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Microscopic Age Changes in the Human Occipital Bone

REFERENCE: Cool, S. M., Hendrikz, J. K., and Wood, W. B., **"Microscopic Age Changes in the Human Occipital Bone,"** *Journal of Forensic Sciences,* JFSCA, Vol. 40, No. 5, September 1995, pp. 789–796.

ABSTRACT: The value of histological examination of the human occipital bone for estimation of age-at-death was assessed. Undecalcified sections of occipital bone from eighteen male Caucasian subjects between the ages of 21 and 70 years were prepared for analysis using polarized light microscopy. The fractional volumes of primary osteons, secondary osteons, osteon fragments, and lamellar bone in both the outer and inner cortical tables were determined. It was found that with increasing age there is a decrease in the fractional volume of primary osteons and a significant decrease in the fractional volume of lamellar bone. The fractional volume of secondary osteons was not found to change significantly with age, while the fractional volume of osteon fragments significantly increases. The microscopic results reflect the continuous process of bone remodeling that is responsible for the variation in cortical parameters with age and is the primary basis for age predicting methods. While observable changes in the occipital bone do occur with increasing age, the amount of random variation in the parameters examined preclude their use for accurate age estimation.

KEYWORDS: physical anthropology, skeletal age estimation, histomorphometry, osteon

At present no accurate method exists for aging the isolated and edentulous adult cranium. In the absence of dental material the stage of cranial suture closure can be used, but this provides only a very rough estimate of age [*1*]. Within the field of forensic science, in particular, there still exists the need to develop a reliable method of estimating age from the edentulous adult cranium.

Since 1965, histologically based techniques dealing with the postcranial skeleton have been used to estimate age [2]. These involve the use of histomorphometry and have achieved age estimations accurate to within ± 5 years of the known age-at-death. It is surprising that such techniques have not been applied to cranial vault bones.

Histological methods based on age-related changes at the microscopic level have provided a useful means of age estimation. Aging is monitored by histomorphometric quantification of the remodeling process reflected in the life of the osteon [*3,4*].

Currently, there are a number of histological age estimating methods available [*5–9*] most of which are modifications of the original method proposed by Kerley [2] and modified by Kerley and Ubelaker [*10*]. Kerley's method [*10*] uses complete transverse sections from the mid-shafts of the femur, tibia, and fibula. This region of a long bone is least affected by cortical bone loss [*11*]. Histomorphological variables are quantified in four microscopic fields tangential to the periosteal surface of bone and the age is estimated using regression formulae. This method allows age estimates to be made to within 5 years for individuals ranging from birth to 90 years of age [2].

Inherent to Kerley's [2] technique is the problem of distinguishing between histomorphological variables. To obviate this problem, Ahlqvist and Damsten [*5*] developed a method based on the sum of the histomorphological variables in a visual field. However, these results proved less accurate than those of Kerley's [2] method [*12*].

Despite having definite applications, the current histological aging methods do not account for individual variations arising from differences in endocrine function, physical activity, disease, trauma, and diet [*13,14*] and between and within the sexes [*11*]. Consequently, the bulk of research has moved to bones such as the mandible, clavicle, and ribs, which are less influenced by environmental factors [*14*].

It is therefore hypothesized that the human occipital bone of the skull, being a nonweight-bearing bone will be less influenced by environmental stresses, and will demonstrate age-related changes at the microscopic level that can be used for age estimation.

Materials and Methods

Human calvaria were obtained from 18 male cadavers undergoing postmortem examination. One subject was subsequently removed from the analysis because examination revealed extreme cortical thickening, leaving a sample size of 17 (Table 1). Age-at-death (in years) and racial origin were recorded from death certificates. Each subject was assigned an identification code number unrelated to his age.

Cores were removed from the left side of the occipital bone for each calvarium using a press drill stand equipped with a 3/8″ Black and Decker type "N" electrical drill. A 16 mm holesaw rotating at 2400 r.p.m. was fitted to the drill and produced cores 12 mm in diameter. Each calvarium was embedded in damp sand to stabilize the bone during the removal of the core and repositioned prior to the removal of each core so that the ectocranial (outer) surface was always perpendicular to the drill. The position for the removal

TABLE 1—*Age distribution of sample.*

Age cohort (years)	Males (*N*)	Mean age (years)	SD (years)
20–40	8	30.2	8.3
50–70	9	61.5	5.7
Totals	17	46.8	17.5

Received for publication 14 Oct. 1993; revised manuscript received 14 July 1994; 21 Sept. 1994, and 30 Jan. 1995; accepted for publication 2 Feb. 1995.

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of each core was standardized from the point lambda using a template. The right side of the occipital bone was unfit for sampling because its inner surface was grooved with the sagittal sinus. Individual cores were stored in sealed plastic vials containing 10% neutral buffered formalin until further processing.

Cores were mounted on an embedding mould using Kerr's "greenstick" dental impression compound and sectioned in the coronal plane as near as possible to the mid-line using a Leitz 1600 Saw Microtome. Sections 60 to 80 μm thick were cut from each core and dehydrated through alcohol, cleared in xylene, and mounted with DePeX on glass slides.

Randomly chosen sections were viewed under a Zeiss polarizing photomicroscope at low power magnification. A camera lucida was attached to the photomicroscope and a 42 point test grid superimposed on to the test area measuring 0.85 × 0.83 mm (Fig. 1).

Microscopic investigations involved examination of four parameters—the fractional volumes of primary osteons, secondary osteons, secondary osteon fragments and lamellar bone.

These fractional volumes were determined using the formula $Va = Pa/Pt$. Where *Va* is the fractional volume of structure "*a*" (primary osteons, secondary osteons, secondary osteon fragments, or lamellar bone), *Pa* is the number of test points falling on structure "*a*," and *Pt* is the total number of test points. ArcSin square root fractional volumes for secondary osteon fragments and lamellar bone were analyzed because of the nature of the probability distributions for these two variables.

Examinations of the ectocranial and endocranial tables were made from three random fields in each section. In each field, the fractional volumes of all four parameters were calculated.

Three preliminary statistical analyses were conducted to compare the two tables with respect to the regression of each of three of the microscopic variables on age. This was done by way of a modified analysis of covariance, where age is a subject covariate. There is no subject factor as such, but there is a split-unit factor within each subject namely the two tables. Values for R^2 (the coefficient of determination) were calculated to assess the percentage of variability attributed jointly to the non-random sources in the analysis.

Secondary analysis involved linear regressions for each ectocranial and endocranial table separately. From the regression output, equations for the prediction of age were generated as well as R^2. A canonical correlation analysis of the three parameters: fractional volume of secondary osteons, of osteon fragments, and of lamellar bone was also conducted.

Mean fractional volumes of the three histological parameters were also examined for correlation with each other, adjusting for any correlation that they would have with age. This was followed by a multivariate canonical correlation analysis, to examine any relationship that the three variables in combination, might have

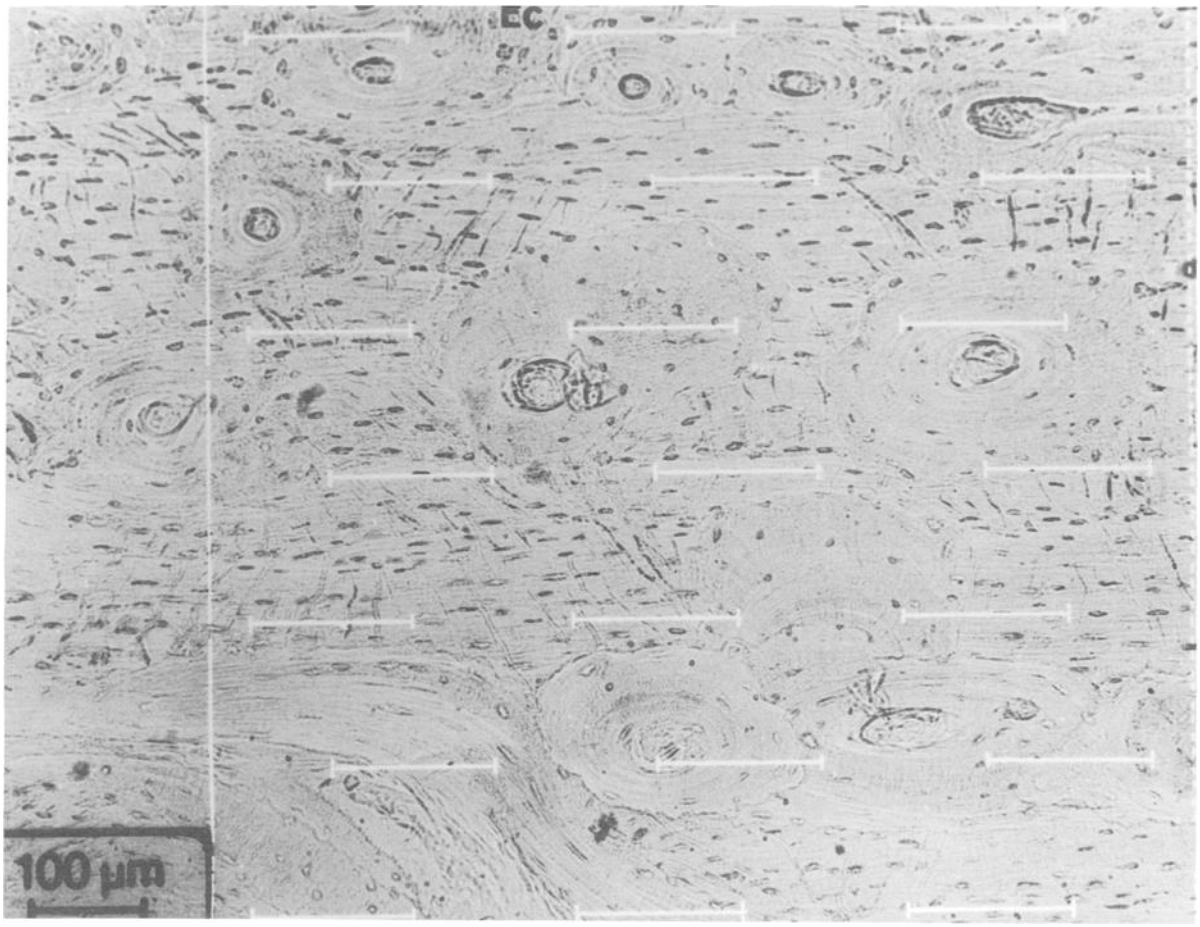

FIG. 1—*Polarized light micrograph of the 42 point test grid used for histomorphometric analysis superimposed on to a test area. The edge of the grid lies over the ectocranial surface of the bone (Ec).*

with age. The combinations examined were all three together, and three combinations consisting of two of the variables at a time. Analyses were done for each table separately.

Results

Primary Osteons

These are formed by the inclusion of small blood vessels within newly forming bone which results from the rapid expansion in diameter of cortical bone associated with young growing bone. Because they are not the product of bone remodeling they do not show a cement line (Fig. 2).

With increasing age the fractional volume of primary osteons was found to decrease in both cortical tables of bone so that after age forty, only one non-zero fractional volume was recorded (Fig. 3). The predominantly zero fractional volume meant that no statistical quantification could be done.

Secondary Osteons

These appear in cross section as vascular canals surrounded by concentric layers of bone that end at cement lines (Fig. 2). Each cement line represents the reversal phase of bone remodeling from one of bone resorption by osteoclasts to that of bone deposition by osteoblasts.

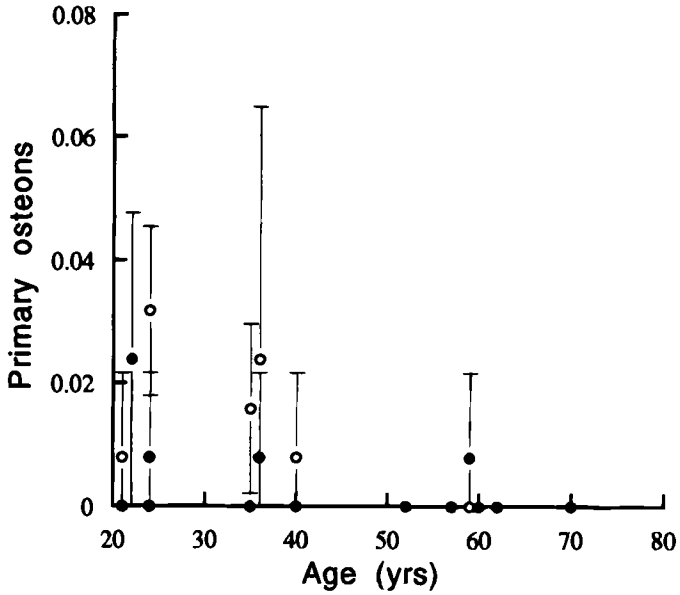

FIG. 3—*Scatter plot of the mean fractional volume of primary osteons versus known-age for the inner and outer cortical tables of bone. Solid dots represent the inner table.*

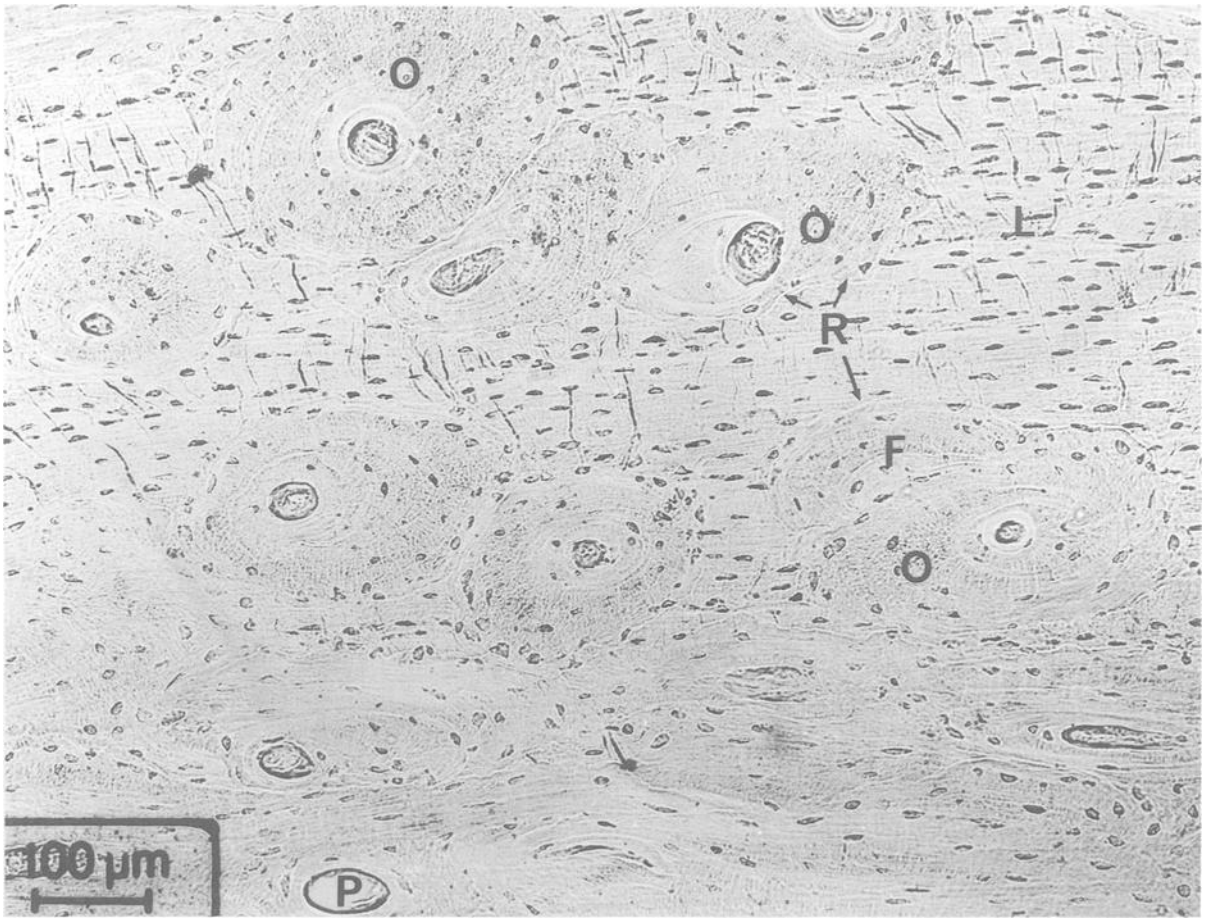

FIG. 2—*Polarized light micrograph of the ectocranial table of bone. Primary osteons* (P) *lack the reversal lines* R *seen surrounding secondary osteons* (O) *and fragments* (F). *Lamellar bone is also present* (L).

As age increases there is no significant change in the fractional volumes of secondary osteons (Fig. 4).

Separate regressions for both cortical tables show that with age, the fractional volumes of secondary osteons decreases non-significantly (Table 2) (Fig. 4). Of this decrease, regression with age accounts for no more than 10.3% of the variation (Fig. 4).

Secondary Osteon Fragments

These are remnants of secondary osteons that remain after they themselves have been largely remodeled (Fig. 2).

With age, the fractional volume of secondary osteon fragments significantly increases (Table 3). This is in response to the continuing bone remodeling that occurs throughout life.

Separate linear regression analysis for both cortical tables shows that despite having significant regression line slopes, the large amount of nonlinear, random variation associated with this parameter makes it a poor age estimator (Fig. 5). At best, regression on age accounts for only 43.6% of the change in secondary osteon fractional volume (Fig. 5).

TABLE 3—*Analysis of split-unit covariance for osteon fragment fractional volume measurements.*

Source of Variation	DF	SS	P > F
Age	1	1.111	0.002[b]
Dev. from regress.	15	1.285	0.0001[b]
Table (Adj)	1	0.080	0.038[a]
Table × Age	1	0.075	0.176 N.S.
Table × Dev from regress.	15	0.558	0.022[a]
Error	68	1.218	
Total	101	4.253	
R^2 (%)	71.4		

NOTE: Results from. DF: degrees of freedom. SS: sums of squares. P > F: probability value. R^2: coefficient of determination.
[a]significant $P < 0.05$
[b]significant $P < 0.01$
N.S. not significant
Tab. (Adj): Comparison of ectocranial and endocranial tables after adjusting for age.

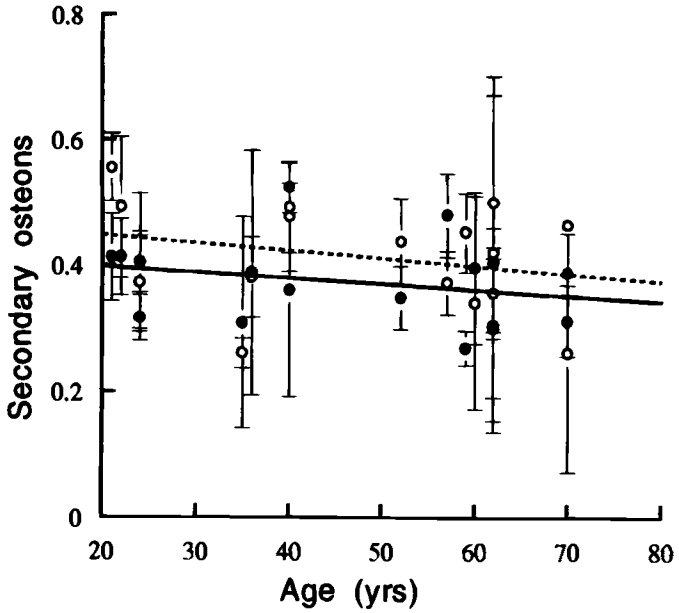

FIG. 4—*Scatter plot of the mean fractional volume of secondary osteons versus known-age for the inner and outer cortical tables of bone. Solid line represents regression for the inner table (Y = 0.416 − 0.0009X, R^2% = 5.6). Dotted line represents regression for outer table (Y = 0.481 − 0.001X, R^2% = 10.3). Solid dots represent the inner table.*

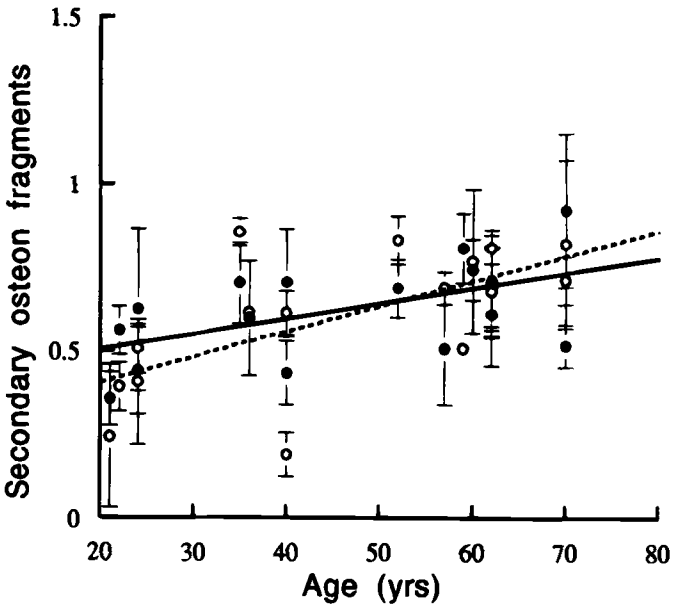

FIG. 5—*Scatter plot of the mean fractional volume of secondary osteon fragments versus known-age for the inner and outer cortical tables of bone. Solid line represents regression for the inner table (*Y = 0.412 + 0.004X, R^2% = 30.1). Dotted line represents regression for outer table (**Y = 0.247 + 0.008X, R^2% = 43.6). Solid dots represent the inner table. * $P < 0.05$, ** $P < 0.01$.*

TABLE 2—*Analysis of split-unit covariance for secondary osteon fractional volume measurements.*

Source of Variation	DF	SS	P > F
Age	1	0.042	0.160 N.S.
Dev. from regress.	15	0.288	0.114 N.S.
Tab. (Adj)	1	0.012	0.321 N.S.
Tab. × Age	1	0.002	0.669 N.S.
Tab. × Dev	15	0.197	0.414 N.S.
Residual error	68	0.846	
Total	101	1.412	
R^2 (%)	40.1		

NOTE: DF: degrees of freedom. SS: sums of squares.
P > F: probability value. R^2: coefficient of determination.
N.S. not significant
Tab. (Adj): Comparison of ectocranial and endocranial tables after adjusting for age.

Lamellar Bone

This is identified by its even, regular, and layered arrangement of compact bone (Fig. 2). It is a prominent feature of the juvenile skeleton and is produced particularly during the rapid increase in cortical thickness of immature bone.

With age, the fractional volume of lamellar bone significantly decreases as more and more bone becomes remodeled and replaced by secondary osteons (Table 4). Separate regression analyses for each cortical table indicates that the fractional volume of lamellar bone in the outer table decreases significantly with age (Table 4)

TABLE 4—*Analysis of split-unit covariance for lamellar bone fractional volume measurements.*

Source of Variation	DF	SS	P > F
Age	1	0.502	0.0116[a]
Dev. from regress.	15	0.912	0.0001[a]
Table (Adj)	1	0.040	0.079 N.S.
Table × Age	1	0.070	0.208 N.S.
Table × Dev from regress.	15	0.603	0.0001[a]
Error	68	0.860	
Total	101	2.965	
R^2 (%)	71.0		

NOTE: DF: degrees of freedom. SS: sums of squares.
P > F: probability value. R^2: coefficient of determination.
[a]significant P < 0.01
N.S. not significant
Tab. (Adj): Comparison of ectocranial and endocranial tables after adjusting for age.

TABLE 5*a*—*Partial correlation coefficients (and probability values) for the fractional volumes of secondary osteon fragments, secondary osteons and lamellar bone taken from the inner table.*

	Osteon fragments	Secondary osteons	Lamellar bone
Osteon fragments		−0.7518 (0.0008)	−0.3536 (0.1791)
Secondary osteons			−0.0371 (0.8914)

TABLE 5*b*—*Partial correlation coefficients (and probability values) for the fractional volumes of secondary osteon fragments, secondary osteons and lamellar bone taken from the outer table.*

	Osteon fragments	Secondary osteons	Lamellar bone
Osteon fragments		−0.5945 (0.0151)	−0.7344 (0.0012)
Secondary osteons			−0.0639 (0.8141)

(Fig. 6). The inner table did not demonstrate a significant change. A large amount of nonlinear, random variability is associated about these regression lines, which makes them poor age estimators. At best, age explains 29.0% of the changes in lamellar bone fractional volume (Fig. 6). Three fields did not seem to be sufficient to estimate the mean parameters precisely for each individual. Coefficients of variation ranged from 20.4% to 32.5% for the two tables and three parameters.

Multivariate Analysis

With the inner table, only the fractional volumes of secondary osteon fragments and secondary osteons were significantly partially correlated with age ($r = -0.7518$, $P = 0.0008$) (Table 5*a*). Their joint canonical correlation with age is $r = 0.6047$, which is just significant ($P = 0.0413$) (Table 6*a*). Age accounts for $R^2 = 36.6\%$ of the variability in the resultant canonical variable which, after re-scaling, is a linear combination of approximately the mean

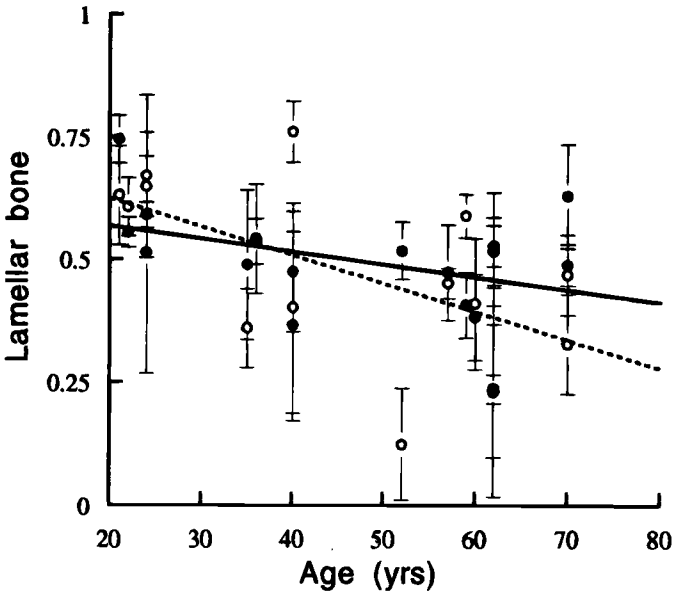

FIG. 6—*Scatter plot of the mean fractional volume of lamellar bone versus known-age for the inner and outer cortical tables of bone. Solid line represents regression for the inner table (Y = 0.619 − 0.003X, $R^2\%$ = 16.0). Dotted line represents regression for outer table (*Y = 0.737 − 0.006X, $R^2\%$ = 32.2). Solid dots represent the inner table. * P < 0.05.*

TABLE 6*a*—*Single correlations and canonical correlations from analysis 1–4 of fractional volumes of secondary osteon fragments, secondary osteons and lamellar bone with age, in the inner table.*

Variable	Probability	*r*	$R^{2\%}$	Eigenvalue
Osteon fragments (OF)	0.0224	0.5486	30.1	n/a
Secondary osteons (SO)	0.3617	0.2366	5.6	n/a
Lamellar bone (LB)	0.1112	0.4000	16.0	n/a
Analysis 1	0.1050	0.6051	36.7	0.5778
Analysis 2	0.0413	0.6047	36.6	0.5764
Analysis 3	0.0645	0.5693	32.4	0.4795
Analysis 4	0.2053	0.4530	20.5	0.2582

NOTE: Canonical variables from analysis 1–4 and raw coefficients:
Analysis 1 = 11.0×OF + 10.7×SO − 0.6×LB.
Analysis 2 = 11.5×OF + 11.3×SO.
Analysis 3 = 6.6×OF + 3.2×LB.
Analysis 4 = 7.6×SO + 8.2×LB.

TABLE 6*b*—*Single correlations and canonical correlations from analysis 1–4 of fractional volumes of secondary osteon fragments, secondary osteons and lamellar bone with age, in the outer table.*

Variable	Probability	*r*	$R^{2\%}$	Eigenvalue
Osteon fragments (OF)	0.0039	0.6603	43.6	n/a
Secondary osteons (SO)	0.2085	0.3209	10.3	n/a
Lamellar bone (LB)	0.0176	0.5675	32.2	n/a
Analysis 1	0.0283	0.7004	49.0	0.9628
Analysis 2	0.0147	0.6727	45.3	0.8266
Analysis 3	0.0178	0.6615	43.8	0.7782
Analysis 4	0.0339	0.6192	38.3	0.6220

NOTE: Canonical variables from analysis 1–4 and raw coefficients:
Analysis 1 = 17.2×OF + 17.3×SO − 9.3×LB.
Analysis 2 = 7.3×OF + 4.0×SO.
Analysis 3 = 5.8×OF − 0.7×LB.
Analysis 4 = 6.2×SO + 6.1×LB.

of the two fractional volumes. This compares with an R^2 of 30.1% ($P = 0.0224$) for the fractional volume of the osteon fragments on their own (Table 6*a*).

With the outer table, the fractional volume of the secondary osteon fragments was partially correlated with that of both secondary osteons and lamellar bone ($r = -0.5945$, $P = 0.0151$ and $r = -0.7344$, $P = 0.0012$, respectively) (Table 5*b*). The canonical correlation of all three with age is $r = 0.7004$, $P = 0.0283$ (Table 6*b*). 49% (R^2) of the variability in the canonical variable is attributable to age, compared with an R^2 of 43.6% ($P = 0.0039$) for the fractional volume of secondary osteon fragments on their own. The canonical variable consists of approximately the mean fractional volume of secondary osteons and secondary osteon fragments plus 0.5 × the fractional volume of lamellar bone. However any combination of two of the histological variables performs equally as well. In particular, a canonical variable consisting of the sum of approximately the fractional volume of secondary osteons and 2 × the fractional volume of secondary osteon fragments, results in a canonical correlation of $r = 0.6727$, $R^2 = 45.3$, $P = 0.0147$ (Table 6*b*).

Discussion

Fractional Volume of Primary Osteons

Primary osteons are formed following rapid accumulation of new periosteal bone. As a result, they are more likely to be found in young, rapidly growing skeletons. The presence of many primary osteons in the bone of animals such as dogs and monkeys during the rapid growth phase of development supports this idea [*15*].

With age, primary osteons may become remodeled and replaced by secondary osteons, producing reduced numbers in the cortical bone layers and eventually, primary osteons may disappear completely [*15*].

The failure of researchers to distinguish primary osteons from secondary osteons has meant they have received scant attention in aging studies [*2,16,17*]. Kerley [*2*] showed that the number of primary osteons in long bone sections was high in infancy and childhood, decreased in adolescence, and disappeared completely around 55 years of age.

The present study on the occipital bone indicates that from age 20 years onwards, there is a continual decrease in the fractional volume of primary osteons in both cortical tables (Fig. 3). After 40 years of age, the fractional volume is essentially zero. Also, there is a higher fractional volume of primary osteons in the outer table than the inner table. It seems reasonable to suggest that the greater prevalence of primary osteons in the outer table is related to the tensile forces associated with nuchal muscle actions, since groups of primary osteons are frequently seen in tubercles and other prominent bony processes [*18*]. This might also be linked to the nutrient needs at insertion sites of muscle tendons.

The decrease in fractional volume of primary osteons with age, as reported in this study, is indicative of the growth processes. Once the cranium reaches its adult form, the rate of surface apposition of bone is reduced under normal conditions. Instead, the process of remodeling, whereby intracortical tissue is replaced in response to physical and environmental conditions, becomes dominant. This 'internal' remodeling is described by Lacroix [*19*] as an essential mechanical and metabolic function necessary for the repair of microdamage and for the storing and releasing of calcium.

Fractional Volume of Secondary Osteons

The secondary osteon, unlike the primary osteon, is formed by a remodeling process involving osteoclastic resorption around a vascular canal and subsequent osteoblastic deposition [*20*]. Osteons produced from this remodeling process represent a durable and lasting record of past remodeling history, which can be used to estimate age [*21*].

In the past, researchers have used counts of osteon number rather than fractional volume to estimate age [*2,3,6,22*]. The percentage of bone covered by osteons has also been combined with that of osteon fragments [*5*]. Clarke [*23*] was the first to use the fractional volume of osteons, fragments and lamellar bone to estimate age in the human parietal bone.

With age, the number of osteons reported in cross sections of human femur increases [*2,3,6,22*]. This increase occurs through continual replacement of lamellar bone at a rate exceeding its formation [*2–5*], resulting in a net decrease in the total amount of lamellar bone [*20*].

In the occipital bone the fractional volume of osteons within the cortical bone layers does not change significantly with age (Table 2) (Fig. 4), a result similar to that obtained by Clarke [*23*] in the parietal bone. Because the fractional volume of osteon fragments significantly increases with age (Table 3) (Fig. 5) there is reason to believe that osteons of the occipital bone do participate in the replacement of aging bone. The stable nature of osteon fractional volume reported in this study may occur if remodeling produces an increased fractional volume of smaller sized osteons, which explains the subsequent increase in osteon fragment fractional volume. The same could be said if remodeling simply replaced pre-existing osteons.

Fractional Volume of Secondary Osteon Fragments

With age, there is an increase in the amount of extra-Haversian bone [*4*]. Clarke [*23*] considers this extra-Haversian bone to be mainly osteon fragments. Sharpe [*20*] reported that "fragments are portions of old osteons that may surround the peripheries of newer osteons. The number of osteon fragments increase as more and more osteons are layed down until, in extreme old age, most of the complete osteons are surrounded by fragments of older ones." Also, Kerley [*2*] noted an increase in the number of osteon fragments with age and Ahlqvist and Damsten [*5*] recorded an increase in the percentage of these structures.

With age, the fractional volume of osteon fragments was found to increase in the occipital bone (Table 3) (Fig. 5). This increase is significant for both the inner and outer cortical tables, and occurs at the expense of lamellar bone (Figs. 5, 6) [*2,5*]. Based on the interpretations of this study, an increase in the number of osteons with age would naturally increase the number of resulting fragments, until a saturation point was reached. At this point the fractional volume of lamellar bone would be low, since for every new osteon produced, an entire fragment would become remodeled, producing no further increase in the fractional volume of osteon fragments.

Fractional Volume of Lamellar Bone

Lamellar bone comprises the great bulk of the immature skeleton [*17,18*]. Studies on long bones indicate that with increasing age, there is a gradual decrease in the amount of lamellar bone as more becomes replaced by osteons and fragments [*2,5*].

Clarke [*23*] found that in the parietal bone there was a significant

decrease in lamellar bone with age, suggesting that intracortical remodeling in the cranium proceeds in a similar manner to long bones. Based on this, it is not surprising that in the occipital bone there is a significant decrease in the fractional volume of lamellar bone with age (Table 4) (Fig. 6). Separate analysis of the two cortical tables of bone indicated that the outer table decreased significantly with age, while the inner table remained essentially unchanged (Fig. 6). Previously, it was suggested that this might result from increased remodeling in the outer table due to muscle tensions acting on this table. Clarke [*23*] suggested the presence of osteoclastic activity in the outer table indicated surface resorption of lamellar bone.

Recently, Clarke [*23*] used a radiographic and histomorphometric analysis to estimate age from the human parietal bone. His results suggest that after 50 years of age a large amount of unexplainable variation occurs in both radiographic and microscopic parameters with age. Consequently, the use of the parietal bone to estimate age is not recommended. It is apparent from the findings of this study that the occipital, like the parietal bone, contains a large amount of random variability making its use for age estimation limited.

Multivariate Analysis

The possibility that the fractional volume of secondary osteons, secondary osteon fragments, and lamellar bone covaried was examined in order to determine whether these parameters could be combined to improve age estimates.

Although slight improvements did result from combining parameters, they were neither sufficient to improve the correlation with known-age nor large enough to support further examination. In fact, it simply highlights the abundant variability in all the parameters examined. Clearly, combining the parameters provides no better estimate of known-age than separate analysis of each parameter.

Summary

The results of this present study indicate that age estimations based on histological parameters are less reliable for the occipital bone than for long bones. This is consistent with the findings of Clarke [*23*], in the parietal bone. With age, the fractional volume of primary osteons decreases to achieve a zero fractional volume after age forty. The fractional volume of secondary osteons in the occipital bone is of little use as a parameter for age estimation as it does not significantly change with age. Also, no significant changes result from separate analysis of both cortical tables of bone, and there is a large amount of unexplained variation in the measurements. The fractional volume of secondary osteon fragments, although it significantly increases with age, contains a significant amount of individual variability and random variation when each cortical table is analyzed separately. Similarly, a significant amount of individual variability and random variation, is present in the measurements of lamellar bone fractional volume for both cortical tables. This reduces the accuracy with which lamellar bone fractional volume measurements can be used to estimate age.

Combining the histological parameters does not significantly improve the correlation between estimated-age and known-age. It is only slightly better than separate analysis of each parameter for both cortical tables.

These results compare unfavorably with Kerley's [*2*] study of long bones of the lower limb, where up to 94% of the variability (number of secondary osteon fragments) associated with estimating age-at-death can be explained using regression. The results of this study of the occipital bone preclude the use of these parameters (both separately and combined) in age estimations.

Acknowledgments

This study was made possible by the use of material from the Institute of Forensic Pathology, Queensland State Health Department. Special thanks to all the staff for their encouragement and support of this research.

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