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TECHNICAL NOTE

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The State of the Art of Bone Identification by Chemical and Microscopic Methods

Traditionally, identification of skeletal remains has been based on distinguishing morphological characteristics.[2] In the past three decades there has been a greater development of techniques used in identification. In part, this effort has been stimulated by the practical need to identify a large number of dead from major wars and by the employment of statistical methods such as multivariate analysis. In addition, new morphological criteria are continually being discovered which will aid the investigator by assigning a racial or sexual classification to bones. Examples of these are tear duct size [*2*] and total subperiosteal area of the second metacarpal [*3*]. Although the latter technique is not fully developed, it offers great promise.

However, more significant is the increasing potential for the utilization of microscopic and chemical analysis of bone. Unfortunately, these techniques, which have been developed mainly by physical anthropologists, have been slow in gaining recognition among pathologists. This paper reviews recent microscopic and chemical techniques for the analysis of bone which may have practical applications for law enforcement and suggests some of their advantages and disadvantages.

Techniques

One of the earlier attempts to use chemical analysis occurred during the 1950s when it was thought that ABO blood types might be obtained by the application of standard blood typing methods to blood protein trapped within the bones. Some researchers thought they were capable of this determination, and an attempt was made to investigate the blood groups of fossil human populations. However, Ezra-Cohn and Cook [*4*] convincingly demonstrated that in such studies the postmortem conditions that influence the results of the blood tests cannot be controlled. In addition, they showed that the residual protein trapped within the cortex of fresh bone is inadequate to give valid results. Recent research in the blood typing of old bone has been centered around the controlling for soil/bone chemistry and the application of multiple tests to determine nitrogen content, residual proteins and protein-polysaccharide complex content. Although still considered by many to not give valid results, reported blood group data are

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[2] Krogman's *The Human Skeleton in Forensic Medicine* [*1*] remains the most complete source for morphological methods of skeletal identification.

beginning to reappear because of improved techniques [5]. Considerable success is achieved in determining ABO blood group classification from fresh bone marrow. However, if the marrow has lost its red color the tests are inconclusive. Because of a growing amount of research and improved methods, there is increasing indication that chemical and microscopic means of identification will provide more useful information in skeletal identification.

The subject's age at death can now be estimated microscopically by studying the haversian canal system of a fragment of bone. This procedure, developed by Ellis R. Kerley [6], has been refined so that it is more accurate than the morphological methods, "to within six years of the true value in 95% of human males" [1], in determining the age of persons older than 40 years. The method involves measuring the number of osteons in a field, obtaining the average number of lamellae per osteon, and determining the average haversian canal diameter. Then these measurements can be compared to tabulated data and an age estimation made. Using these features no racial or sex differences have been noted. If such differences do exist, it is only a matter of time before they are identified and tabulated.

Sometime in the future, studies on the haversian canal system, such as those conducted by D. H. Enlow [8], may also enable the microscopic distinction between human and nonhuman bones. As it stands now such discrimination is possible only between mammal species of very distant relationship.

Questions pertaining to the length of the postmortem interval may arise. Several chemical methods have been devised for dating skeletal remains. For instance, a positive gel-diffusion test indicates that the bone is from a person who has probably been deceased for less than five years. A positive benzidine test indicates the person was dead less than 50 years, as does the presence of both amino proline and hydroxyproline. A bone content of greater than 3.5 g/100 ml of nitrogen indicates an age of less than 100 years [9]. Other chemical techniques reveal older ages, but these are unlikely to be of interest to law enforcement agencies. A big drawback to these techniques is the need to consider the chemical composition of the soil surrounding the remains and the climatic conditions of the area.

Ultraviolet fluorescence can be used with a high degree of success in distinguishing between bones from different bodies in the case where bones are in disarray in a multiple burial. The bones are saturated with a solution containing a fluorescent pigment and are then dried. The site of most pigment uptake is the haversian canal system. Under an ultraviolet source the bones of an individual will have a characteristic color pattern which permits their segregation from the others. That the individual differences in pattern reflect bone architecture is suggestive that at least some of these differences may be the result of age and sex [10]. The success of this technique opens the possibility that future studies will reveal similar methods for the determination of sex and race. Further research along these lines is warranted.

Current promising research on the use of neutron activation analysis of bone for faunal identification is being conducted by members of the Archaeology Department at Washington State University. It is possible such analysis, when developed, will have important applications for forensic science. For instance, individual variation in chemical composition may allow differentiation of bodies by a technique similar to the above ultraviolet fluorescence method.

Conclusion

It can be expected that further expansion of our knowledge concerning the microstructure and chemistry of bone will result in the development of more of these identification methods. In contrast to morphological analysis, which requires substantial

parts of the skeleton, microscopic and chemical techniques can be especially helpful when only a small portion of bone is available to be analyzed. These tests will remain adjuncts to morphological methods since they require special equipment, specialized training, and considerable time and hence will be best conducted in university research facilities by request of law enforcement agencies.

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