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Dietary Reconstruction from Bones Treated with Preservatives

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Pre-Historic diets may be reconstructed from the chemical and isotopic composition of human bone. Collections of human bone from archaeological contexts have been treated with resins, glues, and varnishes to consolidate and strengthen them. The use of such treated bone in chemical analysis for dietary reconstruction is evaluated. Results are presented of a study of a museum collection that had been treated with a common consolidant, Alvar. Treatments to remove Alvar from bone were successful. In addition, for one collagen preparation technique, such pretreatment was not necessary.

Keywords: STABLE ISOTOPE ANALYSIS, DIETARY RECONSTRUCTION, MUSEUM CONSERVATION OF BONE.

Introduction

The reconstruction of pre-Historic human diets from bone chemistry has become well-established during the past two decades (Price *et al.*, 1985). Trace element analysis of bone ash, and stable isotope ratio analysis of carbon and nitrogen from bone collagen were first developed using specimens from modern environments (Comar *et al.*, 1957; DeNiro & Epstein, 1978, 1981; Elias *et al.*, 1982; Schoeninger & DeNiro, 1984). Typically, fresh bone was analysed to establish baseline levels in animals of known diet. The position of humans in various food chains has been estimated by comparing values from human bone with these baseline levels (Schoeninger, 1979; Sillen & Kavanagh, 1982; Schoeninger *et al.*, 1983; Sealy & van der Merwe, 1986; Sealy & Sillen, 1988).

Application of the techniques to archaeological specimens, however, demands consideration of several more sets of variables. Diagenetic alteration of bone must be carefully assessed before choosing samples for dietary analysis (Hare, 1980; Sillen, 1981; Lambert *et al.*, 1985; Nelson *et al.*, 1986; Hanson & Buikstra, 1987; Schoeninger *et al.*, 1989). In addition to the state of preservation of bone at the time of excavation, the treatment of bone after excavation can affect its chemical composition. Specifically,

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treatments to harden and strengthen archaeological bone may contaminate it chemically, restricting the application of analytical techniques.

Problem

Burial in archaeological contexts can affect both the mineral and organic fractions of bone. Often the bone is weakened by degradation so that even though its shape is preserved when surrounded by soil, it collapses upon excavation. This is a frustrating situation for archaeologists and physical anthropologists who want to observe the surface landmarks and morphology of bone which allow determinations of age, sex, and health status. Fieldworkers may brush consolidants directly onto the bone *in situ*, in the attempt to recover it intact from the soil. Once the bone has been removed from the soil and cleaned, consolidants are often routinely applied to strengthen them for shipping, storage, and analysis (Brothwell, 1972; Koob, 1985; Sease, 1987).

A wide variety of substances has been used to consolidate bone, ranging from waxes and gums to modern epoxy resins (Howie, 1985). Among the most popular and widely used have been Alvar (polyvinyl acetaldehyde acetal) and the related polyvinyl acetates (PVAC) and polyvinyl alcohols (PVA), first used in the 1930s (Woodbury, 1936; Garvin, 1956). Alvar is no longer manufactured, and has been replaced for these purposes by acrylic emulsions and colloidal dispersions (Primal & Paraloid, see Koob, 1985). All of these compounds are ultimately derived from petroleum (Leonard, 1970).

These treatments have two possible effects on subsequent chemical analysis. The first is the increased difficulty of cleaning bone covered with consolidants. Normal preparation for chemical analysis involves the removal of degraded surfaces of the bone and the removal of all possible soil contamination by manual scraping and ultrasonic cleaning. If consolidants have been applied before such careful cleaning, soil and root hairs may be glued to the surface or interior of the bones, even though bones may have already been cleaned according to field procedures. If a thick layer of hardened bone and soil must be pared from the bone, it may be necessary to use a larger sample of bone than usual. Very thin or fragile cortical bone, such as that in ribs, may be unusable if they have received preservative treatment.

The second problem is chemical contamination of the sample by the consolidant. In trace element analysis, contamination from consolidants is unlikely, since the bone is ashed at high temperatures (800°C) and the consolidant would be oxidized at the same time as the collagen, fats, and other naturally occurring organic compounds in the bone. In cases where the consolidant itself is contaminated with a metal of interest (e.g. Cu, Sr, Zn, Ba, Mg, Pb, etc.), trace element determinations from treated bone could, in theory, be altered. Small samples of the consolidant should be scraped from the bone and analysed separately to ensure that contaminating metals are not present. For example, in a previous study (Peebles & Schoeninger, 1981) bone had been consolidated using Alvar. An analysis by neutron activation, however, indicated that the elements of interest (Sr and Zn) were present in Alvar at concentrations below that recovered from blanks. Based on this information, the trace element analysis of the bone was initiated. Contamination of trace element determinations by soil is aggravated by the use of consolidants, but can usually be overcome by careful cleaning.

The possible contamination of the organic fraction recovered from archaeological bone (referred to as collagen in this paper) treated with consolidants is a more serious problem. Bone collagen samples are combusted under vacuum to produce gas samples (CO2 and N2) for stable isotope analysis. If a sample of bone collagen is contaminated with a consolidant, any carbon or nitrogen in the contaminating material will contribute to the stable isotope ratios measured for that sample.

The magnitude of the potential effect can be modeled using the empirically determined stable carbon isotope ratios from a consolidant (Alvar) and the bone standard used below. If a bone sample contained 1% Alvar ($\delta^{13}C$–23·9‰) by weight, for example, the resulting collagen residue would contain 5% by weight Alvar (the increase is due to removal of bone mineral). This degree of contamination would change the carbon stable isotope ratio by −0·5‰, a difference greater than the limits of precision of the technique. Larger amounts of Alvar in the sample would produce even larger changes in the stable isotope ratios. Clearly, care must be taken to avoid contamination of the collagen samples by the consolidant.

During a research programme on the paleodiet of a European pre-Historic population, human skeletal material known to have been treated with a specific consolidant was prepared for stable isotope ratio analysis. Several techniques to avoid contamination of the stable isotope ratios were tested using this skeletal sample and modern bone standards.

Materials and Methods

Samples of 20 human skeletons were taken from the Mecklenberg Collection of European Iron Age materials at the Peabody Museum, Harvard University. The skeletal material, dating to the European early Iron Age (*c.* 750–300 BC) is from tumuli at the site of Magdalenska gora in northwestern Yugoslavia. Excavations were carried out before the First World War by the Duchess Paul Friedrich von Mecklenberg, and the material from Magdalenska gora was acquired by the Peabody Museum in 1934 (Hencken, 1978). The bones were moderately well preserved, and records indicate that they were extensively treated in the Peabody Museum with Alvar 770 (Angel, 1968). The bones were allowed to soak in an Alvar solution until saturated, and Alvar was also used as a glue to reconstruct fragmented skulls and long bones. Although all visible deposits of the resin were removed from the surfaces of the bones during preparation for stable isotope analysis, due to the saturation of the bone in Alvar, the remaining cortical bone was assumed to be still contaminated.

Alvar is soluble in acetone as well as several other solvents (Brauer & Horowitz, 1962), so treatment with this consolidant should theoretically be reversible. When bones treated with consolidants are submitted for radiocarbon dating, acetone pretreatments are routine (Mook & Waterbolk, 1985; Protsch, 1986). In one treatment, successful removal of vinyl polymer hardeners is indicated when the acetone decanted no longer forms a white precipitate in water (Mook & Waterbolk, 1985: 40).

In order to assess the effectiveness of procedures for Alvar removal, a series of bone samples were prepared. An homogenized bone powder was used for this purpose. Previously, a standard bone powder had been prepared from fresh pig bone. Over 15 kg of pig bone were cleaned mechanically, the cortical bone was then ground at liquid nitrogen temperatures in a Shatterbox mill, and then sieved through 30 mesh. The stable isotope ratios of the pig bone collagen have been well characterized through its use as a standard in our laboratory. A control sample of pig bone (Table 1, sample C) and a series of pig bone samples with different treatments (Table 1, samples G–L) were prepared for this study.

A sample of pure Alvar was removed from one of the Magdalenska gora bones for stable isotope determination (Table 1, sample N). In addition, a sample of pure, granular Alvar was obtained from the physical anthropology lab at The University of Michigan (Table 1, samples E and F). This sample was used to simulate the effects of applying Alvar to bone in controlled quantities. Alvar was ground to the same mesh size as the pig bone, and a pig bone and Alvar mixture (24% Alvar by weight) was prepared by repeated sieving (Table 1, samples G–J).

The treatment for removal of Alvar followed in this study consisted of adding 20 ml of acetone to a small, weighed amount (less than 0·5 g) of bone powder, and agitating for 20 min. The acetone was decanted and the process repeated with fresh acetone. The bone

Table 1. Treatments to remove consolidant from bone

Sample	Treatment	Sample wt. (g)	%Wt. removed with solvent	Organic residue (g)	% Residue	Delta ^{13}C ‰	Delta ^{15}N ‰
A	Bone treated with ether	0·234	8	0·033	22·6	−12·6	+5·6
B	Bone treated with ether	0·253	9	0·058	22·9	−12·5	+5·8
C	Control	0·273	—	0·038	13·9	−12·4	+5·8
E	Alvar	0·130	—	0·003	1·0	—	—
F	Alvar	0·280	—	0·003	1·1	—	—
G	Bone and Alvar	0·218	—	0·028	18·2	−12·6	+5·8
H	Bone and Alvar	0·281	—	0·031	17·3	−12·5	+5·8
I	Bone and Alvar treated with acetone	0·223	32	0·028	16·6	−12·4	+5·9
J	Bone and Alvar treated with acetone	0·242	30	0·031	16·8	−12·5	+6·2
K	Bone treated with acetone	0·335	13	0·053	18·2	−12·5	+5·7
L	Bone treated with acetone	0·330	11	0·056	19·0	−12·5	+5·5
M	Blank	0	—	0·003	—	—	—
N	Alvar from archaeological sample (MS 2640)	—	—	—	—	−23·9	No N2 produced

Bone and consolidant samples: yields after acetone treatment, yields after collagen preparation, and stable carbon and nitrogen isotope ratios.

powder was then dried to constant weight (Table 1, samples I and J). Weights of the initial bone-Alvar mixture were compared to the final weights to estimate the efficacy of this treatment. Whole, unground bone was treated by soaking 5 g of clean bone pieces in 50 ml of acetone for 24 h, repeating with fresh acetone, and drying to constant weight. Samples were also prepared to evaluate the possible effects of acetone treatment on the bone. Pure pig bone powder was soaked in acetone as above (Table 1, samples K and L).

The choice of technique used to prepare the bone collagen proved to be an important element in the process of dealing with a possible contaminant. At least three techniques for preparing bone collagen are currently in use, each producing comparable results with modern bone samples (Figure 1 and see Tuross *et al.*, 1988). In the most common procedure, powdered bone is demineralized in 1 M HCl, soaked in 0·125 M NaOH, and the collagen is then hydrolized in 0·001 M HCl at 90°C for 10 h (Schoeninger & DeNiro, 1984). Poorly preserved samples, however, may best be demineralized by the other two methods: (a) soaking small chunks of bone in an EDTA solution (Tuross *et al.*, 1988), or (b) in dilute (1%) HCl (Sealy, 1986). The tests for Alvar contamination in this study were designed for the bone powder preparation, although the results of our study are relevant for all three techniques.

Slightly smaller amounts of the modern bone than archaeological bone were used for collagen preparation, because of the higher organic content of the fresh bone. Collagen samples were combusted in sealed quartz tubes according to the method of Northfelt *et al.* (1981), and the resulting carbon dioxide and nitrogen gas was separated and purified cryogenically on a vacuum line. Stable isotope ratios of carbon and nitrogen were determined by mass spectrometry.

Results and Discussion

Acetone appeared to be an effective solvent of Alvar using the treatments described above. Theoretically the treatment by acetone of the bone and Alvar mixture (samples I and J)

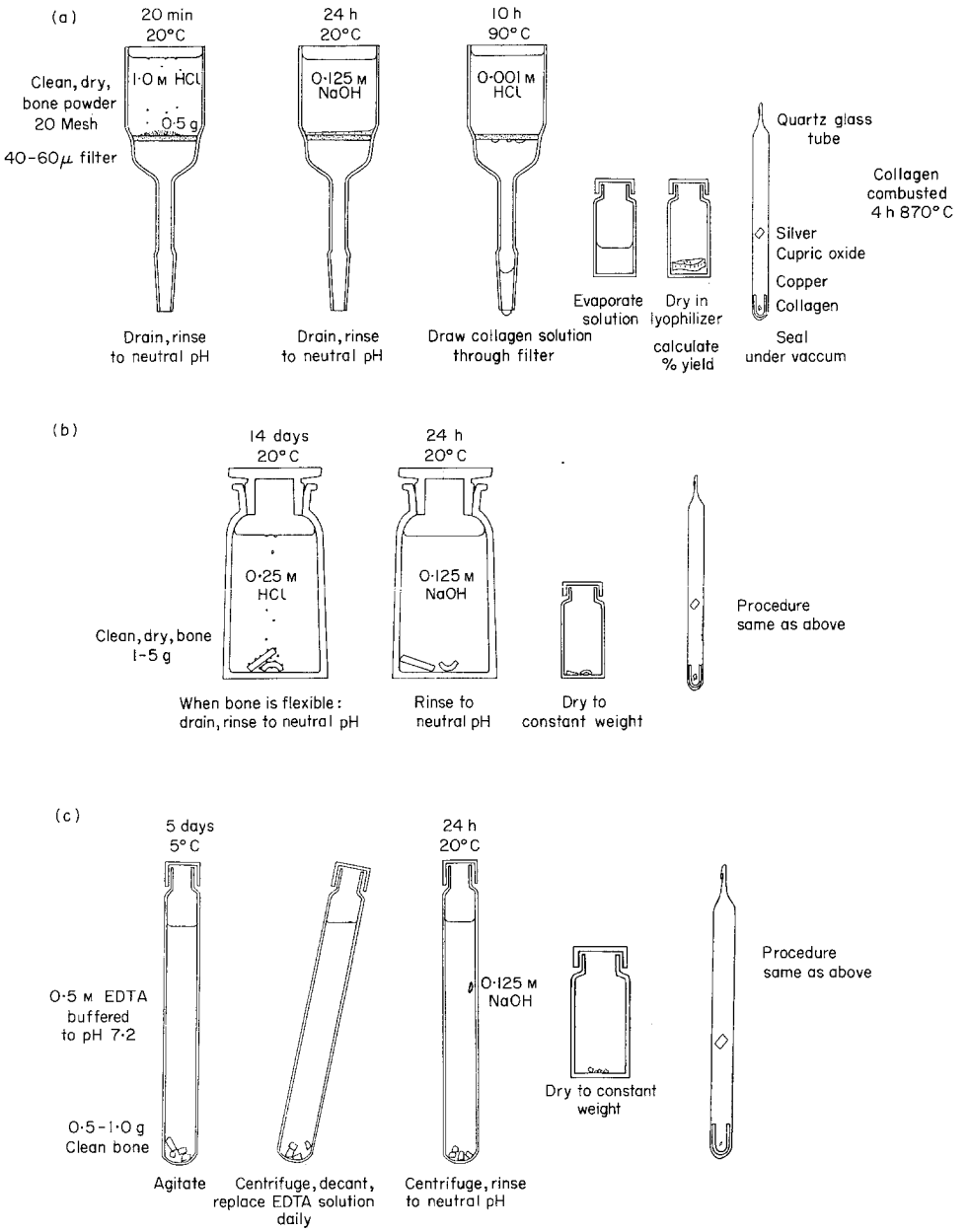

Figure 1. Procedures and equipment used to prepare collagen from bone. In (a), bone powder is the starting material. In (b) and (c), small chunks of clean bone are used. (b) HCl and (c) EDTA preparation of collagen. In (b) and (c), the samples must be free of consolidants and fats before the collagen is prepared.

should have removed 24% of the sample weight, but in these preparations, 32 and 34% of the original sample weight had been lost (Table 1, column 4). This high weight loss resulted from the removal by acetone of not only Alvar but also the naturally occurring

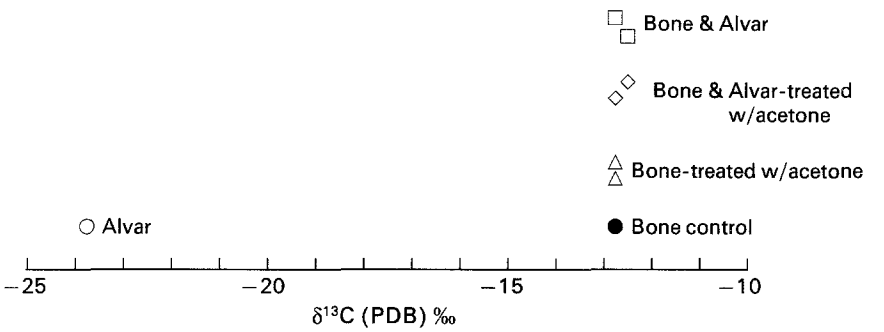

Figure 2. Stable isotope results from bone samples in study, and from Alvar sample. Stable isotope ratios from these samples are not affected by the presence of Alvar in the original bone powder.

fats in the bone. The pure bone samples treated with acetone (samples K and L) showed weight losses attributable to extraction of fats as well. While the acetone pretreatment clearly removed the Alvar from the bone, the necessity of the pretreatment was unclear, given the collagen preparation technique. The bone powder preparation of collagen includes a step where the collagen solution passes through a 40–60 micron (μ) sintered glass filter before it is evaporated to dryness [Figure 1(a)]. The Alvar should not be soluble in the reagents used in collagen extraction, and should be retained on the filter after recovery of the collagen solution. Thus, the extra step of acetone treatment may be avoided if the bone powder preparation of collagen is used.

As a test of the suggestion above, samples of pure Alvar were treated as bone in a collagen preparation (samples E and F). In one of these preparations (sample E) 90% of the original 0·28 g sample was collected from the surface of the filter in a non-quantitative recovery. The remaining 0·03 g could not be collected without abrading the surface of the filter. When solutions from a "collagen preparation" of the Alvar were drawn through the filter and evaporated, the scant white residues weighed only 0·003 g, very close in appearance and weight to residues from a blank preparation made with no sample material (see sample M). When the Alvar-bone mixture was treated as bone to extract collagen, the Alvar was noted on the surface of the filter after the collagen solution was drawn through. These results suggest that Alvar did not pass through the filter, and thus should not contaminate the collagen residues.

Stable isotope ratio determinations were made on collagen from the acetone treated bone (samples I and J) and also from the untreated bone (samples G and H) (Figure 2). A portion of Alvar (sample N) was combusted to determine the stable isotope ratio of the possible contaminant; no nitrogen was produced. All of the treatments produced samples with carbon and nitrogen stable isotope ratios within the normal limits of precision of the technique (N = 7, mean $\delta^{13}C$ − 12·5‰, S.D. 0·07‰). There is no evidence for the presence of Alvar in either collagen residues from the bone-Alvar mixture which had no pretreatment with acetone or in residues from bone-Alvar mix that had been treated to remove Alvar.

The acetone treatment had no independent effect on the stable isotope ratios. As noted above, acetone used to remove consolidants from a bone will also dissolve any lipids in that sample. If collagen from modern bone were contaminated with lipids, the carbon stable isotope ratios would be altered in a negative direction (DeNiro & Epstein, 1978; Tuross *et al.*, 1988). Comparisons of carbon stable isotope ratios from collagen prepared from fresh, untreated pig bone (sample C), pig bone treated with acetone (K and L) and pig bone treated with ether (A and B) show no effect from lipid removal. Ambrose (1987) suggested that the NaOH treatment in the collagen preparation saponified the lipids in

Table 2. Carbon stable isotope ratios of archaeological bone contaminated with Alvar

Sample ms no.	2641	2642
Untreated	−17·4‰	−14·2‰
Treated with acetone	−17·5‰	−13·9‰

Comparisons of stable carbon isotope ratios from two treatments of the same archaeological bone samples. These samples are unknowns, but the similarity of the results between treatments indicate that no Alvar in the untreated sample passes through the filter.

bone, allowing them to be rinsed away before collagen extraction. When powdered bone that had been demineralized and treated with NaOH (as though for collagen preparation), was extracted with ether, significant amounts of lipids (up to 5% of the original bone weight) were recovered. This indicates that the NaOH treatment in the collagen preparation is inadequate to remove lipids from fresh bone. The lack of lipid effect on the isotopic determinations of the samples above may result from the fact that lipids do not pass through the filter after the collagen is hydrolized. This applies specifically to collagen prepared using bone powder. When collagen is prepared from solid bone pieces, bones with high lipid contents should be defatted with a solvent (Tuross *et al.*, 1988). Few archaeological bones, however, would have lipid contents as high as the modern pig bone used in this study.

The results discussed above were all produced using modern bone with high organic content. The bone-Alvar powder might also have slightly different mechanical or chemical properties from archaeological bone to which an Alvar solution had been applied. To investigate this possibility, archaeological samples were also prepared to determine whether comparable results could be obtained (Table 2). Bone powder from two Magdalenska gora samples was used for these tests. Two aliquots were taken from samples known to have been treated with Alvar. One aliquot of each bone powder sample was prepared without pretreatment. The second aliquot was treated with acetone to remove Alvar. Stable isotope ratios of collagen from acetone-treated bone and untreated bone from the same Alvar-contaminated sample are within the range of precision for repeat determinations.

The rest of the archaeological sample from Magdalenska gora was prepared for stable isotope ratio analysis without pretreatment. These stable isotope data are the first obtained from pre-Historic central European populations. The relatively heavy carbon stable isotope ratios in the two samples presented in this paper (and in the majority of the human samples from the site) suggest the importance of a C4 plant in the diet, an unanticipated finding, given the C3 dominated vegetation of temperate Europe (Murray & Schoeninger, 1988).

Conclusions and Recommendations

The experiments in this study were designed to answer specific concerns about a particular archaeological sample and collagen preparation technique. Alvar was not soluble in the acids and base used in the collagen preparation, so the mechanical effect of the filter removed the Alvar from the bone collagen solution. The acetone treatment was also successful in removing Alvar from bone, though the filtering step alone would have been sufficient to purify the samples. As mentioned above, alternative techniques for preparing collagen use solid bone pieces, and the collagen is collected in the form of soft, rubbery chunks which cannot be filtered. When these techniques are used, bone contaminated with Alvar should be treated with acetone before the collagen is prepared.

The Alvar examined in this study is a hydrocarbon that contains no nitrogen, so we had little concern that the nitrogen stable isotope ratios would be contaminated by the presence of Alvar in a bone sample. The results described above confirm this (Table 1, column 8). Other possible consolidating materials, epoxy resins in particular, do contain nitrogen. For each suspected consolidant material, experimental programmes similar to those described here must be carried out. Attention must be paid to the potential contaminations of both the carbon dioxide and nitrogen.

Museum collections of human skeletons are important resources for anthropological research. Dietary reconstruction from bone chemistry allows new information to be gained about populations for which archaeological floral and faunal remains were not recovered. One difficulty in working with museum collections is the frequent lack of documentation on the type of preservative used to consolidate bone, the method of application (surface application, immersion, or vacuum impregnation), and the generality of treatment across the elements of a skeleton. In some archaeological collections different consolidants were used at different times, and different substances were applied serially to the same bones.

Most consolidants are soluble in one or more organic solvents, and where these have been used on bone, a removal treatment can be devised and tested. Simple spot tests may be used to diagnose the preservative material used, and infrared spectra are also available for some of the most common consolidants (Howie, 1985). Application of an insoluble polymer to bone does not necessarily disqualify it from chemical analysis. If the bone is intrinsically well enough preserved to use the bone powder preparation of collagen, it may be possible to extract the collagen solution through a filter in the manner described above, without removing the consolidant.

Diagenetic alteration of bone mineral and collagen from archaeological samples may disqualify them from dietary analysis. Most relatively well preserved samples can be submitted for analysis, even if they have been treated with consolidants. When consolidants have been applied to poorly preserved bone, the combination of methodological constraints may result in an unusable sample.

Dietary analysis from bone chemistry has advanced in refinement and interpretive power so rapidly that sampling procedures that might have been recommended only a few years ago are now outdated. Anthropologists are increasingly able to apply these techniques to poorly preserved skeletal material. Aspects of social and geographic variation which may be studied through bone chemistry are being continually expanded. Given this, it is impossible to predict the field in which skeletons or individual bones may be of interest in the future, and thus excluded from consolidant treatment (contra Koob, 1985). Some laboratories and museums avoid putting foreign substances on a sample of relatively undiagnostic bones (typically ribs) from each skeleton, leaving some untreated bone for chemical analysis. We recommend this policy as a compromise where the use of consolidants cannot be avoided, but feel that as many elements as possible should remain free from such treatments. Obviously, records should be made (and kept) on the details of the consolidating treatments used. Ideally, a pure sample of the substance used should be curated along with these records to facilitate further analysis and treatment, including removal. The potential of bone as a chemical signal for past cultural and ecological relationships should be an integral part of programs for both recovery and curation.

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