CASE REPORT

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The Use of Bone Histomorphometry in Skeletal Identification: The Case of Francisco Pizarro

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ABSTRACT: Two lines of approach are utilized in a histomorphometric analysis of a rib sample from the postcranial remains believed to be those of Francisco Pizarro. Using a newly developed age predicting formula, age at death is estimated to be 62 years. This age agrees with several ages reported in historical documents. The histomorphometry of cortical area/total area (C/T) ratio, mean osteonal cross-sectional area, and mean annual Haversian bone formation rate conform to those of a healthy individual in his early sixties.

KEYWORDS: physical anthropology, human identification, histology, histomorphometrics

Postcranial remains believed to belong to Francisco Pizarro have recently been identified. The purpose of this paper is to present the results of a histomorphometric analysis of rib cortical bone from these remains.

The analysis involved two lines of approach. First, age at death was estimated using a histological method for the rib recently developed in the author's laboratory.[2,3] Next, assuming that a reported age of 64 years for Pizarro at the time of his death is correct, several histomorphometrics were determined for the rib cortical bone of the remains in question. Values were then compared to age associated values reported in the literature for modern rib cortical bone.

Materials and Methods

A several millimetre transverse section was removed from the middle third of the rib, and embedded in Castolite® (Buehler Ltd., Illinois). An approximately 200-μm wafer was then removed with a Buehler Isomet low-speed saw, mounted on a prepared petrographic slide, ground to a final thickness of 50 to 75 μm, and mounted on a standard microscopic slide.

The following histomorphometrics were determined. Area measurements were made with a Merz counting reticule [*1*].

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[2]S. D. Stout, R. Paine, and J. Gavan, "The Estimation of Age at Death Using the Cortical Bone Histomorphology of the Human Rib and Clavicle," unpublished manuscript, no date.

[3]R. Paine, "Histological Aging Utilizing Clavicles and Ribs," unpublished manuscript, 1984.

Total area (T)—The area of bone contained within the periosteal envelope.

Cortical area (C)—The area of bone between the periosteum and endosteum.

C/T ratio—Cortical area divided by total area.

Mean osteonal cross-sectional area—The average area of bone contained within the cement lines of at least 50 complete osteons, excluding the Haversian canal.

Intact osteons—The number of osteons in which at least 90% of the perimeter of the Haversian canal exhibits no evidence of bone remodeling per section (Fig. 1).

Intact osteon density—The number of intact osteons per section divided by the cortical area.

Fragmentary osteons—The number of osteons per section in which evidence of remodeling is present at greater than 10% of the perimeter of the Haversian canal (Fig. 1).

Fragmentary osteon density—The number of fragmentary osteons per section divided by the cortical area.

Total visible osteon creations—The sum of the intact and fragmentary osteon densities, which represents all visible remains of Haversian bone formation since the creation of adult compacta.

Total osteon creations—An indirect measurement of the total number of osteons created throughout the life span of the individual. Calculation of this parameter depends upon the relationship of total visible osteons to the age of the individual, assuming a mean osteonal cross-sectional area of 0.037 mm^2. In those specimens in which the measured mean osteonal cross-sectional area does not equal 0.037 mm^2, total visible osteon creations is multiplied by a correction factor determined by dividing observed mean osteonal cross-sectional area by 0.037 mm^2. Using this derived total visible osteon creations, the total osteon creations per mm^2 of cortex since age 12.5 is determined according to Wu et al [*2*] (Fig. 2).

Effective age of adult compacta—The number of years over which the osteons and osteon fragments visible in a cross section of adult cortex were created. Increase in cross-sectional diameter of long bones with age, and transverse cortical drift, which occurs by periosteal and endosteal appositional or resorptive growth, rapidly remove evidence of the creation of Haversian

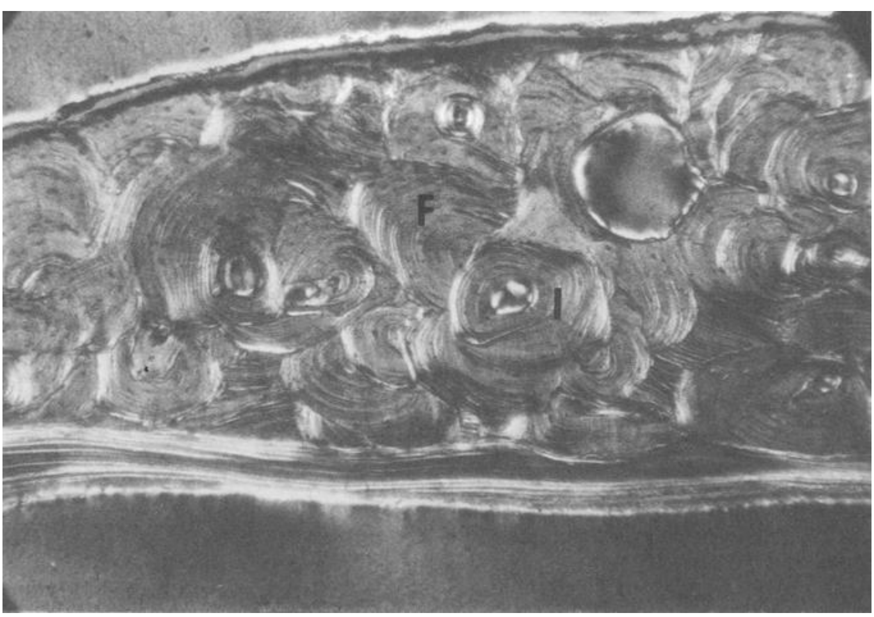

FIG. 1—*Photomicrograph of a transverse 50- to 75-μm-thick cross section of the rib specimen analyzed in this study.* I *and* F *indicate interact and fragmentary osteons (×10 polarized).*

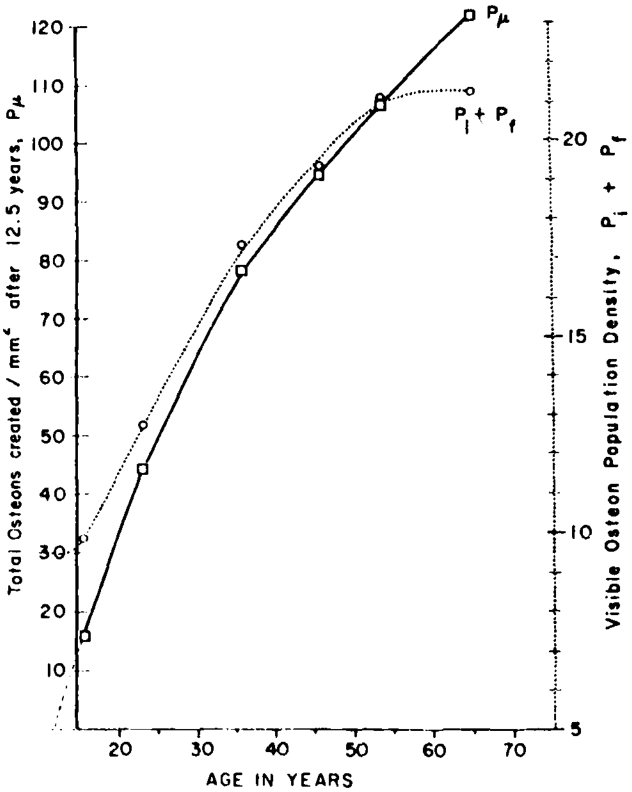

FIG. 2—*Relationship between visible osteons and total osteon creations (from Wu et al* [2]*).*

systems. The earliest point in time at which the parameters used in this study are observable, or the effective age of birth for adult compacta, in the middle third of the cortex of an adult rib, has been determined to be approximately 12.5 years [2]. Effective age of adult compacta, therefore, is computed by subtracting 12.5 years from the estimated or known age.

Mean annual osteon creation frequency—The total osteon creations of an individual section divided by the effective age of adult compacta, which is the rate at which Haversian creations occurred averaged over the effective age of the adult compacta.

Mean annual Haversian bone formation rate—The mean annual osteon creation frequency of each individual is multiplied by the mean osteonal cross-sectional area, the amount of bone added with each new Haversian system.

Results

The histomorphometrics of this rib indicate a healthy individual in his early sixties at the time of death. These results provide additional support to the thesis that the postcranial remains from which the rib was obtained are indeed those of Francisco Pizarro.

Total visible osteon density for the Pizarro rib sample was found to be 25.96 per mm^2. Inserting this value into the predicting equation produces an estimated age at death of 62 years. Table 1 illustrates a test of the formula using values for known ages reported in the literature.

The cortical to total cross-sectional area ratio (C/T) was found to be 0.333. As can be seen in Table 2, this value agrees with that observed in individuals aged 64 years with an age range of 60 to 69 years.

Mean osteonal cross-sectional area was found to be 0.037 mm^2. This measure is too variable to relate to a specific age, but it is interesting to note that it is identical to the mean value reported by Wu et al [2] for a modern sample.

Mean annual Haversian bone formation rate was estimated to have averaged 0.0862 mm^2/mm^2/year over the 51.5 years since the effective birth of the adult compacta was reached. This value agrees with values determined in modern bone biopsies using in vivo tissue time markers such as tetracycline. Wu et al [2] report a mean annual Haversian bone formation rate of 0.095 mm^2/mm^2 year for an age of 58.9 years. When a predicting equation is developed using their mean values and associated mean ages, a mean annual Haversian bone formation rate of 0.079 mm^2/mm^2/year is predicted for an age of 64 years (Table 3).

TABLE 1—*Test of age predicting formula using mean total visible osteon densities and known mean ages reported by Wu et al* [2].

	Total Visible Osteon Density	Age	Predicted Age[a]
	9.8	15.8	15.8
	12.7	23.2	24.1
	17.4	36.0	37.6
	19.3	46.1	43.1
	21.1	53.7	48.3
Pizarro	25.96	...	62.2

[a]Predicting formula is age = −12.239 00 + 2.873 51 (total visible osteon density). $N = 63$, $r = 0.682\ 44$.

TABLE 2—*Cortical to total area ratios.*

Mean age	55	64	75
Age range	30-59	60-69	70-89
C/T[a]	0.36 ± 0.15	0.34 ± 0.1	0.28 ± 0.1
Pizarro	...	(0.33)	...

[a]Based upon Frost [3].

TABLE 3—*Tetracycline-based Haversian bone formation rates for selected mean ages (adapted from Wu et al* [2]*).*

Mean Age	Rate[a] in $mm^2/mm^2/yr$
23	0.177
32	0.138
38	0.116
51.9	0.103
58.9	0.095
64.0[b]	0.079

[a]Integrated from 12.5 years, the effective age of birth for adult compacta in the middle third of the rib.

[b]Estimated by regression of preceding ages and formation rates.

Discussion

The significance of this study lies in its unique use of histomorphometric analysis for the purpose of helping to solve a mystery surrounding the identity of skeletal remains believed to be of historical importance, in this case, the skeleton of Francisco Pizarro.

This study also represents the first time an age estimate has been made using the histomorphology of the rib, made possible with a predicting formula recently developed in the author's laboratory. The major advantage to being able to make histological age estimates using the rib lies in the fact that other available methods require the use of major long bones, primarily the femur, tibia, and fibula [4]. Since these bones are important for other analyses (for example, stature, sex, ethnic origin, and so forth), the destructive nature of an invasive technique such as histology makes it unsuitable in cases where the material is extremely valuable in terms of its uniqueness.

The determination of mean annual Haversian bone formation rate is significant in that it represents a measure of bone formation rate, a dynamic metabolic process, in an individual who died over 440 years ago. Histomorphometric analysis of bone, therefore, has the potential to lead us into an area of investigation that can be termed paleophysiology.

Acknowledgments

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