AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 157:330–338 (2015)

Brief Communication: The Effects of Paraloid B-72 and Butvar B-98 Treatment and Organic Solvent Removal on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ Values of Collagen and Hydroxyapatite in a Modern Bone

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KEY WORDS stable isotopes; bone; Paraloid; Butvar

ABSTRACT Stable isotopes in bones are a powerful tool for diet, provenance, climate, and physiological reconstructions, but necessarily require well-preserved specimens unaltered by postmortem diagenesis or conservation practices. This study examines the effects of Paraloid B-72 and Butvar B-98, two common consolidants used in field and museum conservation, on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values from bone collagen and hydroxyapatite. The effects of solvent removal (100% acetone, 100% ethanol, 9:1 acetone:xylenes, 9:1 ethanol:xylenes) and drying methods (ambient air, vacuum, oven drying at 80°C) were also examined to determine if bones treated with these consolidants can successfully be cleaned and used for stable isotope analyses. Results show that introduction of Paraloid B-72 or Butvar B-98 in 100% acetone or 100% ethanol, respectively, with subsequent removal by the same solvents and drying at 80°C facilitates the most successful removal of consolidants and solvents. The $\delta^{13}C$ values in collagen, $\delta^{15}N$ in collagen, $\delta^{18}O$ in hydroxyapatite phosphate, and $\delta^{13}C$ in hydroxyapatite structural carbonate were unaltered by treatments with Paraloid or Butvar and subsequent solvent removal. The $\delta^{18}O$ in hydroxyapatite structural carbonate showed nonsystematic variability when bones were treated with Paraloid and Butvar, which is hypothesized to be a result of hydroxyl exchange when bones are exposed to consolidants in solution. It is therefore recommended that $\delta^{18}O$ in hydroxyapatite structural carbonate should not be used in stable isotope studies if bones have been treated with Paraloid or Butvar. Am J Phys Anthropol 157:330–338, 2015.

Bone carbon, nitrogen, and oxygen stable isotopes are commonly used in paleontology and archaeology to discern diet (Peterson and Fry, 1987; Ambrose, 1993; Kelly, 2000), water source and provenance (Longinelli, 1984; Luz et al., 1984; Bryant and Froelich, 1995; France et al., 2013), physiology (Ambrose, 1991; Fizet et al., 1995; Kohn et al., 1996), and climate (Heaton et al., 1986; Sealy et al., 1987; Ambrose, 1991; Cormie and Schwarcz, 1996; Grocke et al., 1997; Koch, 1998 and references therein, Zazzo et al., 2002; Levin et al., 2006) of mammals and their surrounding environments, provided no secondary alteration has occurred. Postmortem diagenesis, contamination, or addition of laboratory chemicals and consolidants might alter the isotopes from their original state, thus invalidating interpretations based on these values. While well-established methods exist to monitor postmortem diagensis and contamination of buried bones (DeNiro, 1985; Tuross et al., 1989; Ambrose, 1990; Ayliffe et al., 1994; Hedges et al., 1995; Person et al., 1995; Iacumin et al., 1996; Koch et al., 1997; Lecuyer at al., 1999; van Klinken, 1999; McNulty et al., 2002; Harbeck et al., 2009), the effects of solvents and consolidants on isotope values is less well-understood.

Consolidants are commonly applied to excavated bones to reinforce them for transport or long-term storage in museum and related institutional facilities. These consolidants are found often on bones excavated in previous decades before biochemical testing was ubiquitous, or on recently excavated bones before isotopic testing is pursued. A variety of compounds have been used (Johnson, 1994; Kres and Lovell, 1995; Horie, 2010); the most common are polyvinyl acetal/acetate/alcohol resins (PVAc, PVOH, "Alvar," "Elmer's glue," "AYAx" series), polyvinyl butyral resins ("Butvar"), methyl methacrylate/ethyl methacrylate resins ("Paraloid," "Acryloid"), cellulose nitrate resins ("Ambroid," "Celluloid," "Duco Cement"), and others. Although some formulations have changed over the years (de Witte et al., 1978; Down et al., 1996), the base compounds are the same and contain similar functional groups.

Bone consists of organic and inorganic fractions which may be susceptible to secondary isotope alteration during consolidant application. The organic fraction is composed largely of collagen protein, which is primarily analyzed for carbon and nitrogen isotopes. The inorganic fraction is composed of hydroxyapatite [general formula

Grant sponsor: Smithsonian Institution.

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Received 1 October 2014; revised 19 December 2014; accepted 20 December 2014

DOI: 10.1002/ajpa.22697
Published online 16 January 2015 in Wiley Online Library (wileyonlinelibrary.com).

$Ca_5(PO_4)_3(OH)$], which contains both phosphate ions ($–PO_4$) and structural carbonate ions ($–CO_3$) that substitute for the $–PO_4$ and –OH in the crystal lattice (Elliot, 2002). Oxygen isotopes are analyzed in the phosphates; both oxygen and carbon isotopes are analyzed in the structural carbonates.

Peer-reviewed research on the effects of consolidant treatments and their associated solvents on stable isotopes in bone is minimal. In general, collagen carbon and nitrogen isotopes appear unaffected by application of consolidants or solvents (Moore et al., 1989; Tuross and Fogel, 1992; Takahashi et al., 2002; France et al., 2011), except in cases where solvents are specifically chosen to remove lipids which tends to enrich carbon isotope values (Hobson et al., 1994; Hilderbrand et al., 1996; Hobson et al., 1997; Burton et al., 2001; Post, 2002). Very few studies have examined effects of consolidants and solvents on hydroxyapatite isotope values. Oxygen in phosphates is unaffected by application and removal of PVAc, shellac, and cellulose nitrate (Stephan, 2000; France et al., 2011); carbon in structural carbonates is unaffected by application of PVAc (France et al., 2011). Oxygen in structural carbonates does experience alternation during PVAc application (France et al., 2011).

This study continues the work of France et al. (2011), who examined the effects of PVAc and solvent removal on isotope values in bone collagen and hydroxyapatite. Here the focus is Paraloid B-72 and Butvar B-98, two commonly applied consolidants in museum conservation work which have received little testing in regard to their effects on stable isotope values. Paraloid B-72 (also Acryloid B-72) is an ethyl methacrylate acrylic copolymer [$(C_6H_{10}O_2)_n$]. Butvar B-98, (also PVB) is a polyvinyl butyral resin [$(C_8H_{14}O_2)_n$]. In solution, Butvar B-98 partially hydrolyzes to polyvinyl acetate, similar to the PVAc tested by France et al. (2011) with its associated hydroxyl groups. Paraloid and Butvar are very soluble in acetone and ethanol, respectively, which are the most commonly used solvents due to ready availability. Small amounts of xylenes are often added to the solvent/consolidant mixtures to facilitate dissolution and transport of heavy molecules through the small bone pores. This study replicated these common procedures by using various mixtures of acetone, ethanol, and xylenes to add consolidants and subsequently remove them from bone. Various drying methods were also tested to determine the best means of solvent removal.

MATERIALS AND METHODS

Sample material and preparation

A modern whale rib was sectioned into disks and wedges for subsequent treatment. This bone was almost completely cancellous with a thin layer (~2 mm) of compact bone. The whale rib is more porous than most historic bone and mimics older bone that may experience an increase in porosity during exposure to ground water and subsequent dissolution of bone material. This represents a "worst-case" scenario where consolidants will penetrate easily and coat large surface areas of bone.

The experiment was organized into a complete block design where all possible combinations of consolidant treatment, solvent removal, and drying method were tested (Table 1). Several bone disks were fully submerged for 60 to 90 min in consolidant solutions while controls were left untreated. Consolidant solutions (~10% consolidant by volume) consisted of the following: Paraloid B-72 in 100% acetone, Paraloid B-72 in 9:1 acetone:xylenes solution, Butvar B-98 in 100% ethanol, Butvar B-98 in 9:1 ethanol:xylenes solution. Treated samples were completely dried (at least 48 h) before performing solvent extractions; time between consolidant exposure and solvent removal was often 1 to 2 weeks. Paraloid was removed with 100% acetone or 9:1 acetone:xylenes solution; Butvar was removed with 100% ethanol or 9:1 ethanol:xylenes solution. Consolidant was removed via two 24 h. rinses in solvent (~10 mL/g sample), followed by three five-minute decanted rinses. Consolidant was left intact in some disks as a control. Solvents were removed by ambient air drying (~23°C), oven drying (80°C), or vacuum drying for 24 h.

Collagen, hydroxyapatite phosphate, and hydroxyapatite structural carbonate were extracted from bone samples after consolidant/solvent treatment. All extractions were performed in single batches to minimize variations in processing, which can affect stable isotope values (Garvie-Lok et al., 2004; Crowley and Wheatley, 2014; Pestle et al., 2014). A portion of whole bone was isolated for collagen extraction. Remaining sample was powdered for phosphate and structural carbonate isotope analysis, gas chromatography-mass spectrometric (GC-MS) analysis of solvent and consolidant presence/absence, and Fourier transform infrared spectroscopic (FT-IR) analysis of consolidant presence/absence. Stock pellets of Paraloid B-72 and Butvar B-98 were also available for isotope, GC-MS, and FT-IR analysis.

Collagen was extracted by modified Longin (1971) methods. Approximately 200 mg of whole bone were sonicated in water to remove clinging debris. Samples were demineralized in 0.6M HCl at 4°C for several days (acid replaced daily) until reaction ceased and rinsed to neutrality. In most archaeological or paleontological studies, samples are typically processed to remove humic and fulvic acids. Although our whale rib was not buried and these acids are likely absent, we soaked samples in 0.125M NaOH for 18 h and rinsed to neutrality to mimic this step and facilitate direct comparison to archaeological and paleontological studies. The remaining gelatin was hydrolyzed in 0.03M HCl at 95°C for 18 h to solubilize collagen and the supernatant freeze dried to produce a purified collagen extract.

Structural carbonate was isolated by modified methods of Bryant et al. (1996). Approximately 20 to 30 mg of powdered bone was soaked in 2.5% sodium hypochlorite for 18 h to remove organic phases. Archaeological and paleontological samples are typically soaked in weak acid to remove secondary carbonates deposited during burial. Again, to maintain similarity of treatments, samples were soaked in 1M acetic acid solution buffered with 1M calcium acetate (pH ~4.5) for 4 h. Sample were rinsed to neutrality and dried at 60°C.

Phosphates were isolated by methods of Dettman et al. (2001). Phosphate ions were liberated by soaking ~10 to 20 mg of bone powder in 2M hydrofluoric acid overnight. The supernatant was diluted and buffered with 20% ammonium hydroxide. Silver phosphate (Ag_3PO_4) was precipitated by addition of 2M silver nitrate solution. The precipitate was retrieved by centrifugation, rinsed, and dried at 60°C.

Stable isotope mass spectrometry

All stable isotopes were run on Thermo Delta V advantage gas source mass spectrometers in continuous flow mode at the Smithsonian OUSS/MCI Stable Isotope

TABLE 1. Isotope data

Consolidant applied	Solvent applied	Drying method	Structural carbonate $\delta^{13}C$	Structural carbonate $\delta^{18}O$	Phosphate $\delta^{18}O$	Collagen $\delta^{15}N$	Collagen $\delta^{13}C$
None	None	N/A	−14.2	26.2	19.4	11.5	−18.6
None	None	N/A	−14.2	26.0	19.2	11.4	−18.1
None	None	N/A	−14.3	28.3	18.8	11.6	−17.9
None	None	N/A	−14.0	29.1	20.0	11.5	−19.6
		Average	*−14.2*	*27.4*	*19.4*	*11.5*	*−18.5*
		Stdev	0.14	1.54	0.79	0.10	0.75
None	100% Acetone	Air	−14.5	27.5	20.2	11.9	−17.8
None	100% Acetone	Air	−14.6	27.2	20.2	11.7	−17.8
None	100% Acetone	Oven	−14.5	27.1	19.8	11.7	−18.5
None	100% Acetone	Oven	−14.5	25.9	19.9	11.4	−18.3
none	100% Acetone	Vacuum	−14.4	28.5	20.3	11.6	−18.6
none	100% Acetone	Vacuum	−14.9	26.6	20.3	11.7	−18.0
		Average	*−14.6*	*27.1*	*20.1*	*11.7*	*−18.2*
		Stdev	*0.19*	*0.89*	*0.24*	*0.15*	*0.35*
None	90% Acetone:10% xylenes	Air	−14.1	28.2	19.6	11.6	−18.7
None	90% Acetone:10% xylenes	Air	−14.6	24.2	19.8	11.4	−18.2
None	90% Acetone:10% xylenes	Oven	−14.9	27.4	19.9	11.8	−18.1
None	90% Acetone:10% xylenes	Oven	−14.7	27.6	20.0	12.2	−19.8
None	90% Acetone:10% xylenes	Vacuum	−14.5	27.6	19.5	11.7	−18.1
None	90% Acetone:10% xylenes	Vacuum	−14.5	28.0	20.2	11.6	−18.8
		Average	*−14.5*	*27.2*	*19.9*	*11.7*	*−18.6*
		Stdev	*0.26*	*1.48*	*0.26*	*0.28*	*0.65*
None	100% Ethanol	Air	−14.5	28.2	19.3	11.3	−18.1
None	100% Ethanol	Air	−14.8	27.5	19.9	11.6	−17.9
None	100% Ethanol	Oven	−14.4	27.9	19.9	11.6	−18.5
None	100% Ethanol	Oven	−14.0	28.6	19.5	11.3	−18.9
None	100% Ethanol	Vacuum	−14.3	23.3	20.2	11.4	−19.1
None	100% Ethanol	Vacuum	−14.7	27.7	19.2	11.5	−18.1
		Average	*−14.4*	*27.0*	*19.8*	*11.4*	*−18.4*
		Stdev	*0.31*	*2.11*	*0.39*	*0.15*	*0.49*
None	90% Ethanol:10% xylenes	Air	−13.7	27.9	19.0	11.5	−18.3
None	90% Ethanol:10% xylenes	Air	−14.1	28.9	19.5	11.4	−18.2
None	90% Ethanol:10% xylenes	Oven	−14.0	27.7	19.7	11.5	−18.7
None	90% Ethanol:10% xylenes	Oven	−15.0	25.9	19.6	11.6	−18.0
None	90% Ethanol:10% xylenes	Vacuum	−14.0	28.1	20.4	11.6	−18.8
None	90% Ethanol:10% xylenes	Vacuum	−14.1	27.3	20.4	11.6	−18.4
		Average	*−14.2*	*27.6*	*19.9*	*11.5*	*−18.4*
		Stdev	*0.42*	*0.98*	*0.43*	*0.08*	*0.29*
Butvar B-98 in 100% ethanol	None	N/A	−14.4	28.1	20.1	11.7	−18.7
Butvar B−98 in 90% ethanol:10% xylenes	None	N/A	−14.4	28.0	20.2	11.4	−18.4
Paraloid B-72 in 100% acetone	None	N/A	−14.8	27.3	20.3	11.9	−18.2
Paraloid B-72 in 90% acetone:10% xylenes	None	N/A	−14.9	25.4	19.2	11.6	−18.2
Butvar B-98 in 100% ethanol	100% Ethanol	Air	−14.5	27.2	19.4	11.4	−19.3
Butvar B-98 in 100% ethanol	100% Ethanol	Oven	−14.7	27.6	19.5	11.7	−17.9
Butvar B-98 in 100% ethanol	100% Ethanol	Vacuum	−14.6	26.5	20.2	11.7	−18.0
		Average	*−14.6*	*27.1*	*19.7*	*11.6*	*−18.4*
		Stdev	*0.09*	*0.59*	*0.44*	*0.17*	*0.77*

TABLE 1. Continued

Consolidant applied	Solvent applied	Drying method	Structural carbonate $\delta^{13}C$	Structural carbonate $\delta^{18}O$	Phosphate $\delta^{18}O$	Collagen $\delta^{15}N$	Collagen $\delta^{13}C$
Butvar B-98 in 100% ethanol	90% Ethanol:10% xylenes	Air	−14.8	27.4	20.3	11.6	−18.3
Butvar B-98 in 100% ethanol	90% Ethanol:10% xylenes	Oven	−14.4	23.3	19.1	11.5	−18.2
Butvar B-98 in 100% ethanol	90% Ethanol:10% xylenes	Vacuum	−14.4	27.9	19.5	11.3	−18.7
		Average	*−14.5*	*26.2*	*19.6*	*11.5*	*−18.4*
		Stdev	*0.20*	*2.51*	*0.60*	*0.15*	*0.28*
Butvar B-98 in 90% ethanol:10% xylenes	100% Ethanol	Air	−14.3	27.3	19.9	11.4	−18.4
Butvar B-98 in 90% ethanol:10% xylenes	100% Ethanol	Oven	−14.6	25.8	20.0	11.6	−17.8
Butvar B-98 in 90% ethanol:10% xylenes	100% Ethanol	Vacuum	−14.2	25.6	19.6	11.6	−18.0
		Average	*−14.4*	*26.2*	*19.8*	*11.5*	*−18.1*
		Stdev	*0.25*	*0.91*	*0.19*	*0.13*	*0.32*
Butvar B-98 in 90% ethanol:10% xylenes	90% Ethanol:10% xylenes	Air	−14.3	23.7	19.9	11.7	−18.2
Butvar B-98 in 90% ethanol:10% xylenes	90% Ethanol:10% xylenes	Oven	−14.6	27.1	20.0	11.8	−18.2
Butvar B-98 in 90% ethanol:10% xylenes	90% Ethanol:10% xylenes	Vacuum	−14.6	25.8	19.2	12.0	−18.1
		Average	*−14.5*	*25.5*	*19.7*	*11.8*	*−18.2*
		Stdev	*0.17*	*1.72*	*0.43*	*0.13*	*0.06*
Paraloid B-72 in 100% acetone	100% Acetone	Air	−14.5	28.1	19.0	11.7	−18.9
Paraloid B-72 in 100% acetone	100% Acetone	Oven	−14.7	23.8	18.8	11.7	−18.2
Paraloid B-72 in 100% acetone	100% Acetone	Vacuum	−14.8	26.7	18.6	11.4	−18.9
		Average	*−14.6*	*26.2*	*18.8*	*11.6*	*−18.6*
		Stdev	*0.15*	*2.17*	*0.18*	*0.16*	*0.41*
Paraloid B-72 in 100% acetone	90% Acetone:10% xylenes	Air	−14.9	27.4	20.6	11.7	−18.6
Paraloid B-72 in 100% acetone	90% Acetone:10% xylenes	Oven	−14.7	27.3	18.6	11.6	−18.2
Paraloid B-72 in 100% acetone	90% Acetone:10% xylenes	Vacuum	−14.8	27.3	20.2	11.5	−19.2
		Average	*−14.8*	*27.3*	*19.8*	*11.6*	*−18.7*
		Stdev	*0.11*	*0.04*	*1.02*	*0.10*	*0.52*
Paraloid B-72 in 90% acetone:10% xylenes	100% Acetone	Air	−14.5	27.6	19.9	11.7	−18.0
Paraloid B-72 in 90% acetone:10% xylenes	100% Acetone	Oven	−14.5	27.2	19.4	11.4	−19.5
Paraloid B-72 in 90% acetone:10% xylenes	100% Acetone	Vacuum	−15.0	28.2	19.2	11.7	−17.8
		Average	*−14.7*	*27.7*	*19.5*	*11.6*	*−18.4*
		Stdev	*0.26*	*0.51*	*0.39*	*0.15*	*0.89*
Paraloid B-72 in 90% acetone:10% xylenes	90% Acetone:10% xylenes	Air	−14.4	25.2	20.1	11.7	−19.6
Paraloid B-72 in 90% acetone:10% xylenes	90% Acetone:10% xylenes	Oven	−14.8	27.7	19.9	11.6	−18.7
Paraloid B-72 in 90% acetone:10% xylenes	90% Acetone:10% xylenes	Vacuum	−14.6	29.1	20.5	11.3	−18.4
		Average	*−14.6*	*27.3*	*20.2*	*11.5*	*−18.9*
		Stdev	*0.19*	*1.96*	*0.29*	*0.18*	*0.63*

All isotope values are reported in ‰ notation referenced to V-PDB, V-SMOW, air for carbon, oxygen, nitrogen, respectively. Errors for all δ-values from collagen and structural carbonates are ±0.2‰ (1σ), errors for $\delta^{18}O$ from phosphates are ±0.4‰ (1σ).

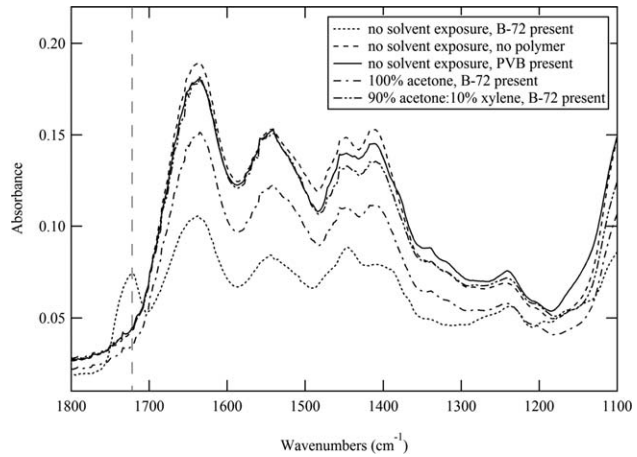

Fig. 1. FT-IR showing removal of acrylic polymer from bone samples. Paraloid B-72 is noted as B-72, Butvar B-98 is noted as PVB. Gray dashed line shows position of acrylic carbonyl peak at 1,722 cm^{-1}. A Butvar-98 consolidated sample spectrum (solid line) is included for reference. A small shift in absorption at approximately 1,155 cm^{-1} is from the major absorption peaks of Butvar-98 but is inconsistent in identifying polymer presence or absence in the infrared spectra from the bone samples.

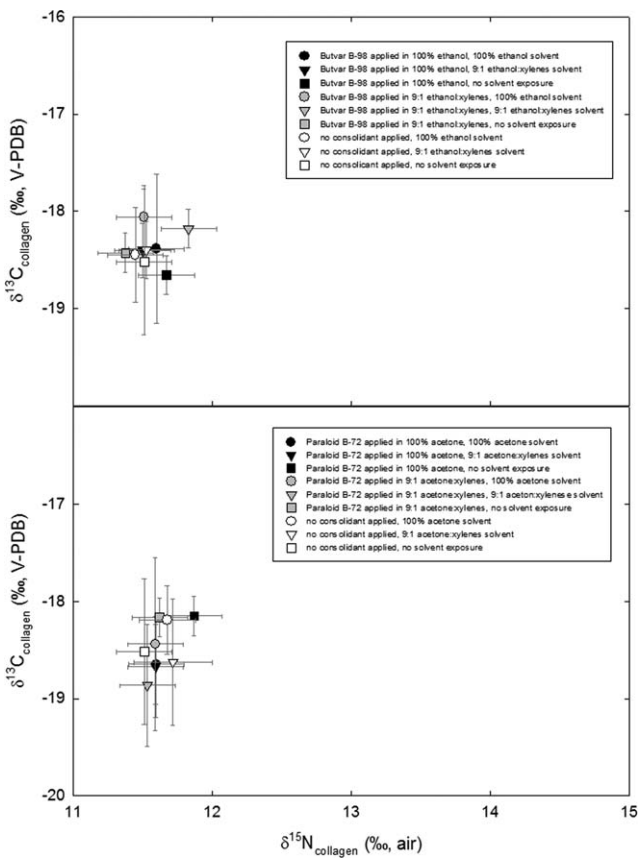

Fig. 2. Average carbon and nitrogen isotope values of bone collagen for different treatment combinations. Error bars reflect the standard deviation (1σ) of the group average, or the analytical error (i.e. $\pm 0.2‰$) in cases where group error is less than analytical error.

Mass Spectrometry Laboratory. Collagen, phosphates, and structural carbonates were run according to their respective methods as concentrated batches to eliminate

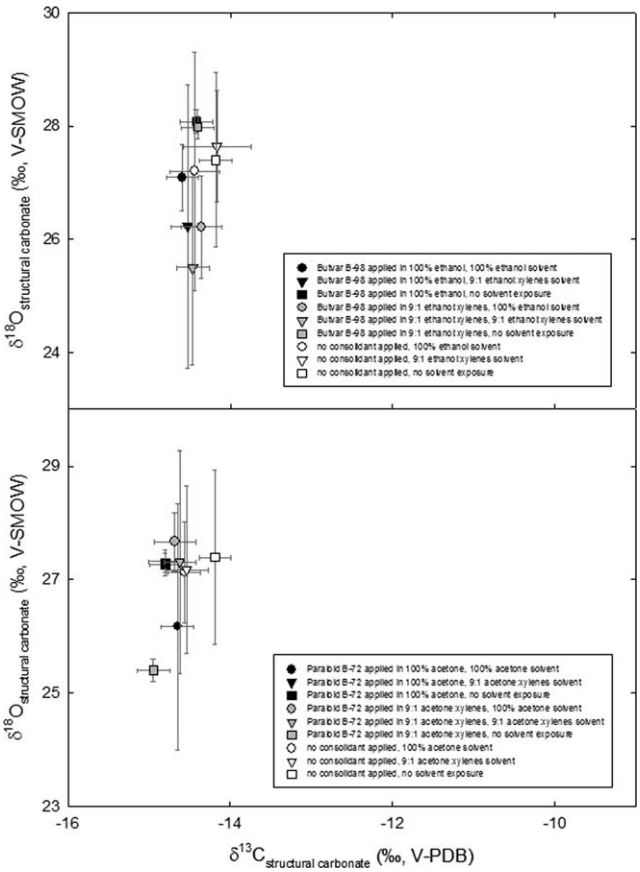

Fig. 3. Average carbon and oxygen isotopic values of structural bone carbonate for different treatment combinations. Error bars reflect the standard deviation (1σ) of the group average, or the analytical error (i.e. $\pm 0.2‰$) in cases where group error is less than analytical error.

instrument (Pestle et al., 2014) and environmental variability. Collagen samples were weighed into tin boats (~500 μg), combusted on a Costech Elemental Analyzer (EA), introduced as purified N_2 and CO_2 gas to the mass spectrometer via a Conflo IV, and analyzed for both carbon and nitrogen isotopes. Silver phosphate samples were weighed into silver boats (~500 μg), thermally decomposed at 1,450°C on a Thermo Temperature Conversion Elemental Analyzer (TCEA), introduced as purified CO gas to the mass spectrometer via a Conflo IV, and analyzed for oxygen isotopes. Structural carbonate samples were placed in exetainer vials, flushed with pure helium gas, and acidified with concentrated phosphoric acid (SG≥1.92) at 25°C for 18 h. The resulting CO_2 gas was introduced to the mass spectrometer via a Thermo Gas Bench II unit and measured for both carbon and oxygen isotopes. The pure consolidant stock pellets were analyzed via the EA and Gas Bench.

All isotope data is reported in standard delta notation:

$$\delta = \left[\left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}}\right] \times 1,000$$

where δ represents an isotope system (i.e. $\delta^{15}N$, $\delta^{13}C$, or $\delta^{18}O$), R is the ratio of heavy to light isotope (i.e. ^{15}N:^{14}N, ^{13}C:^{12}C, or ^{18}O:^{16}O), units are permil (‰), and the standards are atmospheric N_2, V-PDB, and V-SMOW for nitrogen, carbon, and oxygen respectively. Internal

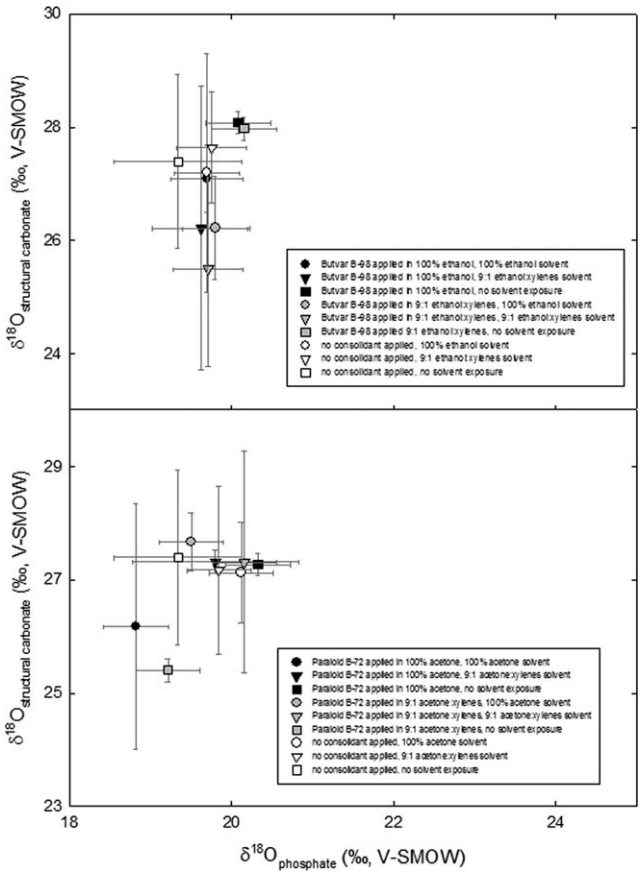

Fig. 4. Average oxygen isotopic values of bone phosphates for different treatment combinations plotted against average oxygen isotopic values of structural bone carbonate for comparison. Error bars reflect the standard deviation (1σ) of the group average, or the analytical error (i.e. $\pm 0.2‰$ for carbonates, $\pm 0.4‰$ for phosphates) in cases where group error is less than analytical error.

standards calibrated to international standards are run with all samples and raw values are corrected by a 2-point linear calibration. Analytical errors based on standards are $\pm 0.2‰$ (1σ) for $\delta^{13}C$ and $\delta^{15}N$ values, $\pm 0.2‰$ (1σ) for $\delta^{18}O$ structural carbonate values, and $\pm 0.4‰$ (1σ) for $\delta^{18}O$ phosphate values.

Gas chromatography-mass spectrometry (GC-MS)

Bone samples were analyzed by GC-MS to determine presence/absence of solvents after treatment and drying. Measured amounts of powdered bone from each treatment combination were sealed in separate headspace vials and analyzed with an Agilent 6890 gas chromatograph with 5975 quadrupole mass spectrometer and an Agilent 7694E headspace analyzer. Samples were heated (50°C) for 5 min before headspace extraction. GC-MS parameters were as follows: sample loop fill 12 s at 55°C, transfer line to GC at 60°C, helium carrier gas at 0.8 mL/min, split splitless inlet in split mode at 70°C with a 20:1 split. Samples were separated on an Agilent J&W HP-5MS, 30 m × 0.25 mm × 0.50 μm column at 25°C for five minutes, then heated to 150°C (10°C/min) and held for five minutes; transfer line to MS was at 200°C. The mass spectrometer used electronic impact, with the ion source at 230°C and the quadrupole at 150°C, measuring mass-charge ratios from 20 to 300 *m/z*. Peaks in the chromatogram were identified using both retention time and the corresponding mass spectrum. Solvent peaks were identified and integrated using Agilent ChemStation software. Solvent peak areas were used to approximate if remaining solvent in samples was ≤1 μL/g.

A selection of reference bone samples and samples treated with Butvar-98 were analyzed with pyrolysis GC-MS to determine the presence/absence of remaining polymer. Samples were placed in quartz pyrolysis tubes and pyrolyzed with a CDS 5150 pyroprobe at 600°C for 10 s. GC-MS was performed on the instrument described above with parameters as follows: helium carrier gas at 1.1 mL/min, split splitless inlet in split mode at 320°C with a 50:1 split. Samples were separated on an Agilent J&W HP-5MS, 30 m × 0.25 mm × 0.50 μm column held at 40°C for 2 min, then heated to 320°C (20°C/min) and held for 13 min; transfer line to MS was at 320°C. The mass spectrometer used electronic impact, with the ion source at 230°C and the quadrupole at 150°C, measuring mass-charge ratios from 29 to 600 *m/z*. The main decomposition peak of Butvar-98, *n*-butanal, was identified using both retention time and the corresponding mass spectrum using AMDIS software v. 2.71; a retention time within 0.1 min and a net mass spectral match of greater than 80 was considered a positive identification. Pyrolysis analysis was presence/absence only; the amount of Butvar-98 in the sample was not quantified.

Fourier transform-infrared spectroscopy (FT-IR)

Powdered sample was analyzed using attenuated total reflectance (ATR) Fourier transform infrared spectroscopy using a Golden Gate ATR accessory in a Thermo Nicolet 6700 FT-IR bench with a DTGS detector. Samples were placed on the diamond window of the ATR and spectra collected from 450 to 4,000 cm^{-1} for 128 scans at a resolution of 4 cm^{-1}. Reference samples of Butvar-98, Paraloid B-72, and untreated bone were analyzed using FT-IR. From France et al. (2011) it is estimated that B-72 concentrations >0.5% could be quantified using the prominent 1,722 cm^{-1} carbonyl peak.

Statistics

Comparison of stable isotope values in different consolidant treatments and solvent removal tests was performed with the Friedman nonparametric test of multiple dependent groups; results were considered significant if $P < 0.05$. This nonparametric version of MANOVA was selected due to relatively small sample sizes for each treatment and potentially non-Gaussian distributions.

RESULTS

Removal of consolidants and solvents

GC-MS indicated that all drying methods left residual amounts of solvent with levels in all but two samples ≤1.0 μL/g. The two samples in question had solvent levels <1.3 μL/g; both were treated with Paraloid B-72, 9:1 acetone:xylene solution and air dried. Comparison of drying methods between similarly treated samples suggests no significant difference in any isotope values (all P values >0.1). For subsequent statistical analyses, all

drying methods were therefore considered equally effective and were grouped together. Samples with the least amount of residual solvent were those oven dried at 80°C with no exposure to xylenes. We recommend that Paraloid or Butvar be applied/removed using acetone or ethanol, respectively, and oven dried (80°C) for best results.

FT-IR spectra from samples treated with solvent to remove Paraloid B-72 showed no peaks at 1,722 cm^{-1}, which suggests $\leq$0.5% consolidant remains (Fig. 1). Butvar B-98 consolidated samples were also examined with FT-IR; however, Butvar B-98 does not have as strong an infrared absorption as the Paraloid B-72 and does not contain a carbonyl peak. In one bone sample treated with Butvar B-98 a baseline shift between 1,239 and 1,130 cm^{-1} was observed. However this peak was not consistently observed in the Butvar B-98 treated samples and is not considered reliable as proof of Butvar removal. Pyrolysis GC-MS of a subset of samples treated with solvent to remove Butvar B-98 indicated that all consolidant was successfully removed.

Collagen isotopes

Average collagen δ^{15}N and δ^{13}C values for each treatment group showed a very narrow range of +11.4 to +11.9‰ (±0.1, 1σ) and −18.9 to −18.1‰ (±0.2, 1σ), respectively (Table 1 and Fig. 2), with no significant differences between any treatment groups ($P > 0.05$ in all cases).

Structural carbonate isotopes

Average structural carbonate δ^{13}C values showed a narrow range between treatment groups of −14.9 to −14.2‰ (±0.2, 1σ) with no significant differences observed ($P > 0.05$ in all cases) (Table 1 and Fig. 3). Average structural carbonate δ^{18}O values exhibited a much greater range of +25.4 to +28.1‰ (±0.8, 1σ). While the statistical tests showed no differences in δ^{18}O values between any treatment groups, several values are clearly outside the bounds of analytical error and differ from the untreated control sample by up to 2‰. This is likely due to the nonparametric statistical test which, while appropriate for small sample sizes and potentially non-Gaussian distributions in this study, reduces the overall power of the test. The most extreme differences between treated samples and untreated controls tend to show a depletion of the former compared to the latter. Additionally, the variation of structural carbonate δ^{18}O is well outside the bounds of analytical error (i.e. ±0.4‰, 2σ) within replicates of some treatment groups. While this is expected for experimental treatments, it is also noted in the control group where $1\sigma = 1.5$‰ ($n = 4$).

Phosphate isotopes

Average phosphate δ^{18}O values showed a range between treatment groups of +18.8 to +20.3‰ (±0.4, 1σ) (Table 1) with no significant differences observed ($P > 0.05$ in all cases). Figure 4 shows phosphate δ^{18}O values plotted against structural carbonate δ^{18}O values as a comparison in variability between the two. While all samples treated with consolidants showed phosphate δ^{18}O values within the analytical error (i.e. ±0.4‰) of the untreated control, the Paraloid B-72 treatments showed slightly higher variability (±0.6‰, 1σ) than the Butvar B-98 (±0.2‰, 1σ).

Consolidant isotopes

The average δ^{13}C values of Paraloid B-72 and Butvar B-98 stock pellets were −31.8‰ and −29.3‰, respectively, which are notably lower than the δ^{13}C values of the bone samples. Stock pellets produced no peaks in the Gas Bench analyses for structural carbonates.

DISCUSSION

Collagen δ^{15}N and δ^{13}C values in the modern whale bone were unaltered by addition of Paraloid B-72 and Butvar B-98, nor were they altered by acetone, ethanol, or xylenes. The stability of δ^{15}N values throughout all treatments is expected given that neither the consolidants nor solvents contain nitrogen thereby presenting no opportunity for isotopic exchange. The covalent carbon bonds in collagen have relatively high dissociation energies and are unlikely to break in organic solvents, which prohibits exchange of atoms or functional groups between molecules. Although the GC-MS and FT-IR analyses show almost complete removal of solvents and consolidants, the collagen extraction procedure itself includes a final step of heated acid hydrolysis, where the collagen is extracted while any remaining solvents or consolidants are volatilized or left behind in solid form, respectively. The stock consolidant pellets themselves have more depleted δ^{13}C values than the extracted collagen, but this depleted signal is not observed in the processed samples, suggesting all consolidant was successfully removed. These findings support previous research which also found no effects of other consolidants or solvents on collagen stable isotope values (Moore et al., 1989; Tuross and Fogel, 1992; Takahashi et al., 2002; France et al., 2011).

The bone phosphate δ^{18}O values were also unaltered by exposure to Paraloid B-72, Butvar B-98, or any solvents used in this study. The phosphate oxygen is part of a double covalent bond with a relatively high dissociation energy compared to oxygen bonds in the solvents and consolidants, making direct atomic exchange unlikely. Neither the consolidants nor solvents contain phosphate ions (–PO_4), thus direct exchange of ionic groups is impossible. These findings support France et al. (2011) and Stephan (2000) who observed no alteration of phosphate δ^{18}O values in bones treated with other consolidants and solvents.

The bone structural carbonate δ^{13}C values were unaltered by exposure to the consolidants or solvents, but the structural carbonate δ^{18}O values did show considerable variation (Fig. 3). The dissociation energy for the carbon and oxygen in the carbonate ion (–CO_3) is relatively high suggesting the ion remains intact throughout treatments, thus making direct atomic exchange unlikely. In a similar manner to phosphate, neither the consolidants nor solvents contain carbonate ions, thus direct exchange of ionic groups is impossible. Furthermore, if the carbonate ion was dissociating or the intact ion was exchanging with solvents or consolidants, it is assumed that both carbon and oxygen would undergo exchange and alteration of isotope values. As this is not the case, another mechanism must be responsible which facilitates isotopic alteration of oxygen, but not carbon.

France et al. (2011) observed a similar phenomenon in samples treated with PVAc where structural carbonate δ^{18}O values showed notable variability compared to δ^{13}C. It was observed that exchange most likely occurred

between labile hydroxide groups (–OH) in the hydroxyapatite mineral and –OH groups in consolidant solutions. During the Gas Bench acidification process, these –OH groups are released with the CO_2 gas and incorporated into the overall isotope value. Butvar B-98 in particular often contains significant hydroxyl content in the form of polyvinyl acetate (PVAc) which readily hydrolyzes to polyvinyl alcohol (PVOH) in solution (Seymour and Carraher, 1988; Lazár et al., 1989; Horie, 2010). Paraloid B-72 can hydrolyze to produce polyacrylic acid which contains a labile –OH group susceptible to exchange with the hydroxyapatite mineral (Seymour and Carrahar, 1988; Horie, 2010).

Recent work by Pestle et al. (2014) suggests that variations in laboratory parameters might also account for high variability in structural carbonate $\delta^{18}O$ values. Although we were able to eliminate such variability in our methods, we saw an unusually high standard deviation in our control group values. The observed structural carbonate $\delta^{18}O$ variability in our treatment groups is most likely due to consolidant exposure, but laboratory environmental conditions may also create minor variations. Regardless, the difference in structural carbonate $\delta^{18}O$ values between treatments is not systematic and one cannot apply any calculated offset to correct for such influences on structural carbonate $\delta^{18}O$. This study therefore recommends that structural carbonate $\delta^{18}O$ values in samples treated with Butvar B-98 or Paraloid B-72 are suspect and should not be used for paleontological or archaeological interpretations.

It should be noted that the drying (or curing) conditions in this experiment were relatively short (i.e. days to weeks) and occurred at ambient temperature ($\sim$23°C). Long-term exposure to unregulated storage conditions (i.e. heat, oxidation, non-visible wavelengths) may cause consolidants to fragment, cross-link, or release volatile compounds (Feller, 1984; Down et al., 1996; Chiantore and Lazzari, 2001; Feller et al., 2007; Down, 2009; Horie, 2010). These changes in structure may result in lower consolidant solubility, but are unlikely to result in labile functional groups exchanging with bone isotopes in a manner much different than that observed in the short term. Some released volatile compounds (such as acetic acid) are slightly acidic (Down et al., 1996; Feller et al., 2007), but are too weak to denature collagen or dissolve hydroxyapatite in bone. While a long-term ageing study is beyond the scope of this experiment, our consolidant treated samples will be stored in our collections. It is our intention to revisit this question and examine changes in consolidant solubility and additional alterations to bone isotope values over time.

CONCLUSIONS

After exposing modern bone to different treatment combinations of Paraloid B-72, Butvar B-98, and different solvents, the following conclusions and recommendations can be drawn:

1. Paraloid B-72 or Butvar B-98 should be mixed in 100% acetone or 100% ethanol solvent, respectively, before coating a bone. The addition of xylenes to the solvent mixture is discouraged because it provides no observable benefit to the process, slightly hinders removal of solvents, and is more toxic.
2. Paraloid B-72 is most successfully removed from treated bones using 100% acetone followed by drying at 80°C for at least 24 h; Butvar B-98 is most successfully removed using 100% ethanol followed by drying at 80°C for at least 24 h.
3. The $\delta^{13}C$ and $\delta^{15}N$ values of isolated bone collagen are unaffected by Paraloid B-72, Butvar B-98, and solvent exposure provided the collagen extraction incorporates a heated acidic gelatinization step.
4. The $\delta^{18}O$ values from the bone hydroxyapatite phosphate are unaffected by Paraloid B-72, Butvar B-98, and solvent exposure.
5. The $\delta^{13}C$ values from the bone hydroxyapatite carbonate are unaffected by Paraloid B-72, Butvar B-98, and solvent exposure.
6. The $\delta^{18}O$ values from the bone hydroxyapatite carbonate are altered by exposure to Paraloid B-72 and Butvar B-98 treatments in an unpredictable manner. These isotope values are altered from their original state and care should be taken in utilizing such data for scientific interpretations.

ACKNOWLEDGMENTS

The authors acknowledge H. Beaubien, Smithsonian Museum Conservation Institute staff, OUSS/MCI Stable Isotope Mass Spectrometry Laboratory, MCI GC-MS Laboratory, and two anonymous reviewers for their consultation, support, and helpful comments.

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