981) also used these whole-bone variables to do a study on the different rates of bone change between two races, U.S. whites and Yupik plus Inupiaq Eskimos. As Thompson's work is the most rigorous, todate, a lengthier treatment of his method appears below.

Age Estimation

The method (1978; 1979) involved the extraction of 0.4cm bone cores with a high speed Dremel drill (Model 33) from the medial mid-shaft of the tibia, the anterior mid-shaft of the femur, from the humerus between the medial side and the deltoid tuberosity at mid-shaft, and from the ulna one third of the way up from the distal end on the lateral surface. The cores were first scanned for their mineral content using absorptiometric analysis (see Cameron and Sorensen 1963: 230-232). The cortical thickness, cortical width, weight and density were measured for whole bone analysis, and then, thin sections were prepared from the cores of 80 um thickness. The sections were ultrasonically cleaned and mounted on slides for histological analysis. Quantification of the microstructures was done following stereological principles after Weibel (1969, in Thompson 1978: 42-46; 1979: 904).

Histological observations were made in four fields adjacent to the periosteum using a 100X phase contrast microscope. An eyepiece micrometer divided into 100 (10 X 10) grid-squares was fixed into the eyepiece. The area of the field on the bone was .992mm^2. the microstructures quantified for the age estimates included the percentage area containing secondary osteons, all Haversian canals, and the secondary osteon lamellae ratio. The ratios taken were of the number of microstructures per field size. The point-counting method was used and osteons that bisected the outer grid were counted on an alternate basis. The aggregate perimeters of the secondary osteons and Haversian canals were quantified using the formula $B_A=(/2)1_L$ (Weibel 1969 in Thompson 1978) - (see Table 7c for a complete list of the histological variables studied).

Thompson (1978) performed 36 step-wise regression analyses on 20 variables, with age as the dependent variable, and obtained an "averaged" standard error of 6.5 years for the right and left femora, 7.5 years for both of the humerii and tibiae, and a standard error of 9.0 years for the right and left ulnae. In his 1979 study, Thompson performed 28 regression analyses on 19 variables and found the most consistently accurate results were obtained for the percentage area covered by secondary osteons. This variable alone produced standard errors of the estimate of 10.57 years for the left ulna, 6.41 years or the right femur (male), and 6.21 years for

the left humerus. The overall estimate for the femora was 7.07 years (Thompson 1979:913).

Sex and Racial Differences

Males and females were studied for their differences using whole-bone measurements on the cores. The male sample (N=64) ranged in age from 50-97 years with a mean age of 71.48 years. The females (N=52) ranged in age from 43-94 with a mean age of 71.94 years. The sample was separated by sex and then each sex was separated by the presence or absence of pathology affecting the cortical bone.

Measurements of cortical thickness and bone mineral content showed a difference between males and females (females have generally less bone mineral than males because of their smaller size). U.S. white males showed a cortical bone loss of 4% per decade over the age of 50, and a bone mineral loss of 6% per decade for the same age group. U.S. white females (over 50 years old) showed a cortical thickness decrease of 8% per decade and a bone mineral loss of 10% per decade (Thompson, 1979:913).

The whole-bone variables were studied by Thompson and Gunness-Hay (1981) on 258 Yupik and Inupiaq skeletons from St. Lawrence Island (N=53), Kokiak Island (N=92), Baffin Island (N=44), and Southampton Island (N=69), and were compared to 116 U.S. white cadaver skeletons and 28 U.S. white forensic cases which were under 50 years of age. Results showed that males had significantly thicker cortices than females ($p=0.05$), and the U.S. white forensic cases (N=28) all exceeded the entire Eskimo sample in cortical thickness. There was a significant difference between the Inupiaq (Southampton Island and Baffin Island) cortical thickness and the Yupik Eskimo (St Lawrence Island and Kodiak Island) cortical thickness, the latter being greater. The cortical thickness of U.S. whites (forensic) under 50 years of age was greater than U.S. whites (cadavers) older than 50, indicating cortical loss with age (see Garn, 1970).

Bone mineral content (Index $=gm/cm^2$) and cortical bone density (gm/cm^3) values were also examined. the only significant difference noted was a lower bone mineral index for the Southampton Island Eskimos. No intragroup sex differences were noted for the bone mineral indices (note sex differences in bone mineral loss for U.S. whites - above) (Thompson and Gunness-Hey, 1981: 2-3).

Similar results were obtained by Thompson et al. (1981: 93-94) showing lower bone mineral values for the Inupiaq Eskimos compared to Yupik Eskimos and U.S. whites. These differences are explained as differences in diet and in activity patterns. A genetic component is suggested by the overall difference in the cortical thickness between the U.S. whites and the Yupik-Inupiaq Eskimos (Thompson and Gunness-Hey 1981:6-7).

Bone remodelling patterns were found to be similar between ancestral populations of Yupik Eskimos and the modern population (St. Lawrence Island skeletons are from the 19th century and the Kodiak Island skeletons date between 700B.C. - A.D.1700). This suggests a genetic continuity within populations related to bone remodelling (Thompson and Gunness-Hey 1981:2-3). If distinct patterns can be isolated using the whole bone

variables, the method may be useful as a race identifier. The whole-bone variables should be recorded before sectioning in order to preserve this information. Age estimates done on the Eskimo skeletons using the regression equations derived from the U.S. whites greatly exceeded the age at death estimates made on the Eskimos using morphological techniques (pubic symphysis degeneration, epiphyseal closure, and cranial suture closure). The difference between the two estimates was greater than the error expected between two populations based solely on morphological estimates (Thompson and Gunness-Hey 1981:5-6). It is evident from these findings that population-specific regression equations are needed (Thompson and Gunness-Hey 1981:7; Slater, in Thompson 1981:93).

Sotaro and Konishi

Work done on male Japanese specimens by Sotaro et al. (1978-1, 1978-2, 1982) indicated that a new series of regression equations was also needed for Japanese samples. Sotaro and Konishi (1982) used ground cross-sections from the mid-shaft of the humerus and femur of 60 Japanese male skeletons ranging in age from 45-102 years. They based their multiple regression equations on the number of osteons/mm^2, the number of interstitial lamellai/mm^2, the average size of the osteon, and the average diameter of the Haversian canals. Observations were made on the anterior edge of the thin sections in a field size of $1.00mm^2$, over three fields (see Tables 7a-7c for a comparison of the techniques).

One discrepancy in their results with earlier findings concerned the diameter of the Haversian canals. Sotaro et al. (1978-2) reported an increase in the diameter with age, whereas, Singh and Gunberg (1970) reported a decrease in mean Haversian canal size with age, and Barer and Jowsey (1967, and Jowsey 1960, in Thompson 1978:17) reported an increase. The reasons for the discrepancy were not discussed in the English summary but may relate to undetected influence from dietary differences or disease factors.

Sotaro et el. (1978-2) applied the regression equations derived by Kerley (1969) and Singh and Gunberg (1970) to their Japanese sample and came up with very inaccurate results (no figures were given - in English). New Regression equations were derived for the Japanese sample and the results from these equations, used on the 1982 series, gave a standard error of 11.5 years for the femora of a "general group" (45-75 years old) and of 8.8 years for the humerii of this same age group. For the "aged group" (75-older) a standard error of 7.3 years was obtained for the femora, and 10.4 years on the humerus (see Table 6 for a comparison of the standard errors obtained by each author). The reason for the discrepancy in the estimates between the two age groups is unknown. The discontinuity in the accuracy shows that there are still problems with their estimation (Sotario and Konishi 1982:33).

APPLICATION OF THE HISTOLOGICAL TECHNIQUE AND COMPARABILITY WITH MORPHOMETRIC METHODS

With archaeological bone the problems of race, sex, disease and possibly geographical location, are compounded by the conditions in which the bone has been deposited. Disturbance of bone by weathering, leaching

or burning are fairly common in archaeological samples. In his 1965 study, Kerley tested the reliability of using his technique on archaeological bone from the Philippines, the Aleutian Islands, Indian Knoll, Kentucky, Virginia and Florida. The spatially scattered specimens represented a chronological range from 5000 B.P. - 500 B.P. and exhibited marked differences in mineralization and leaching by ground water. Kerley found that thin sections prepared from these samples were sufficiently clear to distinguish the histological features. In those cases where mineralization was particularly heavy, it was necessary to use polarized light.

Kerley (1969) applied his technique to 8 forensic cases, of known age at death, that were broken and somewhat burned. He obtained age estimates that were determined to be within ±5 years of the actual ages. These results are encouraging, though the sample was small, and noteworthy because archaeological bone is often burnt. Thompson (1979) was able to estimate the ages of 8 forensic cases to within ±4 years using his core technique.

The 28 forensic cases examined by Thompson and Gunness-Hey (1981, see above) were estimated to within an absolute value of 4.76 years of their actual ages using the number of secondary osteons/mm^2 from femoral sections. One estimate was off by 24 years because of the presence of a healed fracture distal to the site of the section.

In a review of 'the present knowledge' of the preservation of histological structures in archaeological bone, Stout (1978) related that the osteons and their Haversian canals, cement lines and the osteocyte lacunae, are often well preserved and clearly visible (Stout and Teitelbaum 1976a in Stout 1978:601). He adds that post-deposition structural changes in the gross morphology of bone can, and should be, checked by microscopic observation before concluding that the bone has suffered from the affects of pathology or aging (Stout 1978:602).

Following a further line of research, Wu et al. (1970, in Stout 1978:603) essentially inversed the histological aging method to assess rates of bone formation. Metabolic disease can be recognized by their abnormal remodelling patterns. Stout and Teitelbaum (1976b, in Stout 1978) used this method on two Illinois Woodland populations which marked a change from intensive collection to corn agriculture. They found higher rates of bone remodelling in the agricultural population and suggested that it was due to hyperparathyroidism caused by insufficient calcium intake. Stout (1978:603) feels that the histologic method can now be used for studying palaeophysiology as well as for age estimates and determining the state of preservation of histological structures.

Pfeiffer (1980) used the Ahlqvist-Damsten method on skeletal material from the Hind site (AdHk-1), a late Archaic occupation (925 ±75 B.C.). She compared this method to the cranial suture closure technique and the pubic symphysis degeneration aging method. Using 6 adult skeletons she concluded that the microstructural method corresponded in accuracy with the two macroscopic methods. She pointed out that the microstructural method required more time, more money and more experience in bone histology than the histological technique outweighed its advantages. In a

reply to a criticism by Martin et el. (1981:437), Pfeiffer (1981) related the results from a larger ossuary sample which supported Martin el al., showing a significant difference between age estimations based on the pubis aging method and the histological technique. Recently, with better equipment and greater control over the procedures of the technique, Pfeiffer (1983, personal communication) has stated that the histological technique is very useful and has a great deal of potential.

Salter (in Thompson et al. 1981:93-94) also compared age at death estimates using standard morphological techniques against the histological method. She found that the histological age estimations and the morphological age estimations did not correspond. The difference was most marked for those skeletons estimated to be under 30 years of age by morphological methods. The morphological estimates giving age determinations over 30 years old were more similar to estimates made using the histological method (Thompson's core technique). Salter feels that part of this inconsistency is due to the fact that regression equations derived from U.S. whites were used for the histological age estimates of the Eskimo samples. Thompson and Gunness-Hey (1981, see above) also reported a significant discrepancy between age estimates made using morphological criteria versus the histological method. A mean absolute difference of 14.33 years (male) and 15.41 years (female) resulted between the two methods when tested on the Southampton Island skeletons (N=69).

Ubelaker (1974:56-58) compared age estimates using the Ahlqvist-Damsten method against the pubis aging method on 2 ossuary samples. He found that the differences in the age estimates were great enough to affect conclusions that might be made about the demographic profile. For example, Ubelaker (1981:188) explains that the age estimate based on pubic morphology, for ossuary 1, gave an upper age limit of 50-54 years, while femoral remodelling estimates placed the same limit at 65-69 years. For ossuary II, the pubis gave an upper age limit of 45-59 years, and the femoral remodelling estimate placed the limit at 55-59 years. The pubis aging method also showed a higher proportion of individuals in the 30-34 year category. The difference between the demographic conclusions that would be drawn, based on the different results, would significantly affect the demographic profile.

Singh and Gunberg (1970:373) concluded that the histological method was more accurate than the existing morphologic methods. It should be stressed that when choosing which method to use for aging, that the extra time and expense involved with using the histological technique must be weighed against the degree of accuracy required and type of information desired.

Bev Smith working on forensic cases with Pat Kitchen at Michigan State University, stated that the most troublesome cases for aging are those involving individuals between the ages of 30-70. These cases require the use of the histologic method since the gross morphology does not yield accurate age estimations between these ages (personal communication, 1983).

DISCUSSION

As the histological technique developed it was expanded from the use of the femur alone to include all of the major long bones except for the radius. In addition, the mandible has been successfully used. The accuracy obtained by the above authors has varied widely (see Table 6). The best estimates (S.E.E. within ±6 yrs.) have been derived from the number of osteon fragments of the left fibula (Kerley 1965), the number of secondary osteons, and the average number of lamellae/osteon and the average diameter of the Haversian canals considered together for the left femur, left tibia and the mandible (Singh and Gunberg 1970). Note that Singh and Gunberg's samples of femora and tibiae (N=33 for both) were under the optimum sample size of 50+ suggested by Bouvier and Ubelaker (1977). Ahlqvist and Damsten (1969) obtained an age estimate within ±7 years for the percent remodelled bone, but their sample was small (N=20). The Ahlqvist-Damsten variables have been found to be useful when dealing with archaeological bone where the microstructures are unclear. Thompson (1978, 1979) obtained a S.E.E. within 7 years for the 'secondary osteon area' of the right femur and left humerus, and using 36 step-wise regression equations for both right and left femora he obtained an "averaged" standard error of 6.50. Thompson's most accurate estimates were for the 'secondary osteon ratio' and 'Haversian canal ratio' considered together (see Table 6). The different accuracies obtained may be due to the different procedures used by each author. Tables 7a - 7c compare the samples, methods and quantifications made by the various authors discussed above. Sex differences in the microstructural changes with age have not been thoroughly studied. In view of sex difference being indicated in Kerley's (1965) study, sex may not greatly affect the histological estimates. Thompson (1978; 1979; 1980; et al. 1981; and Gunness-Hey 1981) reported sex differences in the rate of bone mineral loss and cortical thickness decrease, which indicates different rates of remodelling but does not quantify the microstructural differences.

SAMPLES USED

Three of the 9 sample variables listed in Table 7a have been used by every author. All authors took care to screen out any pathological bone, the left femur was used and bone samples were taken from the mid-shaft area of the bones. Singh and Gunberg took their mandible sample from the posterior border of the ascending ramus opposite the lingula. Future investigators should choose the same site for extracting a sample from the mandible. The left femur has been consistently used, presumably because it is frquently recoverd in good condition and primarily because bone samples from the femur are easily extracted without causing marked damage (see type of sample bone section below). The left femur has also been used as a convention following Kerley's (1965) initial work. The left tibia has been used by 3 of the 6 authors and the humerus by 2 authors. The other bones (fibula, ulna, and mandible) have only been studied by one author, or pair of authors.

"Race" was not recorded by Ahlqvist and Damsten, nor by Singh and Gunberg. Bouvier and Ubeleker did not specify whether their sample, taken from Kerley's (1965) sample, included any of the "Negroid" subjects. Since it has been shown (Thompson and Gunness-Hey 1981; Thompson et al.

1981; Sotaro et al. 1978-1; 1978-2; 1982) that population-specific equations are needed for age estimation, it is essential that the race of the sample be recorded, and the samples separated accordingly.

The age range varied per author with the mean age leaning to adults over 40. A study sample should cover the adult ages and be of sufficient size to eliminate the large bias created by greater variability. Three of the samples in Table 7a were small (less than 50 subjects). A minimum size of 50 speciments has been recommended by Bouvier and Ubelaker (1977) in order to cover population variability.

METHODOLOGIES

Table 7b lists the factors employed by the different investigators in their methods. Four of the authors took complete cross-sections from the bones they studies. Thompson used his "core technique" (0.4 cm diamter), and Singh and Gunberg extracted 1 cm^2 sections. Another method is used by Pfeiffer who takes wedges from the midshaft. The wedges are approximately 2 cm in diameter on the periosteal surface. The advantage of taking either cores, wedges or other small sections, is that they leave the bone intact so that morphometric measurements may still be taken by future investigators. It is also less offensive than the removal of a section that splits the bone in two.

The thickness of the sections varied from 25um to 80um. Three authors have no data recorded for this factor. The thickness, the magnification, the field shape, and type of light used all affect the clarity of the structures, and to varying extents, the frequency of the variables being counted.

All authors used a 10X ocular lens with a 10X objective lens (=100X). Different magnifications will expose a greater or lesser number of microstructures effecting the regression equations and age estimations. A change in the magnification will also change the size of the field on the bone. Even at the same magnification, different microscopes will produce different field sizes. Kerley and Ubelaker (1978) and Thompson (1978:44) calibrated their microscope to acquire the desired field size. In the studies done above, each author had a different field size for viewing the histological structures. As a consequence, any investigator who uses a specific regression equation/technique to make an age estimation must also have the approximate field size used by the same author, for field size obviously affects the number of microstructures counted. Correction factors do not equalize the quantifications because the spatial distribution of the microstructures varies significantly (Stout and Gehlert 1982:123-125). For example, if Kerley's regression equations are used for an age estimation, then the field size must approximate 1.62 mm in diameter; the field size used by Kerley to produce the equation.

Kerley used a circular field which blurred the microfeatures along the periphery of the field. Subsequent observers (no data for Sotaro and Konishi) used square-ruled grids. The square grids did not have to be moved around in order to see the variables, and the 10 X 10 ruling allowed for the easy counting of the microstructures.

A simple light microscope was used by 3 authors, polarized light was used by Ahlqvist and Damsten and Thompson used phase-contrast microscopy. No data is available for Sotaro and Konishi. Pfeiffer (1983, personal communication) maintains that polarized light helps to demarcate the microstructures. The polarizing filter is adjustable so that the optimum visual properties can be obtained. She has also found that undecalcified sections show the microfeatures more clearly since decalcification eliminates the cement line which marks the osteons. Various stains may be used, depending on which aspect of microanatomy is being studied and on the nature of the sample (see Jowsey 1977:140). Only two authors recorded information on the treatment of the samples. Several methods appear to give equally clear samples.

The location chosen for viewing the fields has, by convention, been situated along the periosteal third of the cortex. Pfeiffer (1983, personal communication) has found that the microstructures often show up more clearly on the endosteal third. This may have been observed by other authors, but it has been avoided because of the more erratic rate of remodelling which occurs along the endosteal surface (Kerley 1965).

Both the positioning of the field on the bone section and the size of the field has been different for every author (no data for Sotaro and Konishi). Different correlations with age were discovered by Bouvier and Ubelaker for the field position. They obtained the best correlations with age for Kerley's laterally situated field ($r=0.971$), and for the posteromedial field of the Ahlqvist-Damsten method ($r=0.966$). The lowest standard errors were achieved by Singh & Gunberg who chose their fields at random (this is not to suggest that this factor produced better results, but is to caution against choosing a field position based solely on a high correlation with age).

Finally, regarding the issue of testing for inter-observer imprecision, Pearson's r, by itself, should not be used. For example measurements made by two observers on the same sample may differ systematically. In this case Pearson's r could be high, yet mean values could be significantly different. The use of the correlation coefficient should be used in conjunction with other statistics, such as t-tests or Anova in order to recognize systematic versus random error (Heathcote 1981).

In summary, only 2 of the 10 methods employed in preparing the samples were consistently used by each author (the 10 X 10 wide field and the use of the periosteal third of the cortex).

VARIABLES STUDIED

The variables examined diverge enormously (Table 7c). The number of secondary osteons was used by 5 of the 6 authors (not by Ahlqvist and Damsten) and, it appears to give the best age estimates. The percent remodelled bone has been used by 3 authors, but it has not yielded reliable results on a sample of adequate size (see Table 6 and Table 8). Thompson's (1978; 1979) quantification of the secondary osteon lamellae ratio is essentially a measure of the amount of remodelled bone, but it is not a percentage because he used the point counting method. The average

diameter of the Haversian canals was used by two authors. the other 15 variables listed were only used by one author, and Thompson alone used 12 of the 21 variables listed.

CONCLUSIONS

The differences in the samples, methods and variables employed by the various authors are considerable. This makes it impossible to determine any sources of error or weak points in the techniques. Stout (1978:602) states that one source of error that is inherent in all the histological techniques used is the insufficient sampling of the histomorphology of the bones. This involves variability in microstructure from bone sample sites (see Frost 1969). Frost (1979, personal communication to Stout 1978) claims that at least 200 osteons or osteon fragments should be counted for an adequate sample. Stout (1978) recorded a decrease in inter-observer error from 20% or more, to less than 10% when counts exceeded 200. this would require, for the histological method, more and/or larger fields. Ubelaker (1981:181-182) states that the error involved in the estimation of age of a prehistorical sample cannot be known. He points out that age estimations made on ancient populations based on equations derived from modern populations, assumes the principle of "uniformitarianism". The process of aging in the past may have been significantly different from what it is today. This also applies to morphometric measurements based on bone growth and development, and rates of ossification and degeneration.

The conclusions presented below are geared to an extraction of the clearest and most accurate methods utilized by the investigators. The proposed standardizations (see Table 9) are not meant to be definitive. They represent this author's opinion, based on the data presented above, of the best criteria for executing the histological technique for aging human bone.

PROPOSED STANDARDIZATIONS FOR USING THE HISTOLOGICAL AGING METHOD

Before taking the bone section, standard morphometric measurements should be carefully made, and any discrete traits noted.

It is felt that the integrity of the bone should be maintained, as much as possible, and to this end the use of Thompson's 'core technique' appears to be the most appropriate. Often there is no power supply in a field situation. In such a case wedges (after Pfeiffer) or Singh and gunberg's 1 cm^2 sections may be taken. After extracting the bone sample, it is advised to do 'whole bone analysis' (Thompson 1978; 1979) to retain information on the bone mineral content, the cortical thickness and the bone density. The samples should be taken from the approximate (3 inches) midshaft of the long bones, following the convention after Kerley (1965; and see the description of the sample zones in Thompson (1978). Samples taken from the mandibles should follow Singh and Gunbert (1970).

The suggestion that small sections be taken from the bone to keep it intact, restricts the side of the bone from which the sample can be extracted (ie. either medially, laterally, posteriorly, or anteriorly). It has been shown (Singh and Gunberg, 1970; Bouvier and Ubelaker, 1977) that counts made on samples from the linea aspera of the femur yielded

unreliable age estimates. Bouvier & Ubelaker, (1977) found that fields situated on the lateral side of the femur produced the best correlations with age, followed by the postero-medial side of the femur. Testing still needs to be done to confirm the superiority of these sampling sites and to locate the optimum sampling position on the other long bones.

Following Pfeiffer's suggestion (1983, personal communication) the thickness of the section may be realistically standardized at 40um - 60um. This is thick enough for archaeological samples which are often fragile, and thin enough for clarity of the microfeatures. Note that Thompson (1978; 1979) obtained clear samples using thin sections of 80um thickness. The range in thickness allows for variation between observers, hopefully without affecting the density/frequency of the microstructures.

The treatment of the bone sample appears to depend on the research and state of preservation fo the sample. A general suggestion, for the histological aging technique, is the use of undecalcified and unstained bone. The sections should be viewed with a simple light microscope fixed with a polarizing light filter for the best visual properties. A X10 ocular lens with a X10 objective lens (X100) should be used. The microscope should be calibrated so that the size of the field on the bone is 2 mm^2. This larger field size is suggested because of Frost's (1969, p.c. to Stout 1978) cautioning note about inherent error due to insufficient sampling of the bone histomorphology (see above).

The use of a square field is suggested for clarity, and point-counting is advised using a square-ruled ocular micrometer. Weibel (1969, in thompson 1978) maintains that the point-counting method is the most accurate for quantifying histological structures.

Four fields should be situated on the periosteal third of the cortex, following the convention, and to obtain a large sample of the bone histomorphology, and be situated adjacent to each other, if possible.

The variable giving the best age estimates (see Table 8 and Table 6) appears to be the number of secondary osteons. This should be calculated per the field size (2 mm^2). The number of osteon fragments, the average number of lamellae/osteon and the average diameter of the Haversian canals should also be quantified. These variables should be dealt with separately and in tandem, the latter treatment usually gives the better age estimates. When the microstrucutres are indistinctive, the percent remodelled bone versus the amount of non-Haversian bone should be quantified.

SUGGESTIONS & STANDARDIZATIONS FOR FUTURE RESEARCH

A definitive study, producing regression equations for all the long bones and the mandibles, has not yet been done (see Table 8 for bones with reliable equations for aging). Such a study, or any subsequent study dealing with this line of research, should include information on the race, sex, clinical history, and type of sample being used. Studies with samples of mixed races and/or sexes, may be useful to archaeologists, or forensic scientists, when race and sex are not determinable. But separate sex and population studies are needed.

An adequate sample size is necessary (over 50, preferably 100+), as well as a broad age range (from 25 yrs. to 80 yrs. would appear to cover the critical adult years where morphologic and morphometric methods are unreliable). The distribution of ages should be as even as possible, and the mean age recorded to check the bias due to skewness.

Naturally, pathological bone should be screened out using gross morphology, radiography and microscopic examination (after Kerley 1965).

Ideally, a definitive study should be done taking into account such problems of standardization and the need for reproducibility as are summarized in these conclusions. Barring this, it is felt that the existing studies can be "cleaned up" and expanded to include standardized procedures. In assessing the results of the methods employed, it is felt that the Ahlqvist - Damsten method, and the Singh - Gunberg method, should be retsted on a larger sample with a more even age distribution, and then compared again to Kerley's method.

A laboratory manual listing the materials and method of execution of the histological technique could include all standardizations and pertinent information for deriving age estimations using this method. Such a manual could also give information on the best variable(s) to employ under restricted circumstances. For example, if only the fibula is available then it is best to estimate the age using the number of osteon fragments (Kerley, 1965). The manual could also include special procedures for handling archaeological samples.

The histological method has proven to be more accurate than standard morphological methods for aging human bone, yielding age estimates of approximately ± 5 years. It also has the advantage of being useful on fragmented bone. However, the histological technique takes more time and involves more expense that the morphological methods. The technique has not yet been standardized, mainly due to the fact that the technique is just completing its experimental stage. As a technique for estimating the age at death, it is most useful between the ages of 30 - 70+ years, when the morphological methods do not give reliable age estimations. Once the procedures have been agreed upon and standardized, it will be possible to prepare a concise manual giving the steps in the use of the technique, and the regression equations for estimating age for each bone studied, which can be used by future investigators. It appears that the technique is useful for more than just estimating the age at death. It has also been applied to the study of palaeophysiology and to forensic medicine and demography, and may even prove useful for indicating ethnogentic background. It appears that it can be developed for other studies in the biological sciences related to remodelling and the mineralization of bone. At present, the full potential of the technique has not yet been explored.

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1981 Bone mineral-osteon analysis of Yupik-Inupiaq skeletons. Am. J. Phys. Anthrop. 55:1-7.

Todd, T.W. and D.W. Lyon
1924 Endocranial suture closure. Am. J. Phys. Anthrop. 7:325-384.

Ubelaker, Douglas H.
1974 Reconstruction of demographic profiles from ossuary samples: A case study from the Tidewater Potomac. Smithsonian Contributions to Anthropolocy No. 18, Washington: Smithsonian Institute Press.

1978 Human Skeletal Remains. Douglas H. Ubelaker, Taraxacum, Washington.

1981 Approaches to demographic problems in the northeast. Chapter 5 in Foundations of Northeast Archaeology. pp. 175-194. New York and London: Academic Press.

Figure 1. Diagram Showing the Remodelling of Long Bones

(Reprinted from Ham, A.W.: J. Bone & Joint Surg., 34-A: 701, 1952, in Ham,A.W. & D.H. Cormack, Histophysiology of Cartilage, Bone & Joints (Chps. 14-16 of Ham, A.W., Histology, 8th ed.) Courtesy of J.B. Lippincott Co., Toronto, and Dr. A.W.Ham & Dr. D.H.Cormack).

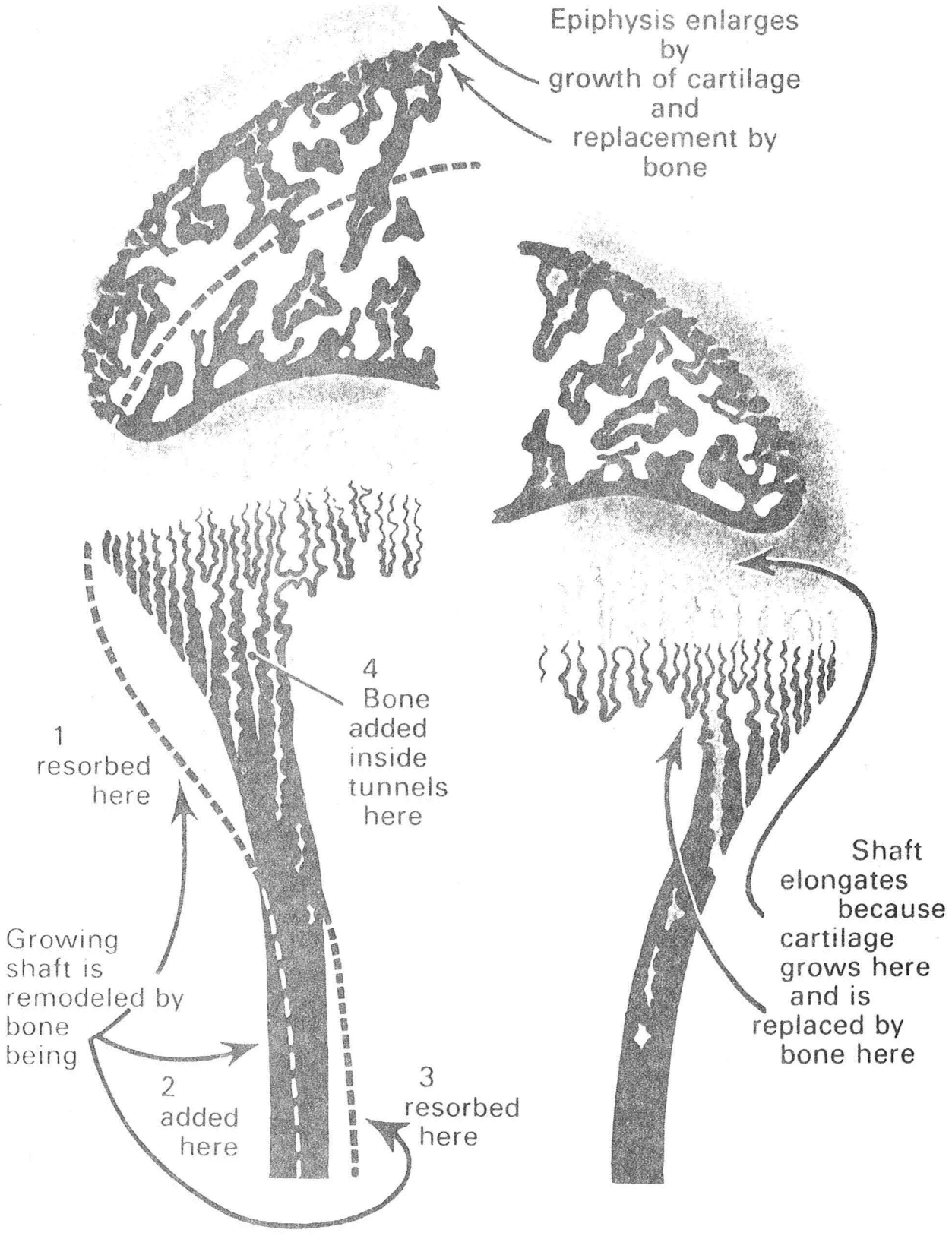

Epiphysis enlarges by growth of cartilage and replacement by bone
4 Bone added inside tunnels here
1 resorbed here
Growing shaft is remodeled by bone being
2 added here
3 resorbed here
Shaft elongates because cartilage grows here and is replaced by bone here

Figure 2. Generalized Parts of an Immature Long Bone

(Reprinted from Bourne, G.H. (ed.), The Biochemistry and Physiology of Bone, 2nd ed., 1971. Courtesy of Academic Press)

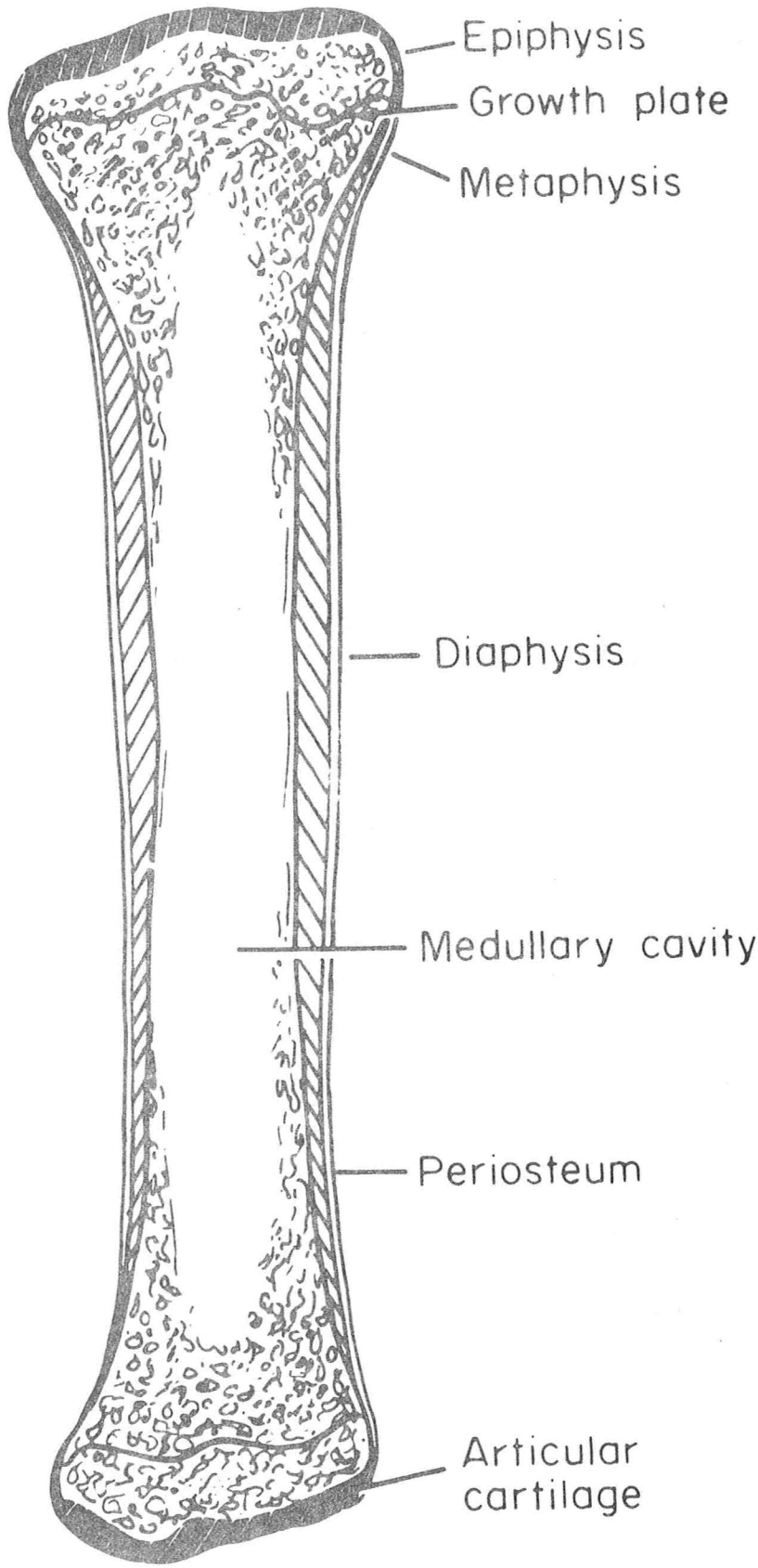

Epiphysis
Growth plate
Metaphysis
Diaphysis
Medullary cavity
Periosteum
Articular cartilage

Figure 3. Three Dimensional Saggittal Section Through the Compact Bone of the Shaft of a Long Bone

(Reprinted from Ham[1a] and Johnson[2], in Harris, W.H. & R.P. Heaney, Skeletal Renewal and Metabolic Bone Disease. Courtesy of Little, Brown & Co., and Dr. Harris & Dr. Heaney).

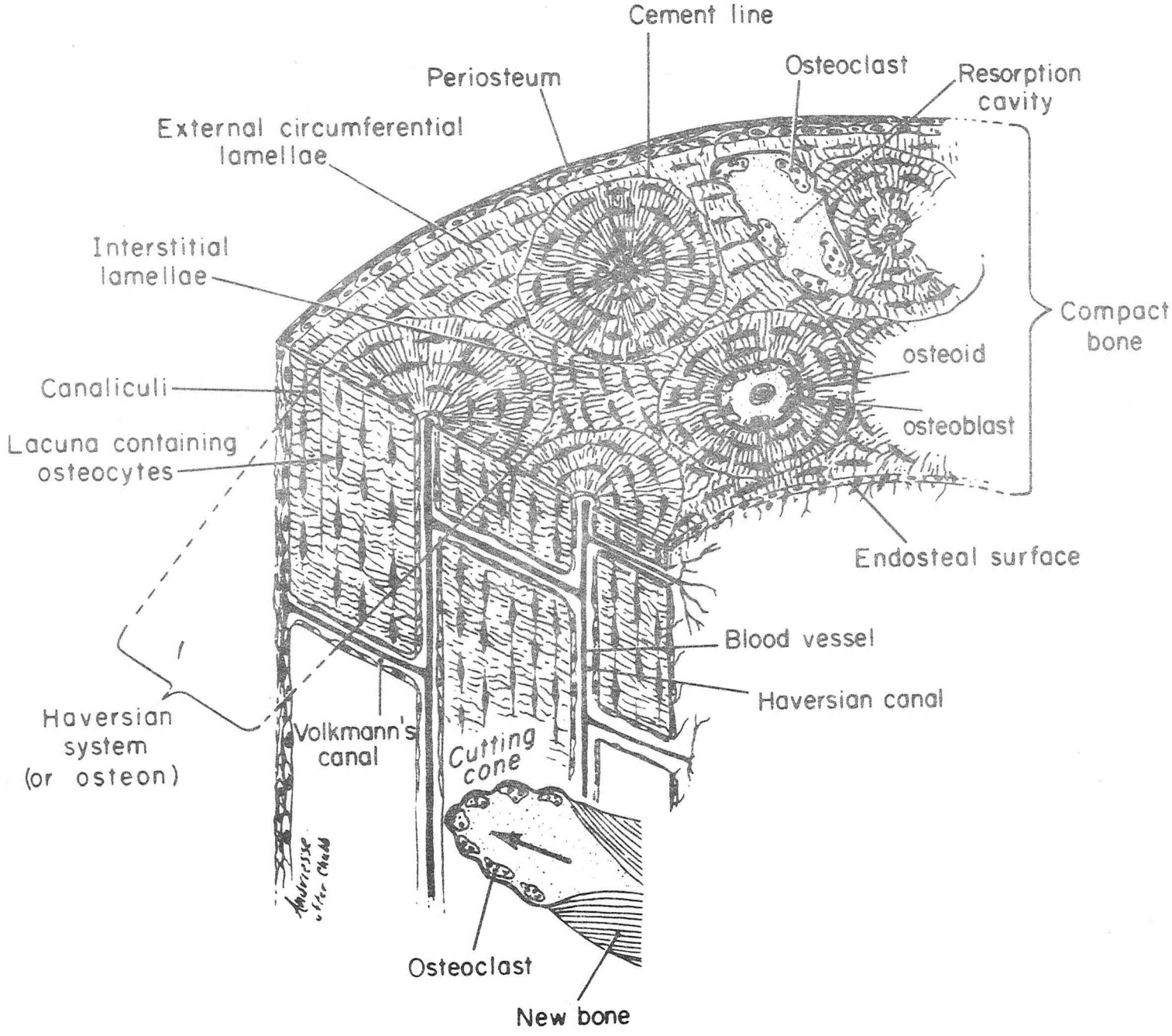

Cement line
Periosteum
Osteoclast
Resorption cavity
External circumferential lamellae
Interstitial lamellae
Compact bone
osteoid
Canaliculi
osteoblast
Lacuna containing osteocytes
Endosteal surface
Blood vessel
Haversian canal
Haversian system (or osteon)
Volkmann's canal
Cutting cone
Osteoclast
New bone

Figure 4. Photomicrograph of a Cross-Section Through a Mature bone, Showing the microstructural Detail

(Reprinted from Ham, A.W. & D.H. Cormack, Histophysiology of Cartilage, Bone & Joints, 1979:405, Fig. 15-25B, first published in Ham, A.W. Histology, 8th ed. Reprint courtesy of J.B. Lippincott Co., and Dr. Ham & Dr. Cormack).

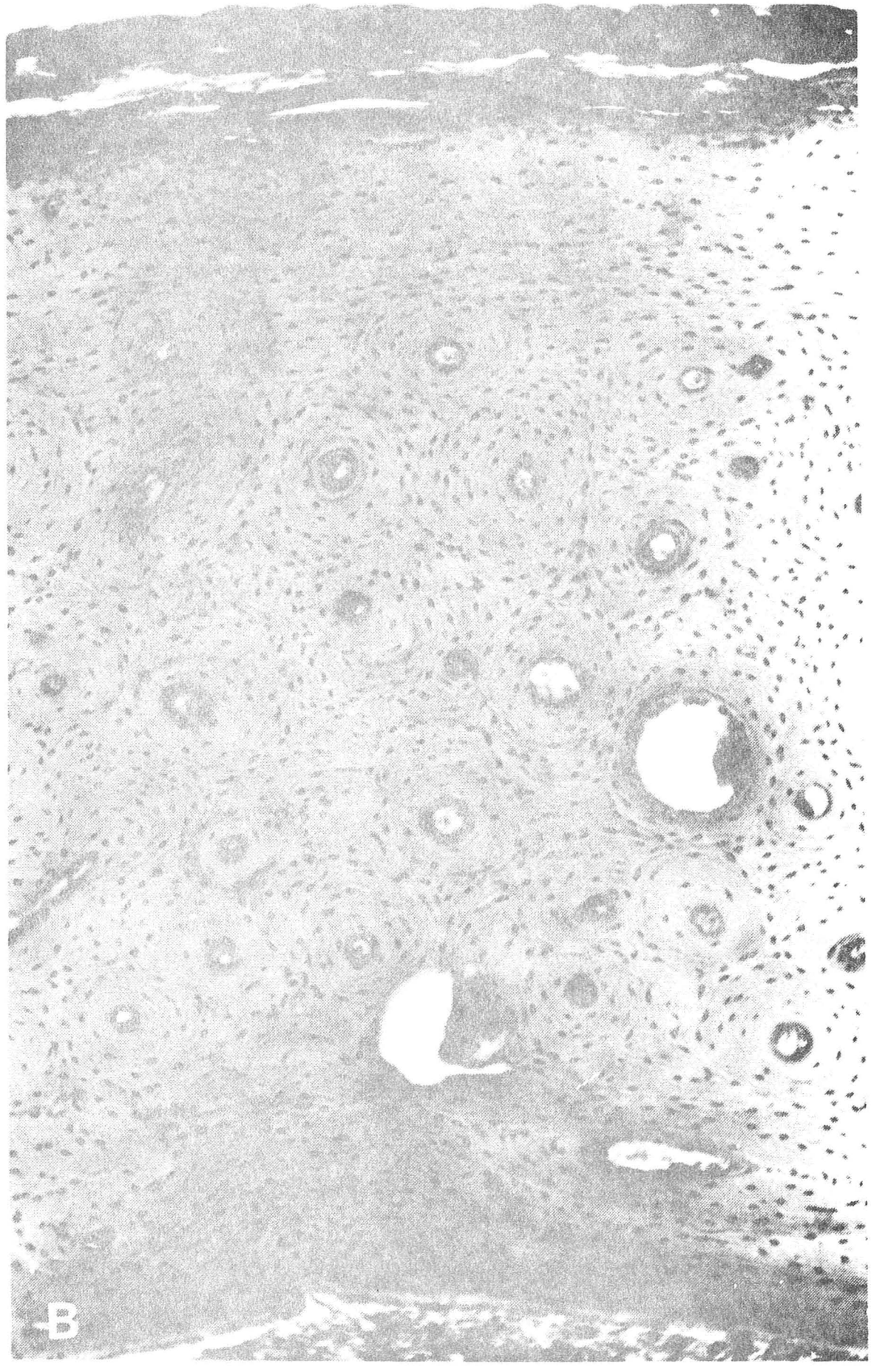

B

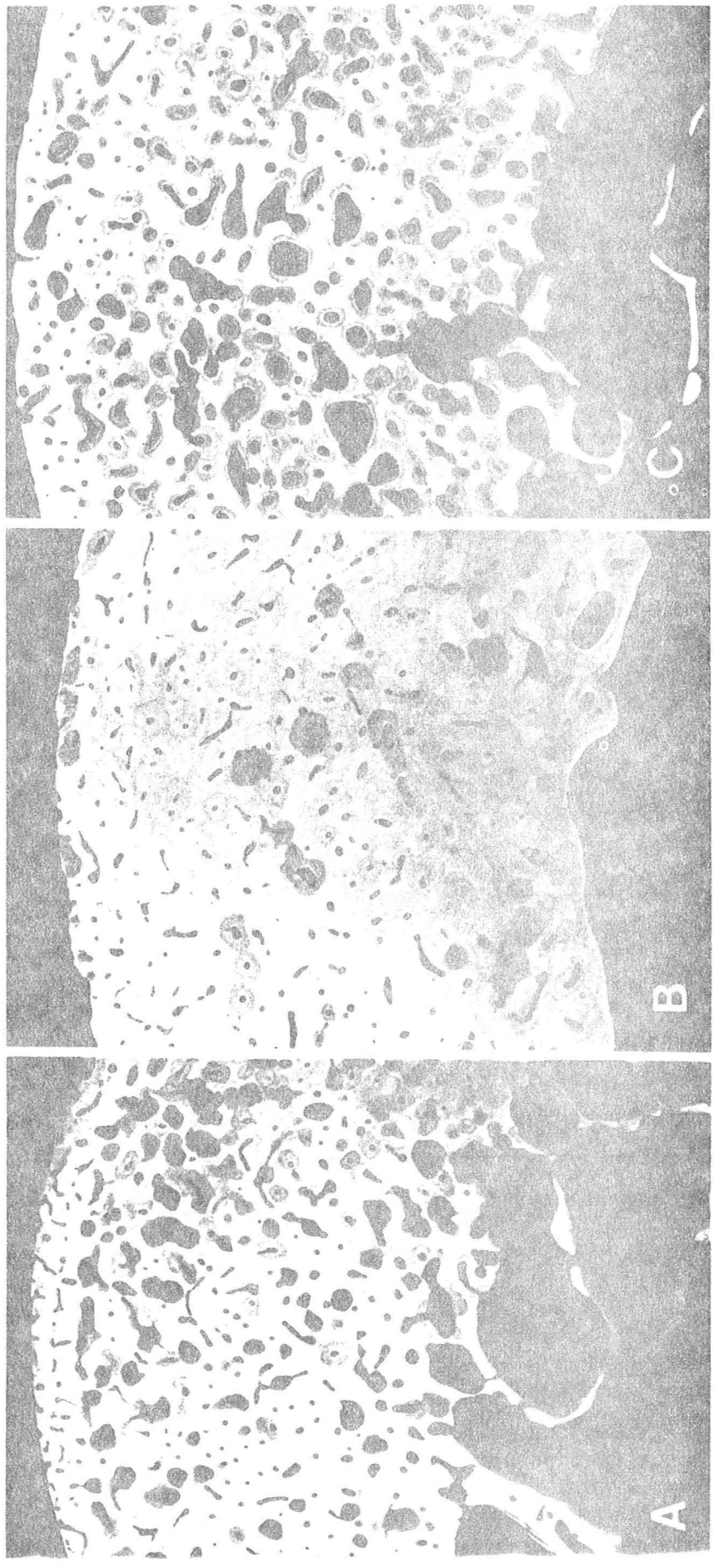
Figure 5. Thin Section of Rhesus Monkey Femur (X50) Showing Microsctructures, the Periosteal layers and the Endosteum.

(Reprinted from Enlow, D.H., Principles of Remodelling, 1963:64, courtesy of Charles C. Thomas).

Figure 6. Microradiographs of Undecalcified Thin Section Taken from the Midshafts of the Femora of 3 people of different ages

A) a 7 year old
B) a 25 year old
C) an 85 year old

(Reprinted from Ham, A.W. & D.H. Cormack, Histophysiology of Cartilage, Bone & Joints, 1979:446, first published in Ham, A.W., Histology, 8th ed. Courtesy of J.B. Lippincott Co., and Dr. Ham & Dr. Cormack).


A
B
C

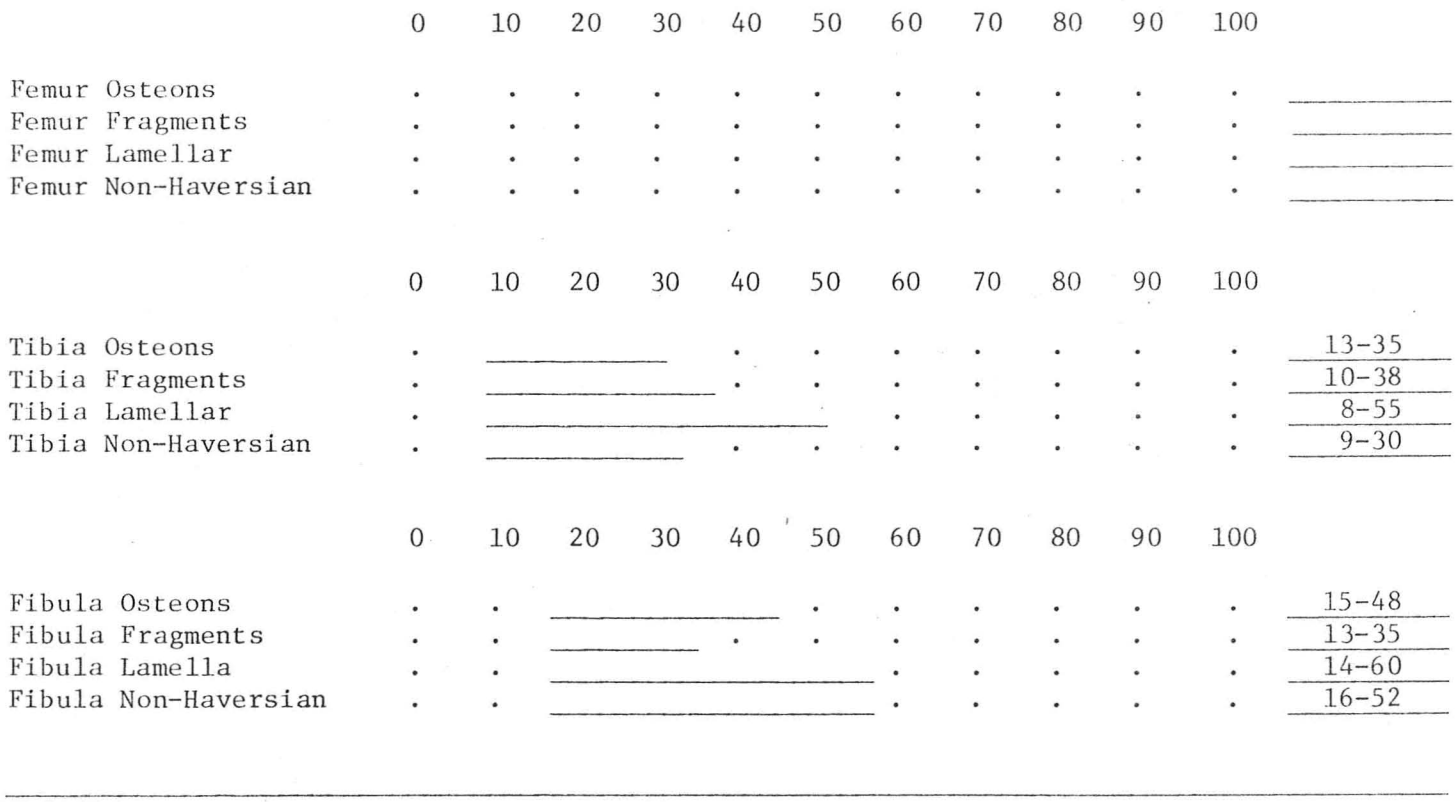


Figure 7. Taken from Kerley (1965:161, Fig. 7). Age Estimate Profile Chart.

This figure shows the age range of the estimate (solid black line and range written to the right), given by the variables listed on the left, for the tibia and fibula. The vertical lines indicate the limits of overlap of the estimates. This provides an age-profile (18-26) which narrows the range of the estimate (16-30). The middle year of the age profile was used as the age estimate.

On limitation that arises in using the age profile chart is that age estimates that do not overlap all the other estimates must be excluded (Kerley, 1965). A theoretical possibility is to get a bimodal distribution which would invalidate the results.

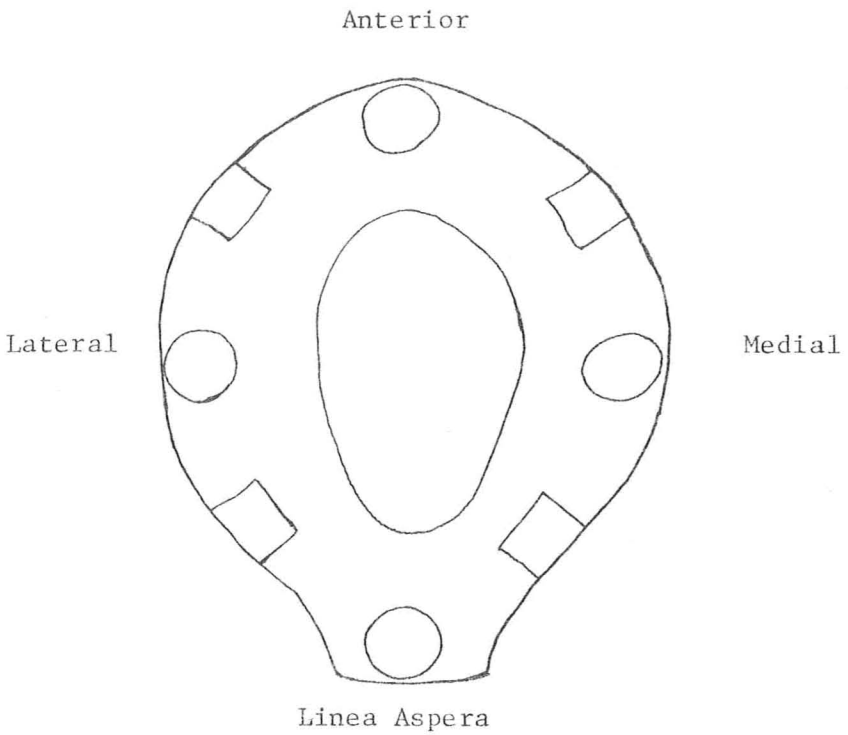


Figure 8. Visual fields used by Kerley (1965) and Ahlqvist & Damsten (1969) on the left femur.

Kerley (1965) Field diameter - 1.62mm

Ahlqvist & Damsten (1969) Field size - 1.00mm^2

Table 1. List of Some of the Various Methods for Estimating the Age at Death of Human Skeletons

METHOD AND MATERIAL	N	AGES	ACCURACY	AUTHOR
Subadult Aging Methods				
Bone Length (femur)[2]	65	5th foetal mo.-18 yrs.	± weeks (at birth)[3]	Stewart (1968)
Appearance of Ossification centres	?	birth - 5 yrs.	± 3 mos.[4]	Stewart (1968)
Epiphyseal Union	?	11 - 30 yrs.	± 4 yrs.[5]	Stewart (1968)
Dental Formation & Eruption	?	5th foetal mo. - 25 yrs.	3 mos.[6]-4 yrs.	Ubelaker (1978)
Morphoscopic Adult Aging Methods				
Dental Attrition	40	21-60	6.29*	Takei (1970)
Cranial Suture Closure	30	28-60	8.93*	Todd & Lyon (1924)
Morphological Observation of the Pubic Symphysis	45	19-70	4.49*	Hanihara (1953)
Morphological Observation of the Pubic Symphysis (males)	349	17-36*	2.15[8]	McKern & Stewart (1957)
Morphological Observation of the Pubic Symphsis (females)	103	13-59	4.77[8]	Gilbert & McKern (1973)
Decrease in Cancellous tissue of the Humeral head	28	30-80	9.65*	Rother et al. (1977)

TABLE 1 cont.....

Microstructural Changes of Compact Bone

METHOD AND MATERIAL	N	AGES	ACCURACY	AUTHOR
Microstructural Changes of Teeth	41	11-69	3.60[7]	Gustafson (1950,1966)
Femur, Tibia, Fibula	67	0-95	5.27[9]	Kerley (1965)
Femur	20	4-89	6.71[10]	Ahlqvist & Damsten (1969)
Mandible, Femur, Tibia	52[11]	46-80	2.58[12]	Singh & Gunberg (1970)
Humerus	20	30-79	8.84*	Rother et al. (1977)
Femur, Tibia, Humerus, Ulna	116	50-97	6.41[13]	Thompson (1978, 1979)
Femur	43 17	45-75 76-102	5.75* 3.63*	Iwamoto & Konishi (1982)
Hemerus	43 17	45-75 76-102	4.40* 5.21*	Iwamoto & Konishi (1982)

---

1. Table 1 is modified and expanded from Sotaro & Konishi, 1982:39, Table 4.

2. Stewart (1968:133) gives the proportionate relationship of femoral lengths of the tibia, fibula, humerus radius and ulna.

3. The accuracy decreases with age and sexual differentiation (Stewart, 1968:133).

4. This data is for male, upper-class whites (taken from Francis & Werle, 1939 in Stewart, 1968) and based on the appearance of the ossification centres in 20% of the case studies. Expertise in X-ray reading and adjustment for socio-economic groups is necessary.

5. Includes various bones from U.S. white males, with the clavicle as the last to fuse. Females fuse 1-2 years earlier for all bones (modified from Krogman, 1939 based on data from McKern & Stewart, 1957 in Stewart, 1968).

6. Age Estimates become less accurate with increasing age.

7. Standard error of the estimate (In Ubelaker, 1974:52). Burns & Maples (1976, in Bass, 1979) have developed a method using multiple regression analysis which is better than Gustafson's (1966) technique.

TABLE 1 cont.....

8. The average accuracy of the estimate for one standard deviation (In Stewart, 1979:157-171).

9. Syandard error of the estimate for the osteon fragments of the fibula.

10. Standard error of the est imate for the present remodelled bone.

11. N for the number of mandibles, there were 40 tibiae and femora.

12. Standard error of the estimate for the mandibles.

13. Standard error of the estimate for the # of secondary osteons/mm^2 (Thompson, 1979).

* Accuracy measured by two standard deviations from the mean (Sotaro & Konishi, 1982).

Table 2. Taken from Bouvier and Ubelaker, 1977: 393, Table 2.

Comparison of Accuracy Between the Kerley Method and the Ahlqvist-Damsten Method for the Femur

	Average Difference from Actual Age	
Age Range of Sample	Kerley Age Estimates	Ahlqvist-Damsten Age Estimates
20-45 years	$\pm$9.93	$\pm$13.87
45-90 years	$\pm$8.05	$\pm$ 7.50
Total	$\pm$8.20	$\pm$ 9.50

Table 3. Modified from Bouvier and Ubelaker, 1977:393, Table 3.

Sizes and Distributions of the Samples Utilized in the Kerley and Ahlqvist-Damsten Studies

Sample	Sample Size	Mean Age	Age Range	Skewness
Ahlqvist-Damsten femora	20	55.45	4-89	-0.581_{NS}
Kerley fibula	25	34.48	0-83	$+0.652_{NS}$
Kerley femora	67	41.55	0-95	$+0.208_{NS}$
Bouvier & Ubelaker femora	40	48.60	11-82	-0.090_{NS}

NS = Not significant at the approximate .05 probability level (Snedecor and Cochran, 1967: Table A6)

Table 4. Correlation and Standard Error of the Estimate for the Mandible, Femur and Tibia, taken from Singh and Gunberg, 1970.

BONE	SAMPLE	VARIABLES	r-VALUE	S.E.E.*
Mandible	N=52 $\bar{x}$ age=64.25 s.d.=12.14	# Osteons Lamellae/Osteon Diam. Havers. Canal	+0.969 +0.950 -0.966	$\pm$2.55
Femur	N=33 $\bar{x}$ age=62.33 s.d.=10.80	# Osteons Lamellae/Osteon Diam. Havers. Canal	+0.945 +0.890 -0.937	$\pm$3.24
Tibia	N=33 $\bar{x}$ age=62.33 s.d.=10.80	# Osteons Lamellae/Osteon Diam. Havers. Canal	+0.919 +0.908 -0.935	$\pm$3.02

* Standard errors of the estimate are for all variables considered together. This gives the best estimate of the true age.

Table 5. Cost/Benefit Analysis of the Histological Technique versus Standard Morphoscopic Methods.

MACROSCOPIC/MORPHOSCOPIC TECHNIQUES	HISTOLOGICAL TECHNIQUE
Time per specimen is short, depending on the technique.	Time per specimen is long. 5 days are needed for the preparation but a few dozen specimens may be prepared at the same time.*
Estimate age to $\pm$10 yrs., less accurage with adults.	Estimates age to $\pm$ 5-8yrs. depending on the technique used and the bone.
Requires only osteometric equipment unless radiography is employed	Requires machinery and chemicals valuing up to 8,000-9,000 dollars to set up a lab.*
Causes no damage to the bone.	Causes some, but little, damage to the bone. The core technique does the least damage.
Requires experience with the various methods to interpret morphology or to take measurements.	Must be able to recognize the histological features and to calibrate a microscope.
Possible to screen-out pathology.	Possible to screen-out pathology.
Useful only on the complete skeleton or with the required region intact.	Useful on fragmented bone with the periosteum to endosteum intact.
Often of little use on weathered, leached, or burnt bone.	May be used on weathered, leached or burnt bone if periosteal to endosteal layers are intact. Also on ancient bone (Neanderthal).
Inter-observer error is moderate for methods if care is taken (not for the pubic symphysis and cranial suture closure, which lack objectivity).	Inter-observer error <u>may</u> be significant even if care is taken. This method is, to date, the most objective.
Terminology and procedures are well-defined.	Terminology is defined, though the definitions of the variables must be checked. The procedures are not completely defined.
Applicable to other biological sciences for studies other than aging.	Applicable to other biological sciences for studies other than aging.

* Information on costs and time for preparation was provided by Pfeiffer, 1983 Personal communication.

TABLE 6. Summary of the Standard Error of the Age Estimates Obtained by the Various Authors for the Bones and Variables Examined.

VARIABLES/AUTHOR	FEMUR		TIBIA		FIBULA		MANDIBLE	HUMERUS		ULNA	
	Right	Left	Right	Left	Right	Left		Right	Left	Right	Left
Kerley (1965)[1]											
# of secondary osteons	-	9.39	-	6.69	-	8.83	-	-	-	-	-
# of Osteon fragments	-	12.19	-	7.78	-	5.27	-	-	-	-	-
% circumferential Lamellae	-	11.78	-	13.62	-	10.85	-	-	-	-	-
# non-Haversian Canals	-	13.85	-	9.63	-	10.70	-	-	-	-	-
Ahlqvist & Damsten (1969)											
% remodelled bone	-	6.71	-	-	-	-	-	-	-	-	-
Bouvier & Ubelaker (1977)											
% remodelled bone	-	9.50	-	-	-	-	-	-	-	-	-
# of secondary Osteons	-	8.20	-	-	-	-	-	-	-	-	-
Singh & Gunberg (1970)[2]											
# of secondary Osteons	-	-	-	-	-	-	-	-	-	-	-
Avg # Lamellae per Osteon	-	3.24	-	3.02	-	-	2.55	-	-	-	-
Avg. diam. Havers. Canals	-	-	-	-	-	-	-	-	-	-	-

TABLE 6. cont....

VARIABLES/AUTHOR	FEMUR		TIBIA		FIBULA		MANDIBLE	HUMERUS		ULNA	
	Right	Left	Right	Left	Right	Left		Right	Left	Right	Left
Thompson (1978, 1979)[3]											
Secondary Osteon Area	6.41	-	-	-	-	-	-	-	6.21	-	10.57
Secondary Osteon ratio & Haversian Canal ratio	7.88	8.65	8.68	9.52	-	-	-	9.46	8.52	10.57	10.17
Avg. SEE/36 Stepwise regressions (both sides)	6.50		7.50		-	-	-	7.50		9.00	
Sotaro & Konishi (1982)[4]											
# of Osteons/mm^2	-	-	-	-	-	-	-	-	-	-	-
# of interstitial Lamellae/mm^2											
Avg. diam. Osteons	11.5/7.3		-	-	-	-	-	-	-	-	-
Avg. diam. Havers. Canals											

1. Kerley's accuracy increased to within 5 yrs., 87.3% of the time, and within 10 yrs., 100% of the time using his age-profile chart. For individuals under 30 years of age the estimate is within 5 yrs., 78.9% of the time.

2. Singh & Gunberg's estimates are listed for all 3 variables considered together. Using a monograph of the 52 mandibles a SEE of 2.58 was obtained in 67% of the cases, and of 5.16 in 95% of the cases.

3. Age estimate errors for the Secondary osteon area are from 1979. The secondary osteon ratio and Haversian canal ratio were the best age predictors in 33 out of 36 step-wise regression analyses.

4. Sotaro & Konishi's SEE's are based on multiple regression analysis of all 4 variables. It is not known if the estimates are for both sides. The first SEE listed is for the age group 45-75 yrs., and the second is for the age group 76+ yrs. The reason for the discontinuity is unknown.

TABLE 7a Comparative List of the Study Samples.

SAMPLE SAMPLE	KERLEY (1965) KERLEY (1965)	AHQVIST & DAMSTEN (1969)	SINGH GUNBERG (1970)	BOUVIER & UBELAKER (1977)	THOMPSON (1978)	SØTARO & KONISHI (1982)
Type	Cadavers	Autopsies	Cadavers	Cadavers	Cadavers	Cadavers
Population (N)	Caucasoid (56) Negroid (11)	n.r.	n.r.	see Kerley(1965)	U.S. white(116)	Japanese (60)
Total N	67	20	40(leg bones) 59(mandibles)	40	116	60
Sex (N)	Male(88 sect.) Female(29 sect.)	n.r.	Male(33-leg, 52 mandibles)Female (7-leg & mandibles	n.r.	Male (64) Female (52)	Male (60)
Ages by Sex	Birth-95 n.r. by sex	4-89 n.r. by sex	Male & Female = 39-87(leg bones) 40-80(mandibles)	20-90 n.r. by sex	Males (50-97) Females (43-94)	Males(45-102)
x̄ ages by sex	41.55[1]	55.45[1]	Males (64.25 - mandibles) (62.33 leg bones)	n.r.	Males (71.48) Females (71.94)	nf. in Englis
Condition	Non-path.	Non-path.	Non-path.	Non-path.	Non-path. All Male All Female Diabetic	Non-path.
Bones	Left Femur Left Tibia Left Fibula	Left Femur	Left Femur Left Tibia Mandible (side)	Left Femur	L&R Femur L&R Tibia L&R Humerus L&R Ulna	Femur (side?) Humerus (side
Region cut	Mid-shaft	Mid-shaft	Mid-shaft Ascending Ramus of Mandible	Mid-shaft	Mid-shaft	Mid-shaft

1. Mean ages taken from Bouvier & Ubelaker (1977:343) for the femora.

TABLE 7b Comparative List of the Methods Used.

METHOD	KERLEY (1965)	AHLQVIST & DAMSTEN (1969)	SINGH & GUNBERG (1970)	BOUVIER & UBELAKER (1977)	THOMPSON (1978)	SOTARO & KONISHI (1982)
Section	Complete cross-section	Complete cross-	1 cm^2 sections	complete cross-	0.4 cm cores	complete cross section
Thickness	n.r.	25 um	30 um - 50 um	n.r.	80 um.	n.r.[1]
Threatment	n.r.	unstained, decalcified	fixed,decalci-fied & stained	n.r.	formalin preserved	n.r.[1]
Microscope light	Light	Polarized light	Light	Light	Phase contrast	n.r.[1]
Counting	By grid area	By grid area	n.r.	By grid area	Point counting	n.r.[1]
Magnification	10 x 10 wide field	10 x 10 wide field	10 x 10 wide field	10 x 10 wide field	10 x 10 wide field	10 x 10 wide field
Field Shape	circular	square-ruled	square-ruled	Tested Kerley vs. Damsten/ Ahlqvist	square-ruled	n.r.[1]
Field Position	Ant., Post., Med., Later.	Ant-medial, Ant-lat., Post-med., Post-lat.	Chosen at Random	Kerley vs. Ahlqvist-Damsten	Adjacent to each other	n.r.[1]
Location cortex	Along Periosteal	Periosteal third	Periosteal third	Periosteal third	Periosteal Third	Periosteal Third
Field size	1.62 mm	1 mm^2	2 mm Diameter	After Kerley, Ahlqvist/Damsten	0.992 mm^2	n.r.[1]

1. It is not currently known if this information was recorded in the text, which is written in Japanese.

TABLE 7c Comparative List of Variables Analyzed

VARIABLES	KERLEY (1965)	AHLQVIST & DAMSTEN (1969)	SINGH GUNBERG (1970)	BOUVIER & UBELAKER(1977)	THOMPSON (1978)	SOTARO & KONISHI (1982
# of secondary osteons	X		X	X	X ($per\ mm^2$)	X ($per\ mm^2$)
# of secondary osteon fragments	X					
% of circumferential osteion perimeter	X					
# of non-Haversian canals	X					
% of remodelled bone		X		X	X	
Avg. # of lamellae per osteon			X			
Avg. Diam. Haversian canals			X		X	
Avg. Diam. Secondary osteons					X	
# of interstitial lamellae/mm^2					X	
Haversian canal Ratio					X	
# of Haversian canals					X	

TABLE 7c cont'd.

VARIABLES	KERLEY (1965)	AHLQVIST & DAMSTEN (1969)	SINGH GUNBERG (1970)	BOUVIER & UBELAKER (1977)	THOMPSON (1978)	SOTARO & KONISHI (1982)
x Ind. secondary osteon ratio					X	
x Ind. Haversian canal ratio					X	
x secondary osteon & Haversian canal Ratios					X	
Aggregate 2nd osteon perimeter					X	
x Ind. Haversian Ratio					X	
x Ind. secondary osteon & Havers. canal Ratios					X	
Aggregate 2nd osteon perimeter					X	
Aggregate Havers canal perimeter					X	
Ind. 2nd osteon perimeter					X	
Ind. Havers. canal perimeter					X	

TABLE 7c cont'd.

VARIABLES	KERLEY (1965)	AHLQVIST & DAMSTEN (1969)	SINGH GUNBERG (1970)	BOUVIER & UBELAKER (1977)	THOMPSON (1978)	SOTARO & KONISHI(1982)
Ratio 1*					X	
Ratio 2*					X	
Ratio 3*					X	

* Ratio 1 = $\frac{\text{Individual Haversian Canal Ratio}}{\text{Individual 2nd Osteon Ratio}}$

Ratio 2 = $\frac{\text{Aggregate Haversian Canal Perimeter}}{\text{Aggregate 2nd Osteon Perimeter}}$

Ratio 3 = $\frac{\text{Individual Haversian Canal Perimeter}}{\text{Individual 2nd Osteon Perimeter}}$

TABLE 8. List of the Lowest S.E.E.'s Obtained for Samples of Adequate Size.

AUTHOR	SEE	VARIABLES	BONE	N	AGE RANGE	SEX & RACE
Kerley (1965)	5.27	# osteon fragments	L. fibula	67	birth-95	M & F 'white' & 'black'
Kerley (1965)	6.69	# secondary osteons	L. tibia	67	birth-95	M & F 'white' & 'black'
Singh & Gunberg (1970)	2.55*	# secondary osteons Avg. # lamellae/ost Avg. diam. Havers. canals	Mandible	57	40-80	M ?
Thompson (1978, 1979)	6.50	36 step-wise regression equations	L & R femur	116	43-97	M & F U.S. 'white'
Thompson (1978, 1979)	6.41	# osteons/mm^2	femur	116	43-97	M & F U.S. 'white'
Thompson (1978, 1979)	6.21	# osteons/mm^2	L. humerus	116	43-97	M & F U.S. 'white'

Singh & Gunberg's extremely low estimate for the mandible sample suggests two possibilities 1) Their method is the best or 2) they were more rigorous in the execution of their technique at each stage. In view of the low S.E.E. that sas obtained, their method should be retested.

TABLE 9. List of the Proposed Standardizations for the Histological Technique.

A) General Standardizations for Histological Aging

1.	Sample size	50+, ideally 100+
2.	Age range	25 yrs. - 80 yrs., with an even distribution
3.	Record information	sample type (eg. cadavers), race, sex, clinical history, age range, x age
4.	Morphometrics	Morphometric measurements should be done, and note any discrete traits
5.	Section	Use cores, wedges or 1 cm^2 sections
6.	Whole bone analysis	Bone mineral content, bone density and cortical thickness should be measured
7.	Zone	Midshift of long bones, follow Singh & Gunberg for mandibles
8.	Position	Testing is needed. Lateral & posteromedial sides give best correlation for femur
9.	Thickness	40 um - 60 um
10.	Treatment	Depends on research and state of preservation generally, undecalcified, unstained
11.	Light type	Simple light microscope with polarizing light filter
12.	Magnification	X10 ocular with X10 objective (=100X)
13.	Field size	2 mm^2, use 4 fields
14.	Field shape	Square. use square-ruled ocular micrometer and point-counting for quantifications
15.	Field location	Along periosteal third
16.	Variables	# secondary osteons/2mm^2; # osteon fragments; Avg. diam. Haversian canals; Avg. # lamellae/osteon. Use % remodelled bone if microfeatures are unclear.
17.	Other	Note any pathological conditions or abnormalities

TABLE 9 cont'd.

B) Suggestions for Future Research

1.	Bones	Reliable age estimates and regression equations are needed for the right fibula, right tibia, right humerus, left and right radius and left and right ulna. A single, standardized study has yet to be done on all long bones. Other bones may also be treated.
2.	Race	population-specific equations needed
3.	Sex	male-female differences in rates of bone remodelling - possibility of sexing by the histological method
4.	Disease studies	for the recognition of diseases by their histomorphology; use in palaeopathology & palaeophysiology
5.	Singh & Gunberg	method should be retested on a larger sample with even age distribution
6.	Ahlqvist & Damsten	method should be retested on a larger sample with even age distribution
7.	Sampling positions	testing needed to determine the optimum number of sites and position on each bone for sampling to obtain best estimates