

The Microscopic Determination of Age in Human Bone

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ABSTRACT The microscopic age changes that occur in human cortical bone were measured by counting the number of osteones, osteon fragments and non-Haversian canals, and estimating the percentage of lamellar bone in four selected 100 power fields in the outer third of the cortex in ground sections from the mid-shaft of the femur, tibia and fibula. Age graphs were derived from 126 specimens covering the age range from birth through 95 years. The best correlation with age by regression analysis was found in the osteon fragment number in the fibula ($r = 0.974$). The use of the regression formula for this factor permits the age to be estimated within $\pm$ ten years of the actual age 95% of the time.

An alternative method utilizes a specially devised profile chart to estimate age from the age change graphs. When this method was checked on 56 additional specimens, 87.3% of the estimates were within $\pm$ five years of the actual age, and all estimates were within $\pm$ ten years.

It was concluded that there are measurable microscopic age changes in the cortex of the major long bones of the leg that can be used to estimate the age of an individual with known accuracy over the entire range from birth to 95 years. This method can be applied to fragmented and incomplete skeletons as well as eroded archeologic skeletons, since only the mid-shafts of leg bones are used.

The traditional methods of determining the age at death of skeletal remains are limited in application by the condition of the remains and in accuracy by the age of the individual. The most useful indicators of age are often missing or obliterated in fragmented, eroded, or incomplete skeletons. In addition, the accuracy of the estimate depends greatly on the experience of the examiner — particularly when dealing with the remains of older individuals. The age at death (Stewart, '54) (Krogman, '62) can be determined with fair accuracy by epiphyseal closure up to age 25 and by the pubic symphysis to about age 50 (McKern and Stewart, '57) (Brooks, '55). Estimates over 50 years of age are based on such intuitive criteria as arthritic lipping and the texture and weight of the bones, or on such unreliable criteria as the closure of the cranial sutures (Singer, '53).

Hansen ('54) and Schranz ('59) have presented methods for determining age from the amount and arrangement of cancellous bone in the proximal end of the humerus, where it decreases with advancing age. However, the reliability of estimates based on this method is undetermined, and the method itself can be

applied only to specimens where the proximal end of the humerus is present and intact.

Amprino and Bairati ('36) and Jowsey ('60) have demonstrated that there are microscopic changes in structure that occur at the midshaft of the femur as age increases. If these changes could actually be used to determine the age at death, they would provide the basis for an excellent supplement to conventional methods of age determination. Only a small piece of the shaft of a long bone would be needed, so that fragmented, eroded, or incomplete skeletons could be aged, as well as those of old individuals.

MATERIAL AND METHOD

In order to quantitate these microscopic age changes and establish a system of age determination based on them, 126 ground cross-sections of the femur, tibia and fibula were examined microscopically. These specimens were removed from the mid-shaft of the bone and were well-documented as to age, sex, and clinical history. Only bone that was distantly removed from any pathology and appeared normal grossly, radiographically, and microscopically was used to establish the age curves. Sixty-

TABLE 1
Age and sex distribution

Bone	Age										
	0	10	20	30	40	50	60	70	80	90	100
Femur	♀			♀					♀		
	*	♂	♂♂	♂					♂♂		
	*	♀	♀♂♂♂♂♂♂	♀♂	♀	♂♂♂♂♂♂	♂	♀♂♂♂♂♂♂	♂♂♂♂♂♂	♂	♂
	*	♀	♀♂♂♂♂♂♂	♂*	♀	♂♂♂♂♂♂	♂	♀♂♂♂♂♂♂	♂♂♂♂♂♂	♂	♂
Tibia	♀	*		♂							
			♀	♂							
			♀♂♂♂♂♂♂	♀♂	♂♂♂♂♂♂	♂		♂		♀	
			♀	♂		♀	♂	♂♂♂♂♂♂	♂♂♂♂♂♂	♂	♂
Fibula	♀	*									
			♀	♂							
			♀♂♂♂♂♂♂	♂	♂	♂	♂	♂	♂	♂	♀

* Sex unknown.

four of the specimens were age 30 or older, and all decades of life from birth through age 95 were represented in the entire series. There were 88 males and 29 females. Sex was not recorded for nine. Table 1 shows the age and sex distribution of the sample.

Preliminary microscopic examination of the slides grouped in chronologic series revealed that the structural changes normally associated with age seemed better spaced to cover the total life span in the outer third of the cortex than in the middle or inner thirds. Also, the outer third is the least affected by resorptive changes in normal bone. From the preliminary microscopic examination it appeared that four factors in particular were consistently associated with age and could be observed quantitatively through a standard laboratory microscope with wide-field eyepieces:

1. The number of osteones.
2. The number of fragments of old osteones.
3. The percentage of circumferential lamellar bone.
4. The number of non-Haversian canals.

Osteones. An Osteon, or Haversian system (fig. 1-a), is easily recognized in cross-section as a vascular canal surrounded by

concentric lamellae, which contain rather evenly spaced osteocytes in their lacunae. Around the entire periphery of the osteon there is a reversal line that marks the area where osteoclastic resorption stopped and was followed by new bone formation. Unless otherwise specified, osteon refers to secondary, remodeled bone.

Fragments. As osteoclasts burrow channels through Haversian bone, fragments of old osteones (fig. 1-b) may surround the edge of the channel and remain after resorption ceases and replacement begins. These fragments increase in number with age as more and more old osteones are partly destroyed. In old age, virtually every complete osteon is surrounded by the fragments of several older ones.

Circumferential lamellar bone. Circumferential lamellar bone (fig. 1-c) is composed of evenly spaced bands, or lamellae, that run parallel to each other around the outer part of the cortex. It appears as birefringent sheets in polarized light and can be distinguished by its long, parallel fibers. Circumferential lamellar bone is a prominent feature of childhood.

Non-haversian canals. All primary vascular channels, including those that have filled in partly with concentric lamellae to



Fig 1a An osteon.

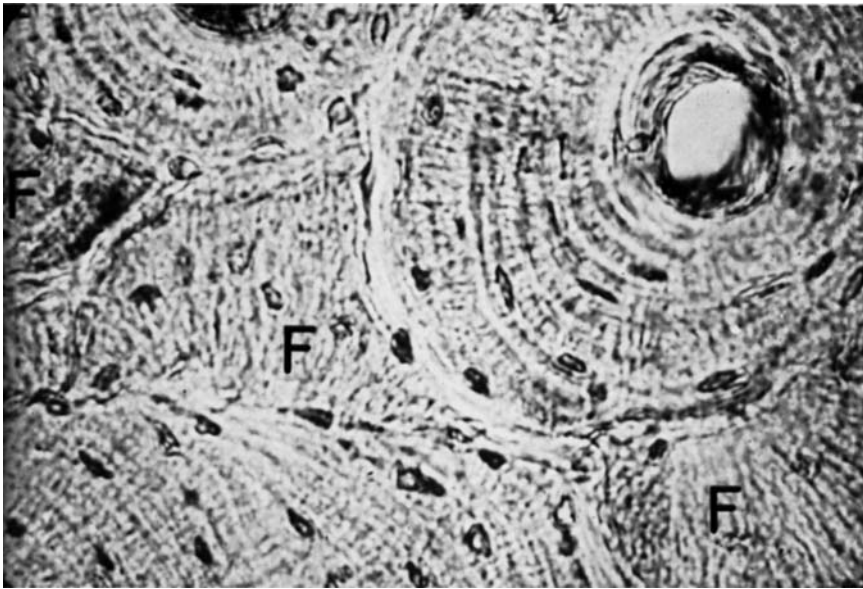


Fig. 1b Fragments of old osteones (F).

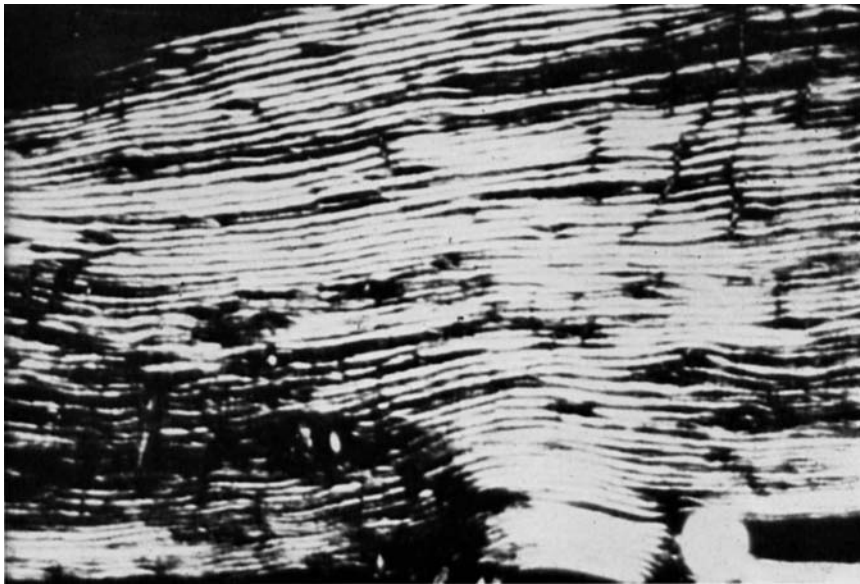


Fig. 1c Circumferential lamellar bone.

form primary osteones or pseudo-Haversian systems, are vascular canals that were formed by the inclusion of small, peripheral blood vessels into the bone by the rapid expansion of the cortex in diameter (fig. 1-d). Since these canals were formed

at the time the surrounding lamellar bone was formed, they are primary and represent areas of unremodeled bone. The secondary osteon is formed in a space left by osteoclastic resorption and represents internal remodeling of the bone. When

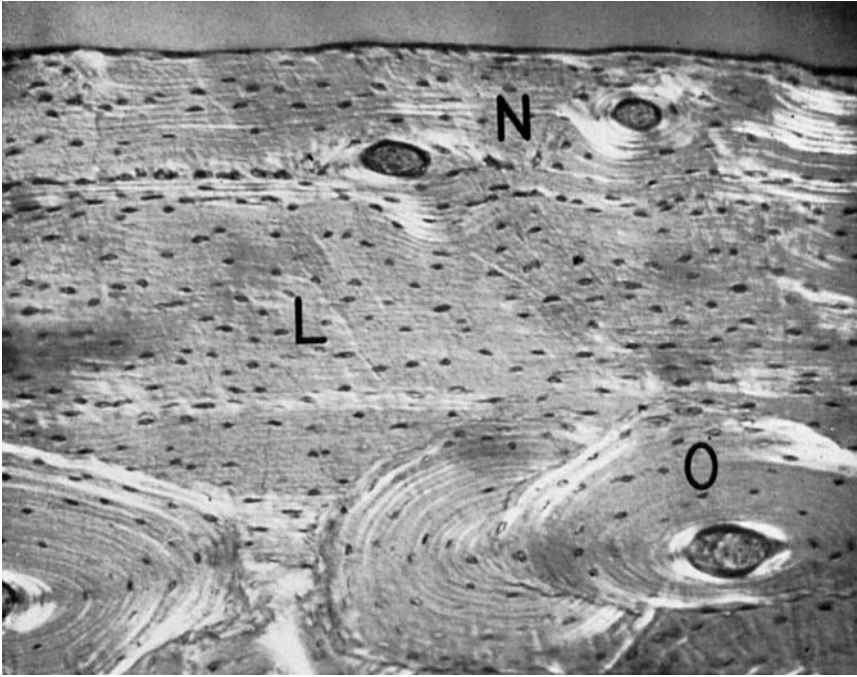


Fig. 1d Non-Haversian canals (N) in circumferential lamellar bone (L); osteones (O).

viewed by polarized light, the primary osteon can be distinguished from the secondary osteon by the lamellar bone surrounding it. The primary osteon has no sharp reversal or cement line around it, and the surrounding lamellae bend smoothly around the outer limits. In areas of strong tendon attachment and in youthful specimens, where the surrounding bone is of a coarse woven texture rather than lamellar, the junction between the concentric lamellae and surrounding bone is vague and poorly defined. In the secondary osteon, the lamellae surrounding it run straight to the reversal line and stop abruptly, where they were bitten off by osteoclasts during the resorptive phase.

Age changes. The life cycle of cortical bone (fig. 2) begins with the rapid formation of radial spicules of coarse woven bone with large spaces in between (fig. 2a). These spaces fill with less-rapidly constructed concentric lamellar bone. As the cortex and medullary cavity expand in diameter, more mature circumferential lamellar bone is formed at a slower rate peripherally, and some lamellar bone is

deposited endosteally. As the cortex expands by the peripheral deposition of layer upon layer of circumferential lamellar bone, small sub-periosteal blood vessels

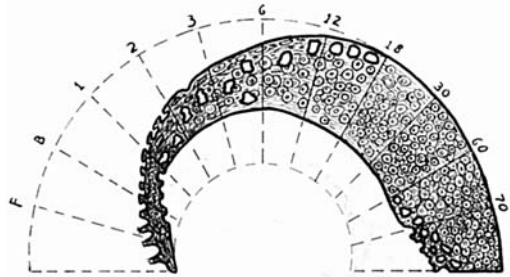


Fig. 2a Cross-sectional age changes in the femoral cortex.

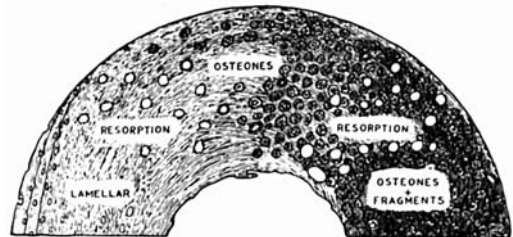


Fig 2b The life cycle of bone.

running along the long axis of the bone are encircled by the advancing periphery of the cortex. Osteoclasts burrow longitudinal channels down through the thickening cortex, which appear as ragged holes in cross-sections (fig. 2b). Osteoblasts fill these resorptive channels with new bone in the form of concentric lamellae, forming osteones. These osteones are in turn partly destroyed by subsequent resorption, and the undestroyed portions remain as fragments surrounding the new osteon that is formed as that channel fills in.

The number of osteones, the number of fragments, the amount of lamellar bone, and the number of non-Haversian canals were examined in a 100-power wide field in each of four anatomic areas in the outer third of each cross-section: anterior, posterior, medial, and lateral (fig. 3). The fields were selected in such a manner that they touched upon the outer edge of the bone and were fairly representative of the particular anatomic area of the section being examined. Each microscopic field covered a circular area of the cross-section that was 1.25 mm in diameter. That was the area covered by a ten-power objective lens combined with ten-power, wide-field oculars.

The total number of recognizable osteones, including those that were partly ob-

scured by the periphery of the field, was counted in each field. Then this number was totalled for all four areas to arrive at a single figure representing the osteon number for the outer zone of that particular section. The same procedure was used for counting osteon fragments and non-Haversian canals. The percentage of each field that was composed of circumferential lamellar bone was estimated, and the four fields were averaged — rather than totalled — to arrive at a figure that was representative of the outer zone lamellar bone.

RESULTS

The composite value for each outer zone factor in all four fields was plotted against age for each bone, using intact normal specimens only. The age change graphs were essentially similar for all three of the major long bones of the leg, although the actual numbers varied slightly from bone to bone. In the femur (fig. 4) the osteones increased in number with an increase in age from birth through age 95. The number of fragments of old osteones in the outer third of the femur increased with advancing age, also. The percentage of circumferential lamellar bone was high in childhood and decreased with advancing age. While it was virtually absent in some specimens, it was present in others up through age 95. Following much the same pattern as lamellar bone, the number of non-Haversian canals was quite high in infancy and childhood, decreasing in adolescence and disappearing completely around age 55. The same patterns of age change were found in the tibia (fig. 5) and in the fibula (fig. 6).

The data were subjected to regression analysis, both linear and curvilinear as appropriate. All showed significant correlations between age and the outer zone factors. The most significant correlation was afforded by the number of osteon fragments in the outer third of the fibula. The regression formulae for estimating age from the value of each outer zone factor, the standard error of the estimate, and the correlation coefficient are shown in table 2 for the three major long bones of the leg. In general, the number of osteones and osteon fragments were better correlated

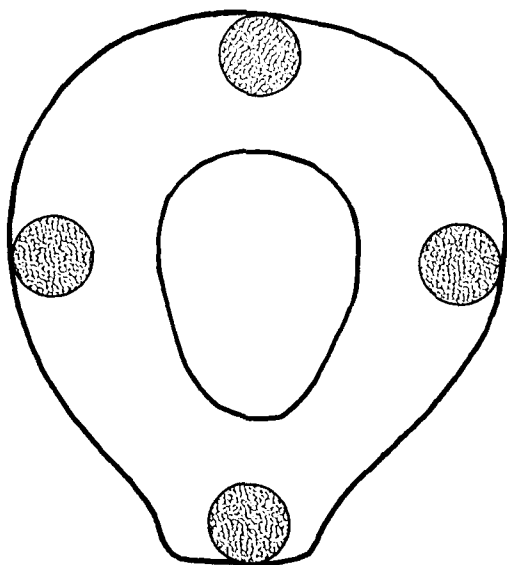


Fig. 3 Location of four 100-power fields.

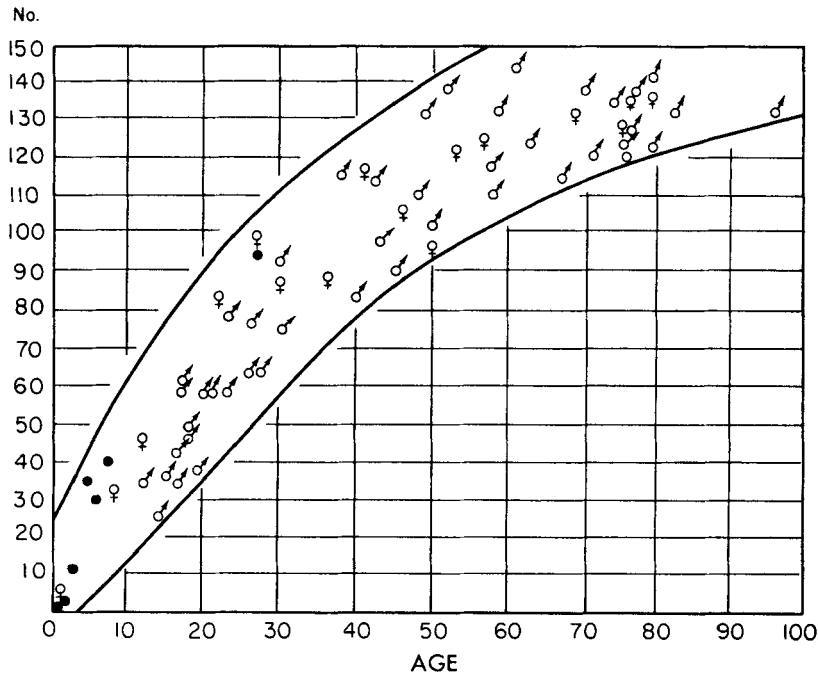


Fig. 4a Femoral osteones.

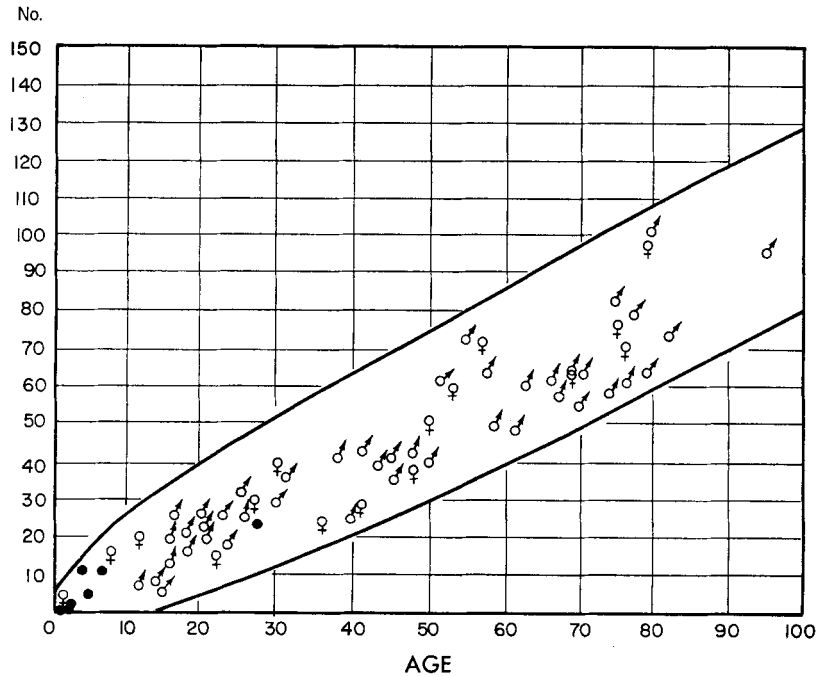


Fig. 4b Femoral fragments.

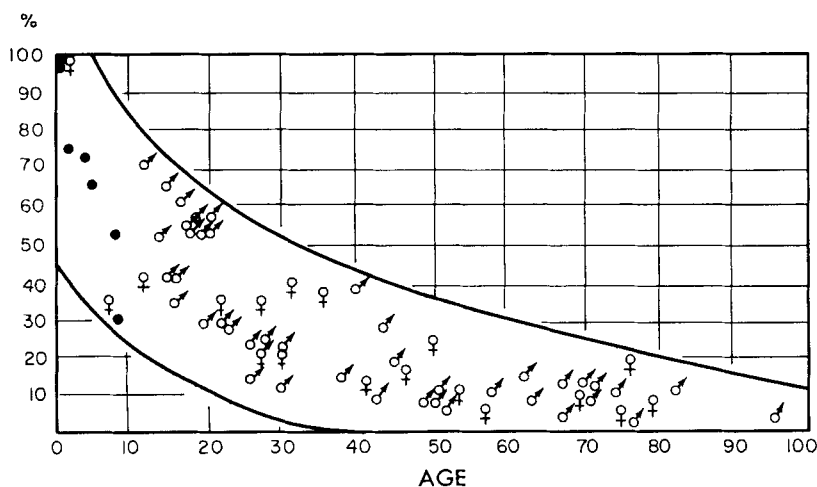


Fig. 4c Femoral lamellar bone.

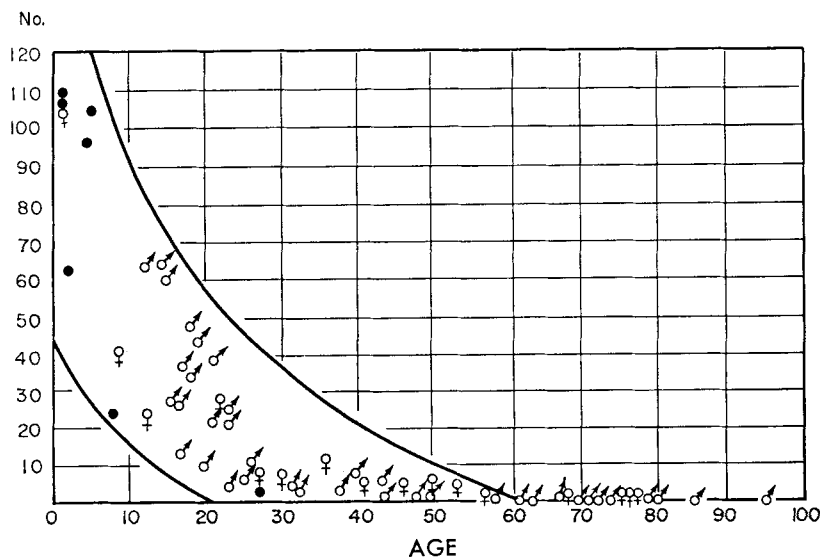


Fig. 4d Femoral non-Haversian.

with age than were lamellar bone or non-Haversian canals.

When the regression formulae in table 2 are used to estimate age from outer zone factor values and two standard errors of the estimate are used as the limits of confidence, the actual age should fall between the plus or minus two standard error limits 95% of the time. These limits of confidence are rather good for the number of fragments in the fibula, having a range of

± 10.54 years. However, the range between the limits of confidence was fairly wide for the rest of the factors in all three bones. No single factor other than fibular osteon fragments had a range of confidence that was sufficiently narrow to afford a particularly accurate estimate of the age by itself.

Since the selection of fields and counting of fragments are inevitably somewhat subjective in nature, the use of multiple factors might afford greater reliability than

PLATE 1

EXPLANATION OF FIGURES

- 1 Section of the skin of the ischial callosity showing the phosphorylase and amylo-1,4-1,6-transglucosidase of the epidermis. ca. $\times 100$.
- 2 Alkaline phosphatase reactive capillaries of the subepidermal arcades in the papillary layer of the ischial callosity. The arrow indicates a sinusoid which also has an endothelium reactive for alkaline phosphatase. ca. $\times 100$.
- 3 Section from the chest showing both organized nerve end-organs and nerve fibers which contain acetylcholinesterase. ca. $\times 100$.

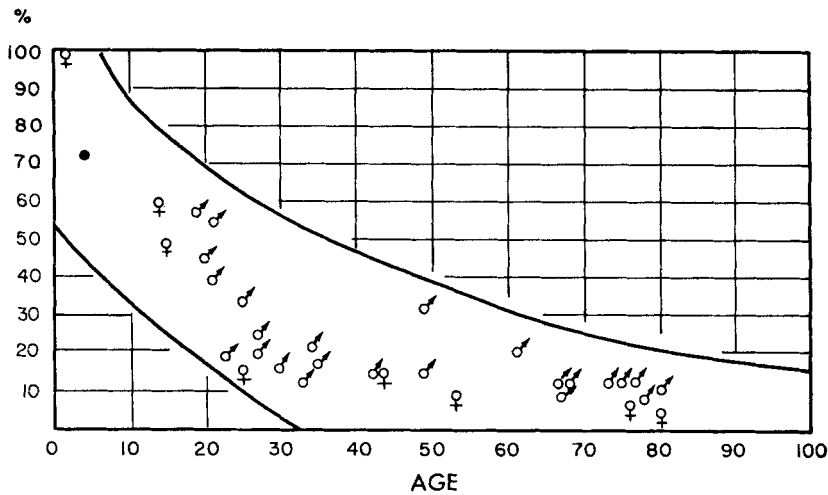


Fig. 5c Tibial lamellar bone.

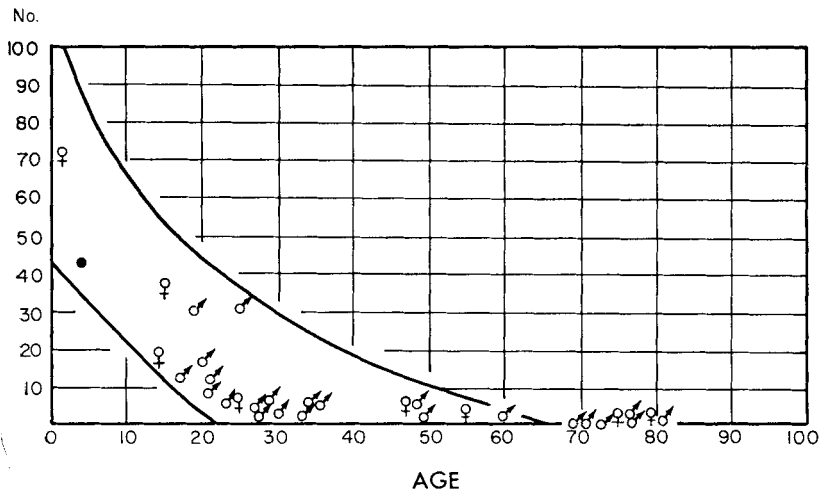


Fig. 5d Tibial non-Haversian.

the space at the right. When this was done for all four factors, only a certain portion of the age scale was covered by parts of all age range lines. Vertical lines were drawn to delineate the range of overlap. All ages not falling between the vertical lines were excluded, since they fell outside the absolute observed range of one or more of the four outer zone age change factors. This procedure can be applied to one, two, or all three of the major leg bones in combination.

Using the year that was midway between the vertical lines of exclusion as the estimated age, the accuracy of the estimate was checked on 56 additional specimens covering the range from birth through 82 years of age. For the entire range, 87.3% of the estimates were within ± 5 years of the actual age, and *all* were within ± 10 years. In the 12 specimens where two or more bones from the same individual were used, all estimates were within ± 5 years of the age also. The accuracy was better

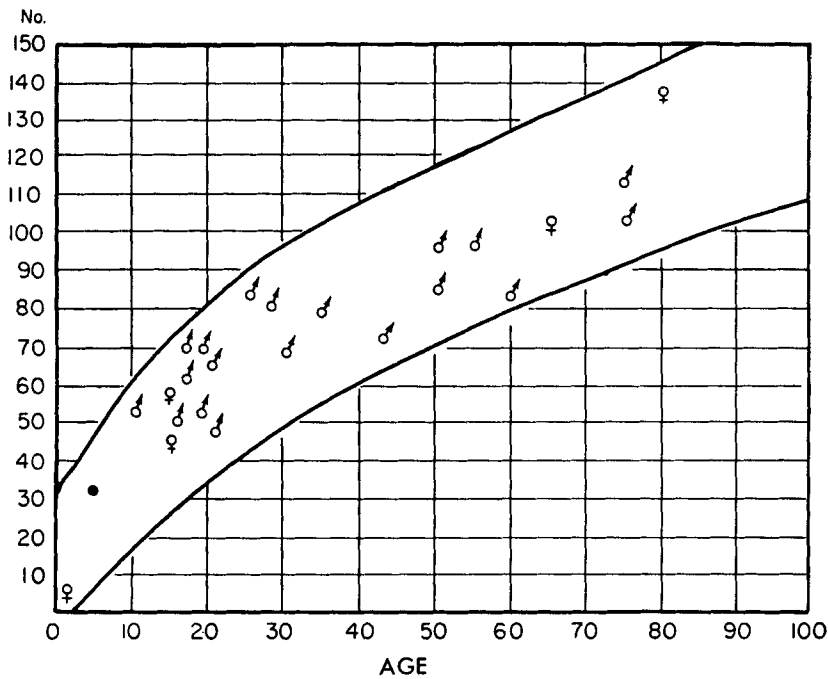


Fig. 6a Fibular osteones.

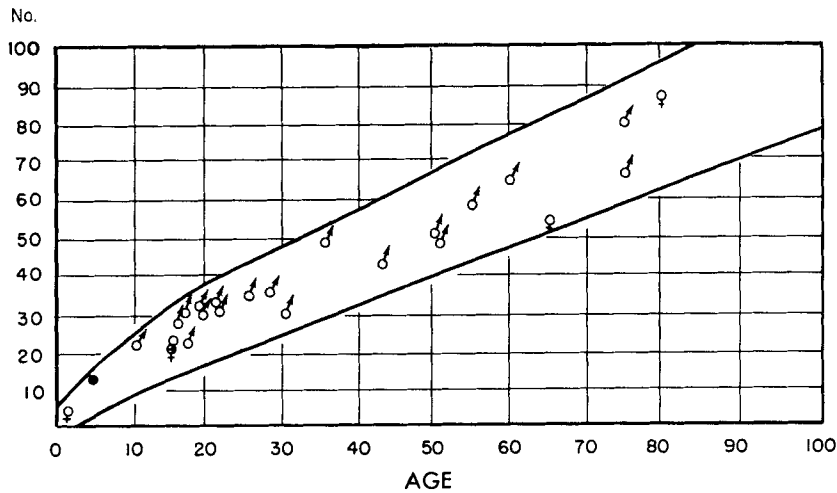


Fig. 6b Fibular fragments.

for younger specimens than it was for the older ones, being 91.7% within ± 5 years for those under 30 years of age and 78.9% for all those 30 and over. This widening of the range of accuracy in old age was a natural result of the greater variations between biologic and chronologic age in the

upper age ranges that can be seen on the age graphs (figs. 4-6), and of the increased dependence on only two of the factors (osteones and fragments).

The age ranges for the various factors can be determined from the observed absolute ranges on the special age curves

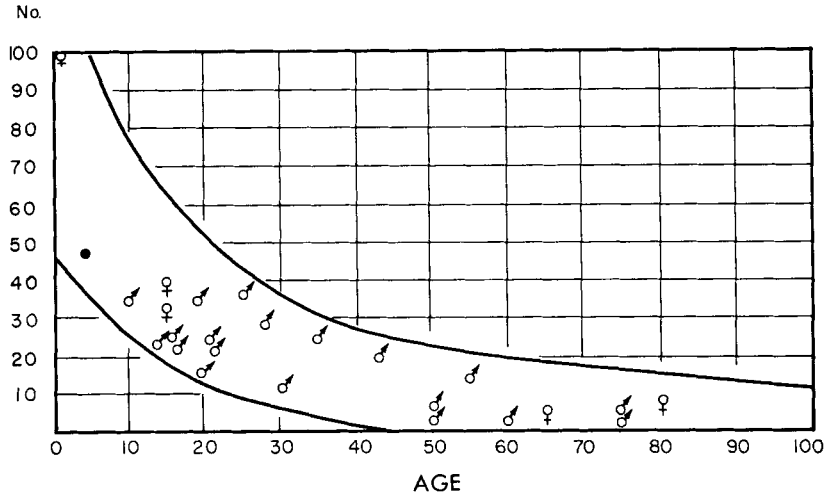


Fig. 6c Fibular lamellar.

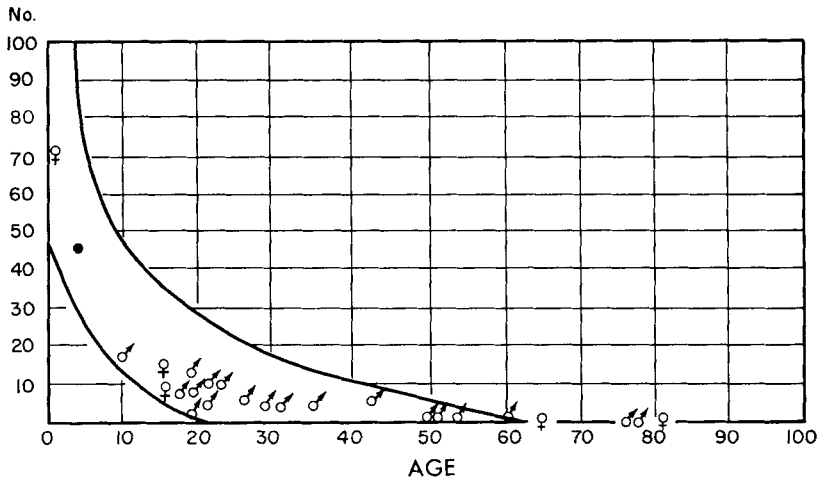


Fig. 6d Fibular non-Haversian.

TABLE 2
Regression formulae for estimating age (Y) from factor value (X)

Factor	Regression	Correlation	Standard error
Femoral osteones	$Y = 3.473 + 0.144X + 0.003X^2$	$\rho = 0.922$	9.39
Femoral fragments	$Y = 8.786 + 0.834X$	$r = 0.864$	12.19
Femoral lamellar	$Y = 79.455 - 2.427X + 0.023X^2$	$\rho = 0.870$	11.78
Femoral non-haversian	$Y = 57.811 - 1.728X + 0.013X^2$	$\rho = 0.815$	13.85
Tibial osteones	$Y = -10.082 + 0.634X$	$r = 0.925$	6.69
Tibial fragments	$Y = -7.061 + 0.931X + 2.210X^2 - 2.538X^3$	$\rho = 0.947$	7.78
Tibial lamellar	$Y = 76.338 - 1.794X + 0.011X^2$	$\rho = 0.816$	13.62
Tibial non-haversian	$Y = 70.270 - 10.944X + 0.647X^2 - 0.011X^3$	$\rho = 0.790$	9.63
Fibular osteones	$Y = 2.366 - 0.538X + 0.018X^2 - 0.001X^3$	$\rho = 0.922$	8.83
Fibular fragments	$Y = 1.328 - 0.058X + 0.034X^2$	$\rho = 0.974$	5.27
Fibular lamellar	$Y = 69.108 - 2.208X + 0.015X^2$	$\rho = 0.881$	10.85
Fibular non-haversian	$Y = 55.241 - 4.300X + 0.050X^2$	$\rho = 0.879$	10.70

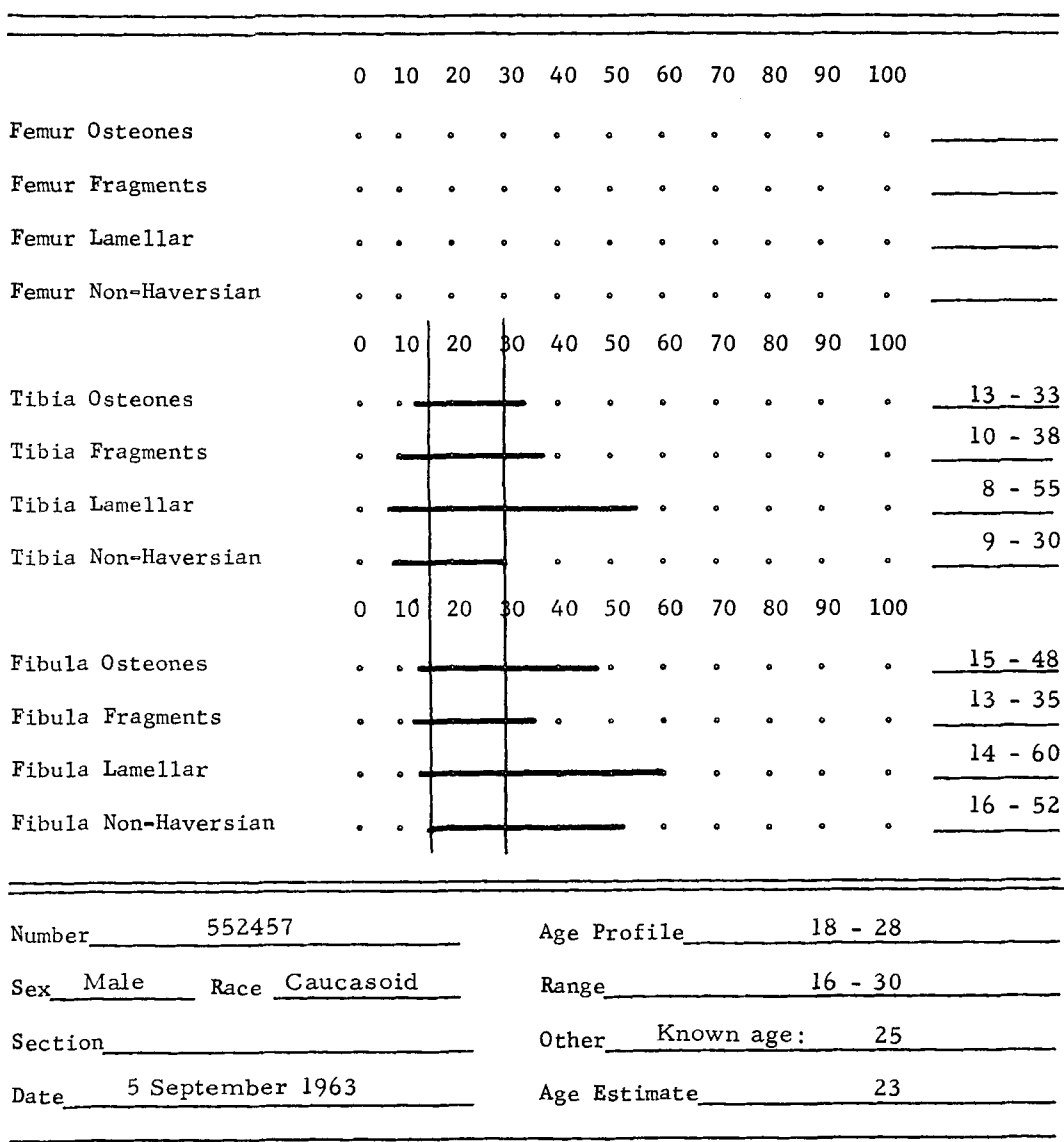


Fig. 7 Age estimate profile chart.

(figs. 4-6) or the age estimate can be derived from the regression formula for the number of fibular osteon fragments. Either method yields known accuracy and reliability that compare favorably with standard methods of age determination for the difficult specimens over age 30. The regression formulae offer excellent reliability when two standard errors of the estimates are used as the limits, but the ranges

of such estimates are too wide to afford any accuracy in estimating age, except for the fibular fragments. In some cases, greater accuracy can be achieved by using the profile chart (fig. 7) to narrow the range of the estimates obtained by the regression formulae. This is particularly useful when all of the factors are used in two or more bones. When there is sufficient variation in the ranges of the in-

dividual regression formula age estimates to narrow the range of the over-all estimated age on the profile chart, there is an increase in accuracy with no loss of reliability, since the narrower estimate is still within the two-standard error limits of all the regression estimates used.

DISCUSSION

Every effort has been made to keep this method of estimating the age at death by microscopic examination as simple and easily-repeatable as possible, and to eliminate variations not related to age. The use of four fields in separate anatomic areas of the cross-section minimizes the likelihood of basing the age estimate on a single, atypical field, and the use of a standard laboratory microscope and 100-power fields enhances the comparability of similar data obtained in the future by others for estimating ages by the method presented here. The basic microscopic structures utilized, the osteon and lamellar bone, are readily identifiable, and counting instead of measuring eliminates variations in ocular reticules or other measuring devices.

One problem in applying this method and the age graphs in figures 4-6 is determining what constitutes a "recognizable osteon" as opposed to a fragment. It is impractical to use only osteones which have in no way been encroached upon by others, since the outer margins of osteones are often irregular and may be difficult to distinguish in some cases. A workable definition of a recognizable osteon is one that is easily distinguishable over 80% or more of its area and has the canal intact. Fragments include any osteones that have discernible encroachment by subsequent generations of osteones and are exemplified by arcs of concentric lamellae between newer osteones (see fig. 2).

While simplicity and repeatability were sought in establishing this method of age determination, equal attention had to be given to its versatility of application. This microscopic method of age determination utilizes any or all of the major long bones of the leg, which can be articulated with the axial skeleton through the hip joint to establish continuity with the spine and skull. The mid-shaft area of the diaphysis was chosen because it is anatomically

identifiable, survives most conditions of burial or injury better than the ends of bones do, and had been shown to have age changes in the microscopic structure (Amprino and Bairati, '36). It should be pointed out that slides need not be made from the *exact* middle of the shaft, since there is a block in the mid-shaft area that is about three inches in length and is homogeneous with regard to microscopic age changes. Thus, badly fragmented bones can be used as long as a cross-section can be obtained from anywhere in the area of the middle of the diaphysis.

Archeologic specimens from the Philippine and Aleutian Islands, Indian Knoll Kentucky, Virginia, and Florida were examined by the microscopic method in order to test its applicability to archeologic material that had been subjected to a variety of burial conditions. These specimens ranged in postmortem age from 500 to around 5,000 years. In spite of marked differences in mineralization and leaching by ground water, thin ground sections of all the specimens afforded sufficient clarity and detail for the use of this method. There was enough variation in the mineralization of these specimens, however, to suggest that some archeologic material will not yield adequate detail by normal light microscopy. In such cases, polarized light or microradiographs may give better structural detail than ordinary light microscopy.

Disease processes might well affect the reliability of the microscopic method, since many diseases disrupt the microscopic structure of bone. Sections of 78 pathologic specimens were examined for microscopic age changes. Twenty-four common disease processes were represented in such major categories as trauma, inflammation, cardiovascular, atrophy, neoplasm, developmental, and metabolic diseases. In areas of discernible pathology the microscopic structure of the bone was disturbed and the age changes could not be assessed. However, unless there was gross or microscopic evidence of pathology in the area from which the slides were made, the reliability of the microscopic method of age determination was not affected, with one exception. In Paget's disease, the number of fragments was excessive, far exceeding the number of osteones, and age could not

be estimated although pathologic changes were slight in the area from which the sections were made. In general, the ages of normal looking bone — even from individuals with skeletal pathology — could be estimated with the same reliability as bone from normal individuals.

Inconsistent estimates did not occur in any of the normal specimens or specimens from normal appearing areas of the skeleton. That is, all ages were never excluded by the overlapping of the four factors on the profile chart, nor did age estimates of one bone completely exclude those for another bone in the same individual. If it should happen that the four factors overlapped in such a manner that no age range remained for an estimate, that particular specimen would clearly be beyond the application of the microscopic method presented here. The same would be true if the age range for one bone did not coincide in any way with the age range for another bone from the same individual. In that case, however, one might seriously consider the possibility that the remains of two or more individuals have been commingled.

Sex differences in the specimens did not affect the microscopic age changes utilized by this method. Statistical correlations between sex and the outer zone age factors ranged from $r = 0.022$ for osteones in the femur to $r = 0.391$ for non-Haversian canals in the fibula. Although there were few specimens other than Caucasoid in the sample, race did not seem to have any appreciable effect on age changes either. Slides of 11 Negroid specimens were distributed in a random manner on the age graphs. The Mongoloid specimens tested by this method were all archeologic, and the ages were not documented. However, the estimates derived by microscopic age changes were in agreement with those based on gross age changes. It should be possible, then, to apply this microscopic method of age determination to fragments

of bone of any age without having to know the sex or race of the individual.

The skeletal age of an individual can be determined within the limits of the method presented at all age levels and throughout adult life. While gross methods are more accurate for determining the skeletal ages of children and adolescents, the microscopic method can be applied to all ages through 95 years. In this respect, the age graphs presented represent norms for skeletal age throughout the normal human life span. Such norms provide a base line for comparison with pathologic specimens from amputation or autopsy to determine the relationship between bone age and chronologic age in various pathologic conditions.

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