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# A Microscopic Comparison of Fresh and Burned Bone

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**ABSTRACT:** Examination of the microstructure of human bone is useful in estimating age at death in forensic science cases. This technique has been tested and is well accepted, however samples of burned bone may complicate analysis because of possible changes in the microstructure occurring during the burning process. In a comparison of fresh and burned ground thin sections taken from midshaft femorae of eight dissecting-room cadavers of known age and sex, this study finds significant shrinkage of microstructural elements through the burning process. These results are compared to previous work on the subject, which found the microstructural elements to increase through the burning process.

**KEYWORDS:** physical anthropology, osteon, age determination, burned bone

Examining the microstructure of human bone is useful for estimating age at death in forensic-science cases [*1*]. Kerley demonstrated that the ratio of remodeled to unremodeled bone in the cortical third of a long bone shaft varies proportionate to the age of the sample. Numerous researchers have suggested additional techniques refining Kerley's method [*2–6*]. While this technique for estimating age is well accepted, samples of burned bone may complicate analysis because of possible changes in the microstructure occurring during the burning process.

Studies at both the macroscopic[2] [*7*] and microscopic levels [*8–12*] generally agree that under conditions of extreme heat, bone shrinks, splits, and cracks. Of the more recent microscopic studies, Shipman et al. [*11*] present Scanning Electron Microscope (SEM) photomicrographs of bone that has been burned at various temperatures. Bradtmiller and Buikstra [*8*] are specifically concerned with the effects of extreme heat on the microstructure of cortical bone, particularly as these effects apply to microscopic techniques for the estimation of age at death. Bradtmiller and Buikstra note a wide variation in shrinkage reported in other studies (2 to 25% reduction from the original fresh bone) with experimentally controlled cases at 3 to 5%. Shrinkage, as pointed out by these authors, ". . . is a very important consideration . . ." when adapting Kerley's method (or any known microscopic examination technique) to estimate age in burned bone.

They went on to report that the shrinkage noted in their study did not seriously affect the osteon counts used for aging the individuals they tested. They found, however, that osteon size actually increased, rather than decreased by shrinking, as would have been predicted by previous work on the subject [*8*].

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[2]Burns, K. R., "Linear Effects of Drying and Burning on Human Bones," presented at AAFS meeting Feb. 1988.

This article compares ground fresh and burned femoral midshaft thin sections under controlled conditions to assess the consistency of Bradtmiller and Buikstra's shrinkage results. In addition, this paper demonstrates the adaptability of Bradtmiller and Buikstra's techniques for estimating age at death to equipment more likely available in most physical anthropology laboratories. Bradtmiller and Buikstra's study used sophisticated microradiography and photographic enlargement for measurements of the bone microstructure; in contrast, the research for this paper was carried out on standard laboratory equipment, and measures the microstructural elements directly.

## Materials and Methods

Femoral midshaft segments from eight dissecting-room cadavers of known age and sex were cleaned of soft tissue. Thin sections were made using an Isomet (TM) rotary sectioning saw, then degreased in acetone and mounted with epoxy on glass slides. Specimens were ground to approximately 25 to 30 μm thickness and polished on 600 grit emery cloth. The femoral segments, measuring between 3.5 cm and 7 cm in length were then burned at between 1000 and 1500°F in a propane-fired kiln for 30 min and allowed to cool slowly in the kiln. The burned segments were stabilized in polyvinyl acetate resin and cast in blocks of Evercoat (TM) casting resin. Thin sections were made within 1 to 2 cm of the face cut on the bone segments (now burned) from which the fresh sections were made. Taking the thin section from this point in the burned sample is believed to avoid potential damage to the surface of the bone occurring during the burning process, while minimizing the possibility of inappropriate comparison of heterogenous samples taken from more diverse parts of the bone. These thin sections were then ground until they could be examined under a light microscope. Light can pass through the black burned material at a thickness of less than 25 μM.

Sixteen slides were then examined under a conventional binocular light microscope using a 1 $mm^2$ calibrated ocular micrometer. Measurements were made on 102 complete osteons from each slide, in two perpendicular dimensions as outlined in Singh and Gunberg [5]. Complete osteons were defined as those in which the canal is clearly visible, and which were not encroached upon by subsequent remodeling. The dimensions of the Haversian canal of each osteon were measured in this manner as well. This produced a sample size of 1636 osteons, with $n = 816$ for a fresh sample group and $n = 820$ for the burned sample group. Circular and nearly circular osteons were chosen so that the mean of the two diameter measurements was actually representative of the osteon. An attempt was made to measure the number of concentric lamellae per osteon, although when measuring the burned material this count was often not practical as the interfaces between lamellae were not consistently distinguishable.

For the statistical analysis, used values were defined as:

1) Mean Osteon Diameter (OSTDIAM), osteon size by mean diameter.

2) Mean Canal Diameter (CANDIAM), canal size by mean diameter.

3) Osteon Index (OSINDEX), a ratio, computed by dividing the mean canal diameter by the mean osteon diameter.

4) Osteon Area (OSTAREA), computed using ½ the mean diameter as the radius, in the formula a = (pi) r (squared).

5) Canal Area (CANAREA), using the same formula as #4.

6) #4 minus #5 (OMINUSC), similar measure to #3, but reflecting the measured change in area in square units.

The Index ratio (Variable #3) is computed to negate the difference in actual osteon size and to address the relationship between the whole osteon and the canal running through it. (For example, if two resorptive spaces are of mean diameter 0.20 mm and 0.40 mm, and have secondary redeposition of concentric lamellae yielding canal diameters of 0.05 mm and 0.10 mm respectively, the index for both these osteons will be 0.25.) Higher index values should be indicative of less completely filled Haversian canals, while lower values indicate more completely filled, possibly older canals. Variable #6, on the other hand, is a direct measure of area, and represents the calculated surface area of the osteon minus the surface area of the cross cut canal. Excessively ovoid osteons (probably not cut perpendicular to the plane of the sectioning saw) and those appearing to exhibit considerable osseous "drift" [12], were not measured.

An SPSS/PC+ program was used to compare fresh and burned material on the basis of the six values described above. Following the basic descriptive statistics (mean, standard deviation, range, frequency of occurrence), a T-test was performed comparing the means of the fresh versus burned subsamples.

## Results

Visual assessment of the burned material at low (40 times) magnification suggests that canal size is markedly increased in many instances. Results of measurements taken at 100 times magnification and computed relative to other dimensions such as osteon diameter and index ratio confirm this observation. Extreme heat causes statistically significant changes ($P < 0.01$) in measurements of the bone microstructure. In this study osteon diameter for the sample means decreased through the burning process by 16.7%, while canal diameter increased by 10.5%. This change is reflected in the index ratio change from 0.29 to 0.39, indicating an increase in the canal dimension relative to the overall osteon dimension. This may imply either a decrease in the area of the osteon taken up by concentric lamellae through shrinkage or burning away of the lamellae themselves, or the burning away of dessicated soft tissue within the canal. This value was not determined, as individual lamellae were many times indistinguishable in the burned bone.

Table 1 gives descriptive statistics for the variables "osteon diameter," "canal diameter," "osteon index ratio," "osteon area," "canal area," and "osteon area minus canal area" for comparison in "fresh" and "burned" groupings. The SPSS T-test shows statistically significant ($P < 0.01$) differences between the two groups for the means of osteon diameter, osteon index, osteon area, and osteon area minus canal area, and in

TABLE 1—*Descriptive statistics for fresh vs. burned subsamples.*

| Variable | | Mean | SD | Variance | Range | Min | Max |
|---|---|---|---|---|---|---|---|
| **Fresh sample, *n* = 816 osteons.** | | | | | | | |
| OSTDIAM | + | 20.07 | 7.53 | 56.73 | 47.50 | 8.5 | 55.5 |
| CANDIAM | @ | 5.79 | 3.32 | 11.00 | 29.00 | 1.0 | 30.0 |
| OSINDEX | * | .29 | .11 | .01 | .84 | .59 | .90 |
| OSTAREA | * | 360.87 | 274.60 | 75 406.44 | 2368.73 | 50.3 | 2419 |
| CANAREA | # | 34.99 | 50.50 | 2 550.61 | 706.11 | 0.79 | 706.9 |
| OMINUSC | * | 325.87 | 245.28 | 60 146.20 | 1697.08 | 14.92 | 1712 |
| **Burned sample, *n* = 820 osteons** | | | | | | | |
| OSTDIAM | + | 16.72 | 6.77 | 45.83 | 42.00 | 3.0 | 45.0 |
| CANDIAM | @ | 6.40 | 3.29 | 10.45 | 19.50 | 2.0 | 21.5 |
| OSINDEX | * | .39 | .13 | .02 | .85 | .15 | .99 |
| OSTAREA | * | 255.39 | 217.24 | 47 193.38 | 1582.93 | 7.07 | 1590 |
| CANAREA | # | 40.72 | 47.37 | 2 244.37 | 359.96 | 3.14 | 363.1 |
| OMINUSC | * | 214.66 | 189.14 | 35 772.20 | 1438.55 | .01 | 1436 |

* $P < .000$, pooled and separate variance estimates $p < .000$.
\+ $P < .002$, pooled and separate variance estimates $p < .000$.
\# $P < .068$, pooled and separate variance estimates $p < .018$.
@ $P < .845$, pooled and separate variance estimates $p < .000$.

the variance estimates for all values. Measurements in Table 1 are given in increments of 1 = 10 microns, (1 = 10). Area is given in (1 = 10).

**Discussion**

The primary difference between the results in the present study and those of Bradtmiller and Buikstra [8] is that while they found an overall increase in the size of the osteons through the burning process, I find that osteon size actually decreases quite significantly with burning. This may be attributable to mechanical differences between the two temperature and time duration strategies used in burning the material. In contrast to the method outlined above, Bradtmiller and Buikstra burned their specimens in an electric oven for ". . . several minutes until it seemed likely that the bone had reached the same temperature as the oven" (600°C). "At that point the oven was switched off, but the bone was allowed to burn out and cool at room temperature." Bradtmiller and Buikstra do not indicate whether the specimen is completely calcined. If it is being burned with the flesh on, as their methods section indicates, there may not have been sufficient time to completely burn the bone.

Bradtmiller and Buikstra offer a number of possible reasons for the increase in osteon size, and suggest that the 10 cm difference between the fresh and burned sample sources (on the same bone) is the most parsimonious explanation. However, their results may be due to incomplete burning, as they point out. Because bone may expand slightly before it shrinks, Bradtmiller and Buikstra's results may exemplify such a point in the burning process, and be valid only for incompletely burned material. Van Vark [11] doesn't record shrinkage until the 700 to 900°C temperature range, and goes on to state that no significant differences in shrinkage results were noted between samples burned with flesh on and those defleshed prior to burning. The bone samples used in this study were well burned before being allowed to cool in order to study changes in the broader range of fresh to completely calcined. It is believed this would more accurately assess osseous shrinkage in crematory or otherwise calcined skeletal remains.

Another possible explanation for the observed osteon size increase in Bradtmiller and Buikstra's sample could be related to confusion between the most prominent cementum line and the actual osteon boundary. Osteons in the burned bone are distinguishable primarily on the basis of clearly demarcated cementum lines separating the filled resorptive canal space from the surrounding bone, whether it is of the circumferential lamellar type or is fragmentary remodelled osteons. On the other hand, in fresh bone, some processes (osseous drift, for example [12]) appear to be traceable through the shape of the osteon, and the differentiation between the cementum lines of a viable osteon and those of a remodeled osteon becomes vague. When the bone is burned, a particular cementum line other than the one (theoretically) chosen prior to burning as the boundary for that osteon may become more prominent, giving the appearance of increased osteon size to the particular structure being measured. This differential misidentification of cementum lines would have to be carried to colossal proportions, however, in order to account for the overall increase in osteon size as reported by Bradtmiller and Buikstra. To address possible osteon size differences in the spacially separated sections of a bone [8], thin sections were taken from within 1 to 2 cm of each other, rather than 10 cm or more as previously reported. Another possible explanation for a size increase is scaling error caused by measuring an enlarged photographic representation of a microradiograph.

For the sake of replicability, these procedures were carried out on standard laboratory equipment using supplies locally available, such as the casting resin, which was procured from a nearby hobby shop. A local lapidary shop turned out to be a very good source of inexpensive saw blades and water base cutting lubricant. If a circular sectioning saw is not available, cutting and grinding sections by hand is a viable alternative. This procedure is tedious and time consuming, suggesting that if an individual wishes to research thin sectioning techniques, a saw and polisher should be a practical requirement. The 1 mm square ocular micrometer is used to make counts and measurements because, as explained by Alquist and Damsten [2], variation between microscope field of view size and the problems encountered in reading at the edge of the field of view are eliminated by the use of this standardizing device.

Shrinkage, as reported by Bradtmiller and Buikstra [8], does not seem to seriously affect estimation of age by osteon count. The example they give is based on a mathematical reduction, however, rather than observed shrinkage, since their osteons expanded. While this appears to pose a reasonable model with which to compare empirical results, until actual observations are made, it is probably premature to minimize the effects shrinkage may have. If shrinkage does indeed cover the reported range of variation, it seems logical that under some conditions the estimation of age would be affected. Bradtmiller and Buikstra [8] apparently base their results on the analysis of a single femur section. Results of the present study are based on observations carried out on eight femoral sections.

Obviously a much larger sample size and more study will be necessary before any solid conclusions can be drawn. Future study would profit from research in interobserver variability in estimating age by observations of quantitative histology, possibly as a continuation of Bouvier and Ubelaker [13]. In order to address the problem of homogeneous distribution of osteons through the length of the diaphysis, a number of femora, fibulae, and other long bones could be sectioned at 2 cm, 3 cm, or 4 cm intervals and studied.

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