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CAN WE GET A GOOD RADIOCARBON AGE FROM "BAD BONE"? DETERMINING THE RELIABILITY OF RADIOCARBON AGE FROM BIOAPATITE

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ABSTRACT. The study of the radiocarbon age of bone bioapatite was initiated by necessity to date archaeological artifacts, which often contain little or no collagen as a result of poor preservation. Contamination of the organic fraction in the process of the burial or during museum preservation treatment generally prohibits the use of the collagen fraction for dating. Our investigation has shown that the pretreatment of bone with diluted acetic acid following a proscribed technique allows the separation of the bioapatite fraction from diagenetic carbonates. We have successfully used this technique to prepare and date samples of bone and of tooth enamel and dentin, with varying degrees of preservation condition, and from time intervals ranging from a few hundred ^{14}C yr to greater than 40,000 ^{14}C yr.

INTRODUCTION

Since the early days of radiocarbon dating, the analysis of bone material has been a problem due to frequent discord between the dates of the bone material and associated charcoal, and/or between different fractions isolated from a single bone. Early studies were skeptical of ^{14}C dates obtained from bone apatite due to possible contamination with secondary carbonates from surrounding deposits (Tamers and Pearson 1965; Hassan et al. 1977). Recent publications show that such problems still exist and suggest that they are caused by both the poor preservation of collagen and the difficulties in removing contaminants from the bone extracts used for dating (Koch et al. 1997; Collins et al. 2002; Ambrose and Krigbaum 2003).

Today, most methods of bone preparation for ^{14}C dating are designed to extract and purify (with varying degrees of success) a fraction of the organic residue. In general, the goal of these methods is to isolate collagen or some individual compounds such as protein or amino acids of collagen. However, for badly preserved bones, the problem becomes acute, as the bones often do not contain enough collagen even for accelerator mass spectrometry (AMS) dating, or the collagen fraction was strongly altered during the burial. The use of bioapatite for the dating and paleodietary studies led to an active discussion in the early 1980s. Schoeninger and DeNiro (1982) considered it impossible to use this kind of reconstruction for paleodiet. However, others (e.g. Tieszen and Farge 1993) have thought that it is possible.

More than 20 yr ago, it was suggested that carbon from bioapatite in fossil bone should provide reliable dietary and environmental information (Sullivan and Krueger 1981). The validity of such information depends on the lack of alteration of the isotopic composition of the carbon in bioapatite by exchange with carbonates of soil solution, groundwater, or atmospheric carbon dioxide. It also depends upon the ability of the analyst to remove any deposits of secondary or diagenetic carbonates that may be present in the bone. Different ways were investigated to separate bone bioapatite from secondary carbonates, i.e. by heating in an oxygen atmosphere in specific steps to separate of CO_2 fractions from different sources (Haas and Banewicz 1980; Surovell 2000), fast treatment with strong acids (Hedges et al. 1995), and the step treatment with weak acids (Saliège et al. 1995; Balter et al. 2002).

The mineral fraction does not usually undergo microbiological decomposition, but may be exposed to isotopic exchange with environmental carbonates. However, there is a possible precipitation of secondary apatite either inorganic or under microbial mediation. If this happens, no treatments will have any effect as the carbonate is trapped in the crystals (Zazzo et al. 2004a,b).

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Minerals in bones and teeth usually survive much better than the organic fractions of collagen and lipids. Collagen tends to undergo microbiological decomposition, hydrolysis, dissolution, and denaturation over archaeological and geological timescales, so that only in exceptional conditions, such as burial in permafrost, is collagen found to survive without significant changes into the Pleistocene. The material's survival is usually far shorter for the bones buried in warmer regions. In contrast, the mineral fraction of bones and teeth could be preserved quite well or it could alter and stabilize, thus recording the changes of fossilization.

Bioapatite belongs to the group of hexagonal calcium phosphates, of which hydroxyapatite, $[Ca_{10}(PO_4)_6(OH)_2]$ is closest in structure to biological apatite. It differs from geological apatite by the high degree of isomorphic substitutions and absorption of carbonate and small crystal size, properties that each result in a poorer crystallization of bone bioapatite (LeGeros and LeGeros 1984).

The carbonate substituted within bioapatite maintains its carbon isotope signature in both stable and radioactive isotopes. Carbonate occurs in several locations in the crystals, as absorbed ions on the surface and within the crystals. Substitutions are mainly in the phosphate position and most likely in the hydroxyl position. The absorbed carbonates are more labile, but substituted ones are much more stable (they are actually structural carbonates) and contribute to saving the original isotope composition. Thus, they could be used for ^{14}C dating.

Enamel bioapatite is different from bone bioapatite. In bone, the organic fraction consists mainly of collagen and remains high at about 20–30%. In contrast, enamel contains very little organic material, generally <1%. Enamel is more crystalline and has fewer substitutions, less distortion, and greater crystal size. It also exhibits higher-order structures and differs in the type and quantity of organic matrix. Unlike enamel, bone apatite is formed in close association with collagen fiber, which is regulated by the deposition and orientation of apatite (Eisenmann 1984).

The problem thus becomes one of separating the diagenetic carbonates without significantly destroying the bioapatite content. Lee-Thorp (Lee-Thorp and van der Merwe 1991; Lee-Thorp 2000) was one of the first researchers who began to use the mineral fraction of bones and teeth for stable isotope dietary and climate reconstruction studies. She has shown that carbon isotope ratios on bioapatite reflected those of the integrated diet and climate changes during the Pleistocene.

This paper discusses alternative techniques for preparation of mineral carbon fraction from the bioapatite of bone. We also compare the mineral and organic fractions for bones and teeth of different ages and differing degrees of preservation. It is concluded that the technique can be applied to samples in varying states of preservation and practically over a broad whole range of ^{14}C dating.

MATERIALS AND METHODS

Proper procedures for the preparation of bone samples for isotopic analysis are critical for obtaining reliable data. In general, it must be assumed that bone samples have undergone some alteration or contamination in their natural environment, and also that the excavation process may be have added modern contaminations as a result of handling and/or preservation procedures.

Materials

Monk seal samples (UGAMS-1879 and -1882) were collected in the undersea Bel Torrente Cave, one of the most interesting submarine karst resurgences in the Gulf of Orosei, central-east Sardinia. Human rib samples (UGAMS-2797, -2798, 2803, and -2804) were found in Stone Age shell middens in the Western Cape of South Africa. Human bone (UGAMS-2831) was collected from Belle

Glade burial mound in south Florida, USA. Mammoth femur fragments samples (UGAMS-2684 and -2947) were collected in clay deposits in Texas, USA. Burnt bison bone fragments (UGAMS-1935) were sampled from clay matrix near Glendive, Montana, USA. Unidentified tooth sample (GX-27521) from carbonate deposits was collected in the Stone Age El Miron Cave in Cantabrian Cordillera of northern Spain.

Bone and tooth samples from Saharan Africa (UGAMS-2837, -2838, -2840, -2842, and -2847) belonged to domesticated cattle (submitted by Savino de Lernia). Samples UGAMS-2837 and -2838 were collected in the early 1960s and stored in a museum, where they were likely treated with some kind of preservatives, possibly resin. The other samples were collected in 2007 and never were treated with preservatives. The bison bone sample UGAMS-2295 has been excavated by Christine Chataigner from a layer of the early Upper Paleolithic period of site Kalavan-2 in Armenia. Dog tooth samples have been excavated on the island of Martha's Vineyard, Cape Cod, Massachusetts, USA.

Smith et al. (2007) have developed a classification of bone preservation degree based on multi-parameter analyses, such as percent of collagen, mineral crystallinity index, cracking index, Oxford histological index, 3 types of porosity (based on the pore size), bulk density, and skeletal density. However, it is not always possible to analyze all those parameters, and we are going to use only some of them. For this study, we chose the bone and tooth samples with differing degree of preservation.

The preservation has been estimated based on the collagen concentration in the bone. We have suggested 4 degrees of bone preservation based on the collagen content: well-preserved bone samples of about 10% collagen or higher, relatively well-preserved samples have >1% collagen, poorly preserved samples have <1% collagen, and extremely poorly preserved samples have no collagen at all. We also estimated the collagen preservation based on the stable isotope data. For example, if a normal $\delta^{13}C$ value on collagen for C_3 eaters is in the range –18 to –21‰, then collagen is still good for the dating. However, if it is depleted compared to his range, that means it was separated collagen (i.e. material contaminated or exchanged with younger organics) and this material could not be dated at all.

Samples prepared by Geochron Laboratory are identified by the GX laboratory prefix. Those samples prepared by the Center for Applied Isotope Studies are identified with the UGAMS prefix.

Physical Cleaning

The bone sample for analysis is first thoroughly washed, using an ultrasonic bath, and wire brushed to remove external material. If secondary deposits or identification marks remain, the bone is physically scraped or sanded to remove such extraneous material. If the sample is large enough, the bone is split and the marrow cavity is stripped out. Any introduced dirt, secondary materials, etc. are discarded.

Chemical Cleaning

The carbonates in fossil bone may be contaminated by secondary carbonates, precipitated in the process of burial either as pore-filling cements or as bicarbonates absorbed to the surface of crystals. To remove these diagenetic carbon compounds, the bone is soaked overnight in 1N acetic acid. The samples are then washed free of acetic acid by repeated decantation. Loose or extraneous material is discarded and the bone sample is dried at ~70 °C. After drying, a sample of 1–3 g is selected for further preparation. The bone is gently crushed to small fragments <1 mm but not to a fine powder,

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for further cleaning. The powdering of the poorly preserved bone leads to complete dissolving of collagen and destruction of structure bioapatite. The bone fragments are again reacted with 1N acetic acid in a 250-mL Erlenmeyer flask. The flask is periodically evacuated to remove air and/or CO_2 from micropores, after which the flask is returned to atmospheric pressure to force fresh acid into microspaces of the sample. The first evacuation may be fairly violent since both absorbed air and carbon dioxide from the external diagenetic carbonates will be removed. The nature of this reaction is a qualitative indication of $CaCO_3$ contamination. This process of evacuation and repressuring is continued at about 20-min intervals until no substantial release of gas (as fine foamy bubbles) occurs, even at the vapor pressure of water. The process of evacuation and repressurization to atmospheric pressure should be repeated at least 4–5 times, the last reaction being overnight (20+ hr) under atmospheric pressure, because the reaction of acidification of bioapatite by acetic acid could continue in the vacuum. In this case, the reaction destroys both diagenetic carbonates and carbonates of bioapatite.

If after overnight reaction, under vacuum only large bubbles are observed, then there are no more diagenetic carbonates, but they are primarily water vapors at such low pressures. The reaction of acetic acid and bone should not be allowed to stand much more than 72 hr since the bioapatite will slowly react with acetic acid and the legitimate bioapatite carbon may be depleted or lost altogether.

Seriously contaminated samples (~50% $CaCO_3$) should be treated longer with pumping each day; such treatment should result in enough bioapatite remaining for reliable analysis. However, daily evacuation for more than 6 days will start to deplete the carbon content of bioapatite, due to its slow conversion to brushite ($CaHPO_4$).

Once the evolution of fine gas bubbles has ceased (virtually an end point to the reaction), it can be assumed that all secondary or surface exchanged carbonates have been removed. The completely cleaned bone sample is then washed free of acetic acid by repeated soaking and decantation with demineralized water, then vacuum-dried. The sample is now ready for isotopic analysis of carbon in bioapatite, for collagen extraction, Sr isotope analysis, or trace element analysis.

For analysis of carbon isotopes in bioapatite, approximately 100–500 mg of the cleaned bone powder is transferred to a vacuum flask and evacuated to remove air. The bone is then reacted under vacuum with about 10 mL of degassed 1N HCl. The reaction is usually completed within 20 min or even less and can be monitored to determine whether or not collagen pseudo-morphs have sunk to the bottom. If the bone is suspected of being highly altered, this reaction should be performed at freezing point, 0 °C in an ice bath, to improve collagen recovery.

The released CO_2 is purified by cryogenic separation and collected in sealing tubes for AMS and stable isotope analyses. Bioapatite content in the modern bone is about 1% and about 0.8% in the modern enamel. However, in archaeological samples after treatment with acetic acid this value should be in the range 0.4–0.7%; therefore, 100 mg of bone should yield about 0.4–0.7 mg of carbon. If yields are much higher, it is probable that diagenetic carbonates were not completely removed.

For further analyses of collagen for $\delta^{13}C$ and $\delta^{15}N$, we used a standard procedure involving closed-tube combustion with copper oxide at 575 °C for 6 hr and then cryogenic separation of carbon dioxide and nitrogen for analyses (Bocherens et al. 1991). Koch et al. (1997) have tested the effect of the treatment with oxidizers, such as NaOCl and H_2O_2, followed by acid treatment, but they did not find any improvement versus the acid treatment only.

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For AMS analysis, the cleaned carbon dioxide was catalytically converted to graphite using the method of Vogel et al. (1984). The graphite $^{14}C/^{13}C$ ratio was measured using a 0.5MV Pelletron AMS instrument. The sample ratios were compared to the ratio measured from oxalic acid standard OXI (NBS-4990) to calculate ^{14}C age.

RESULTS AND DISCUSSION

The ^{14}C ages of the well-preserved bone from South Africa are shown in the Table 1 (UGAMS-2797 through UGAMS-2804). They exhibit a yield of collagen from 5 to 10%. The analyses of both fractions show good agreement, with differences not exceeding 2 σ of standard deviation. These results allow us to conclude that the pretreatment of the mineral fraction completely excluded contaminants, such as secondary or diagenetic carbonates.

Table 1 ^{14}C age of the different bone fractions. $\delta^{13}C$ is relative to PDB standard.

				Collagen		Bioapatite	
Lab #	Taxon	Location	Preservation	$\delta^{13}C$ (‰)	^{14}C age (yr BP)	$\delta^{13}C$ (‰)	^{14}C age (yr BP)
UGAMS-2797	human	South Africa	well	–13.1	3800 ± 40	–10.0	3650 ± 40
UGAMS-2798	human	South Africa	well	–15.0	3190 ± 40	–12.2	3160 ± 40
UGAMS-2803	human	South Africa	well	–12.5	2470 ± 40	–10.5	2380 ± 40
UGAMS-2804	human	South Africa	well	–12.4	2340 ± 40	–10.4	2220 ± 40
UGAMS-1879	monk seal	Sardinia, Italy	poor	–11.1	4420 ± 50	–7.5	6000 ± 50
UGAMS-1882	monk seal	Sardinia, Italy	poor	–10.4	5860 ± 60	–7.2	6940 ± 50
UGAMS-2831	human	Florida, USA	poor	–19.7	1780 ± 50	–12.0	1410 ± 40
UGAMS-2837	sheep/goat	Sahara, Africa	poor	–8.4	6350 ± 50	–1.7	6640 ± 50
UGAMS-2838	sheep/goat	Sahara, Africa	poor	–19.4	6980 ± 60	–13.9	7480 ± 50
UGAMS-2295	bison	Armenia	poor	–19.9	42,040 ± 400	–9.0	29,230 ± 110
UGAMS-2684	mammoth	Texas, USA	ext. poor	none	none	–6.7	36,700 ± 210
UGAMS-2947	mammoth	Texas, USA	ext. poor	none	none	–6.6	31,360 ± 100

Seal bone samples collected from an undersea cave (UGAMA-1879 and -1882) were poorly preserved and contain only ~0.6% of collagen. The collagen yielded reasonable stable carbon isotope values; however, the ^{14}C ages for the both samples are significantly younger than the bioapatite data. This rejuvenation could be explained by biodegradation of most of collagen and possible bone contamination by absorption of foreign organic matter on the porous bone surface. The described treatment of diagenetic carbonates has allowed removing them completely. The bioapatite age is in good agreement with the estimation of the sea level at this time (De Waele et al. 2009).

The samples UGAMS-2831 and -2295 were collected in a cave environment saturated with carbonate solutions. As a result, we can see recrystallization and suspect possible isotopic exchange in the structural bioapatite carbonates, although it is impossible to eliminate with chemical treatment.

The samples collected from the arid environment of north Africa (UGAMS-2837 and -2838) were also poorly preserved. The bones had been weathered physically by sand and wind and were very porous with a collagen content of <1%. Because isotopic exchange in such dry conditions is very unlikely, we can better trust the dates of bioapatite fractions, which are correlated with the stratigraphy and dates on other materials, but the collagen dates exhibit rejuvenation due to biodegradation processes.

The Late Pleistocene samples from Texas (UGAMS-2684 and -2947) were extremely poorly preserved and contained almost no collagen. The organic collagen-like compounds separated from the bone were depleted in ^{13}C (about –25‰) and have a concentration of organic carbon lower than

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0.05%, so the organic fraction was not dated at all. The ages of bioapatite fractions are in good agreement with the stratigraphy.

We have also dated the organic fraction of charred bone and compared these dates with bioapatite ages (Table 2). Because all bone samples were charred at relatively low temperature, they have still contain about 1% of carbon. The samples UGAMS-2842 and -2847 are black in color, whereas UGAMS-1935 is a dark brown color.

Table 2 Charred bone via bioapatite. $\delta^{13}C$ is relative to PDB standard.

			Charred bone		Bioapatite	
Lab #	Taxon	Location	$\delta^{13}C$ (‰)	^{14}C age (yr BP)	$\delta^{13}C$ (‰)	^{14}C age (yr BP)
UGAMS-1935	bison	Montana, USA	−15.7	25,170 ± 230	−6.4	25,370 ± 220
UGAMS-2842	sheep	Sahara, Africa	−7.7	5630 ± 40	−1.0	5710 ± 50
UGAMS-2847	sheep	Sahara, Africa	−11.1	5550 ± 40	−1.4	5550 ± 40

Two different portions of bone have been treated. One portion was used to separate bioapatite as described above, and another portion was treated with the standard acid-alkali-acid (AAA) treatment as would be done for charcoal samples. The dating results of both fractions yielded very good agreement, because carbonized collagen is quite stable and resistant to microbiological decomposition, even in the case of Late Pleistocene bone with an age of ~25 kyr. As shown by Zazzo et al. (2009), in a laboratory experiment, the cremated bones are more stable due to the recrystallization of bioapatite at high temperatures of cremation into larger and more well-structured crystals. These crystals are very similar to enamel crystals, with dense packing that protects them from further exchange with diagenetic carbonates. This finding was used to explain the reliability of Holocene ^{14}C dates (Lanting et al. 2001).

Table 3 represents ^{14}C ages obtained on different fractions of the same tooth. The tooth enamel has a denser structure, with the bioapatite crystals significantly bigger than bone bioapatite crystals. The enamel bioapatite absorbs less carbonate on the surface because of larger crystals and the isotope exchange reaction is much less likely for the enamel. As a result, the samples of tooth enamel should be better preserved and more reliable for ^{14}C dating. The first 4 samples (GX-27335, -27625, -27626, and -27627) are dated at younger than 1000 ^{14}C yr and show good agreement between both the collagen and bioapatite fractions. These samples have each been dated using the bioapatite in the whole tooth, i.e. a mix of dentine and enamel.

Table 3 ^{14}C age of the different tooth fractions. $\delta^{13}C$ is relative to PDB standard.

				Dentine collagen		Dentine apatite		Enamel	
Lab #	Taxon	Location	Preservation	$\delta^{13}C$ (‰)	^{14}C age (yr BP)	$\delta^{13}C$ (‰)	^{14}C age (yr BP)	$\delta^{13}C$ (‰)	^{14}C age (yr BP)
GX-27335	dog	Cape Cod, USA	well	−15.8	430 ± 40	−11.2	400 ± 40	n/a	n/a
GX-27625	dog	Cape Cod, USA	well	−11.3	600 ± 40	−7.2	610 ± 40	n/a	n/a
GX-27626	dog	Cape Cod, USA	well	−19.2	310 ± 40	−14.3	350 ± 40	n/a	n/a
GX-27627	dog	Cape Cod, USA	well	−8.6	770 ± 40	−12.5	820 ± 40	n/a	n/a
GX-27521	bison	El Miron, Spain	poor	−20.1	10,390 ± 50	−11.9	10,740 ± 40	n/a	n/a
UGAMS-2681	llama	Argentina	poor	−20.7	7830 ± 50	−12.8	7980 ± 50	−13.3	8060 ± 50
UGAMS-2840	sheep	Sahara, Africa	ext. poor	none	none	−1.7	5340 ± 50	−0.4	5350 ± 50

For tooth samples with a UGAMS prefix, the bioapatite and dentin were physically separated and treated as separate samples. As in the case of the bone samples, there is a good agreement between both fractions for the well-preserved relatively young tooth samples.

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The Late Pleistocene tooth sample GX-27521 was collected in El Miron cave in carbonate deposits and has been relatively well preserved with a concentration of collagen just above 1%. However, the stable isotope values are very reliable for the both fraction, and the difference between them is typical of C_3 eaters. The ^{14}C ages differ by 350 ^{14}C yr, but this difference is in the range of 2 σ for calibrated dates.

The tooth sample UGAMS-2840 was collected in the extremely arid conditions of north Africa and has been poorly preserved. It did not contain enough collagen even for AMS analysis and was thus dated only on the bioapatite fraction. Both the dentine and enamel bioapatite fractions demonstrate the same ^{14}C age even though their stable isotope composition differs.

The tooth sample UGAMS-2681 was poorly preserved with only about 0.5% of collagen. However, all 3 fractions—dentin collagen, bone bioapatite, and enamel bioapatite—show ^{14}C ages within the range of 2 σ of standard deviation.

The sample preparation technique is very important for getting reliable ^{14}C dates from bone material. The use of treatment by a diluted acetic acid with periodic evacuation and repressurization to atmospheric pressure gave the best results. In the cases of well-preserved bone from South Africa and Cape Cod, the ^{14}C dates on bioapatite and collagen are in good agreement with each other.

Despite the opinion (Tuross et al. 1989; Hedges et al. 1995; Zazzo et al. 2004a) that degradation of both organic fraction of collagen and mineral fraction of bioapatite proceed simultaneously, the results of ^{14}C dating of poorly preserved bone show that the original bioapatite could be separated and reliable dates be obtained for this fraction, although the collagen fraction is degradated and contaminated in the process of burial. We can observe that even for the monk seal bones collected in the undersea Bel Torrente Cave, the bioapatite fraction yielded reliable dates.

We cannot state, however, that bioapatite is absolutely unchangeable in any case. In certain conditions (i.e. in the environment) with the solutions saturated with carbonate, the preservation of original bioapatite could be destroyed by chemical or microbiological exchange with the carbonates of the solution. We can see this example for the bone from the Upper Paleolithic site Kalavan-2 in Armenia, where the date of bioapatite is significantly rejuvenated compared to the collagen fraction.

CONCLUSION

The proper pretreatment of bone and tooth samples permits the separation of diagenetic, secondary carbonates from bioapatite carbonates if their structure has not been degraded completely and exposed to isotope exchange. Tooth bioapatite usually is better preserved than bone bioapatite, with larger and are more stable crystals of bioapatite.

Burned bone samples are reliable material for ^{14}C analyses. The bioapatite fraction along with the fraction of carbonized collagen may be used for dating with the proper pretreatment, which allows the removal of contaminants.

In the case of samples derived from carbonate environments, the possibility of isotope exchange is increased, especially in changing conditions of wet and dry seasons. Thus, reactions of dissolution and precipitation occur and affect the carbon isotope composition by rejuvenation of the ^{14}C age. On the other hand, if old carbonate material is present it could be leached from the deposits and the ^{14}C age of the samples may seem older as a result of exchange with old, “dead” carbonates.

Most Holocene samples exhibit reliable ^{14}C ages on the bioapatite fraction. Late Pleistocene samples have shown reliable results even for extremely poorly preserved bone in the case of samples derived from a non-carbonate environment.

These results have implications for the dating of museum collections where samples are often preserved using natural and/or synthetic glues. If casein was used for bone preservation, the bone cannot be dated using the collagen fraction, as the glue and collagen have an almost identical organic structure and it is extremely difficult to distinguish these chronologically different organic phases. In such cases, only the bioapatite fraction can be used for ^{14}C dating.

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