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RADIOCARBON AND STABLE ISOTOPE ANALYSES OF ARCHAEOLOGICAL BONE CONSOLIDATED WITH HIDE GLUE

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ABSTRACT. We tested a simple method for removing a collagen-based glue preservative from bone destined for radiocarbon and stable isotope analyses. The method is sufficient for bone samples from which only stable isotope measurements are required. For ^{14}C dating, such samples of age less than about 10 ka can be adequately dated, but for older samples, the circumstances must be carefully evaluated.

INTRODUCTION

We recently encountered circumstances in which glue had been used as a consolidant for archaeological bone specimens. Fourier Transform Infrared (FT IR) analysis showed that this preservative was the old woodworker's "hide glue" which was universally used until the advent of modern adhesives. It was manufactured by rendering soluble the collagen of animal hide or bone and then drying it. The woodworker had only to dissolve it again for use, as it remains water-soluble (cf. Flexner 1995).

Ancient bone treated with this material presents a difficult problem if radiocarbon or stable isotope measurements are required. The general procedure used to extract bone collagen for isotopic measurement is the same as that used in making the glue, and yields a very similar product. Is it possible to adequately remove the glue from the bone so that the autochthonous bone collagen can be extracted for isotopic measurement?

Because extracted bone collagen and hide glue are chemically very similar, separation must be based on their physical properties. In our usual method of bone collagen extraction (e.g. Brown 1988), we use the fact that the intact collagen trimer is insoluble, while the monomer is soluble. We discard any soluble material from the initial acid dissolution of the bone sample, and then render the residue soluble by heating in mild acid. This is followed by ultra-filtration to select only large collagen fragments. In principle, this procedure should remove the glue at the initial dissolution. One might also wish to soak the preserved bone in water before dissolution to bring the glue and any degraded autochthonous collagen into solution, which can then be discarded.

METHODS AND RESULTS

To test these possibilities, a sample of European hide glue was obtained from a retired cabinetmaker who had leftover stock from the 1960s. We prepared this glue for use by dissolving the pellets in equal parts water (in this case ultra-pure) at 60 °C to yield a viscous liquid.

Four similar fragments of a clean, dry, recent bone were obtained. Two were coated with a layer of glue (~1–2 mm thick) and allowed to cure at room temperature for three days; the other two were left untreated as blanks. All were then weighed and soaked in water at room temperature with periodic mixing. After 24 hours they were rinsed thoroughly with water and dried under vacuum. The soaking solutions and rinse water were pooled and freeze-dried to recover the soluble materials.

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The blanks showed no difference in weight or appearance before and after treatment, indicating that no autochthonous collagen was removed. Examination of the glue-coated samples after treatment revealed no visible signs of glue remaining on the bone surface. The mass of residue recovered from the wash solution was very slightly greater than the mass of glue applied, due to small bone flakes transferred during washing. Within weighing accuracy, it thus seems that a layer of hide glue on a bone can be quantitatively removed by soaking in water without affecting autochthonous collagen.

A much more sensitive test of glue removal is to compare the ^{14}C ages of uncoated, very old bone samples with some which have been glue-coated. We had in the lab a poorly preserved mammoth bone sample which dates from about the ^{14}C limit. A portion was removed, and most of its surface coated with glue as above. After curing, several sub-samples were taken using a low-speed drill. The first (Mam-1) was from an area not coated with glue. About 1–2 mm of the outer surface was removed by milling and the sample taken by drilling the underlying bone. The second (Mam-1b) was a "worst-case" situation taken by drilling through the glue layer into the bone material below, keeping all the drillings. The third and fourth sub-samples (Mam-1c and Mam-1d) were also taken from a glue-coated area, but the glue-coat and outer 1–2 mm of bone were removed (by milling) before taking samples. Mam–1b and –1d were soaked to remove any residual glue prior to collagen extraction. Collagen was extracted from Mam–1 and Mam–1c without this initial glue removal treatment.

All samples were demineralized by sonicating for 20 minutes in 0.25 N HCl. The insoluble protein residue was heated in 0.01 N HCl at 60 °C with gentle rocking to render the protein soluble. After about 20 hours of heating, these solutions were filtered (GF/A, Whatman Corporation) to remove any large particulates, then ultra-filtered with a nominal 30 kiloDalton MW device (Millipore Corporation). The <30 kD fraction was again ultra-filtered using a 10 kD device. These fractions were separately freeze-dried. Table 1 shows the sample weights and product yields.

No measurable product was recovered in the <30 kD, >10 kD fractions. The >30 kD extracts were the white, fine-pored sponge-like material that characterizes well-preserved high molecular weight collagen. The glue-treated samples gave slightly higher yields than the blanks, but the differences were no larger than is often found for different samples of the same bone, especially for bones as old as this.

From each collagen extract and for the glue itself, an aliquot of 1–2.5 mg was transferred to a baked quartz tube with approximately 80 mg of CuO and a few milligrams of silver powder. These tubes were evacuated, sealed and combusted at 900 °C. The resulting CO_2 was purified by cryogenic distillation and then submitted for accelerator mass spectrometry (AMS) dating. The remainder of each extract was used for $\delta^{13}C$ measurements. Carbon and nitrogen concentrations were also determined as a test of extract quality. The results are given in Table 2.

The glue contained bomb carbon in the amount anticipated (Levin et al. 1985). For the mammoth samples, the extract yields, C and N concentrations, the C/N ratios and the $\delta^{13}C$ value are those expected for bone collagen from this animal. While this indicates that the mammoth collagen was well preserved, these concentrations and isotope ratios provide little information on the amount of remaining contaminant, as the glue is likely to be very similar.

The sensitive ^{14}C data clearly show that the final extracts obtained from Mam-1b, -1c, and -1d contained glue residue. If we accept the age of the untreated sample as the true age of the mammoth (it makes little difference to this analysis if it is older) then the extract from Mam-1b, for which the glue layer was deliberately included in the sampling process, contained about 1.3% glue; e.g. the treatment removed almost 99% of the contaminant. The amount remaining in sample Mam-1c was almost a factor of ten lower, about 0.15%. The same result was obtained for Mam-1d, indicating that

Table 1 Collagen extraction results

Sample ID	Bone + glue (mg)	Bone – glue (mg)	>30 kD collagen (mg)	% Yield
Mam-1	29.6	N/A	2.5	8.4
Mam-1b	40.1	33.7	3.6	10.7
Mam-1c	30.4	N/A	4.0	13.2
Mam-1d	28.0	25.7	2.8	10.9

Table 2 AMS and stable isotope results

Sample ID	CAMS number	C conc (%)	N conc (%)	C/N ratio	$\delta^{13}C$ (‰ vPDB)	$\Delta^{14}C$	^{14}C age (BP)[a]	Glue treated?
Glue	68202	43.4	15.6	2.8	–19.2	596.7 ± 7.4	—	N/A
Mam-1	55932	45.0	15.9	2.8	–20.2	–996.2 ± 1.0	44,800 ± 2200	No
Mam-1b	55934	44.8	14.7	2.9	–20.6	–983.7 ± 0.6	30,050 ± 320	Yes
Mam-1c	62004	44.4	15.7	2.8	–20.9	–994.0 ± 0.8	41,000 ± 1100	Yes
Mam-1d	68203	44.0	15.7	2.8	–20.5	–994.0 ± 0.9	41,040 ± 1270	Yes

[a]Conventional radiocarbon years (Stuiver and Polach 1977)

the glue did penetrate into the bone interior, and that the extra treatment step did not eliminate any more contaminant than did our usual collagen extraction. Perhaps there was a small, residual amount of insoluble collagen in the glue.

From these data, one can evaluate the magnitude of the problems to be faced in making isotopic measurements on bones coated with hide glue. This simple treatment removed most, but not all, the glue. The consequences for ^{14}C dating depend on the age of the sample. At 1.3% contamination with this 1960s glue, samples with true ^{14}C ages of 20 ka, 10 ka, and 1 ka would appear to be respectively 1650, 445, and 80 ^{14}C years too young. At 0.15% contamination, the corresponding age differences are 220, 55, and 10 years, the latter well within usual measurement uncertainty. These values provide worst-case rules of thumb, since pre-bomb hide glue would be of lesser consequence, and recent glue is not likely to have been used for this purpose.

For stable isotope measurements, the consequences of glue treatment are not significant. The $\delta^{13}C$ and $\delta^{15}N$ values of glues are not likely to differ from that of the bone collagen by more than about 10–15‰, and so the influence of the glue would be in the range of 0.1–0.2‰ even at the higher contamination level. For bone samples from which only stable isotope measurements are required, these methods of glue-removal appear to be adequate.

CONCLUSIONS

For stable isotope measurements, a simple soaking in water, followed by our standard collagen extraction protocol, appears to provide adequate preparation for hide-glue coated bones. For ^{14}C dating, the situation is not so clear-cut, and will depend on the nature of the question to be answered. Certainly, a careful evaluation of the specific circumstance will be required, but analysts should not immediately dismiss the prospect of dating such samples.

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REFERENCES

Brown TA, Nelson DE, Vogel JS, Southon JR. 1988. Improved collagen extraction by modified Longin method. *Radiocarbon* 30(2):171–7.

Flexner B. 1995. *Woodwork* 35:46–52.

Levin I, Bernd K, Schoch-Fischer H, Bruns M, Münnich M, Berdau D, Vogel JC, Muunnich KO. 1985. 25 years of tropospheric ^{14}C observations in central Europe. *Radiocarbon* 27(1):1–19.

Stuiver M, Polach H. 1977. Discussion: reporting of ^{14}C data. *Radiocarbon* 19(3):355–63.