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4/19/11

Bring me more beer: Haversian system formation rates for a Nubian population and intervals
between periods of tetracycline ingestion

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An abstract of
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of Emory University in partial fulfillment
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Department of Anthropology

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Abstract

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By Amanda Winburn

The study summarized in this paper is two-fold in nature. The Haversian system formation rates for the Nubian Christian-group 21-R-2 cemetery (604 ± 46 C.E.) are determined, and potential variation within the rates based on sex and age at death are analyzed. The information regarding Haversian system formation rates is then used in to address the discovery that the Christian-group exhibits tetracycline labeling bound into the cement of its osteons (Bassett et al. 1980). This second phase represents the development and application of new methods to determine the time intervals represented between identifiable tetracycline labels. The principles of modern-day tetracycline studies are applied to this archaeological population in order to determine the time intervals between tetracycline ingestion within the population. The two-fold nature of this paper, then, demonstrates a hybrid methodology that is new to the field of bioarchaeology.

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ACKNOWLEDGMENTS

My sincere appreciation goes to George Armelagos, without whom this thesis would never have been possible. George introduced me to the field of bioarchaeology, and his enthusiasm has inspired many students to pursue Honors Theses, publication, and study of the field at the graduate level. My own graduate and professional experience with bioarchaeology will always reflect George's influence.

Additionally, thank you to Sally Gouzoules for help throughout the process and to Dietrich Stout and Arri Eisen for read-throughs and suggestions. Your input has been most valuable.

Lastly, my appreciation goes to my wonderful dog, Koda, who sat patiently with me on many a night and rarely made me feel guilty for not playing more fetch.

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I. INTRODUCTION

Bioarchaeology is a field of continuous research and development (Armelagos 2003; Larsen 1987; Larsen 2002). New techniques that can be applied to skeletal analysis within an archaeological context are regularly examined, with the validity of such techniques presented to the bioarchaeological community for collaborative use.

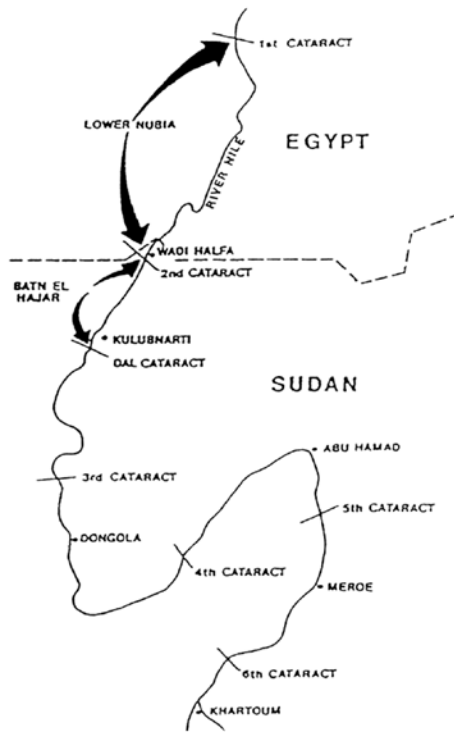
The study summarized in this paper is two-fold in nature, with the end result presenting a new method for bioarchaeological analysis. The Haversian system formation rates for the Nubian Christian-group 21-R-2 cemetery 558-650 C.E. are determined, and potential variation within the rates based on sex and age at death are analyzed. The information regarding Haversian system formation rates is then used to address the discovery that the population exhibits tetracycline labeling bound into the cement of its osteons (Bassett et al. 1980). This second phase represents the development and application of new methods to determine the time intervals represented between identifiable tetracycline labels. The principles of modern-day tetracycline studies are applied to this archaeological population in order to determine the time intervals between tetracycline ingestion within the Christian-group. The two-fold nature of this paper, then, demonstrates a hybrid methodology that is new to the field of bioarchaeology.

II. LITERATURE REVIEW

The Christian-Group

The population used in the present study was recovered from cemetery 21-R-2, which has been radiocarbon dated between 550 to 1450 C.E. The 21-R-2 cemetery group comes from the site of Kulubnarti, one hundred miles south of Wadi Halfa and situated in the extreme northern region of Upper Nubia between the Second and Dal Cataracts of the Nile River. Figure 1 details the location of Kulubnarti. The local region is considered the *Batn el Hajar* (Belly of Rock); a reference to the harsh environment (Armelagos 1969; Bassett et al. 1980; Martin et al. 1984; Mulhern 2000; Mulhern and Van Gerven 1997).

The Nile River in the Belly of Rock region is covered with outcroppings and rapids separating numerous tiny islands. The site of Kulubnarti, including detail on outcroppings near the site, is shown in Figure 2. In response to the harsh environment, the population within the 21-R-2 cemetery was comprised mostly of local farmers who, unlike their neighbors in Nubia, did not practice irrigation agriculture. The local population practiced seluka agriculture, wherein a stick was used to make holes within wet soil in which seeds were deposited. It was common for populations in this geographic region to live in small-scale farming villages focusing on the staple crops of wheat, millet, barley, sorghum, lentils, beans, peas and dates with pigs, sheep and cattle providing protein (Mulhern 2000; Van Gerven 1981; Van Gerven 1990; Van Gerven et al. 1995; Van Gerven et al. 1981).



Top: Figure 1. Map detailing Kulubnarti and surrounding areas (Van Gerven et al. 1995).

Bottom: Figure 2. Photograph of Kulubnarti (Anderson 2008).

The dry, arid climate of Lower Nubia, receiving an average of less than 1 mm of rain per year, ensured desirable preservation of skeletal remains (Martin 1979; Mulhern and Van Gerven 1997). In addition to having a dry climate, the geographic area maintains regular high temperatures which provide a combination of factors that resulted in well-preserved burials. Many population remains from this region contain hair and mummified soft tissue with no indication of embalming having occurred (Armelagos 1977; Armelagos et al. 1984; Bassett et al. 1980). The remains contain collagen levels comparable to those found in extracted biopsy or autopsy material, as identified in Bassett's analyses of hydroxyproline, proline, and mucopolysaccharides (Baker 1992). The excellent preservation of the population skeletons, further exhibited in the non-occurrence of birefringence, indicates that secondary Haversian systems can be found intact (Armelagos 2001). Figure 3 represents a Meroitic individual demonstrating the excellent preservation of remains within this geographical area. Figure 4 details birefringence within remains from the local area. Well-preserved and intact secondary Haversian systems provide ideal source material on which to apply new research.

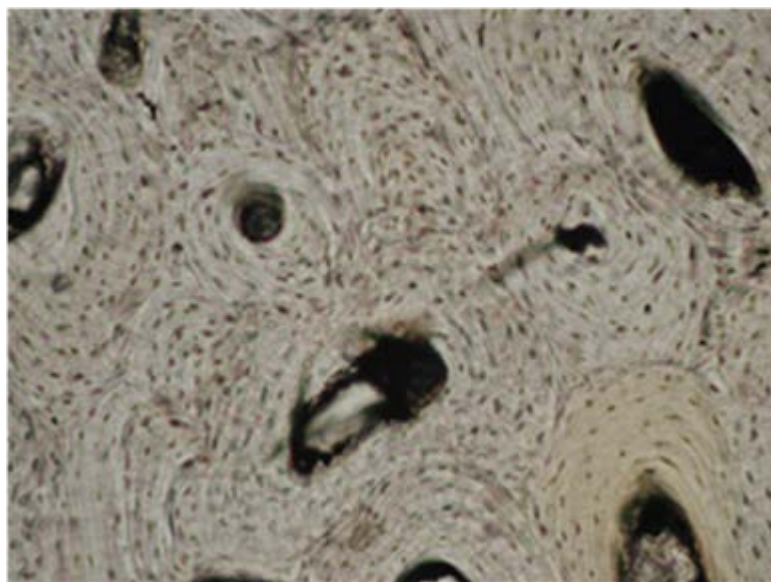
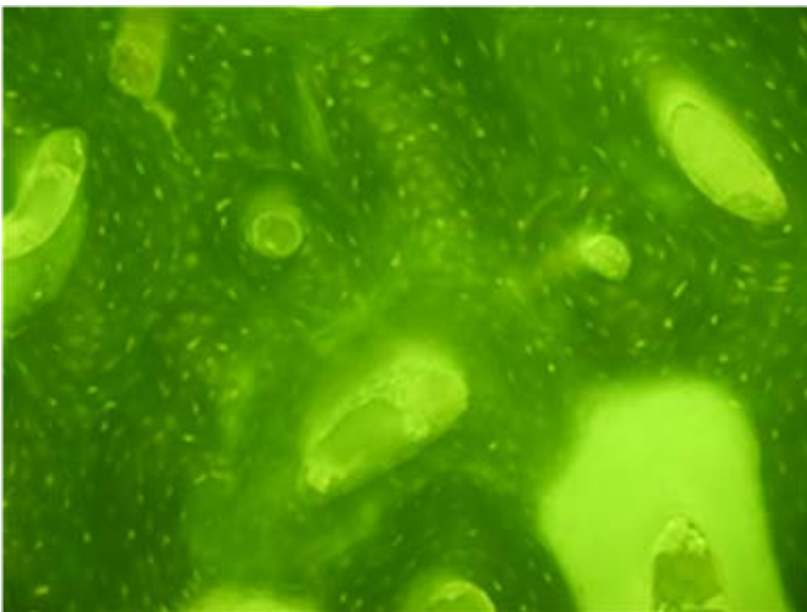
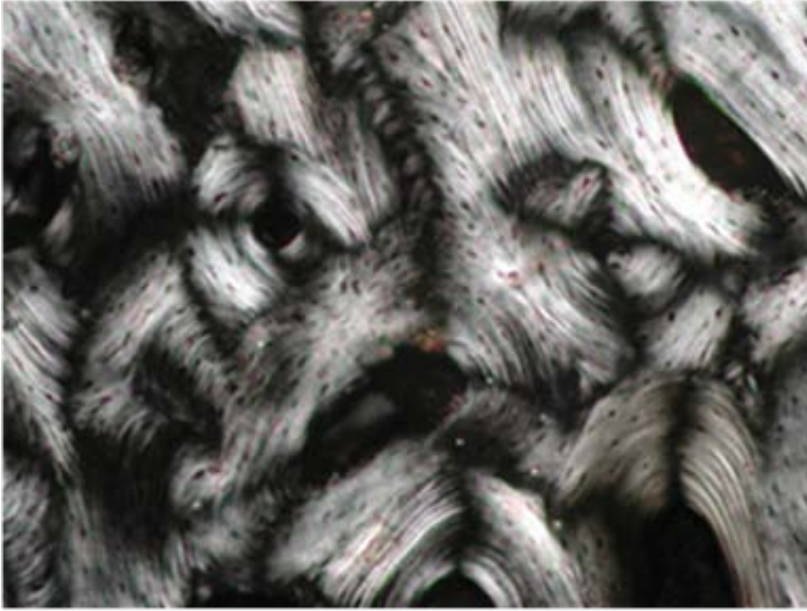


Figure 3. An undecalcified thin section of bone from a Meroitic specimen.



Top: Figure 4. Polarized view of a thin section showing birefringence indicating well preserved collagen.

Bottom: Figure 5. Tetracycline-labeled bone showing in vivo incorporation.

The populations of Kulubnarti have been well-documented. Previous research on this population includes paleopathological studies focusing on lesions found on the remains, macroscopic and microscopic examination of compact and trabecular bone, analysis of dentition, and study of cranial morphological variation (Armelagos 1969; Bassett et al. 1980). Much of the research on this population, however, involves tetracycline labels bound into the cement of the population's osteons. Figure 5 exhibits tetracycline-labeling within an osteon.

The tetracycline labels found in the osteons of the 21-R-2 skeletal remains result from the unique local manufacturing process for beer and bread (Armelagos et al. 2001; Hummert and VanGerven 1982; Nelson et al. 2010). The manufacturing process, involving grain stored in mud bins for long periods of time, cultivated *Streptomyces*, a bacteria that can produce nonsynthetic tetracycline. The dry, arid environment of Lower Nubia provided an ideal host for *Streptomyces* to develop; between 60 and 70 percent of the bacteria in the soil of Sudanese Nubia is *Streptomyces* (Bassett 1980). As many of the population's staple crops were made from grain, tetracycline became a common occurrence in the local diet.

Tetracycline

Tetracyclines are a group of antibiotics rather than a particular single product (Chopra 2001).

The extraction of tetracycline from a nearby Nubian population (the NAX-group) demonstrates that ancient tetracyclines are comparable to the modern product (Nelson et al. 2010).

Tetracyclines are stable tissue markers, and labeling with the antibiotic does not disturb cell population dynamic parameters. The antibiotic is effective against Gram-positive and Gram-negative bacteria, as well as against some viruses, spirochetes, and *Rickettsiae* (Bassett et al. 1980). Tetracycline is fluorescent under a diagnostic ultraviolet or blue light within undecalcified

tissue; decalcifying the tissue removes the fluorescence (Frost 1963; Frost 1969; Frost et al. 1961). This fluorescence is referred to as the tetracycline “label.”

The tetracycline label is created when tetracycline binds with the calcium present at the calcification front during the first stage of osteon mineralization in new bone matrix (Bassett et al. 1980; Frost 1969; Frost et al. 1961). These permanent tetracycline labels, created in the very center of new bone formation, are best viewed in undecalcified thin sections of approximately 20-100 μ thick (Frost 1969). As a result of the beginning stage of osteon mineralization of organic matrix correlating with the synthesis rate of new matrix, the labeling of mineralization can be indicative of the rate of protein synthesis (Frost 1969).

The tetracycline needed to form a label within a Haversian system should be regularly ingested, but does not need to necessarily be large amounts. The bind that occurs in the first stage of osteon mineralization indicates that tetracycline is an active ion chelator and forms permanent calcium and protein compounds that produce yellow-green fluorophors (Bassett et al. 1980). The fluorophors thus record cellular behavior and can be utilized in the analysis of bone dynamics (Frost 1963). Only a small amount of tetracycline is required for this kind of useful labeling; 0.5-2 g of tetracycline will cause fluorescent rings in Haversian systems when viewed under ultraviolet light at 490 \AA (Nelson et al. 2010).

There is a large amount of information that can be gathered from tetracycline labeling. Modern tetracycline studies use the label to measure the rate of bone formation at the level of the Haversian system and the speed of formation at the osteoblastic level. Frost indicates that once the number of bone-forming centers is known, one can compute the number of new osteoblasts created from the mesenchymal cell pool and the rate of bone formation averaged over the sample in its entirety (Frost 1963; Frost 1969).

Tetracycline labeling within the population

Tetracycline ingestion among the population in study indicates a level high enough to impact disease, health, and demographic patterns and must have occurred through multiple doses.

Bassett demonstrates that tetracycline may explain low rates of infectious disease found among Nubian populations (Bassett et al. 1980). Armelagos indicates that 9.2% of the osteons and 6.0% of the bone from the population exhibited enough tetracycline to inhibit trabecular bone loss (Armelagos et al. 2001).

Nelson extracted tetracycline samples from sample remains and noted that the extent of the labeling contradicts any theory for a one-time dosage or contamination of food. Osteon mineralization occurs in periods of ~80 days; this population received regular tetracycline during these mineralization periods. Nelson found equivalent retention times of the chromatograph peaks between samples from this geographic area and anhydrotetracycline, and equivalent mass spectra results. He also notes that direct visualization of the population sample consistently maintained extremely high tetracycline levels (Nelson et al. 2010). To accurately understand the relationship between tetracycline and the labeling found within the Christian-group, the formation and resorption rates of Haversian systems need be clarified.

Haversian systems: formation and resorption

An osteon is a blood vessel with concentric layers, referred to as lamellae, of mineralizing bone encircling it (Stini 1995). Canals, termed canaliculae, link osteons with other osteons. This entire system is referred to as a Haversian system. Haversian systems are displayed in Figures 3, 4 and 5.

Each Haversian system includes openings that are occupied by bone cells, referred to as osteocytes. The function of the Haversian system is to transport nutrients and waste products. As a result of the constant flow of nutrients into and out of the Haversian system, the system is remodeled every 80 to 120 days through a uniform remodeling process of activation, resorption and formation (Stini 1995; Stout and Lueck 1995). Haversian system formation rates are the prime concern of this research. It should be noted, however, that this research concerns *secondary* Haversian system formation rates. Thus, the methodologies of this paper incorporate necessary resorption rates and the entire remodeling process.

Haversian systems consistently maintain identical cellular organization throughout the bone and the remodeling process (Jaworsky and Lok 1972). Formation begins with a newly forming osteon as a cylindrical cavity in bone that fills centripetally with bone deposition. Frost writes that this initial formation stage is first marked by local mesenchymal cells producing osteoclasts and osteoblasts, and refers to this stage as “activation” (Frost 1969). During activation, osteoclasts first resorb approximately 0.05 mm^3 of local bone. The osteoclasts are followed by budding capillaries and osteoblasts, which are responsible for the amalgamation of the organic substances that make up bone and secrete osteoid, a collagen-fiber containing substance (Schultz 2001). Osteoblasts are embedded, along with osteocytes, in the newly developing bone matrix (Jaworsky and Lok 1972). It is during this time that tetracycline labeling occurs (Frost 1963).

The osteoblastic deposition is measured by administering tetracycline to the individual in two distinct instances, usually with the interval between the instances set at 10 days. The bone is then biopsied, undecalcified cross sections are made, and the amount of bone between the two tetracycline markers is determined (Frost 1963). It is important to note that the remodeling

described above, beginning with osteoclastic resorption and ending with osteoblastic formation, occurs consistently on the bone surface area. Haversian system formation rates, however, are measured as volume. The rates as volume are able to be measured from surface area activity as a result of the inclusion of a time marker in the measurements (Frost 1969). To be able to calculate these measurements, total osteon density must also be known.

Imaging software

The fact that the initial osteoclastic bone resorption does not fully engulf the primary osteon allows us to see partial, older osteons known as fragmented osteons (Stini 1995). By utilizing the amount of whole and fragmented osteons, density can be calculated for the entire osteon. Subjective bias in counting whole and fragmented osteons can be removed with the aid of imaging software such as Image Pro-Plus®.

The commercially-available Image Pro-Plus® software allows one to calibrate the program and take multiple measurements, either automated or manual. This program has been consistently used in academic research, including in multiple quantification studies (Rawlinson et al. 2005). Rawlinson et. al., a pilot study utilizing Image Pro-Plus®, found that "...the methodology, alignment apparatus and computer software used enabled accurate and reproducible repeat images of dentate skulls to be acquired in a simulated clinical environment, and also on patients" (Rawlinson et al. 2005). The study further went on to indicate that the software was very accurate with regards to consistent measurements on repeat images.

III. MATERIALS

The wide variation between bones, with regards to formation rates, density, etc., necessitates the regulation of which bone is used in the current research. By standardizing the sampling site, the results of each individual's Haversian system formation rates can be comparable to the rates of other individuals (Frost 1963). This study exclusively uses thin sections of rib bones from the middle third of the sixth rib. Rib bones remodel more often and more quickly than other bones such as radii, tibiae, and metacarpals. This results in greater labeling of Haversian systems within rib bones (Frost 1969). Additionally, rib bones experience uniform stresses across age groups, genders, class standing, etc., unlike other bones such as femurs with varying mechanical usage (Mulhern 2000).

Rib thin sections from the cemetery 21-R-2 were used exclusively for this study, with a total sample size of 20 individuals. Sex and chronological age at death were established for previous research at the University of Colorado using well-preserved soft tissue and pelvic morphology for the determination of sex and seriation of multiple components (i.e. pubic bone morphology) in determining age at death (Mulhern 2000; Van Gerven et al. 1981). Samples from individuals whose chronological age at death was less than 14 years were excluded from study in order to account for the age of compacta for rib bones. Additionally, individuals with an age at death greater than 51 were excluded from study due to the difficulty in establishing a precise chronological age at death. The age at death thus ranged from 15 to 49 years and was equally divided between adolescents and adults, as displayed in Figure 6.

Additionally, the thin sections used in this study were prepared during research at the University of Colorado. The methodology for this preparation is published by Mulhern (2000).

Each specimen was coated with epoxy, allowed to dry, and sawed into ¼ to ½ inch slabs.

Mulhern writes,

“The specimen was then impregnated with Epoxylite 301 and isopropyl alcohol under a strong vacuum with alternating atmospheric pressure. Small sections (less than ¾ inch in diameter) were dried in an oven. All specimens were ground using 600 grit and then dried on a hot plate for 1-4 hr. Each section was mounted using Epoxylite 301. After hardening, excess epoxy was removed by sawing or grinding, using an oil bath saw or oil grinder. Each section was then ground with 320 grit in alcohol or Pella A oil to about 70 µm. Grinding was finished in a 600 grit alcohol or oil slurry to about 40 µm” (2000).

The methodology for generating the thin sections used in the present research thus follows established scientific norms.

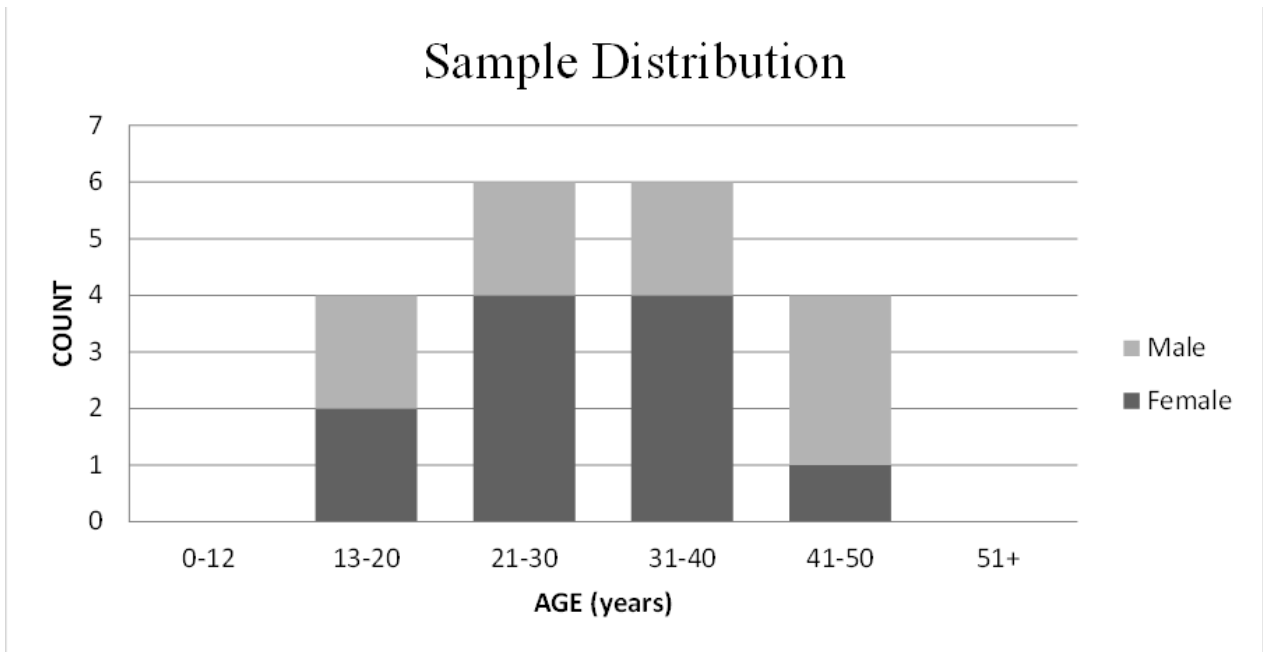


Figure 6. Age and sex distribution for samples used in the present research.

IV. CALCULATING HAVERSIAN SYSTEM FORMATION RATES

Methodology

The thin sections were analyzed under ultraviolet light with a Leica DMRB microscope at N PLAN 5x/0.11, 10x/0.22, 20x/0.40, and 40x/0.65 magnifications. Images of the thin sections at these magnifications were analyzed with Image Pro-Plus® software. Photographs of the images were taken at 5x/0.11 magnification, which the Image Pro-Plus® software was calibrated to take measurements at. A SONY color video camera 3CCD ExwaveHAD and CMA-D2 camera adaptor were used between the microscope and computer software. Additionally, the ultraviolet light was provided by a miller BG 40 quartz IREM 10050 ultraviolet box.

The methodology for determining mean annual bone formation rates for an archaeological population has been expressed in Frost and Wu (1967), Wu et al. (1970), Stout and Teitelbaum (1976), Frost (1987b), Stout and Paine (1994), Stout and Lueck (1995), and Mulhern (2000). The methodology for the present research continues in this tradition, adhering most strictly to the standards set in Stout and Lueck (1995). The following measurements and calculations were taken for each rib thin section:

Mean osteonal cross-sectional area (A_h), defined as the average amount of bone within the cement lines of complete secondary osteons.

Mean osteonal cross-sectional diameter (D_h), calculated from the mean osteonal cross-sectional area using the following formula:

$$2\sqrt{(A_h/\pi)}$$

Intact osteon density (P_i), a count of the osteons in a mm² section with 90% or more of the Haversian canal unremodeled.

Fragmentary osteon density (P_f), a count of the osteons in a mm^2 section with between 10% and 90% of the Haversian canal unremodeled.

Osteon population density (OPD), the combined intact osteon density and fragmentary osteon density for a mm^2 area, determined using the formula:

$$P_i + P_f$$

Accumulated osteon creations (AOC), the number of osteons accumulated during lifespan. This calculation required a scaling operator (β) that was first outlined by Frost (1987b); this asymptote function has been applied to an archaeological population by Stout and Paine (1994), among others. The calculations are as follows:

$$\text{AOC} = \beta \times \text{OPD}$$

$$\beta = (1 - \alpha^x)^{-1}$$

$$x = 3.5 \text{ (determined for the sixth rib by (Frost 1987b))}$$

$$\alpha = \text{OPD}(\text{asymptote})^{-1}$$

$$\text{OPD asymptote} = k((D_h)^2)^{-1}$$

$$K = (\text{asymptote})(D_h)^2 .$$

Stout and Lueck (1995) determined k to be 1.7 based on the suggestion of Frost (1987a) to use an independent sample of rib cross sections from older individuals. K is currently used as a universal for the sixth rib, and 1.7 is used in the present study (Frost 1987b, Stout and Paine 1994, Stout and Lueck 1995).

Mean activation frequency (μ_{rc}), the number of osteons generated annually in a mm^2 area, as identified by the following formula:

$$\text{AOC}/(\text{chronological age} - 12.5 \text{ years})$$

Net bone formation (${}_{net}V_{f,r,t}$), the total amount of secondary osteon formation that has occurred during lifetime. This calculation is expressed in mm^2/mm^2 , and was determined by the following formula:

$$\text{AOC} \times A_h$$

Mean annual bone formation rate ($V_{f,r,t}$), the calculated bone formation rate per year, as expressed in $\text{mm}^2/\text{mm}^2/\text{year}$. The following equation was used for this calculation:

$$\mu_{rc} \times A_h$$

That concludes the use of Stout and Lueck's methods and allows comparisons of bone formation rates between the 21-R-2 cemetery group and four other populations, three archaeological and one modern.

Results

The sample-wide results for annual bone formation rates are described in Figure 7. Following the determination of the sample-wide results, the sample was separated into age groups and sexes, and the rates re-calculated, to identify any potential variation between age groups and sexes. As demonstrated in Figure 8, the formation rates between females and males are similar to an extent that the small variation need not be addressed. There is mild variation between the age groups, shown in Figure 9. This variation, however, is age-associated. OPD, AOC and ${}_{net}V_{f,r,t}$ increase between the younger age groups and the older age groups; these results are expected with an increase in age and are also reflected in the bone formation rates across age groups for other studies (Mulhern 2000; Stout and Lueck 1995). Additionally, the decrease in μ_{rc} and $V_{f,r,t}$ are age-associated, expected, and reflected in the same studies.

Independent Groups T-Test for Means were calculated between the differing age groups. With a confidence interval of 90%, the T-Test between age groups 15-20 years and 41-50 years demonstrated 6 Degrees of Freedom and a 2-Tail Confidence Level of 99.89%. With a confidence interval of 95%, the T-Test between age groups 21-30 years and 41-50 years showed 8 Degrees of Freedom and a 99.84% 2-Tail Confidence Level. The comparison between age groups 31-40 years and 41-50 years exhibited a 95% Confidence Level with 8 Degrees of Freedom and a 99.84% 2-Tail Confidence Level.

An Independent Groups T-Test for Means was also calculated between the results for males and females. This comparison involved a 95% Confidence Level with 18 Degrees of Freedom and a 53.27% 2-Tail Confidence Level. This Confidence Level demonstrates that the male and female differences are not significant.

Figure 7. Descriptive statistics for the sample population¹

Variable	Christian-Group
Age (years)	30.1 ± 2.28
OPD (#/mm ²)	16.8 ± 0.78
A _h (mm ²)	0.038 ± 0.0017
D _h (mm)	0.221 ± 0.022
AOC (#/mm ²)	19.4 ± 1.21
μ _{rc} (#/mm ² /year)	1.6 ± 0.31
_{net} V _{fr,t} (mm ² /mm ²)	0.744 ± 0.051
V _{fr,t} (mm ² /mm ² /year)	0.062 ± 0.011

¹Values are mean ± Standard Error. OPD, Osteon Population Density; A_h, Mean Osteonal Cross Sectional Area; D_h, Mean Osteonal Cross-Sectional Diameter; AOC, Accumulated Osteon Creations; μ_{rc}, Mean Activation Frequency; _{net}V_{fr,t}, Net Bone Formation; V_{fr,t}, Mean Annual Bone Formation Rate.

Figure 8. Descriptive statistics for samples separated for comparison by sex¹

Variable	Female	Male
# individuals	11	9
Age (years)	29.1 ± 2.49	31.4 ± 4.46
OPD (#/mm ²)	16.9 ± 0.73	16.7 ± 1.56
A _h (mm ²)	0.036 ± 0.0017	0.041 ± 0.0030
D _h (mm)	0.215 ± 0.005	0.228 ± 0.009
AOC (#/mm ²)	19.3 ± 1.36	19.5 ± 2.20
μ _{rc} (#/mm ² /year)	1.787 ± 0.528	1.47 ± 0.268
netV _{f,r,t} (mm ² /mm ²)	0.701 ± 0.059	0.797 ± 0.087
V _{f,r,t} (mm ² /mm ² /year)	0.064 ± 0.018	0.059 ± 0.010

¹Values are mean ± Standard Error. OPD, Osteon Population Density; A_h, Mean Osteonal Cross Sectional Area; D_h, Mean Osteonal Cross-Sectional Diameter; AOC, Accumulated Osteon Creations; μ_{rc}, Mean Activation Frequency; netV_{f,r,t}, Net Bone Formation; V_{f,r,t}, Mean Annual Bone Formation Rate.

Figure 9. Descriptive statistics for samples separated for comparison by age at death¹

Variable	15-20 years	21-30 years	31-40 years	41-50 years
# individuals	4	6	6	4
Mean Age (years)	17 ± 0.91	24.5 ± 1.06	34.8 ± 0.98	44.7 ± 1.87
OPD (#/mm ²)	13.25 ± 1.31	16.2 ± 1.33	18.16 ± 1.14	19.5 ± 1.55
A _h (mm ²)	0.037 ± 0.0056	0.040 ± 0.0036	0.041 ± 0.0023	0.036 ± 0.0034
D _h (mm)	0.216 ± 0.016	0.224 ± 0.010	0.227 ± 0.009	0.212 ± 0.010
AOC (#/mm ²)	13.8 ± 1.44	19.1 ± 2.05	21.4 ± 2.05	22.5 ± 2.63
μ _{rc} (#/mm ² /year)	3.6 ± 1.07	1.6 ± 0.20	0.973 ± 0.107	0.698 ± 0.068
netV _{f,r,t} (mm ² /mm ²)	0.513 ± 0.077	0.728 ± 0.055	0.817 ± 0.095	0.810 ± 0.134
V _{f,r,t} (mm ² /mm ² /year)	0.128 ± 0.035	0.064 ± 0.016	0.040 ± 0.005	0.025 ± 0.004

¹Values are mean ± Standard Error. OPD, Osteon Population Density; A_h, Mean Osteonal Cross Sectional Area; D_h, Mean Osteonal Cross-Sectional Diameter; AOC, Accumulated Osteon Creations; μ_{rc}, Mean Activation Frequency; netV_{f,r,t}, Net Bone Formation; V_{f,r,t}, Mean Annual Bone Formation Rate.

V. MEASURING INTERVALS BETWEEN TETRACYCLINE LABELS

Methodology

The approximate time intervals between tetracycline labels reflect the individual's chronological age at death and activation rates. In order to maintain comparable rates within each individual, all of the interrupted labels used in this research occurred in structurally intact secondary osteons, i.e., osteons that developed within the same formation rates that the individuals were experiencing at time of death.

The following calculations and methods were designed and used in the present research as a result of the population's unique tetracycline labeling. An example of an interrupted tetracycline label is found below in Figure 10.

Mean daily activation rate ($_{Daily}\mu_{rc}$), the number of osteons generated daily in a mm² area. Calculate the mean daily activation rate by first *multiplying the chronological age with 365.25*, thereby establishing the chronological age expressed in days. With that value, the mean daily activation rate was calculated using the following formula:

$$AOC / (\text{chronological age expressed in days} - 4565.625 \text{ days})$$

Mean daily formation rate ($_{Daily}V_{f,r,t}$), the average bone formation rate per day, as expressed in mm²/mm²/day. This rate was calculated using the following:

$$_{Daily}\mu_{rc} \times A_h$$

The mean daily formation rate was calculated for each sample and was then compared to the amount of bone found between tetracycline labels for the same sample. The amount of bone found between tetracycline labels was determined by measuring the area of the osteon within the outer (older) label and subtracting the area of the osteon within the inner (younger) label, demonstrated in the following:

Area within outer label – Area within inner label;

thus generating an area between the two tetracycline labels in mm^2 . This measurement is demonstrated below in Figure 11.

Growth between tetracycline labels (G_{TL}); to find the growth between tetracycline labels, the area between labels (expressed in mm^2) was multiplied by the $\text{Daily}\mu_{rc}$ with the result expressed in $\text{mm}^2/\text{mm}^2/\text{day}$.

The final calculation was dividing the mean daily bone formation rate by the growth between tetracycline labels, expressed in the formula:

$$\text{Daily}V_{f,r,t} / G_{TL}$$

The result of this calculation is the approximate number of days between the two tetracycline labels within the osteon.

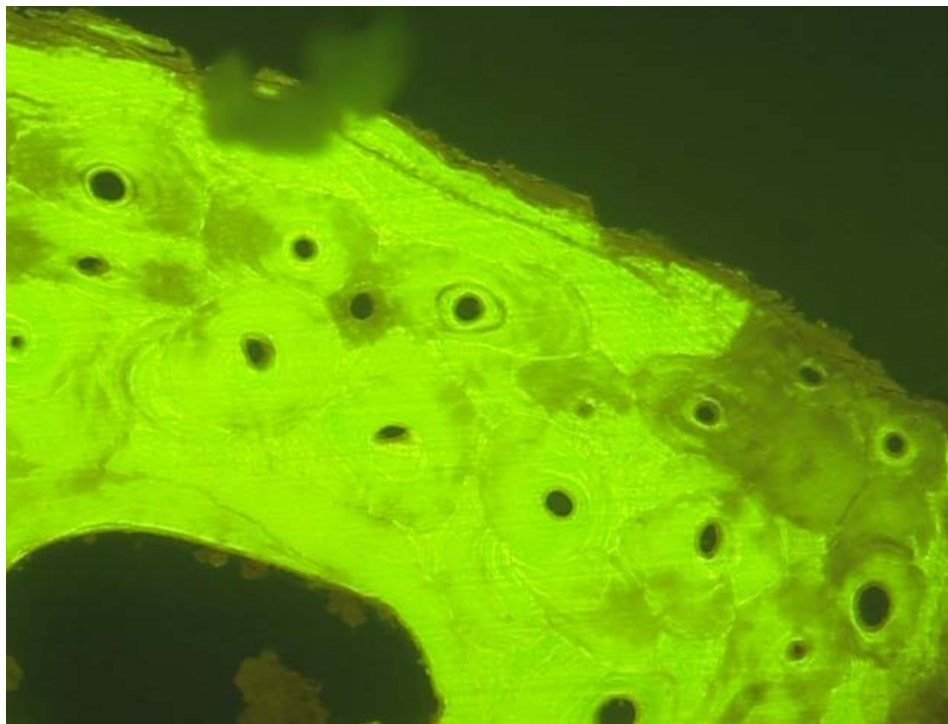


Figure 10. A photograph of a sample individual exhibiting interrupted tetracycline labeling.



Figure 11. The diagram represents an osteon with interrupted tetracycline labeling. The area of 'B' was subtracted from the area of 'A' in order to determine the area between tetracycline labels.

Results

The intervals between tetracycline labels range from 3.3 days to 40 days, as displayed in Figure 12.

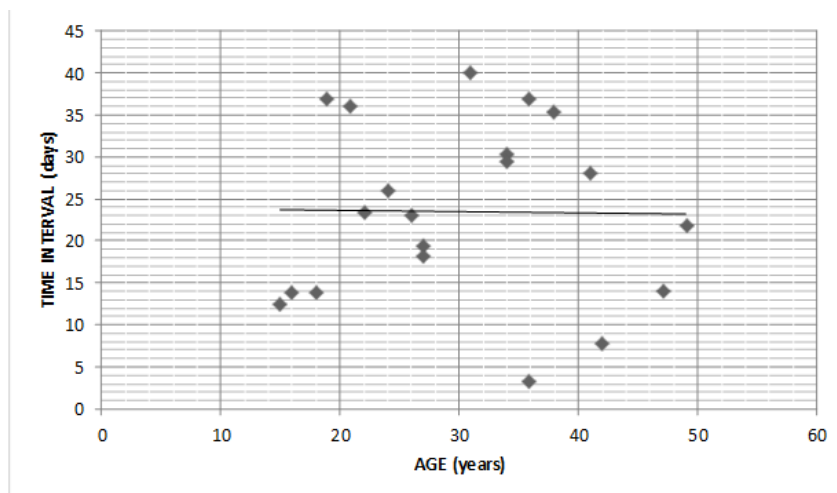


Figure 12. Approximate number of days between periods of tetracycline ingestion set against age in years. The overlaid line is a linear regression trendline.

VI. DISCUSSION

Contextualizing Haversian system formation rates

Research into the Haversian system formation rates from Kulubnarti is benefitted by a comparative analysis between formation rates of the 21-R-2 cemetery group, three other archaeological populations, and a modern sample. The Haversian system formation rates of the three archaeological populations (Windover, Gobson and Ledders) and modern sample have been calculated by Stout and Lueck (1995).

The Windover population was located in east-central Florida during the Early Archaic period. The population has been radiocarbon dated to between 6,900 and 8,120 B.C.E. The population ingested deer, freshwater fish, marsh vertebrates and plant resources. The Gibson population represents a site in the Lower Illinois River Valley that has been dated to between 50 B.C.E. and 400 C.E., placing it in the Middle Woodland Period. The Gibson population practiced harvest collecting. The Ledders group is also from the Lower Illinois River Valley, and has been dated to 1,000 C.E., thereby representing the Late Woodland Period. Lastly, the modern sample represents the middle third of the sixth rib from 45 individuals of known age, sex and race (Stout and Lueck 1995).

The OPD and AOC rates are low for the 21-R-2 cemetery group comparatively. The A_h and D_h rates are high for this population comparatively. The $\mu_{rc, net}$, $V_{f,r,t}$ and $V_{f,r,t}$, are, however, directly on the linear trendline. These three rates are arguably the most informative, the $V_{f,r,t}$ being the desired calculation for both the present research and that of Stout and Lueck (1995).

The comparison of the Kulubnarti Haversian system formation rates to those of four other populations supports the idea of a change of Haversian system formation rates over time. Stout and Lueck (1995) introduce this idea, and demonstrate support for it through their own

comparison of Haversian system formation rates between the Windover, Gibson and Ledders groups and a modern population. The authors call for formation rates from additional populations to be considered in relation to other archaeological populations to further demonstrate the change in Haversian system formation rates through time. The rates of the 21-R-2 population demonstrate support for Stout and Lueck's (1995) claim.

Time intervals between tetracycline labels

Clear interruption between labels of tetracycline were difficult to find within the osteons of the 21-R-2 cemetery group. The labels that were located represented relatively short periods of time (between 3 and 40 days, as shown in Figure 12) wherein the individual did not ingest tetracycline. It is anthropologically significant that this population was ingesting tetracycline at such a consistent level; tetracycline-carrying beer thus played a common role in daily, weekly, or monthly life.

There were no evident differences between time intervals between ingestion and sex of the individual. Figure 12 shows the differences in time intervals associated with age. A linear regression line indicates that time intervals between ingestion were not consistent based on one's chronological age at death.

VII. CONCLUSION

The 21-R-2 cemetery group bone formation rates fit well within the established bone formation rates for the Windover, Gibson and Ledders populations, as well as with that of the modern sample. The combined formation rates demonstrate a linear trend throughout time of change in formation rates, a concept advocated by Stout and Lueck (1995).

The calculated time intervals between two tetracycline labels for each individual demonstrate consistent ingestion of tetracycline. No perceivable difference was found between sex or age groups, indicating widespread use throughout the group. Based on this analysis, tetracycline, and the beer that carried it, played an important role among these individuals.

By calculating Haversian system formation rates, comparing them to four other populations, and using the Haversian system formation rates to determine time intervals between tetracycline ingestion, further applications of tetracycline to archaeological populations can be identified. Potential direction for future research into tetracycline and the individuals of Kulubnarti could include determining more precise ages for individuals older than 51 years at death, thus allowing these individuals to be included into current results for the Christian-group. Additionally, the 21-R-2 individuals older than 51 years could give valuable insight into the role of tetracycline in inhibiting bone loss among the elderly.

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APPENDIX

Visual Representation of Thesis



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