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ABH Antigen Typing in Bone Tissue

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ABSTRACT: Results obtained from the ABH grouping of bone tissues by the absorption-elution procedure and by a recently described two-dimensional absorption-inhibition procedure are reported. Neither the elution nor the inhibition procedure alone yielded uniformly correct results. A combination procedure consisting of the use of both absorption-elution and two-dimensional absorption-inhibition is proposed for bone ABH grouping. When elution and inhibition were used in combination, specimens yielding concordant results with both techniques were correctly grouped.

KEYWORDS: forensic science, genetic typing, human identification, musculoskeletal system, ABO blood group system, bone grouping, absorption-inhibition, absorption-elution, paleoserology

Forensic scientists are called upon to attempt to identify human skeletal remains in a variety of situations, including mass disasters as a result of natural phenomena, air crashes, fires and explosions; the identification of the human skeletal remains of military personnel recovered from wartime theaters; cases involving the finding of human skeletal remains thought to belong to a person previously reported missing; and cases involving the identification of skeletal remains in criminal matters where mutilation or significant destruction of the body followed or accompanied the homicidal death.

A number of methods are used to identify human remains depending on the circumstances and the condition of the remains. The most common of these include: identification by direct facial recognition; identification less directly by the recognition of individualizing scars, marks, or other special features; matching of fingerprints with premortem fingerprints; and matching of dentition by a forensic odontologist with premortem dental X-rays.

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In many situations, however, these methods cannot be used because of extensive putrefaction or destruction of the remains, the unavailability of appropriate premortem records, or the lack of identifying features or markings on the remains as a result of natural or intentional destruction. Under such circumstances, other more indirect methods must be used to assist in the identification.

Such indirect methods include the following: use of clothing and belongings; findings at autopsy that yield information about the medical history; analysis of skeletal remains by forensic anthropologists to estimate sex, age, time since death, race, and stature; attempted reconstructions of facial features from skulls by physical anthropologists; hair comparisons in cases where premortem hair exemplars can be obtained; and blood grouping. In those cases where the remains are solely skeletal, or where only small fragments of bone are found, no information is generally available from clothing or belongings. The ability of forensic pathologists, anthropologists, or odontologists to make or assist in making an identification depends in great part on whether or not the skull is recovered, whether or not it contains teeth, and what other bones are recovered and available for analysis. In situations in which other methods are not useful, blood grouping of bone fragments can be a powerful tool in assisting in identifications as well as in excluding possible misidentities.

The properties of the ABO system, first described in human blood in 1901, are well known [*1–4*]. In addition, it has been known for decades that the ABH antigenic receptors that characterize the system not only occur on the surfaces of blood cells but are widely distributed throughout human tissues [*5,6*]. This knowledge has been exploited for medicolegal identification purposes for many years [*7–9*] primarily by procedures devised originally for the grouping of ABH antigens in bloodstains.

Application of blood-grouping techniques to bone tissue has yielded mixed results, particularly when aged bones, compact bone, or bones subjected to putrefactive processes are analyzed. The earlier investigators used absorption-inhibition procedures in accordance with the general practices for dried blood grouping [*10–13*]. Reliable results were rarely obtained in those studies, a result caused in no small part by the lack of sensitivity of the procedure. More recently, absorption-elution techniques have been used in attempts to determine the ABH antigens of bone tissues [*14–19*]. The percentage of incorrect results in these studies has been so high, however, that the procedure cannot be regarded as reliable. Greater but still limited success has recently been reported [*19*] using a semiquantitative elution technique that we and others have previously described and recommended for the evaluation of antisera for bloodstain grouping and for the grouping of blood group antigens other than ABH in bloodstains [*20–24*].

In this paper, we describe a combination procedure for the ABH typing of bone, involving extractive absorption-inhibition and direct absorption-elution procedures, and report the results obtained with these procedures on a series of fresh and older bones. A systematic procedure for the examination and typing of bone tissue is also proposed.

Materials and Methods

Bone specimens were first examined visually and microscopically, classified as to type, then measured and weighed. Obvious trace materials, hair, tissues, and blood were removed. Small specimens were prepared for grouping by washing in cold running water for 2 h. The washed bones were then rinsed twice in cold distilled water.

Specimens were then immersed in a small quantity of ether to remove greasy and fatty materials. The ether was removed by aspiration, and the samples washed in a small amount of ethanol before drying in a gentle stream of air or nitrogen. Each was then crushed into small fragments and divided into two separate samples. One of these samples, representing 10 to 15 mg, was further divided into three equal specimens for absorption-elution with anti-A, anti-B, and anti-H. The remaining sample was boiled in minimal distilled water for 1 to

2 h to produce an extract. This extract was filtered, and any additional fatty material was removed. The extract was then evaporated to an appropriate volume for use in the inhibition tests. Figures 1 and 2 summarize the sample preparation steps.

Absorption-elution was performed in glass tubes following the procedure described previously [*25*]. Absorption-inhibition was performed by the two-dimensional technique [*26*] using three selected dilutions of antisera or anti-H lectin corresponding to starting titers of 32, 8, and 2, and using 50 μL as one volume.

Anti-A and anti-B were standard, polyclonal blood grouping antisera of human origin from Ortho (Raritan, New Jersey) or Dade (through American Scientific Products, Boston, Massachusetts). Anti-H was prepared from *Ulex europaeus* seeds (F. W. Schumacher, Sandwich, Massachusetts) following the method of Kind [*27*], or it was obtained from Dade. Affinity-purified anti-H was also used (UEA I, Miles Laboratories, Naperville, Illinois) in some experiments [*28*].

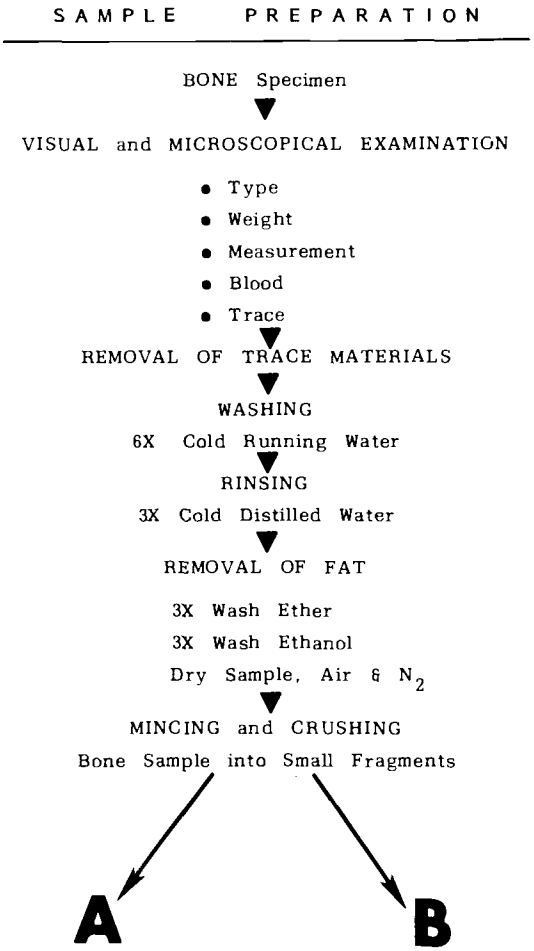

FIG. 1—*Preparation of bone sample for grouping procedures.*

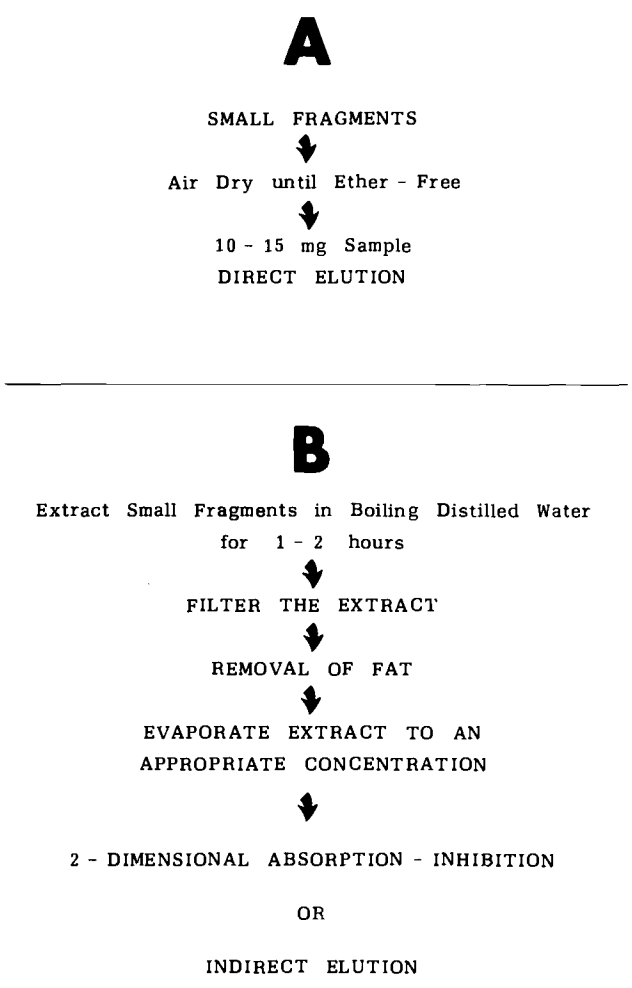

FIG. 2—*Separation of bone sample into specimens for grouping by the absorption-inhibition and absorption-elution procedures.*

Results and Discussion

Results obtained using the elution technique are shown in Table 1. An "older" specimen is one that is six months old or older. Among the fresh specimens, 19 Type A bones were correctly typed, one specimen from a B individual gave results consistent with an O, and A antigen was detected in 5 of 33 bone specimens from O individuals. Among the older specimens, one specimen from an A individual and one from a B individual grouped as AB. Three bone specimens from O individuals yielded B antigen results. In addition, the older specimens showed a higher fraction in which no antigen was detected.

The results of the two-dimensional inhibition tests on a series of fresh and older bone tissue samples are shown in Table 2. There were no incorrect results with the fresh samples using inhibition. All twenty-one specimens yielded grouping results corresponding to the blood group. With older bones, one specimen from a B individual was incorrectly grouped as AB. However, this particular specimen was correctly grouped as Type B in the absorption elution test. Not surprisingly, no antigen was detected in four of the older specimens.

TABLE 1—*Results obtained with fresh and older bone tissues by absorption-elution testing for ABH antigens.*

Type of Bone	Known Blood Type	Number Tested	Number Yielding Indicated Conclusion[a]					
			A	B	H	AB	NAD	Inc
Fresh	A	19	19	...	...	...	...	...
	B	19	...	18	1	...	...	...
	O	33	5	...	27	...	...	1
	AB	0	...	...	...	...	...	...
Older	A	10	4	...	...	1	5	...
	B	10	...	5	...	1	4	...
	O	19	...	3	10	...	6	...
	AB	2	...	...	...	1	1	...

[a]NAD = no antigen detected; Inc = inconclusive.

TABLE 2—*Results obtained with fresh and older bone tissues by two-dimensional absorption-inhibition testing for ABH antigens.*

Type of Bone	Known Blood Type	Number Tested	Number Yielding Indicated Conclusion[a]					
			A	B	H	AB	NAD	Inc
Fresh	A	6	6	...	...	...	...	...
	B	4	...	4	...	...	...	...
	O	11	...	...	11	...	...	...
	AB	0	...	...	...	...	...	...
Older	A	10	9	...	...	...	1	...
	B	10	9	...	...	1	...	...
	O	15	...	...	12	...	3	...
	AB	2	...	...	...	2	...	...

[a]NAD = no antigen detected; Inc = inconclusive.

Specimens yielding inconclusive or no results do not present an interpretation problem, since no group can be inferred. Specimens giving incorrect results represent a major problem in interpretation, however, and one which has been seen in every study to date regardless of the technique used. These data, along with those from previous studies, indicate that neither elution nor inhibition testing alone yields uniformly correct results from bone tissues, especially as the specimens get older.

But, by combining two-dimensional inhibition and elution tests and assigning a blood group only to those specimens that yield concordant results with both, the correct type can be obtained. All the specimens in our study that have yielded the same grouping results by both two-dimensional inhibition and absorption elution techniques have been grouped correctly. Reliable bone grouping, therefore, at present appears to require the combined application of both techniques, and only those results that are concordant by both procedures are interpreted as conclusive in terms of assigning consistency with a blood group. This point is illustrated in Fig. 3, which represents the overall approach to bone grouping diagramatically.

In all cases, we kept track of whether the specimen tested originated from spongy or compact bone, but this parameter did not materially affect the results.

A series of very old bone specimens from mummies or museum collections was also grouped using the proposed procedure. Nineteen of twenty-six specimens tested yielded con-

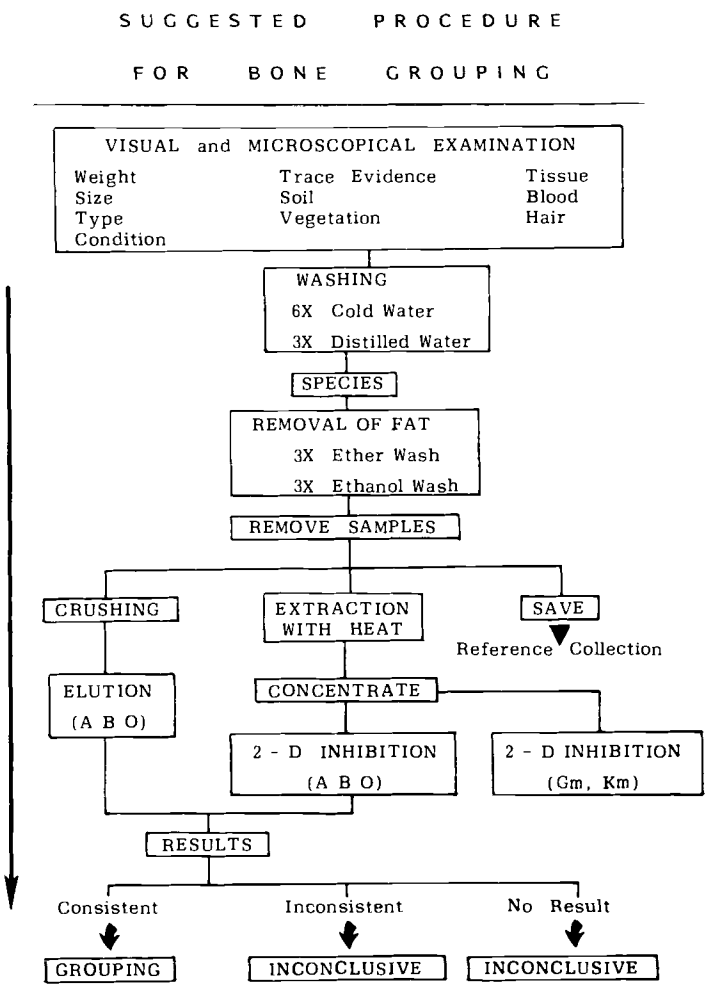

FIG. 3—*Overall procedure for bone tissue grouping and interpretation of the results.*

clusive results, but it is obviously not possible to determine whether the results were correct because of the lack of corresponding blood group information.

Further studies on the ABH grouping of bone tissue are continuing in our laboratories. The feasibility of determining immunoglobulin allotypes from bone tissue represents a part of these continuing experiments. In addition, preliminary results indicate that high molecular weight DNA suitable for possible restriction fragment length polymorphism analysis with DNA probes can be extracted and recovered from human bone tissue. Additional experiments on DNA isolation and analysis are being pursued.

Acknowledgments

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