Bruce Bradtmiller,[1] *M.A. and Jane E. Buikstra,*[2] *Ph.D.*

Effects of Burning on Human Bone Microstructure: A Preliminary Study

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REFERENCE: Bradtmiller, B. and Buikstra, J. E., **"Effects of Burning on Human Bone Microstructure: A Preliminary Study,"** *Journal of Forensic Sciences*, JFSCA, Vol. 29, No. 2, April 1984, pp. 535-540.

ABSTRACT: The microscopic determination of age at death in human bone is a widely used technique in forensic anthropology. Despite its use, little attention has been given to the reliability of microscopic aging when the subject has been burned, either at the time of death, or after death. This preliminary report examines some of the variables of the burning process that may affect the age estimates. Preliminary conclusions are: (1) bone burned at 600°C retains all of the structures necessary for microscopic aging and (2) bone shrinkage, widely reported in the literature, does not appear to have significant effect on the age estimate. A research plan is outlined that will address some of the questions left unanswerable in the present report.

KEYWORDS: physical anthropology, human identification, burning, microscopic age determination, osteon aging

This preliminary report explores whether, and how, bone microstructure changes under conditions of burning. The present work, and more extensive investigations to follow, have a forensic science application in the determination of age at death from human cortical bone. This important method was developed by Kerley [*1,2*] and has inspired several other microscopic methods in the nearly 20 years since the first publication on the subject [*3-6*]. Despite the widespread use of many of these microscopic methods, little attention has been given to how the techniques might be affected by burning (however, see Ref *7*). The work presented here addresses two aspects of burning on bone microstructure. These are: (1) whether osteons and other microstructural elements of human cortical bone change appearance to impair or prevent their correct identification when the structures are counted and (2) whether the osteons or other structures change in size.

Little research has been done on the effects of burning on bone microstructure; however, inferences can be drawn from other work on bone and the burning of bone. Various researchers have found that burning produces deep transverse splitting [*8*] and flaking off of the outer layers of cortex [*7*]. Cracking itself should not pose more than mechanical problems in making the thin section and adjusting the field size to account for the cracks. If the outer third of the cortex has flaked off, however, Kerley's method, at least, loses considerable precision. Hermann [*9*] discovered that in very hot fires, 700 to 800°C for example, the bone mineral crystals can fuse. The disruption of bone mineral can have greater or lesser significance, depending on the preparation techniques being used. Under polarized light,

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Presented at the 35th Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, OH, 15-19 Feb. 1983. Received for publication 17 May 1983; accepted for publication 25 Aug. 1983.

[1] Physical anthropologist, Anthropology Research Project, Inc., Yellow Springs, OH.

[2] Professor, Department of Anthropology, Northwestern University, Evanston, IL.

Hermann [9] found many of the structures unrecognizable. He notes, however, that with using microradiography, even completely cremated material (that is, burned over 800°C) maintains its appearance.

Bone shrinkage was also observed in Hermann's [9] experiments, and in the work of several other investigators [10-13].[3] The shrinkage commonly reported presumably occurs as a direct result of the fusion of mineral crystals that Hermann [9] observed at 700 to 800°C. Under that temperature he found a shrinkage of 1 to 2%. Other published works report shrinkage of up to 25% [10,12], but these works lack important experimental controls. Swegle's work[3] benefited from rigorously controlled conditions and produced shrinkage of 0 to 5%, with most cases around 3%. The amount of shrinkage in bone is a very important consideration in using Kerley's osteon aging technique on burned human bone. Shrinkage of bone microstructures may result in more of those structures in a given microscopic field. Different numbers of osteons and osteon framents will, of course, yield different age estimates for the burned and unburned bones.

Materials and Methods

The ideal conditions to test these ideas would allow the examination of a bone thin section both before and after burning. Unfortunately the burning of thin sections is so dissimilar to the ordinary conditions of burning whole bones that the results would be meaningless. The next best circumstance, and the procedure followed here, was the burning of parts of a bone and comparing them to unburned parts of the same bone. The femur of a dissecting room cadaver was chosen for the experiment. Two chunks of the bone, each approximately 10 cm long were burned. On the first of these, the flesh was removed before burning. On the second, the bone was burned with the flesh still surrounding the bone. The thin sections used in the analysis were taken from the midpoint of the burned bone. This strategy approaches as closely as possible the conditions in burning whole bones. A thin section was also taken from the nonburned bone. We wished to examine the effects of burning dry bone as well. This situation does not occur frequently in the forensic science context, but is a matter of interest in archeological analysis. The sampling procedure was repeated and part of a femur from an archeological source with no provenience was burned. The remainder was unburned. Unfortunately the archeological bone had become demineralized in the ground, and neither the burned nor the unburned thin sections were interpretable.

The burning took place in a small electric oven; while the exact temperature of the bone as it burned is not known, the temperature of the oven was brought to about 600°C and allowed to remain there several minutes until it seemed likely that the bone had reached the same temperature as the oven. At that point the oven was switched off, but the bone was allowed to burn out and cool at room temperature. The specimens generally burned in the oven for about 30 min after the oven was switched off.

From both the burned and the unburned specimens 3-cm blocks were taken from the midpoint of the bone and embedded in methyl methacrylate polymer.[4] After hardening, 100-μm thin sections were cut from the blocks. The thin sections were microradiographed at low kilovoltage for an hour and developed by standard techniques. The recognizability of the various structures can easily be assessed by examining the microradiographs under light microscopy, or by viewing photographs. Photographs were taken of three sites on the micro-

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[3] M. Swegle, "A Study of Experimentally Cremated Bone," unpublished manuscript, Bloomington, IN, 1979.

[4] Embedding bone specimens in methyl methacrylate is not a standard practice in making slides for use in microscopic aging techniques. The burned bone was quite brittle and broke frequently. After embedding in the methyl methacrylate polymer, which actually permeates the bones, sectioning was routine and presented no further problems. Other embedding media may work as well, but this one can be recommended without reservation.

radiograph and were used in the measurement of osteon diameters. The diameter of each complete osteon in each photograph was measured in two perpendicular dimensions, so that one diameter was the maximum dimension. The simple mean of the two diameters is taken as the diameter of the osteon. Osteon measurements from bones that were not burned, burned with the flesh on, and burned with flesh removed were analyzed using the SPSS analysis of variance (ANOVA) procedure.[5]

Results

Figures 1 and 2 show that all structures are easily identifiable in burned bones, whether the flesh was removed or left on the bones. The temperature of this experiment did not reach Hermann's [*9*] critical level, but his works show that microradiographically the bone structure remains unchanged even above 800°C. Thus one conclusion from this preliminary project is that burned bone still has the appropriate elements necessary for microscopic aging [*1,2*].

The analysis of osteon size produced unexpected results. The osteons from the burned bone was uniformly larger than those from the unburned bone. This is contrary to what would be expected given the previous work on the topic. Further the ANOVA with all three groups contrasted (Table 1) has a significant F at 0.001. The subsequent tables show ANOVAs contrasting the nonburned osteons with the burned osteons of each type. The nonburned bone is not significantly different from the defleshed burned bone (Table 2), but the nonburned bone is highly significantly different, at 0.0001, from the fleshed burned bone (Table 3). The latter case is more frequently seen in a forensic science context. Several explanations may be offered for the larger size of the osteons in the burned bone.

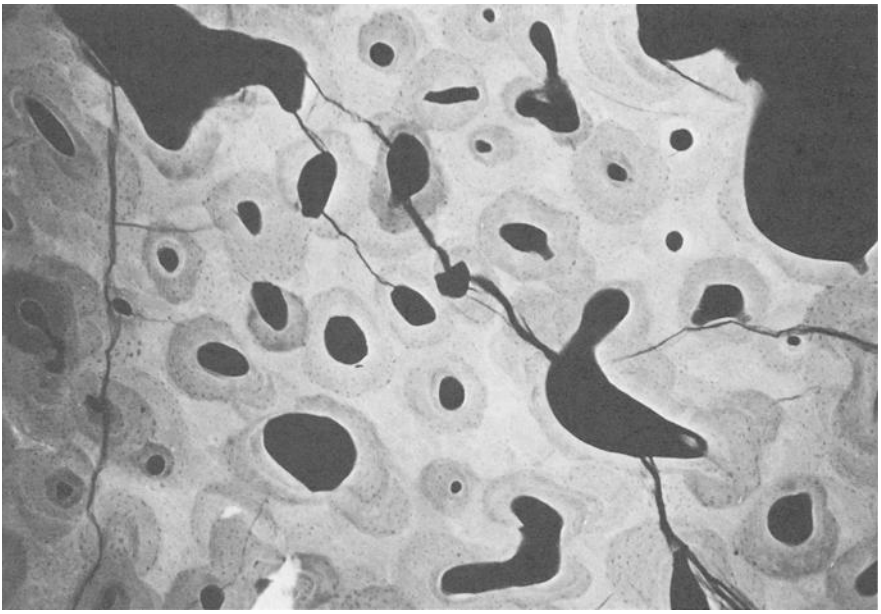

FIG. 1—*Photomicroradiograph of a femur burned with the flesh on. See text for details of preparation. Note that structures are clearly delineated.* ×*40.*

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[5] SPSS = Statistical Package for the Social Sciences.

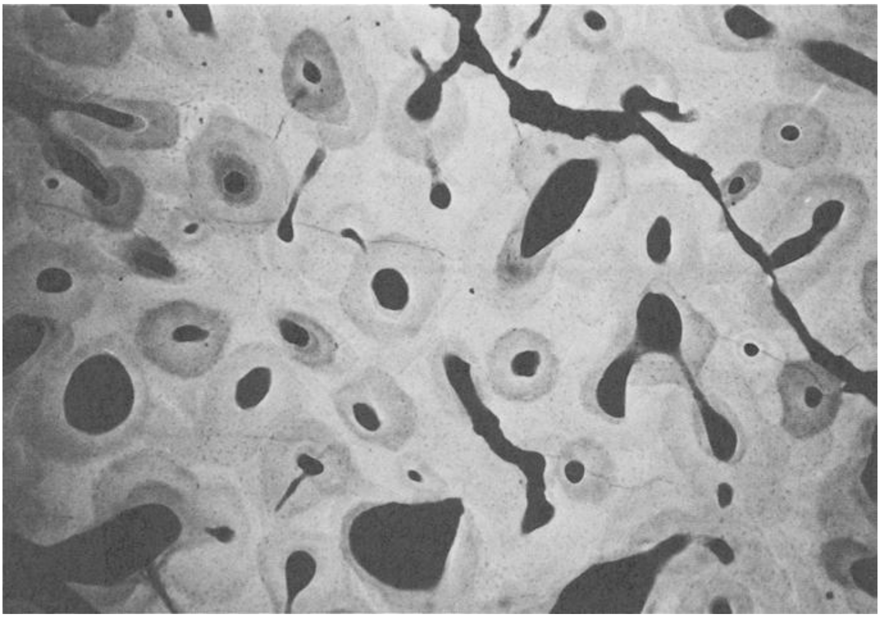

FIG. 2—*Photomicroradiograph of a femur burned with the flesh removed. Same bone as in Fig. 1.* ×*40.*

TABLE 1—*Osteon size ANOVA—three-way contrast.*[a]

Source	Sum of Squares	Mean Square	F	Significance of F
Between	569.24	284.62	7.21	0.001
Within	7538.65	39.47	...	...
Total	8107.89	...	...	...

[a] $N = 194$.

TABLE 2—*Osteon size ANOVA—nonburned versus defleshed.*[a]

Source	Sum of Square	Mean Square	F	Significance of F
Between	101.29	101.29	2.35	0.128
Within	6171.68	43.16	...	...
Total	6272.97	...	...	...

[a] $N = 145$.

1. The bone may expand slightly before it shrinks, and in this case the bone stopped burning before shrinkage.
2. The bone may shrink in its external dimensions, but because of some rearrangement of microstructural elements the osteons themselves actually increase in size.
3. The bone, and the osteons with it, actually shrink as expected, but the shrinkage is not apparent here because of a sampling problem.

TABLE 3—*Osteon size ANOVA—nonburned versus fleshed.*[a]

Source	Sum of Squares	Mean Squares	F	Significance of F
Between	569.18	569.18	16.89	0.0001
Within	4278.32	33.69	...	...
Total	4847.50	...	...	...

[a] $N = 129$.

As noted in the Methods section, chunks of bone about 10 cm in length were burned, with the thin sections taken from the midshafts of those chunks. Thus the thin sections from which osteons were measured were actually about 10 cm apart. It may be that over such distances on the femoral shaft there are osteon size differences in the living individual. This explanation is presently the most parsimonious.

In an effort to explore the effects of uniform bone shrinkage on Kerley's osteon aging technique [*1,2*], a recent forensic science case was reexamined. This individual was found partially skeletonized in July 1982 in the city of Chesapeake, VA. The case was referred to Dr. David Glassman of Virginia Polytechnic Institute, who sent a femur sample to the osteon counting laboratory at the University of Tennessee. Using the actual counts from this case, the age was computed in the ordinary way. Then, each of the counts was increased by 9.75%, the shrinkage in area associated with the 5% linear shrinkage reported by Swegle.[3] When the age of the individual was recomputed using the increased counts (Table 4), the difference was only 2.0 years. This demonstration assumes that structures are uniformly distributed throughout the cortex. Under conditions of actual bone shrinkage, decrease in the size of the structures would probably not produce the exact increases seen here. Further, age differences will vary with the age of the individual. Nevertheless, this demonstration shows that with 5% overall shrinkage, the alterations in the age estimate may be fairly small.

Conclusions

This is quite clearly a preliminary investigation, and any conclusions drawn from it must necessarily be quite tentative. Nonetheless, it does suggest some directions for future research. First, it may be worthwhile to retest Hermann's critical level of 800°C to verify if microstructures are still readily discernible. This may be tested by incremental increases in temperature. It should also be determined if microradiography is the only technique that can be used on those completely burned specimens. Second the shrinkage issue needs much more work. A two-phase examination is proposed here. In the first phase, a series of sections should be taken from both the right and left side of the same individual to determine the extent of osteonal size variation between the two sides. If it can be demonstrated that there is

TABLE 4—*The age of the individual was recomputed using the increased counts.*

	Original Data	9.75% Increase
Osteons	74	81
Osteon fragments	39	45
Lamellar bone	14	15
Non-Haversian canals	5	5
Age estimate	42	44

minimal side-to-side variation, then the right side can be burned and tested against the unburned left side of the same individual. Any additional variation in osteon size could be attributed to the burning. In this way precise midshaft sections can be used which will eliminate the problem of distance between sections on the same bone. After that proposed research is completed, microscopic aging methods can be used on burn cases with considerably more confidence than is now possible.

Acknowledgments

We gratefully acknowledge the assistance of: Carol R. Cottom, Mark Swegle, and Steven A. Symes in the preparation of this report. The specimens were burned in facilities provided by Joseph B. Lambert, Department of Chemistry, Northwestern University, with the assistance of Sharon M. Vlasak of the same department. The microradiography was carried out in the laboratory of Robert A. Schlenker, Center for Human Radiobiology, Argonne National Laboratory, with the kind assistance of his staff. Partial funding for the project was provided by The Graduate School, Northwestern University.

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Address requests for reprints or additional information to
Bruce Bradtmiller
Anthropology Research Project, Inc.
503 Xenia Ave.
Yellow Springs, OH 45387