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The Use of Histological Techniques in the Determination of Cultural and/or Environmental Processes in Archaeological Skeletal Populations

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THE USE OF HISTOLOGICAL TECHNIQUES IN
THE DETERMINATION OF CULTURAL AND/OR
ENVIRONMENTAL PROCESSES IN ARCHAEOLOGICAL
SKELETAL POPULATIONS

by

Randy L. Parshall

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Randy L. Parshall

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INTRODUCTION

In many instances, it is possible to infer certain cultural practices or environmental processes which have affected the gross morphological structure of archaeological skeletal collections without the aid of a microscope. For example, longitudinal cracks may frequently be observed in bones which have remained in the open long enough to become weathered (Tappen, '69). It is also possible to determine whether skeletal remains have been cremated or not from an analysis of the calcined and uncalcined remains of the skeleton. Furthermore, it has been demonstrated that from an analysis of cremated skeletal material it is often possible to determine whether it was cremated in the flesh or whether it was a dry bone cremation (Baby, '54; Binford, '63).

Human and animal skeletal remains are often found in midden deposits. Each, to some extent, may have been affected by some cultural and/or environmental process. Although macroscopic evaluations do, occasionally, allow anthropologists to say something of the cultural practices of the people under consideration or environmental processes which may have affected gross morphological bone structure, these processes are often difficult to determine due to the fragmentary nature of such remains. Therefore, it would be of value to the archaeologist analyzing skeletal material to know exactly what histological changes occur in bone that has been burned, boiled, or weathered (frozen and thawed) so that they can better interpret the significance of this type of

bone found at archaeological sites. Since these processes affect the gross morphological structure of bone, it was hypothesized, by the researcher, that these processes may also affect the microscopic structure of the bone. Although similar research has been done concerning the effects of heat on living bone, in vivo, no such studies had been done on archaeological bone (Enlow, '73: personal communication). For these reasons, the present study was undertaken.

The histological structure of the long bones of the three animal species was examined after being subjected to various cultural and/or environmental conditions. These three forces or processes which may change the gross histological structure of bone will be dealt with as follows:

1. The burning of a long bone with flesh on and off.

This process should simulate, among other things, the cooking of meat over an open fire; bone thrown into a fire either partially or totally defleshed; cremated bone which has not been completely calcined; or bone in which the flesh has been partially or totally decomposed and later burned, either intentionally or unintentionally.

2. The boiling of a long bone both with the flesh on and off.

This process should simulate the technique of boiling meat; the boiling of partially defleshed bones in such preparations as soups and stews; or bone placed for any other reason in boiling water.

3. The freezing and thawing of a long bone both with the flesh on and the flesh off as one aspect of the weathering process.

This process should simulate the effects of an animal that died and the carcass left to decompose; or an animal that was totally or partially skinned

and the bones discarded and left to the elements. Freezing and thawing should partly simulate the histological effects of weathering on a long bone that has not proceeded too far, since this type of weathering per se would probably only affect outer bone appearance and not destroy the internal organic matrix.

MATERIALS AND METHODS

The long bone materials used in this study were from the white-tailed deer (Odoicoileus virginianus), the domestic dog (Canis familiaris), and the raccoon (Procyon lotor). Although the exact ages of these animals were not known, they were all known to have been adults since in all three cases the epiphyses had united to the diaphyses. In the case of Odoicoileus virginianus only the left rear metapodial was available while all of the long bones were available from Canis familiaris and Procyon lotor.

In order to simulate cultural practices and/or environmental processes that may affect the histological structure of bone the following techniques were employed. Burning was simulated by baking the bones in an oven at a temperature of 350° for a period of approximately one-half hour. Since one of the major effects of burning a bone is to drive the moisture from it, the heating of a bone in an oven at a controlled temperature should simulate the same process. A second set of bones was placed in a pan of boiling water for approximately one hour. A third set was placed in a freezer for approximately twenty-four hours and removed for the same period of time. The latter process was repeated over a two-week period of time.

For the white-tailed deer, the metapodial was first scraped clean of flesh and then four adjacent cross sections, approximately two to three millimeters wide, were taken from the midshaft of the diaphysis. Three of these sections were subjected to the

previously mentioned experimental techniques while the fourth, one of the inner two sections, was kept as a control section. Although these small sections may show exaggerated reactions, basically the reactions would be similar to those one would expect to find if whole long bones had been used.

In the case of the dog and the raccoon, the long bones were subjected to the various techniques both with flesh on and flesh off the bone, to see if intact flesh did, in fact, have any effect on any microscopic changes that may have taken place. The ways in which the various long bones were treated are as follows:

- 1) The tibia was scraped clean and then used as the control specimen;
- 2) Two femora, one with flesh intact and one completely defleshed were roasted;
- 3) Fleshed and defleshed radii were boiled for one hour;
- 4) Two humerii, one with flesh on and the other with flesh off, were subjected to alternate periods of freezing and thawing over a two-week period.

After each bone had been treated, a small section, approximately two to three millimeters in thickness, was taken from the center of the midshaft of the diaphysis. These sections were then ground, permanently mounted and subsequently examined under the microscope. Sections were taken from the midshaft because this area has the greatest amount of compact and the least amount of cancellous bone, and since different regions in the same bone or different bones of the same skeleton may show different structural designs (Enlow, '66: 94; Enlow, '68: 13).

Each section which had been experimentally treated was then

compared to its respective control section. These sections were each compared at magnification powers of X 30, X 60, and X 105. These comparisons were first made under ordinary light magnification and then under polarized light. Polarized light was used because it was discovered that it had the effect of making the individual bone structures, particularly lamellae and cement lines, more distinctly visible.

After these comparisons had been completed, those sections which had been experimentally treated with the flesh intact were compared to their respective sections which had been treated with the flesh removed. These comparisons were made to see if intact flesh had an effect on the results.

The preparation of thin undecalcified bone sections can be done rapidly, inexpensively, and dependably using Frost's ('58) method (see Appendix A). Three useful modifications of this method were discovered that aided in the preparation of higher quality slides. First, during grinding, it was found that detergent suds added to the water on the carborundum paper had the effect of washing the section and keeping unnecessarily large amounts of grit from permeating it. The inexperienced should be cautioned to use suds and not straight dishwashing detergent since the latter will have the effect of an adhesive rather than a washing agent.

Second, it was discovered that most preparations ground on 360 and finally 400 grit carborundum paper had a tendency to be somewhat scratchy, thus obscuring parts of the section.

It was subsequently found that by grinding the 20 μ thick section for a few minutes on 600 grit carborundum paper these scratches would disappear and a much better slide resulted. For a good clear slide this step is essential.

Finally, it is possible for a curled section to crack when it is permanently mounted; however, curling of a section can be eliminated by using the following method. After the section has been washed and rinsed, instead of setting it out to air dry, the section can be placed between two sheets of bibulous paper with a substantial weight placed on it. The bibulous paper will absorb the water and the weight will prevent the section from curling, thus eliminating any hazard of it cracking during mounting.

After the section is dry, it can either be dry mounted or permanently mounted. If the section is to be permanently mounted, one should be careful not to use mediums which are acid or which become acid over a period of time, because there will be a gradual release of carbon dioxide with subsequent elevation of the cover slip. Permunt or Harleco synthetic resins are two satisfactory mountants (Frost, '59: 144).

DISCUSSION AND RESULTS

There are a number of different structural components of bone; however, bone from different sources will differ in the proportions of fibers, crystals, and cement in the matrix, the arrangement of fibers, and the size, density, and patterning of the osteocytes. For these reasons, several different types of bone can be distinguished (Pritchard, '72:2). In order to explore changes which may have occurred in the experimentally treated bone, it is first necessary to distinguish what type of bone was present in each animal. This step was taken since histological structure varies between species, by age in the individual, and by bone within the individual. Individual bones may, in fact, show different structural patterns in various parts of the same bone (i.e. the proximal end of the bone may not show the same histological structure as the midshaft of the same bone) (Enlow, '66: 94; Enlow, '68: 13).

The bones of most artiodactyls, including deer, are basically plexiform in structure. Plexiform bone is formed by a process of lamellar compaction within the spaces of nonlamellar cancellous bone. It is formed in such a manner that a three-dimensional network or plexus of radial, longitudinal, and circumferential canals are present. It is the strikingly uniform, regular, and symmetrical arrangement of this network which serves to characterize this canal system (Enlow, '68: 24).

In many skeletal elements, this pattern may remain as an adult tissue and subsequent secondary development may follow this pattern; however, in some bones this plexiform tissue pattern may be partially or entirely replaced by Haversian tissues (Enlow and Brown, '58: 205, 214, and 227).

Although Haversian systems or secondary osteons were first described in human bone, this term does not simply apply to human bone. A Haversian system is secondary bone development in which the resulting structure contains a Haversian canal, containing a minute artery, vein, and nerve filament, which in turn is surrounded by concentric lamellae. Within the lamellae are lacunae, containing the osteocytes in life, connected to each other and to the Haversian canal by a fine network of canaliculae. These structures can be circular, elliptical, or ovoid in appearance and are demarcated from each other by highly noticeable cement lines (Windle, '68: 122-123).

The long bones of the raccoon, Procyon, all possess a basic reticular vascularization pattern. Reticular bone is formed in the same manner as is plexiform bone; however, vascular canals of the former are arranged in an unorganized, irregular network as opposed to the highly uniform, regular, and symmetrical arrangement of the latter. In the majority of the bone tissue regions, reticular bone has been replaced by secondary osteons although indications of the former vascular reticulum may remain (Enlow and Brown, '58: 197).

A typical plexiform structure, similar to that of most

artiodactyls, frequently characterizes the deposition of primary tissues on the outer bone surfaces of the compacta of long bone diaphyses of the dog. This regular, organized network of vessels is primary in structure and scattered secondary osteons usually develop within the system. The inner areas of the compacta, however, are almost always replaced by secondary osteons (Enlow and Brown, '58: 197).

After determining the type of bone representative of each animal, each bone section which had been treated experimentally was compared with its respective control specimen. In each case, they were first compared microscopically using ordinary light and later compared under polarized light. Next, those sections which had been experimentally treated with the flesh intact were compared with those which had been treated under identical conditions with the flesh removed. These sections were compared in the same manner previously described.

When comparing sections, a number of different areas were observed for any possible changes that may have occurred. Those changes looked for are as follows: 1) possible breaks in the cement lines demarcating Haversian systems; 2) possible rearrangement of the concentric lamellae within each Haversian system; 3) possible rearrangement of the lacunae within the lamellae; 4) possible rearrangement in the tissue structure of plexiform or reticular bone; 5) significant increases in the area between Haversian system cement lines and/or the networks of vascular canals in plexiform and reticular bone; and

6) significant increases or decreases in the average size of Haversian systems from the same bones.

When magnified under ordinary light, no significant differences between those sections which had not been treated were observed. Similar results were also obtained when the same sections were compared with the use of polarized light. It was also observed that there were no significant differences between those sections which had been treated with the flesh intact as opposed to those which had been treated with the flesh removed.

CONCLUSIONS

The results of this study warrant the conclusion that it is not possible to determine the various cultural and/or environmental processes previously discussed through the use of histological techniques, at least as far as these processes were simulated and the sections compared in this study. Therefore, contrary to the original hypothesis, the histological structure of experimentally burned, boiled, and frozen and thawed long bones does not change.

These conclusions, however, should not rule out the possibility of using histological techniques in the determination of cultural and/or environmental processes in archaeological skeletal collections. In doing further research into this area, the following suggestions may prove to be helpful. First, it may be beneficial to vary the amount of time involved in the boiling and burning of long bones. Instead of boiling bones for only one hour, this process should be done over a longer period of time, perhaps as long as two or three days. Similarly, the time involved in burning a bone should be increased.

In conjunction with burning, it may also be beneficial to vary the temperature. This variation should include decreases as well as increases in temperature, providing these are done with a subsequent variation in the length of time each is cooked.

Second, it may be beneficial to compare a large series of sections from a single species which have been subjected to a single experiment to control sections. For example, left femora

from a large number of animals of the same species would be burned while the other would be kept as a control. This large sample should then be checked for changes under both regular and polarized light.

Third, another technique which may be useful for determining cultural practices and/or environmental processes would be the amount of refraction shown by sections compared under polarized light. Again, only one cultural practice should be observed. Also, the same species and the same bones should be used in all such research. The loss of moisture during burning or the possible addition of moisture and/or minerals during boiling, for example, may in fact affect the refractive index of a particular specimen.

Finally, if any of the above suggestions are followed and the sections are viewed under polarized light, then a method will have to be devised and used to control, with greater accuracy, the thickness of each section. Not only should sections be taken from the same species, the same bone, and the same place in each bone, but also each section should be as close to the same desired thickness as possible. This fact becomes of particular importance if one is trying to measure the amount of refraction and use it for part or all of the basis of determining differences between compared sections, since the thickness of each section affects the amount of refraction. This is especially true of sections that are magnified with polarized light.

This study should provide the basis from which other studies applying histological techniques to an analysis of skeletal

populations can be done. Until such time as these studies are completed and further results become available, it can be concluded that it is not possible to determine the various cultural and/or environmental processes previously discussed through the use of histological techniques.

APPENDIX A

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A Rapid Manual Method for the Preparation of Thin Undecalcified Bone Sections

Special supplies

Carborundum abrasive paper with waterproof adhesive; grit No. 320, 360, or 400.

A piece of flat glass about 10 x 12 inches upon which to place the paper.

Detergent. The type available in grocery stores for kitchen use is adequate. Dilute to about 1:500 with water for use.

A fine-toothed hack saw; 32 or more teeth to the inch.

Technique

Saw 1 - 2 mm. slabs of the desired orientation. Saw slowly to avoid production of heat and cracks. If the local effects of sawing heat are a problem the section can be sawed thicker and subsequently ground to the desired thinness, thus avoiding the heated zone. The thinner the slab, the less subsequent grinding will be required.

Place a sheet of the abrasive paper on the plate glass, abrasive up. Moisten liberally with water. The plate glass is necessary to produce a flat grinding surface. Using the fingers, move the section over the abrasive surface with relatively light pressure in a circular motion. The paper cuts very quickly. The saw marks will be found to be ground out in less than 1 min. Repeated use of the paper removes the largest points of its abrasive grit and this used paper should be saved for later on, when slower grinding action will be needed. Keep the fingers clear of the abrasive surface.

When the saw marks are ground off one side of the section, turn it over and grind the other side similarly. The second surface may be a little difficult to grind on a perfectly fresh sheet of paper but will grind freely on a slightly used piece. If the section sticks, even on the used piece, the points of abrasive are still too large. Tear off a small corner of the paper and lightly rub it over the central surface of the large sheet to reduce the size

of the abrasive grit exposed.

When the section is too thin to manage with the fingers, which is again in about a minute, a strip of paper of about 1/2 inch in width and long enough to wrap once around a 3" x 1" glass slide should be torn from the edge of a fresh sheet. Wrap this strip around the slide, abrasive side out. Place the section down on the used, large sheet and place the slide with the strip of paper wrapped around it on top of the section. Because the abrasive wrapped around the slide is perfectly fresh, it exposes sharp points to the bone in contact with it and sticks. Since the surface of the large sheet of paper underneath is considerably finer, due to use, the section will move across it when the slide is pushed around with the fingers. Again, using light to moderate pressure, grind with circular motion. The paper still cuts rapidly and the section should be turned from time to time to grind an equal amount on both surfaces. Check often to keep the surfaces parallel.

Sections thinner than 30 μ are produced by identical manipulations but on used paper with #400 grit which cuts slower and more evenly. The abrasive paper strip wrapped around the slide should be a fresh piece of the same grit. When the section begins to get transparent, it will be about 75 μ thick. The grinding stroke should be circular and large enough to span most of the width and length of the paper. About one cycle/sec is a good speed. Faster grinding develops considerable heat and produces lateral stresses large enough to crack the section. Thin sections are particularly prone to such cracks. Between 50 - 200 gm pressure/cm² of section surface is ideal. Too much pressure will cause thin sections to disintegrate.

At 75 μ a section appears mottled white. At 50 μ it has the appearance of wet ground glass. At 20 μ or less it is so transparent that it is almost invisible. Use liberal amounts of water during grinding....

When the above is completed, the section should be placed in a 50 ml flask with detergent solution and shaken vigorously for a minute or so to remove debris which inevitably adheres to the surface, otherwise an extremely dirty mount results. The section should then be

rinsed, after pouring off the detergent, by reshaking with several changes of distilled water....

It can then be dried in air at room temperature or up to 50° C. The section will curl slightly because it dries faster from the upper surface (Frost, '58: 273-276).

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