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# Genetic Markers in Human Bone: II. Studies on ABO (and IGH) Grouping

**REFERENCE:** Lee, H. C., Berka, K. M., Folk, N. L., Pagliaro, E. M., Carroll-Reho, J., Brubaker, T. L., and Gaensslen, R. E., **"Genetic Markers in Human Bone: II. Studies on ABO (and IGH) Grouping,"** *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 3, May 1991, pp. 639–655.

**ABSTRACT:** A combination absorption-elution, two-dimensional absorption-inhibition procedure was used to determine the ABH antigen composition of a series of human bone specimens of known ABO type that had been aged up to nine months under dry and humid conditions at ambient temperature, 37°C, and 56°C; at ambient temperature in dry and wet soil; and buried in soil outdoors. Grouping data for the separate elution and inhibition testing, as well as for the combination procedure, are given. The combination method was found to be a highly reliable procedure for bone tissue ABH typing. Some data on microbial contaminants of human bone specimens aging in soil, and their effects on ABH typing results, are presented. No direct correlation between the properties of microbial contaminants and specific changes in the ABH antigenic composition of aging bone tissue specimens could be ascertained. Data on IGH antigen determination and on the quantitation of immunoglobulin G (IgG) in human bone tissue extracts indicated that immunoglobulin levels were typically too low to expect routinely successful Gm antigen testing results. However, these factors can sometimes be determined in fresh bone tissue extracts, particularly if the extracts are concentrated.

**KEYWORDS:** physical anthropology, musculoskeletal system, human identification, genetic typing, bone grouping, paleoserology; ABO blood group system; absorption-elution; absorption-inhibition; IGH antigens; Gm system; IGH allotypes, two-dimensional absorption-inhibition

There has been interest in determining ABO blood groups from human tissues other than blood for over 50 years. The presence of water-soluble ABH blood group substances

Presented in part at the 39th Annual Meeting of the American Academy of Forensic Sciences, San Diego, CA, 16–21 Feb. 1987; the 12th International Congress, International Society for Forensic Haemogenetics, Vienna, Austria, Aug. 1987; and the 41st Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, OH, 19–24 Feb. 1990.

This work was supported in part by Grant No. 87-IJ-CX-0041 to R.E.G. and H.C.L. from the National Institute of Justice, U.S. Department of Justice, Washington, DC. The opinions expressed and conclusions reached in this work represent the views of the authors and do not necessarily reflect the official position of the U.S. Government, the U.S. Department of Justice, or the National Institute of Justice. Received for publication 6 July 1990; accepted for publication 24 July 1990.

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in body fluids (saliva, semen, liver bile, and other fluids), which are detectable by routine absorption inhibition tests, was recognized in the 1920s [*1*]. This characteristic was later shown to depend on a simple Mendelian dominant gene that came to be called "secretor" [*2*]. It also became clear that ABO group substances (corresponding to the blood group of the same individual) were detectable in various human organ tissues, including bone [*3–7*]. The development of reliable methods for determining ABO types from tissue has been prompted by the interests of two major independent fields: legal medicine and physical anthropology. The medicolegal interest derives from the possibility of using ABO grouping as one means of putative identification in cases involving human remains that cannot be identified by recognition, fingerprints, or dental records. There is a reasonable likelihood that a person's ABO type has been determined during life and that a record can be found. The interest of physical anthropologists derived from the idea of using ABO blood group distributions determined from older, to medieval, to even ancient human remains as a method of tracing human migrations through the ages and of determining possible historical relatedness between various human subpopulations. Although the purposes of tissue and bone ABO grouping by medicolegal specialists and physical anthropologists are different, the methods used are similar or identical, and both groups have a common interest in the development of more reliable grouping methods [*8*].

A large literature has grown up around this subject, more of it devoted to anthropological questions than to identification.

The physical anthropology literature on ABO group determination focuses primarily on two types of material from human remains: mummified tissue and bone. Systematic efforts to group mummified tissue, especially from ancient human remains, began in the 1930s, and there are several more recent reports as well [*9–16*]. There is a larger literature on the ABO grouping of older to ancient human bone, beginning with the work of Candela in 1937 [*17–20*]. Generally speaking, the anthropological bone grouping reports cover skeletal material from three broad age groups: decades to a few hundred years old (often recovered from excavated cemeteries), medieval material, and ancient skeletal remains. The use of bone typing and the ABO results obtained to help answer anthropological questions has been called "paleoserology," and the extensive literature and findings have been thoroughly reviewed by Borgonini Tarli [*21,22*]. In our earlier work on bone grouping [*23*], we tested 26 ancient bone specimens and obtained conclusive results with 19 of them. Interesting as this work may be, there is obviously no way to know whether the results obtained are correct or not.

Of greater interest in the medicolegal context are studies involving fresh or artificially aged/exposed bone in which the blood groups of the donors were known. These studies provide a basis for judging the accuracy and reliability of the typing procedures. Earlier investigators utilized absorption-inhibition (AI) procedures, in accordance with the general practice for dried blood grouping [*13,24–26*]. The results obtained in those studies were not very reliable. Part of the problem with the earlier work is attributable to the fact that anti-H reagents were unavailable, and Group O reactions could not be positively diagnosed.

In addition, there was considerable methodological variability in the inhibition tests. More recently, absorption-elution (AE) tests for the ABH antigens [*27–29*] were widely adopted for bloodstain ABO typing [*30,31*] and have been preferred in efforts to determine the ABO group of bone tissue [*32,33*]. Lengyel [*34,35*] reported a high percentage of successful results with fresh bone tissue using fluorescein-labeled antibodies in an immunohistochemical technique on thin sections. Immunohistochemical procedures have been successfully employed in determining ABH and Lewis antigens on a variety of fresh and decomposed human tissues [*36–39*], burned human tissue [*40*], and body fluids of forensic interest [*41*]. However, Berg et al. [*42*] reported little success with Lengyel's method in bone and some other tissues. Hauser et al. [*43*] tested a series of bones

artificially aged in air, soil, and water by AE and found a significant number of nonspecific reactions that yielded incorrect results. Concomitant microbiological studies were conducted on the soil and immersed specimens in an effort to correlate the nonspecific reactions with microbial activity. While there was no doubt that the microorganisms exhibited A and B activity, the spurious results could not be perfectly correlated with the activities of the bacteria isolated from particular specimens. Hauser [*44*] conducted a further study of similar specimens using a modified semiquantitative elution technique, which 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 [*45–49*]. He reported that, at least in some cases, the spurious reactions were distinguishable from the specific ones on the basis of eluate titer.

In general, correct results have been obtainable in controlled grouping studies on fresh bone tissue by AE in all or the majority of specimens. However, grouping tests on bone tissue specimens that were aged or environmentally exposed, or both, have yielded an unacceptably high percentage of incorrect results, to the extent that the neither AI nor AE procedures alone can be regarded as reliable testing methods for the determination of blood group. Berg and collaborators [*42*] suggested that grouping results obtained from other than fresh or carefully preserved bone tissue specimens, and especially results from older or ancient specimens, be interpreted with great caution.

Several years ago, we developed a two-dimensional absorption inhibition (2-D AI) test that was more sensitive than those in routine use [*50*], and accordingly more useful in testing body fluids or tissue extracts having low ABH antigen content in relation to usually encountered secretor semen or saliva specimens. We applied this method, in combination with AE, to the ABO grouping of bone tissue [*23*] and obtained very promising results. In this communication, we report the results of extensive studies on the application of the combination AE-2-D AI procedure to ABO grouping in a series of bone specimens aged for up to nine months under a variety of environmental conditions.

## Materials and Methods

Bone specimens from 88 different individuals were visually and microscopically examined, classified as to type, and then measured and weighed. Obvious trace materials, hair, adhering tissues, fat, and blood were removed.

Corresponding postmortem bloods, or bloodstains prepared on clean cotton cloth from postmortem blood, were available and were used to determine the ABO (and Lewis, in the case of whole blood) groups. Within the 88 specimens were 29 (33%) of Group A, 13 (14.8%) of Group B, 40 (45.5%) of Group O, and 6 (6.8%) of Group AB. Whole blood Lewis typing could be used in 31 specimens as an indication of ABH secretor status; the results suggested 28 secretors and 3 nonsecretors.

Each bone was divided into ten smaller subspecimens, thus producing ten identical series of the available bones. One series was tested with neither aging nor environmental exposure as a "zero time, no exposure" control. The remaining nine series were aged under the following conditions: room temperature, dry; room temperature, humid; 37°C, dry; 37°C, humid; 56°C, dry; 56°C, humid; in dry soil inside at room temperature (RT); in wet soil inside at RT; and buried outdoors in the ground. Specimens from each of these nine series were collected at 1, 2, 4, 6, and 9 months, and either tested right away or frozen until they could be tested.

Small bone specimens were prepared for grouping by being washed in cold running water for 2 h, rinsed twice in cold distilled water, immersed in a small quantity of ether to remove greasy and fatty materials, and, finally, immersed in a small amount of ethanol before being dried in a gentle stream of air or nitrogen. The specimen was then immersed in liquid nitrogen and crushed into powder or small fragments with a hammer or hydraulic

press. Approximately 200 to 300 mg of powdered bone was extracted in approximately 600 to 700 μL of phosphate-buffered saline (PBS), at pH 7.2, in a shaking water bath at 95°C for 2 h. Two hundred milligrams of bone powder extracted in 600 μL PBS is adequate for ABO antigen testing.

Proportionally more material is used if the extract is also to be used for IGH antigen testing, or if a portion of the solid material is to be used for DNA isolation [*51*]. The 95°C extraction step has no discernible untoward effects on the DNA isolated from powdered bone solids. A 450-μL extract was used for two-dimensional absorption-inhibition (2DAI) testing (see below). After removal of the extraction fluid, the solid material was dried at RT or 37°C and used for AE tests.

Absorption-elution was performed in glass tubes following a previously described procedure [*47–49*] on three equal samples of dried bone solid. A total of 15 mg is sufficient for AE testing, although the results were not materially affected if larger quantitites of bone solid were tested. In some experiments, eluates were titrated by placing 20 μL of eluate in the first tube of a row, then successively transferring 40 μL down a row of tubes containing 20 μL of phosphate buffered saline (PBS), at pH 7.4, to which was added 0.3% BSA, producing PBS containing bovine serum albumin (PBSA). This titration by "½" rather than the usual doubling dilutions produces antibody dilutions corresponding to 1 to 1, 1.5, 2.25, 3.38, 5.06, 7.6, 11.4, and 17.1 in the first 8 tubes. In routine AE tests, we score an agglutination result as positive for an antigen if the agglutination strength was 1+ or greater.

Two-dimensional absorption-inhibition [*50*] was performed as previously described using three dilutions of antisera or anti-H lectin, corresponding to starting titers of 32, 8, and 2, and using 50 μL as one volume. The 2DAI tests were set up in V-bottom microtiter plates, but the well contents were transferred to Boerner or ring slides for the addition of cells and for rotation and reading. Aliquots were removed from each well after overnight incubation of the plate at 4°C and tested for agglutination.

If conclusive results were obtained (see below), the second dimension titration was not carried out. In 2DAI tests, an antigen was interpreted as detected if agglutination results from the first dimension test yielded − − − or + − − (where "+" represents any degree of agglutination). For any other reaction pattern, second-dimension titration and detection was done. In complete two-dimensional tests, a titration score was computed for the specimen as well as for uninhibited controls, using a previously described scoring system [*47,48*]. Secretor saliva specimens from individuals of blood groups A, B, and O, a nonsecretor saliva specimen, and a saline control were included as known controls in every 2DAI test. Accordingly, there was more than one "uninhibited control" for each antigen being tested (for example, in the case of A antigen, the anti-A results in the nonsecretor, B secretor, O secretor, and saline controls each represent a separate uninhibited control). Uninhibited control scores were averaged. There is an additional advantage to having more than one "uninhibited control" for each antigen; namely, it serves as an indicator of reproducibility in the 2DAI test scheme. An antigen was interpreted as detected if the specimen showed a score reduction of 25 or greater compared with the average score of the uninhibited controls.

A complete, combination bone grouping test was interpreted on the basis of the results obtained from the separate AE and 2DAI tests. The antigens detected in an AE test are consistent with a particular blood group (for example, an A + H or A antigen result implies Group A); similarly, the antigens detected in a 2DAI test are consistent with a particular blood group.

If the results of the separate tests were consistent with the same blood group, we interpreted them conclusively. If the results of the separate tests were not consistent with the same blood group, we interpreted the overall test result as "nonconcordant" and no blood group was assigned to the specimen. Combination tests were always interpreted

as nonconcordant (NC) if either of the separate AE, 2DAI tests, or both yielded inconclusive (INC), no antigen detected (NAD), or no result (NR) results. Results are INC if an eluate or inhibition test sample lyses the cells, NAD if neither A, B, nor H gave a sufficiently strong eluate agglutination or inhibition score reduction to be considered conclusive, and NR if A, B, and H give INC results in the same test. The interpretation scheme for the combination grouping test is shown diagrammatically in Fig. 1.

Polyclonal anti-A and anti-B blood grouping antisera of human origin were obtained from Ortho (Raritan, New Jersey) or Immucor (Norcross, Georgia). Monoclonal anti-A and anti-B was obtained from Immucor or from Chembiomed (Edmonton, Aberta, Canada). Anti-H was prepared from *Ulex europaeus* seeds (F. W. Schumacher, Sandwich, Massachusetts) following the method of Kind [*52*]. Affinity-purified anti-H (UEA I, Polysciences, Warrenton, Pennsylvania) [*53*] and a monoclonal anti-H (Analytical Genetic Testing Center, Denver, Colorado) were also employed in some experiments. Human reagent red blood cells of Groups $A_1$, $A_2$, B, and O were from Immucor. $A_1$ cells were used to detect eluted anti-A in AE, while $A_2$ cells were used in the anti-A testing wells in 2DAI. Group O cells were papain-treated following the procedure of Boorman et al. [*54*].

Tests for selected IGH and KM antigens were carried out on 10-μL aliquots of PBS extracts, or extracts that had been concentrated, using previously described methods [*55*].

Some bone tissue extracts were assayed for immunoglobulin G (IgG) concentration by radial immunodiffusion using testing gels from Miles Laboratories (Naperville, Illinois).

A small quantity of material closely adhering to some bone specimens recovered from soil after nine months was streaked onto sterile agar plates containing growth media suitable for aerobic bacteria, anaerobic bacteria, and fungi. Plates were incubated for several days at 28°C or until visible colonies appeared. Specimens of colonies were transferred to slant tubes containing appropriate media for further growth, and later, in some cases, to appropriate nutrient broth for additional growth and recovery of the organisms.

## Results and Discussion

Results obtained with all the control specimens, that is, prior to any aging or environmental exposure, are shown in Table 1. These results are in accord with our previous findings [*23*] and with those of many other investigators who have conducted either AE or AI testing separately on bone tissues. Although conclusive results were obtained only

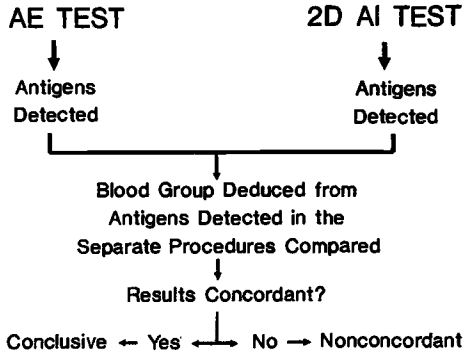


FIG. 1—*Interpretation scheme for combination AE-2DAI test.*

TABLE 1—*Summary of grouping tests on control specimens.*

| Grouping Results | AE, *N* (%) | 2DAI, *N* (%) | Combination, *N* (%) |
|---|---|---|---|
| Correct | 81 (92) | 65 (73.8) | 61 (69.3) |
| Incorrect | 7 (8) | 13 (14.8) | 0 |
| INC/NR/NAD/NC | 0 | 10 (11.4) | 27 (30.7) |

with about 69% of the specimens using the combination procedure, all of them were correct. Throughout these studies, our concern has been with defining a procedure that uses techniques familiar to most forensic serologists and that yields correct results when the results are conclusive. A significant number of specimens were expected to, and did, yield NC (nonconcordant) results. Specimens yielding INC (inconclusive), NAD (no antigen detected), or NR (no result) results in either AE or 2DAI tests with any antigen were scored NC for the combination test result.

We expected that specimens would yield fewer correct results by AE and 2DAI, and thus fewer correct combination results, as they aged under various environmental conditions. We also expected that some environmental conditions would cause greater numbers of incorrect, INC, NR, and NAD results than others, with a corresponding decrease in the percentage of specimens that could be correctly grouped by the combination procedure. These expectations were largely borne out by the data. Increases in the number of NR, INC, and NAD results in AE and especially in 2DAI tests were accompanied by decreases in the number of correct results obtained by considering the combination of the two. The percentages of total specimens giving incorrect results are shown in Fig. 2 as a function of the individual environmental conditions, as well as for all specimens tested. Detailed results of all the testing are shown in Table 2. The data from Table 2 were used to form the pictorial representation seen in Fig. 2*b*. It is apparent from Table 2 and Fig. 2*a* that bone specimens buried in moist soil at ambient laboratory temperatures (SW series) or in the ground out of doors (G series) yielded significantly higher percentages of incorrect results with either AE or 2DAI testing. Thus, the percentage of overall incorrect results decreases if SW or G series results, or both, are removed from the totals (Fig. 2*b*).

The percentages of specimens showing INC or NAD results in separate AE and 2DAI tests, and NC or NR results by combination testing, as a function of environmental condition and aging time are depicted diagrammatically in Fig. 3. For the most part, more specimens yielded this category of results in the 2DAI test than in the AE test.

The percentage of specimens that yielded NC results by the combination testing procedure tended to be less a function of aging time over the nine-month period than of the environmental condition (Fig. 3*c*). In general, specimens maintained under humid conditions yielded fewer conclusive results than comparable ones kept under dry conditions. And, as noted above, specimens maintained in wet soil or in the ground yielded the lowest percentage of correct results using the combination procedure.

The percentages of specimens yielding correct results by separate AE or 2DAI testing and by the combination test procedure are shown in Fig. 4. Looking at the data in this way reinforces the points made earlier concerning the effects of aging and of exposure to various environmental conditions. It is worthy of mention that we observed no correlation between the ability to obtain conclusive antigenic testing results either by AE or by 2DAI and the secretor status of the specimen donor, as inferred from red-cell Lewis typing. Others have noted a strict correlation between secretor status and the

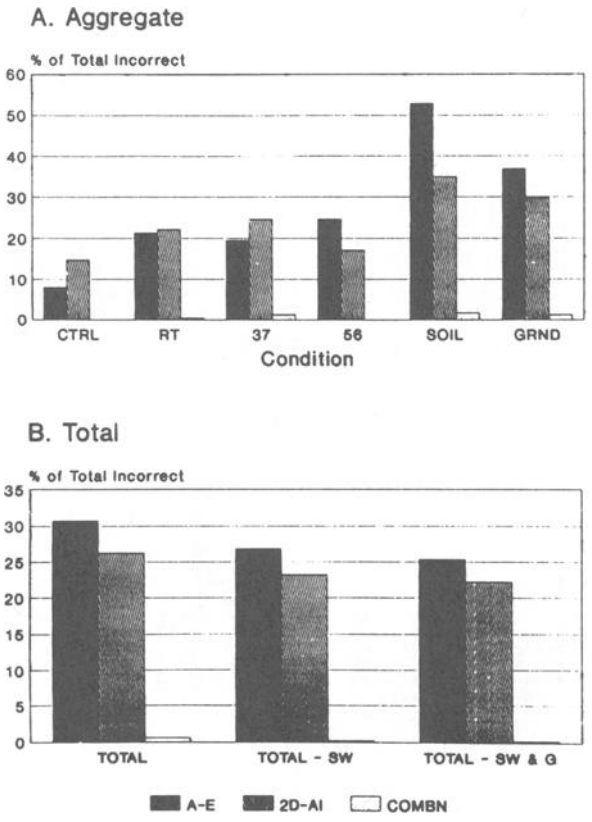


FIG. 2—*Percentage of specimens tested yielding incorrect results:* (a) *according to environmental exposure condition;* (b) *all specimens. SW = wet soil; G = buried in the ground.*

TABLE 2—*Summary of all ABO typing tests in bone specimens.*

| Method | Total, *N* (%) | Total − SW, *N* (%)[a] | Total − SW and G, *N* (%)[b] |
|---|---|---|---|
| Elution | | | |
| Correct | 1003 (65.4) | 924 (69.5) | 827 (71.4) |
| Incorrect | 471 (30.7) | 356 (26.8) | 293 (25.3) |
| INC/NR | 60 (3.9) | 49 (3.7) | 38 (3.3) |
| 2-D Inhibition | | | |
| Correct | 675 (44.0) | 618 (46.5) | 568 (49.1) |
| Incorrect | 402 (26.2) | 308 (23.2) | 257 (22.2) |
| NAD/NR | 457 (29.8) | 403 (30.3) | 333 (28.8) |
| Combination | | | |
| Correct | 490 (31.9) | 464 (34.9) | 439 (37.9) |
| Incorrect | 9 (0.6) | 3 (0.2) | 2 (0.2) |
| NC/NR | 1035 (67.5) | 862 (64.9) | 717 (61.9) |

[a]SW = wet soil. This column shows all results except those for samples in wet soil.
[b]G = buried in the ground. This column shows all results except those for samples in wet soil and specimens buried in the ground.

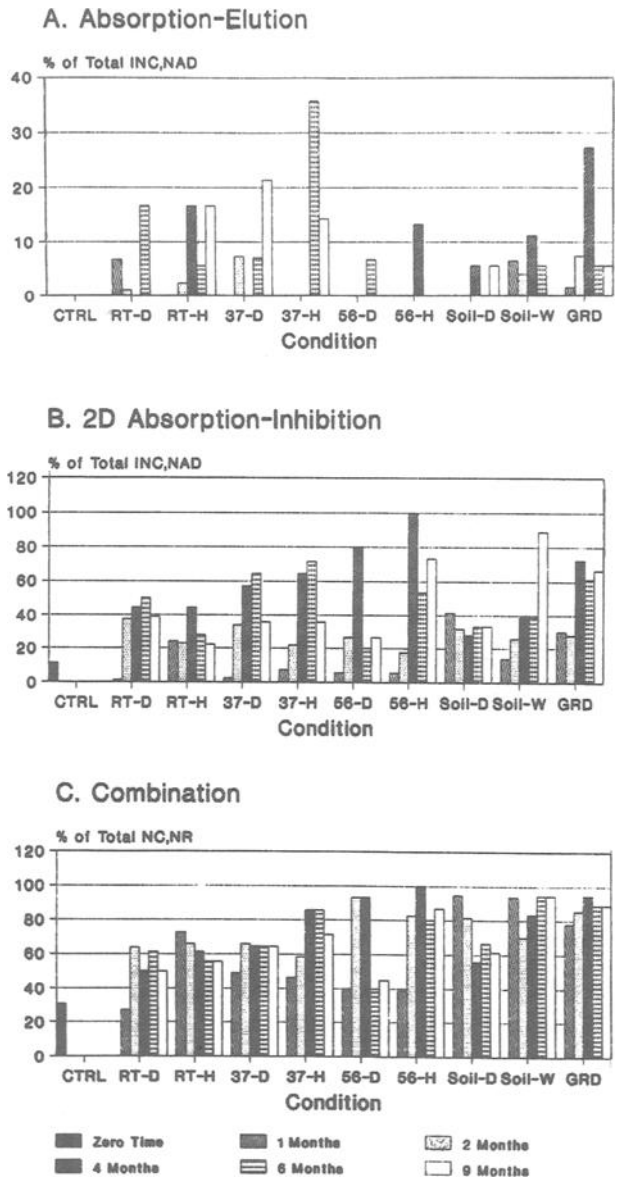


FIG. 3—*Percentage of specimens tested yielding inconclusive, no, or nonconcordant results as a function of aging time and environmental exposure condition:* (a) *AE testing,* (b) *2DAI testing,* (c) *combination procedure.*

ability to detect ABH antigens in bone tissue extracts, especially when using inhibition typing methods.

It was of interest to look at the data in terms of the ABO group distribution in the tested series and compare it with the ABO group distribution of the correctly typed specimens. Such a comparison might indicate whether the testing procedures showed any bias toward a particular ABO group. Figure 5 compares these ABO group distributions

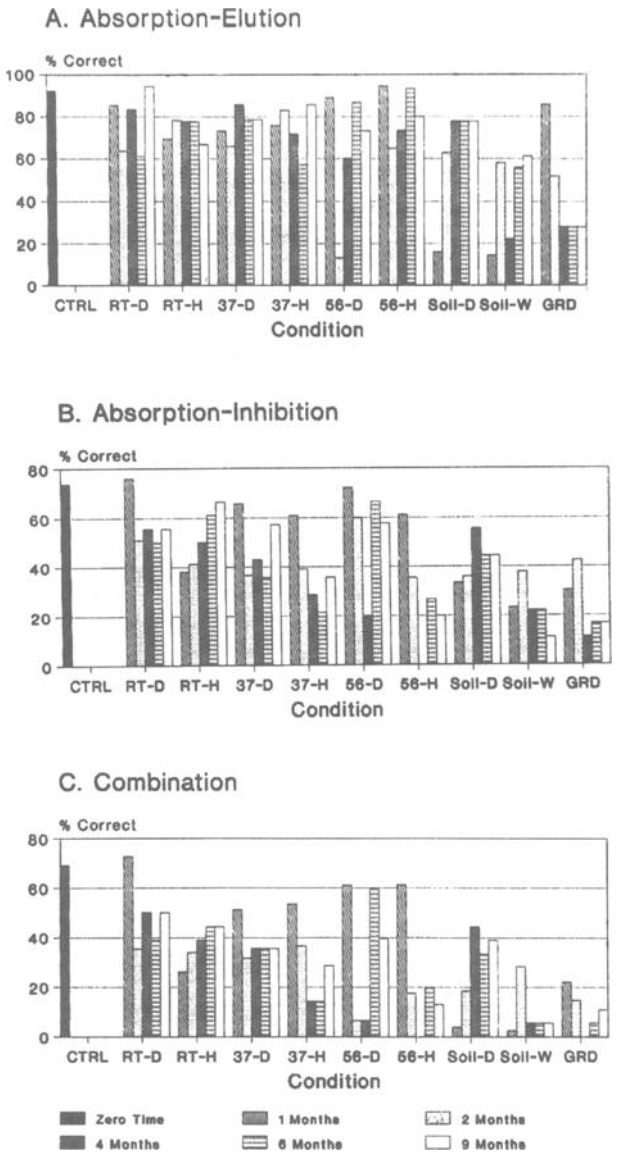


FIG. 4—*Percentage of specimens tested yielding correct results as a function of aging time and environmental exposure condition:* (a) *AE testing,* (b) *2DAI testing,* (c) *combination procedure.*

as a function of environmental condition for separate AE and 2DAI tests as well as for the combination procedure.

Little, if any, bias toward any particular group was apparent in the AE test results (Fig. 5*a*), but a bias toward Group O becomes apparent in the 2DAI test results (Fig. 5*b*), and it is more pronounced in the 37°C, 56°C, soil, and ground series than in the RT or control specimens. This apparent bias is, of course, reflected in the combination procedure test results (Fig. 5*c*). A similar trend is evident in a comparison of actual ABO group distributions with those of correctly grouped specimens for the entire group of

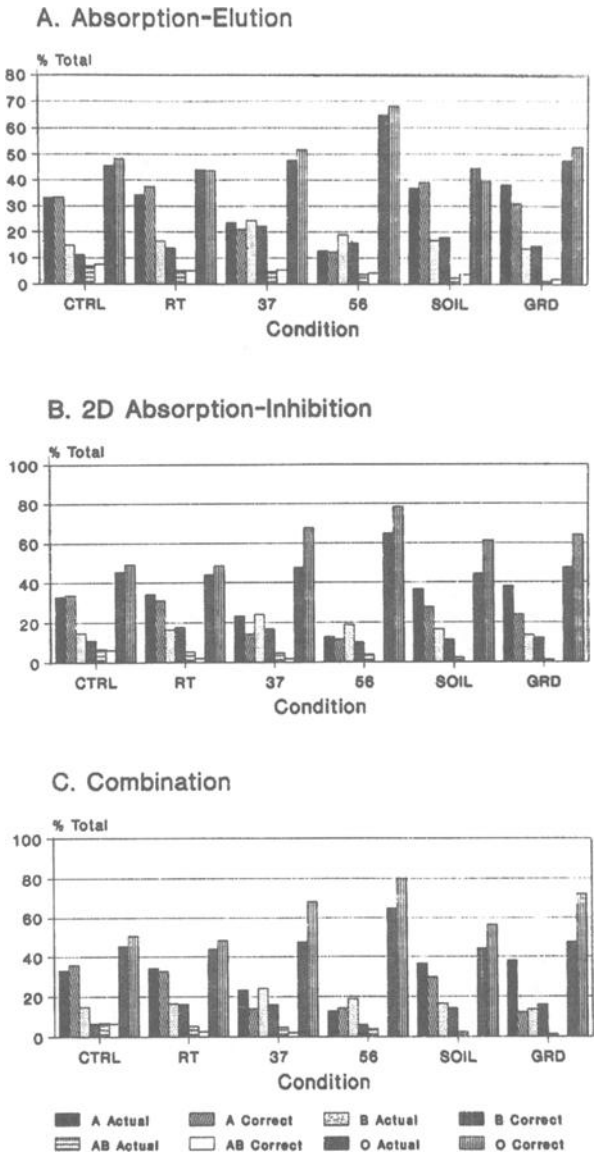


FIG. 5—*Distribution of ABO types in subsamples of specimens compared with distribution of ABO types in correctly grouped specimens of the same subsample, arranged according to environmental exposure condition:* (a) *AE testing,* (b) *2DAI testing,* (c) *combination procedure.*

specimens (Fig. 6). This figure (like Fig. 2*b* and Table 2) also indicates the distribution relationship for all specimens without the SW series, and for all specimens without the SW or G series. The data indicate that Group O specimens are disproportionately represented in the "correct" distribution, that this effect is attributable almost entirely to an apparent bias in the 2DAI testing, and that the bias toward Group O occurs at the expense of Groups A, B, and AB on a roughly equal basis. This finding suggested that bone specimens from donors of blood groups other than O appear to stand a greater chance of being adversely affected by aging and exposure to various environmental

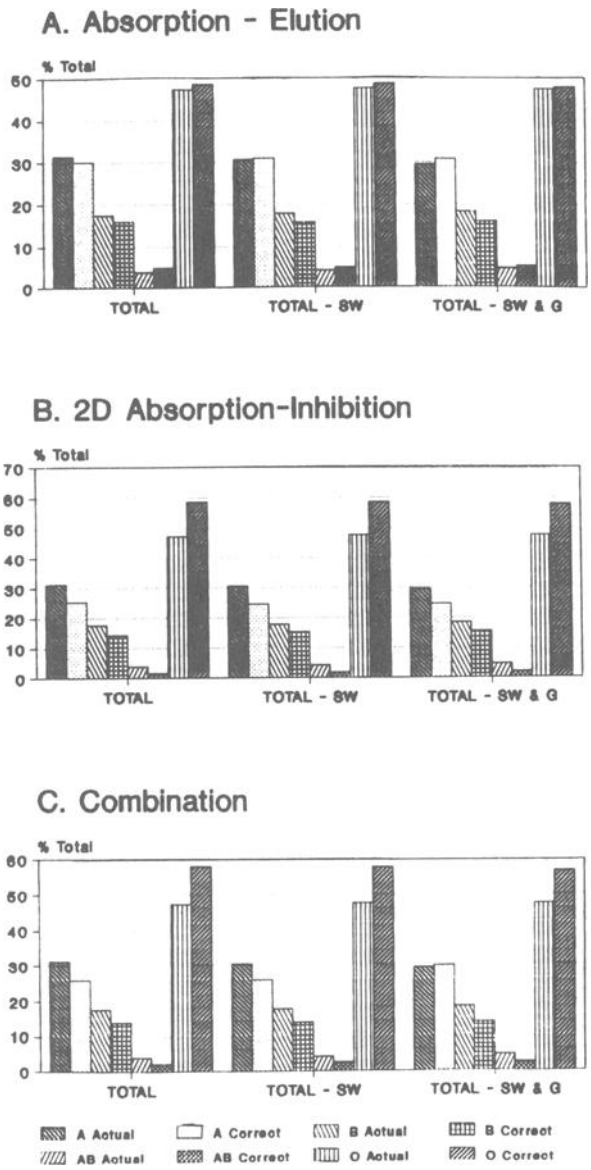


FIG. 6—*Distribution of ABO types in total and subsamples of specimens compared with distribution of ABO types in correctly grouped specimens of the total or same subsample.* (a) *AE testing;* (b) *2DAI testing;* (c) *combination procedure. SW = wet soil; G = buried in the ground.*

conditions, and it prompted some additional investigation into possible explanations for the observations.

An obvious possible explanation for inconclusive or spurious ABH testing results is that microbial activity in the specimens, especially those aged under environmental conditions that might be expected to promote bacterial growth and activity, was in some way responsible for alteration of the antigenic activity. ABH antigens have long been known to represent widely distributed cell surface structures in the biological world [*56–58*].

The effect of actual or apparent microbial activity in biological specimens on subsequent ABH antigen testing is well known and has been previously investigated in various types of clinical [*59*] and forensic specimens [*60–62*], including human tissues [*63,64*] and bone [*43*]. At least in theory, microbial contaminants of human tissues could affect ABH antigen determinations in two ways. First, if the contaminating organisms have A-like, B-like, or H-like antigens, or some combination of them, these "acquired" antigens might be detected in grouping tests. Second, some microorganisms have enzymes capable of hydrolyzing immunodeterminant terminal sugars and thus altering ABH blood group specificity [*65*]. The most important of these enzymes are galactosidases and *n*-acetyl-glucosaminidases, capable of converting B and A immunodeterminant end groups, respectively, to H. Group B red cells have been converted to Group O in vitro using an appropriate enzyme [*66*].

We isolated a number of cultures of *Pseudomonas* microorganisms from several bone specimens that had been in wet soil or in the ground for six to nine months. Growth media designed to promote the propagation of anaerobic forms and fungi failed to yield any viable cultures. The isolated organisms were tested directly for ABH antigens using the AE procedure. Of the seven cultures tested, one showed A, B, and H results (the A reaction was weak), and another showed a weak H reaction. To test the possibility that microbial hydrolytic enzymes capable of altering ABH antigen composition might be present in the isolated microbes, reagent red cells of Groups A, B, and O were incubated with bacterial cell culture supernatants, or with resuspended bacteria that had been ground with alumina to ensure cell breakage, at 37°C for periods up to 48 h.

Table 3 shows the results of a typical experiment in which treated and untreated red cells of comparable concentration were used as test cells in the titration of the same anti-A, anti-B, and anti-H. The antisera were adjusted to a consistent titer at the outset.

TABLE 3—*Titration of constant titer anti-A, anti-B, and anti-H with reagent A, B, and O cells following incubation of cells with bacterial culture supernatants.*

| Specimen | Test With | Titer (Score) |
|---|---|---|
| 1B2C[a] | α[b] | 256 (73) |
| | β[b] | 256 (65) |
| | anti-H | 128 (63) |
| 2B2B | α | 256 (73) |
| | β | 256 (71) |
| | anti-H | 64 (57) |
| 3B1A | α | 256 (70) |
| | β | 256 (71) |
| | anti-H | Lysis |
| 3B1A1 | α | 64 (57) |
| | β | 128 (63) |
| | anti-H | Lysis |
| Culture medium broth | α | 128 (63) |
| | β | 256 (71) |
| | anti-H | 128 (63) |
| Saline | α | 128 (62) |
| | β | 128 (65) |
| | anti-H | 128 (65) |

[a]Arbitrary culture designation.
[b]Abbreviations: α = anti-A; β = anti-B.

Significant hydrolytic activity capable of altering ABH antigen composition would have produced corresponding significant decreases in the titers and scores of incubated specimens in comparison with controls. There was a slight reduction in A activity in A cells incubated with one culture, and a slight decrease in H activity in O cells incubated with another. These experiments reaffirmed the well-known fact that microorganisms infecting human tissue can and do themselves exhibit ABH-like antigens detectable in AE tests. Further, the possibility of microbial enzymatic activity capable of altering ABH antigen composition cannot be ruled out. In carefully analyzing all the grouping data, we were not able to correlate consistent or predictable changes in blood group antigen composition with environmental conditions or with the time of aging. Further, the results of limited investigations of blood group antigen and antigen-altering enzyme activities of the infecting microorganisms could not be directly correlated with specific antigenic changes observed in the specimens. These latter findings are in general agreement with those of Hauser and collaborators [*43*].

Several experiments were conducted to determine whether the AE test could be rendered more specific with bone tissue by performing the test semiquantitatively and whether monoclonal antibodies would provide greater specificity than their polyclonal counterparts. If the eluate from an AE test is titrated, a relative measure of the quantity of antibody bound to the antigen can be obtained [*45–48*].

Some success with this approach has been reported by Hauser [*44*]. Using a titration procedure slightly modified from that described by Hauser, eluates from several different bone specimens were examined, following absorption with monoclonal and polyclonal anti-A and anti-B, and with crude *Ulex* anti-H and UEA I. Representative results are shown in Table 4. The specimens for these experiments were selected on the basis of

TABLE 4—*AE testing with eluate titration on selected specimens.*

| Known Group | Testing With[a] | Titration Score | Interpretation Method 1[b] | Interpretation Method 2[c] |
|---|---|---|---|---|
| AB | PC α | 25 | | |
| | β | 30 | AB | AB |
| | AH | 31 | | |
| AB | PC α | 21 | | |
| | β | 10 | AB | A |
| | AH | 47 | | |
| A | PC α | 33 | | |
| | β | 10 | AB | A |
| | AH | 54 | | |
| A | MC α | 10 | | |
| | β | 2 | A | O |
| | UEA I | 51 | | |
| O | PC α | 10 | | |
| | β | 28 | AB | B |
| | AH | 41 | | |
| O | MC α | 0 | | |
| | β | 36 | B | B |
| | UEA I | 64 | | |

[a]Abbreviations: PC = polyclonal; MC = monoclonal; AH = crude anti-H lectin from seed extracts; UEAI = affinity-purified anti-H lectin I.

[b]Results were considered conclusive if score ≥ 10.

[c]Results were considered conclusive if score ≥ 15.

their having shown a false positive or false negative result in routine AE testing. Both false positive and false negative results can be obtained using monoclonal or polyclonal anti-A and anti-B and crude *Ulex* anti-H or UEAI. Two possible interpretation schemes are shown in the table. The data showed, however, that no interpretation scheme could be defined that would enable all false positive and false negative results to be detected. In these limited studies, monoclonal antibodies were equally as reactive with "false" antigens as with indigenous ones. UEA I showed no superiority over crude *Ulex* seed anti-H lectin in these experiments.

Several experiments were conducted to explore the possibility of detecting IGH (Gm) antigens in extracts of bone tissue. In preliminary studies, we had some success in demonstrating selected G1m and G3m antigens in bone tissue extracts prepared essentially as described above for ABH testing by 2DAI. Testing for IGH antigens was more successful in extracts that had been concentrated between 10-fold and 20-fold. Tests on eleven unconcentrated bone tissue extracts for a number of IGH antigens known to be present in the corresponding blood by an inhibition technique yielded no results in the present series of experiments, however. Radial immunodiffusion (RID) tests were used to quantitate IgG levels in several randomly selected bone specimen extracts.

Extracts prepared essentially as described above for the ABH 2DAI testing were used in these experiments, as well as 10-fold concentrated extracts. No IgG could be detected by the RID tests in any of the specimens. The RID procedure had a lower detection limit of about 1 μg for IgG, and 10 μL of specimen was tested. Thus, the concentration of IgG in the bone tissue extracts tested was less than 100 ng/μL. Failure to detect IgG in the 10-fold concentrated extracts suggests that the IgG concentration in the original extracts was less than 10 ng/μL. If serum has an IgG concentration of about 5.5 mg/mL, a 1:1000 serum dilution would have about 5.5 ng/μL IgG. The fact that G1m factors can sometimes be detected in 1:1000 serum dilutions suggests that the IgG concentration in the bone tissue extracts tested in these experiments was less than 5.5 ng/μL. Thus, although it is sometimes possible to detect IGH factors in bone tissue extracts that have been concentrated 10-fold or more, it does not appear that conclusive results can be expected on a routine basis.

The extensive studies on the combination AE-2DAI procedure for bone ABH typing presented here show that it is the most reliable procedure developed thus far for the purpose. With bone specimens that have aged under various environmental conditions, the percentage of specimens yielding conclusive results is expected to be relatively low. The data indicate, however, that conclusive results are nearly always correct, the key criterion from the point of view of forensic science. Caution should be exercised in the interpretation even of conclusive results from bone tissue specimens that have aged underground or in any type of moist soil. Of the environmental conditions included in the present studies, these were the most detrimental to subsequent successful ABH bone typing.

*Acknowledgment*

We thank Dr. M. S. Schanfield of the Analytical Genetic Testing Center, Denver, Colorado, for assistance with IGH and KM testing aspects of this work.

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