

## TECHNICAL NOTE

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# Dietary Health Does Affect Histological Age Assessment: An Evaluation of the Stout and Paine (1992) Age Estimation Equation Using Secondary Osteons from the Rib\*

**ABSTRACT:** An age at death estimation equation that uses rib histological variables presented by Stout and Paine was used to evaluate a skeletal population of individuals with a known age at death and cause of death from either malnutrition or the niacin deficiency disease pellagra. The sample was comprised of 26 autopsied black South Africans. Histological analysis of mounted thin sections involved the microscopic measurement of cortical area and a count of the number of intact and fragmentary secondary osteons for the entire cross-section of the rib. Rib osteon population density values were then calculated for each case. It was found that this equation under-aged individuals on average by 29.2 years. Overall, secondary osteon size and Haversian canals tended to be larger than expected, while cortical bone area was less when compared with a control population. The implications of these findings are critical given that many of the skeletal remains examined by forensic anthropologists come from marginalized backgrounds, including malnutrition. This research suggests that measurements based on healthy cases may not be useful in an analysis of individuals with poor diet and health. It is argued that new standards for histological age assessment methods need to be created that account for variation in the health status of individuals examined by forensic anthropologists.

**KEYWORDS:** forensic science, malnutrition, age predication, secondary osteons

Questions concerning the accuracy of age predicting methods using histological criteria have been posed and subjected to re-evaluation by many skeletal biologists (1–11). Research to date has demonstrated that most methods have a rather small error range in predicting age, with the Stout and Paine equation being noted as the most accurate of the techniques (6,7). We recently evaluated the Stout and Paine (1) rib histological age estimation equation (1) on a known skeletal population of 20th century South African blacks from the Raymond Dart skeletal collection (12). This sample population of 26 autopsied individuals is housed at the University of Witwatersrand Medical School in Johannesburg, South Africa. Age at the time of death and cause of death are recorded for each case. All of the individuals in this sample had died from dietary deficiencies, either nonspecific general malnutrition or pellagra.

Pellagra, a niacin/tryptophan deficiency disease, is characterized by the four “Ds” symptomology of dermatitis, diarrhea, dementia, and eventual death. It has often been associated with the maize-based diets of impoverished and nutritionally marginalized

populations. Endemic pellagra has been well documented historically in northern Italy and Spain (18th–19th centuries), southern U.S. states (early 20th century), and in South Africa (mid-to-late 20th century) (13).

Many of the skeletal cases examined by forensic anthropologists tend to be individuals who had marginalized lifestyles (e.g., transients, homeless, a history of substance abuse, neglect and/or physical abuse) that compromised their diet and health status. Forensic anthropologists working with mass burials in international human rights cases often encounter undernourished populations, too. For that reason this study set out to examine if the chronic dietary deficiencies represented by these South African blacks might also affect the age assessment potential of the Stout and Paine’s equation (1).

## Methods

Histological data from the ribs of 26 individuals whose cause of death was either general malnutrition or pellagra were gathered from the Dart Collection. No macro-lesions or pathologies were noted on the sample ribs. To prepare the ribs for histological analysis we used the methods outlined by Stout and Paine (1) and had bone wafers prepared by embedding them in epoxy before they were ground down to a thickness of 75  $\mu\text{m}$ . Cortical area and the number of intact and fragmentary secondary osteons were read for the entire cross-section of the cortical bone. Similar to the original work performed by Paine (14), the entire cross-section of the rib was read. This was carried out to avoid sampling error or bias during the collection of osteon data.

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TABLE 1—Known age and estimated age for the black South African sample.

Case No.	Age	OPD	Estimated Age	Difference in Years
A1917*	38	7.84	15	23
A2319*	40	13.29	20	20
A3225*	45	13.70	20.9	24.1
A2735*	47	6.90	15	32
A3141*	50	16.96	24.5	25.5
A2382*	52	20.10	29	23
A1419*	67	11.99	19	48
A1351*	70	16.70	24	46
A2153*	75	10.73	18	57
A2469*	89	14.19	21	68
A2393	16	5.31	13.5	2.5
A2933	29	10.80	18	11
A3421	30	14.79	22	8
A2212	38	13.84	21	17
A466	39	12.10	19	20
A1558	39	14.70	22	17
A162	40	12.30	19	21
A3696	43	14.78	22	21
A730	46	14.37	21.6	24.4
A2455	50	14.19	21	29
A980	50	12.58	20	30
A2863	58	16.67	24	34
A3546	60	19.46	28	32
A2919	64	13.33	20.5	43.5
A3131	65	18.21	29.6	35.4
A3423	65	12.28	19.4	45.6
Mean	50.2	13.54	21.0	29.2

\*Indicates individuals who died from pellagra.  
OPD, osteon population density.

In creating osteon population density (OPD) values, intact and fragmentary secondary osteons were examined. These features are defined by their Haversian canal morphology as suggested by Stout and Paine (1). Fragmentary secondary osteons have 10% or less of the canal present, while intact osteons have 10% or more of the canal present.

Secondary OPD was calculated by using the two variables cortical area and the number of secondary osteons (intact and fragmentary counts are combined) for each rib sample. This number was then used to estimate the age of each individual using the rib equation (1):

$$\ln \text{age} = 2.343 + 0.050877 \text{ rib (OPD)}$$

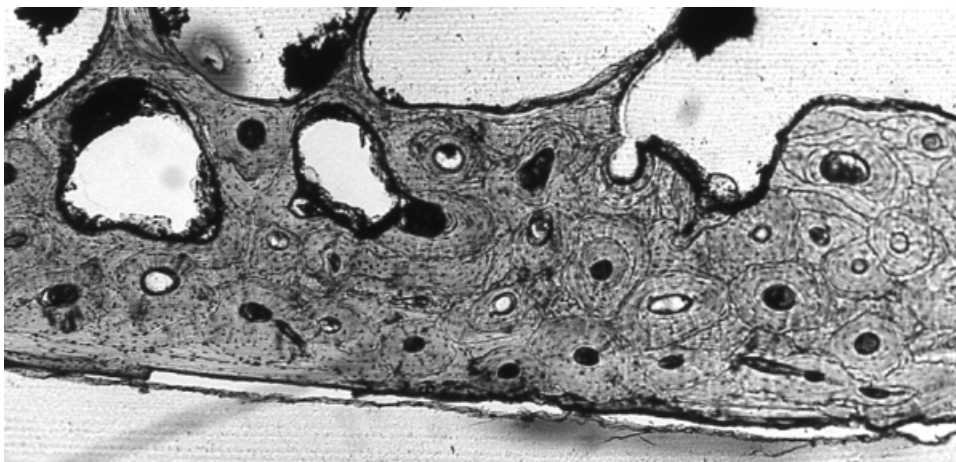


FIG. 1—Rib microanatomy, intact secondary osteons seen in a 70-year-old female who died from pellagra. Notice there are very few fragmentary osteons seen in this photo. A gray filter was used to take this photo.

The histological data recorded from the Raymond Dart skeletal collection was then compared with data from the RRP rib collection. The RRP rib collection was created during a 1981–1982 research period (14) and consisted of samples taken from autopsies conducted by Dr. Jay Dix of the Boone County Medical Examiner's Office in Missouri. Age, sex, as well as manner and cause of death were available for each of the RRP samples (1). About 15% of the RRP collection was identified as African American whereas the remaining 85% were of European descent. To date there are no known significant differences in the skeletal histology of human populations.

## Results

The rib equation offered by Stout and Paine (1) under-estimated the age of all 26 individuals in the sample. They were under-aged by an average of 29.2 years (see Table 1). The pellagra subsample was under-aged by 36.7 years whereas the nonspecific malnutrition subsample was under-aged by 24.4 years. For example, rib sample A1351, a 70-year-old female, showed very few fragmentary osteons (Fig. 1). This number of fragmentary osteons is unusual for someone of her age, and she was under-aged by 46 years.

Another rib sample (A3225), showed how low the OPD could be in this population by exhibiting very few secondary osteons (Fig. 2). This 45-year-old female pellagrin was under-aged by 24 years.

We also found that secondary osteon size and Haversian canals tended to be larger than expected for the mean age of these sample groups, while cortical bone area was less in those suffering from malnutrition and pellagra compared with the RRP control population (Table 2). There was no statistically significant difference in osteon area among these groups but, there were significant differences in cortical area and Haversian canal area between the two sample groups, 95% CI.

## Discussion

There are several possible explanations for our results. Metabolic disorders and dietary deficiencies have been suggested as causing either increased bone turnover rates leading to the production of the presence of more secondary osteons per area of

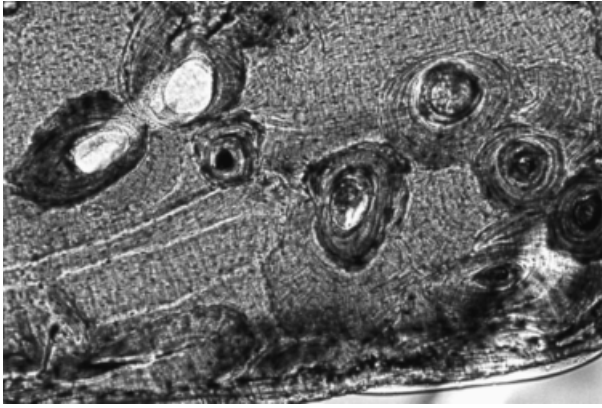


FIG. 2—Rib microanatomy, a low density of secondary osteons seen in a 45-year-old female who died from pellagra. Blue and gray filters were used to create this photo.

bone than what is considered to be normal, or can lead to lower bone turnover rates resulting in fewer secondary osteons per volume of bone than what is expected for a given age.

For example, Wu et al. (15) and Robling and Stout (10) mention that osteogenesis imperfecta (brittle bone disease) is related to a lower than expected OPD value, specifically in adults. Metabolic problems like hyperparathyroidism may lead to an increase in the OPD value (10). Both conditions will lead to abnormal secondary osteon counts creating inaccurate age estimations using current histological formulas.

Ericksen (16) attempted to deal with this issue by collecting histological data that included individuals with the known disorders diabetes and chronic renal disease while producing age predicting equations using histological criteria. The usefulness of attempts like this have been put into question by Robling and Stout (10) who claim that the equations and their usefulness are questionable since a disorder may lead to age predicting equation biases with too many or too few osteons per area of cortical bone for a given age. Thus, these equations may be less useful when attempting to predict the age of healthy individuals via histological criteria. We do not disagree with this assessment.

However, we believe that a closer look at how standards using “typical” or “healthy” individuals affect our ability to determine age at death of those who do suffer from metabolic and dietary related disorders is required. Our findings tell us that the implications of a chronically malnourished diet in cases encountered by forensic anthropologists is critical to understand why histological methods might work well for some cases and fail in others. It must be considered also as part of the overall initial macroscopic evaluation of the skeleton for well-established diet and health-related skeletal pathologies.

Like many standards that have been created, Stout and Paine (1) paid little attention to the effect of diet on bone turnover rates and

TABLE 2—Cortical area and Haversian system differences among the RRP rib collection, pellagra, and nonspecific malnutrition.

Sample Population	Mean Age	Cortical Area*	Osteon Area*	Haversian Canal Area*
RRP	28	22.36	0.0396	0.00144
Pellagra	51	13.65	0.0421	0.00243
Malnutrition	47	18.36	0.0435	0.00219

\*Measurements are recorded in mm<sup>2</sup>.

the formation of OPD values. The original rib equation, based on the RRP control population, was worked out using rib samples from individuals that, on the face of it, had a better diet potential than the South African blacks from the Raymond Dart collection. Of the 40 individuals from the RRP collection used to create the rib equation, 26 of them died of accidental or violent circumstances unrelated to dietary problems or other related metabolic disorders (1). One individual’s death was attributed to obesity and the others were related to metabolic problems, such as cystic fibrosis, cancers, and heart problems. Given the general health status of the RRP collection, it seems very likely that this rib equation is not well suited for age estimation of individuals with dietary deficiencies.

**Conclusion**

In light of our recent findings, we argue that the Stout and Paine (1) rib formula did not come close to estimate the age of autopsied samples from the Dart collection. We recommend that the focus of future efforts to create histological standards based on current skeletal populations used to estimate age at death be directed toward research that takes into account the health status and diet. This also means that we must forgo the traditional typologies used to create standards, those via race or ethnic groups. It appears to us that these populations may not appropriately address the real biological concerns of differential turnover rates among individuals. For future, the creation of histological age-estimating formulas specific to dietary and health-related problems is one of the critical directions that our work on rib histology is leading us.

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