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Paleoserology Techniques Applied to Skeletal Identification

The purpose of this article is to present a brief history of bone blood-grouping, to review paleoserological methodology, and to discuss the logic employed in several styles of reporting this blood group information. Included in the discussion will be additions in technique employed in my studies of archeological bone samples.

Historic Review

The history of paleoserology goes back to the initial work of the Boyds [*1,2*], Matson [*3*], and Candela [*4*] during the 1930s. Blood groups were based upon the absorption test method and involved dried, powdered muscle tissue against specific antisera of "known titer." The earliest published accounts of absorption tests using cancellous bone powder are found in the works of both Boyd [*5*] and Candela [*6*] in 1939.

During the late 1950s, a reinvestigation of bone blood-grouping methods was undertaken. Due to the desire to apply human population genetics to the understanding of present human variability, natural history of past populations, and identification of individuals, the collection and summarization of biochemical traits gained priority in anthropological research. Although received with mixed emotions, theoretical involvement of serological data in the anthropological concepts of population identification and race was presented in W. C. Boyd's text of 1950 [*7*]. In this publication Boyd briefly referred to blood group information in archeology. Also, sporadic blood group data on past peoples were reported during this period and still were mainly based upon muscle tissue-typing, as in Gilbey and Lubran's Egyptian and South American Indian mummy data for A, B, H, and Rh antigenicity [*8,9*]. In 1956, Thieme et al typed the 7000 years old Midland, Texas cranial fragments for the ABO system [*10*].

The interpretation and logic of laboratory methods appeared valid only until 1957. Within a time span of two years, 1957–1959, several publications cast doubt upon the interpretation of bone blood group results. An Army research and development command report of research by Thieme, Otten, and Wheeler [*11*] surveyed both dried tissue and cancellous bone-typing in respect to laboratory error and sample contamination. A subsequent publication of their work appeared in the *American Journal of Physical Anthropology* and strongly pointed out the unreliability of typing aged bone [*12*]. It is essential to note that this report dealt with known ABO-grouped cadaveral bone that had been buried no

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longer than three years—not archeological bone. Unfortunately, many anthropologists failed to distinguish between these two time levels of aged or old bone and simply recall the warnings of these workers and tend to discount previous and subsequent work. A negative impression is reinforced by the short discussion of blood-grouping in Krogman's widely-used reference in human skeletal identification [*13*], which to many stands for all bone in time.

Despite the common assumption that one cannot blood type old human bone, significant methodology is in recent literature, particularly in the area dealing with archeological techniques [*14–17*].

Methods

Although serological identification of bone is not routinely described in medicolegal laboratory procedures, the techniques now employed are similar to those utilized in the ABO(H) blood group identification of dried blood stains [*18*]. The procedures usually combine the techniques of the basic antibody ten-tube serial dilution titration with either absorption or inhibition antigen-determination tests.

The absorption test consists of a standardized quantity of sample plus the addition of an exact quantity of known-titered antiserum. The antiserum, after an incubation period at a specified temperature, is then removed and re-tested for its agglutinating capacity against specific A, B, and O red blood cells. The rationale is that if the antiserum is not able to agglutinate the RBC at its original strength, then there has been an absorbing of its strength by a specific antigen or antigen-like chemistry (Fig. 1*a*). This procedure has historically utilized either human serum agglutinins or immune commercial anti-A and anti-B sera. Anti-H serum, specific for O antigenicity, was introduced procedurally in 1950. The most common anti-H is the Lectin (plant extract) *Ulex europaeus* [*2,5,6*].

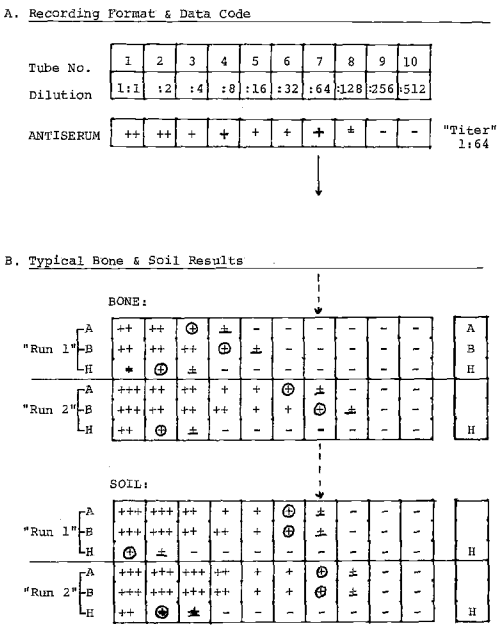

FIG. 1—*Titration recording format and typical bone/soil absorption examples (Cochiti Pueblo skeletal remains).*

Early paleoserology recognized that cancellous bone samples appeared to display more sensitive results than dried muscle samples. Tests of bone and muscle under parallel conditions were reported by Candela [*6*], and although both Boyd [*5*] and Candela used similar techniques, they did disagree as to the accuracy, sources of laboratory error, and contamination in samples. At one point, Candela devised a "micro-slide" technique for reading and interpreting the end-point of absorption or inhibition titers. The slide method was not routinely utilized in subsequent years in preference to test-tube observation of agglutination ranges [*19*].

There also have been attempts to satisfactorily blood type compact bone for the ABO system. The results were usually negative or uncertain for any significant determination of group-specific substances [*20*].

It is generally assumed by workers that positive results in the ABO(H) absorption tests reflect the ABO(H) blood-group substances phenomenon in body fluids, namely the serological "secretor status" of an individual [*21*].

Considering the above methods, then, the awareness of contamination possibilities which can result in non-specific absorption is of prime importance and can present a major flaw in bone blood-typing. Known blood type cadaveral bone has been buried, dried, left in open containers, and burned to obtain a better understanding of sample degrading and contamination effects [*12*]. Also, controls for non-specific absorption from plant and animal sources have been suggested by Gray and depend upon variations in extract preparations [*22*]. A major source of contamination is the Forssman positive A-like antigenicity found in plant and animal extracts.

With these interpretive considerations in mind, Glemser's methods in the 1963 edition of *Science in Archeology* presented several additional controls over sources of error [*15*]. She introduces the rationale of several rerun tests of the same sample with replacement of fresh antisera. The basis for absorption specificity is a three-or-more dilution tube drop in antiserum titer. Despite this control, Glemser states the unknowns in paleoserology as follows:

> In spite of the need for clarity on a vital issue, the effects of the environment on antigen stability are not sufficiently well known. It may be expected that in tissues exposed to the leaching action of water in the soil, water-soluble antigens would be removed, and that in consequence the diagnosis of blood group would depend on the alcohol-soluble fraction. The rate at which the water-soluble fraction is removed is not known; neither have studies been carried out on the effects of pH of soil on antigen stability.

This attempt to have some understanding of the general influences of soil/bone chemistry is one of the several trends in current research investigating further feasibility of paleoserology. Of significance is the use of multiple laboratory techniques such as nitrogen and collagen values as protein indicators, precipitation tests and repeated Boyd-Candela techniques, plus fluorescent antibody studies for antigen determination. Usually this work can compare both fresh autopsy and ranges of aged and archeological bone samples [*16,24*].

Lengyel's [*24*] investigation of nitrogen content and the protein-polysaccharide complex suggests that if the latter falls below 0.02 mg/100 g then blood group determination is unreliable. Conversely, if the amount is in excess of this concentration it is justifiable to assume A or B, or both specificity in bone. Negative results may mean that the bone sample is of an O person, the person was a non-secretor, or there has been a technical error [*16*].

Still the primary problem is to obtain a better understanding of change in bone tissue and chemistry over time. Chemico-histological studies have been reported. Most crucial to paleoserology is the awareness that it is not simply a matter of bone chemical, tissue, and protein decomposition that occurs in time, but that the polysaccharides may degrade and also change in type. This change, if it is in the nature of alterations in sugar composition, can result in a different sugar-based specific antigenicity that would be identified in absorption tests as a different blood type [*16,17*]. This, plus the other factors mentioned here, present to date a multiplicity of considerations to be made in future bone serological identification.

Current Research

Combining the medicolegal technique of parallel testing blood-stained and unstained cloth with the multiple testing of the same samples, I have been engaged in testing archeological bone powder against parallel tests of burial soils. The procedural sequence is as follows:

(1) Cancellous bone (thoracic vertebra or femoral head) is pulverized to talcum-powder consistency.

(2) Soil sample (secured from the burial strata or preferably within inches of the burial remains) is also pulverized.

(3) 0.5-g bone or soil sample is added to two sets of three 10 by 75 mm tubes.

(4) 0.2-cc antiserum (titered at 1:64) for anti-A, anti-B, anti-H are added to their respective A, B, and O tubes.

(5) Samples are refrigerated at 4 to 5 C for 12 h.

(6) Refrigerated test tube sets are agitated hourly.

(7) Tubes are centrifuged and antisera decanted.

(8) Antisera are re-titered against 2 percent suspensions of A, B, and O RBC. This is "Run 1."

(9) Fresh antisera are added to the *original* sample tubes.

(10) Repetition of steps 4 to 7. This is "Run 2."

(11) Decanted antisera of Run 2 are observed for absorption of original titer.

(12) When the bone sample causes absorption three tubes or more below the standardized antiserum titer, it is considered group-specific *only* if the absorption did not occur in "Run 2" of the soil sample.

Figure 1*b* presents typical bone sample absorption titrations personally recorded from Cochiti Pueblo archeological samples. Note the non-specific absorptions in Run 1, then specific absorption in Run 2 for H. Likewise the soil sample presented may be judged as containing H antigenicity. Further research over the past several years has allowed the application of bone/soil testing in a population sample. A series of samples from the 30,000 to 10,000 years ago levels of Niah Cave, Borneo, have been typed [*23*]. Representative test results are shown in Fig. 2. Here, the serological assumption is that Run 2 demonstrates antigen specificity in the bone sample for A and H and no specificity in the soil sample. Therefore, only the A group-specific typing would be regarded as significant if both bone and soil contained H. If H alone were apparent in the bone sample and not in the soil, the bone would be typed as Group O.

Discussion

There are presently three levels of investigation and reporting bone blood group identification results. First is the straight report of paleoserological information as an assumption of skeletal blood group determination. Here, A, B, AB, and O categories are given in

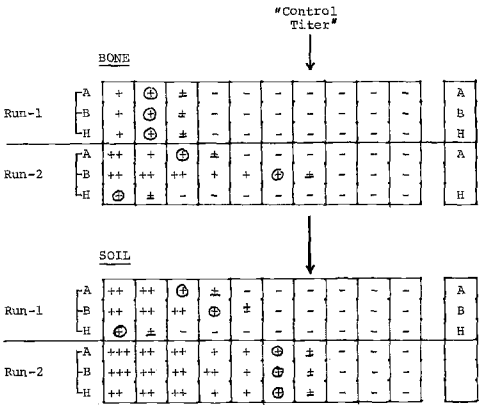

FIG. 2—*Selected example of applied bone/soil parallel tests (Niah Cave, Borneo).*

individual sample counts or percentages, or both of occurrence in a population sample [*25,26*]. Second, one can report the results of bone and soil group-specific ABO(H) absorption tests. This data is advantageous in that it presents an added control underlying the bone type assumptions. For example, the investigator's Niah Cave Series demonstrated that 85 percent of the recorded bone types were based upon the occurrence of group substance in the bone sample but not in the parallel test of the related soil sample. No significant absorption for the ABO(H) system for bone and soil together occurred in 4 out of 45 parallel tests. What few positive absorptions there were in the soil samples were only representative of H specificity [*23*]. Third, there is some security in the nitrogen assay as it indicates protein content in a single bone sample. This value is published along with the blood group determination of individual samples [*27*].

If at all possible, a researcher or expert witness will support his findings with as many tests as his training and laboratory facilities will allow. We are still at the point where both negative and positive laboratory results can only enhance the future accuracy and potential of this effort. Therefore, any further bone blood-grouping done can only benefit the further application of this interest in forensic anthropology.

Summary

(1) Absorption and inhibition tests for ABO antigenicity in human cancellous bone were performed as early as the 1930s.

(2) In the late 1950s several publications dealt with the unreliability of blood-grouping bone.

(3) Paleoserology, especially in anthropology, is still considered by many not to give accurate or valid information due to non-specific absorption, contamination, and possible technical error.

(4) In the 1960s there was interest and work in the nitrogen content, protein-polysaccharide complex, and residual proteins found in old bone.

(5) At present, multiple tests are performed and usually involve fresh autopsy cancellous bone and samples from several periods in time.

(6) Recently reported blood group data can be found in several publication formats such as single skeletal or population sample blood group counts or percentages or both; results of parallel bone and soil typing; and comparative nitrogen content in blood samples that have been blood-grouped.

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