BIOAPATITE ^{14}C AGE OF GIANT MAMMALS FROM BRAZIL

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ABSTRACT. We investigated the radiocarbon age and stable isotope composition of bioapatite from bone, enamel, and dentine material from 3 different species of extinct mammals in South America. Most samples of *Eremotherium laurillardi*, *Toxodon platensis*, and *Notiomastodon platensis* were collected in natural depressions located in the northeastern Brazilian provinces of Sergipe, Bahia, and Rio Grande do Norte. All samples studied were devoid of collagen, which had decomposed as a result of high microbiological activity in this tropical region. We have instead analyzed the bioapatite fraction of the samples, which was relatively well preserved even in these harsh tropical conditions. The mineral fraction of bone and tooth material does not usually undergo microbiological decomposition but may be exposed to isotopic exchange with environmental carbonates. The problem thus becomes one of separating the diagenetic carbonates without destroying the bioapatite. We offer a technique for removing the secondary diagenetic carbonates by treatment with diluted acetic acid in a vacuum. We also demonstrate that proper pretreatment of bone and tooth samples allows the separation of diagenetic carbonates from bioapatite, as long as the carbon in these samples has not degraded completely. Bone, enamel, and dentine samples from individuals of the 3 mammalian species were dated using this technique and were compared to results by other researchers from the literature. Date ranges for the species presented were in good agreement with prior research. A comparison with other dating techniques such as U/Th and ESR shows the reliability of the treatment described and the feasibility of ^{14}C dating the bioapatite fraction given certain conditions. In 2 cases, we dated bone enamel and dentine samples from the same individuals of *N. platensis*, with results between 14 and 21 ka. Results from dating samples of *T. platensis* are between 11.5 and 13 ka. The oldest tissue in both cases was dentine. The dating of enamel and dentine from the same species did not show regular differences; however, more often the dentine material was older. The oldest date, ~22.5 ka for *E. laurillardi*, was obtained on the bioapatite fraction of dentine.

INTRODUCTION

Since the advent 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 expressed a skepticism with ^{14}C dates obtained from the bone apatite of samples because of possible contamination with secondary carbonates derived 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 the collagen, or other individual compounds such as protein or amino acids of collagen. However, for poorly 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 may have been strongly altered during the burial. The use of bioapatite for dating was actively discussed starting in the early 1980s, when Sullivan and Krueger (1981) suggested that carbon from bioapatite in fossil bone may provide reliable dietary and environmental information. It was acknowledged that the validity of such information would depend 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 would also depend upon the ability of the analyst to remove any

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deposits of secondary or diagenetic carbonates that may be present in the bone. Schoeninger and DeNiro (1982) concluded that it was not feasible to use this technique for paleodietary studies, although many others (e.g. Tieszen and Farge 1993) believed that it may have useful application.

Different ways to separate bone bioapatite from secondary carbonates have been suggested by various researchers. Haas and Banewicz (1980) and Surovell (2000) proposed the heating of samples in an oxygen atmosphere in specific steps to separate the CO_2 fractions from different sources. Hedges et al. (1995) proposed a quicker treatment with a strong acid. Balter et al. (2002) suggested a step treatment with weak acids.

The mineral fraction of bone material does not usually undergo microbiological decomposition, but may be exposed to isotopic exchange with environmental carbonates. The precipitation of secondary apatite, either inorganic or under microbial mediation, is also possible, rendering any treatments ineffective because the carbonate is trapped in the crystals (Zazzo et al. 2004).

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 Pleistocene-age collagen found to survive without significant changes. Furthermore, the survival of collagen is usually significantly diminished for bones buried in warmer regions. In contrast, the mineral fraction of bones and teeth may be very well preserved, or it may alter and stabilize, thus recording the changes of fossilization.

The extinction of many large mammals in the Americas (such as *Toxodon platensis*, *Notiomastodon platensis*, and *Eremotherium laurillardi*) at the Late Pleistocene-Holocene boundary is widely discussed in literature. The reasons for these mass extinctions have attracted considerable interest and discussion (Barnosky and Lindsey 2010; Prescott et al. 2012). Comparing the previous glacial-interglacial cycles with the Late Pleistocene extinction of megafauna, we can see that these last losses were characterized by their magnitude and rapid rate, especially in South America. The main reasons for the megafauna extinction that are widely recognized include climate change and the inability of large animals to survive in the new conditions of the Late Pleistocene, and/or a change in food availability. Others have suggested that the massive extinction of many South American species was due to the impact of the expansion of early humans in the New World at approximately the same time (Martin 1984; Barnosky and Lindsey 2010; Dantas et al. 2012). The influence of both factors was also discussed by Barnosky et al. (2004), with a focus on the debate about the dates the mammals last appeared (i.e. the youngest date for these species). However, little attention has been focused on the quality of the dates themselves and the possibility of contamination, which quite often occur in the case of bone samples.

MATERIALS AND METHODS

Samples were collected in several “tanks,” or natural depressions, on Neo-Mesoproterozoic lithotypes in the northeastern Brazilian districts of Sergipe, Bahia, and Rio Grande do Norte. Tanks are characterized by numerous fractures resulting from physical and chemical erosion, and contain sediments transported by seasonal rains, including the remains of animals and plants accumulated during the dry season. Sediments in these depressions are estimated as being of the Late Pleistocene and Holocene ages.

The materials studied were part of a scientific collection of the Laboratório de Paleontologia at the Universidade Federal de Sergipe, Laborátorio de Geologia da Universidade Estadual do Sudoeste da

Bahia and of the Museu Câmara Cascudo at the Universidade Federal do Rio Grande do Norte. Bone and tooth samples were derived from 3 different species *Eremotherium laurillardi* (Figure 1C), *Notiomastodon platensis* (Figure 1B), and *Toxodon platensis* (Figure 1A). Analyses were performed on the bioapatite fraction due to poor collagen preservation, using a technique developed at the Center for Applied Isotope Studies (CAIS) at the University of Georgia (Cherkinsky 2009).

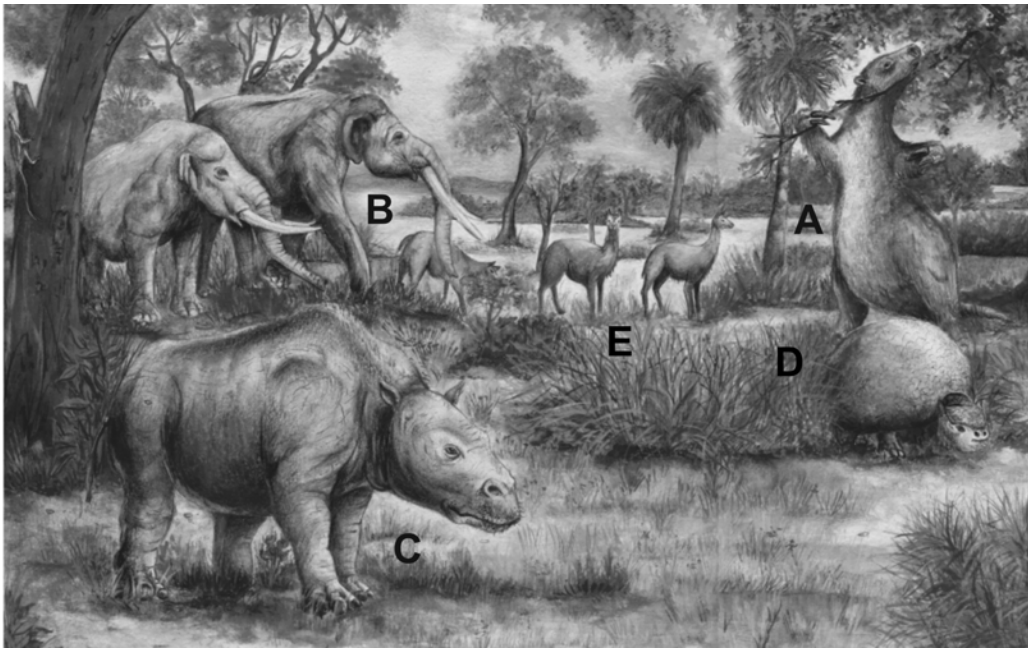

Figure 1 Reconstruction of the South American giant mammals: (A) *Eremotherium laurillardi*; (B) *Notiomastodon platensis*; (C) *Toxodon platensis*; (D) *Glyptodon* sp.; and (E) *Palaeolama major* (image courtesy of Marcelo Viana, 2011).

Procedures for the preparation of bone samples for isotopic analysis are critical for obtaining reliable data (Koch et al. 1997). In general, it must be assumed that bone samples have undergone some alteration or contamination in their natural environment. The carbonates in fossil bone may be contaminated by secondary carbonates, which have been precipitated during the process of burial either as pore-filling cements or as bicarbonates absorbed to the surface of crystals.

To remove 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 approximately 70 °C. After drying, a sample of 1–3 g is selected for further preparation. The sample is gently crushed into small fragments of <1 mm for further cleaning. The crushed sample is then reacted with 1N acetic acid in the 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 nature of this reaction is a qualitative indication of $CaCO_3$ contamination. This process of evacuation and repressuring is continued at ~20-min intervals until no substantial release of fine, foamy gas bubbles occurs. The process of evacuation and repressurization to atmospheric pressure should be repeated at least 4–5 times, the last reaction being overnight (20+ hr). 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 washed free of acetic acid by repeated soaking and decantation with demineralized water and is then vacuum-dried. For analysis of the carbon isotopes in bioapatite, approximately 100–500 mg of the cleaned bone powder is transferred to a special vessel with an arm for 100% phosphoric acid. The vessel is then evacuated and the acid mixed with the samples, initiating a reaction that is usually completed within 30 min at 60 °C. The released CO_2 is cryogenically purified and collected in sealing tubes for accelerator mass spectrometry (AMS) and stable isotope analyses. Bioapatite should have a carbon content of about 0.4–0.7%.

The resulting CO_2 is then cryogenically purified from the other reaction products and catalytically converted to graphite using the method of Vogel et al. (1984). Graphite $^{14}C/^{13}C$ ratios were measured using the CAIS 0.5MeV accelerator mass spectrometer. Sample ratios were compared to the ratio measured from a reference sample of oxalic acid I (NBS SRM 4990). The sample $^{13}C/^{12}C$ ratios were measured separately using a stable isotope ratio mass spectrometer and expressed as $\delta^{13}C$ with respect to PDB, with an error of <0.1‰.

The efficiency of this method of bioapatite purification was described in Cherkinsky (2009), which demonstrated the consistency of the results from the dating of the bioapatite fraction and collagen obtained from the same bone. For example, for a bison bone from El Mirón Cave in Spain, we obtained nearly identical dates for the bioapatite and collagen, at 10,740 ± 50 and 10,390 ± 50 uncalibrated BP, respectively. We have also compared the dates of bioapatite and charred bone collagen for a Pleistocene bison bone from Montana, with results of 25,370 ± 220 and 25,170 ± 230 uncalibrated BP, respectively. The vast majority of Holocene pairs are likewise in good agreement.

RESULTS AND DISCUSSION

Available chronological data indicates that *E. laurillardi*, *N. platensis*, and *T. platensis* were present in the Brazilian intertropical region during a long period of humidity at the end of the Pleistocene between 10 and 40 ka (Auler et al. 2006; Barnosky and Lindsey 2010; Dantas et al. 2012). Table 1 presents a compilation of results of stable isotope measurements and ^{14}C dates from several sources for bone and tooth samples from these mammals.

The *E. laurillardi* samples analyzed by CAIS were of dentine (UGAMS-9432) and bone (UGAMS-9435), and contain no collagen. The ^{14}C dates obtained by CAIS for this species range from 18,580–18,850 to 26,640–27,690 cal BP in Brazil. Rossetti et al. (2004) dated *E. laurillardi* bone using the collagen fraction and obtained a much younger date of 13,110–13,330 cal BP due to very poor preservation. Doubts about this date stem from a much older date they obtained from a wood sample collected from the same stratigraphic horizon, which was dated to 41,570–43,110 cal BP. It is possible that the very poorly preserved bone had no original collagen and the date obtained was based on extracted collagen-like material. The authors, however, explained this discrepancy by proposing a resedimentation of the wood fragment. However, the stable isotope ratio shows some contamination, as it should be in the range –18‰ to –22‰ for C_3-consuming animals. Instead, the ratio was –26.9‰ for the analyzed organics.

Auler et al. (2006) dated *E. laurillardi* bone using the U series technique. Three speleothem samples were collected above, between, and below large bones. Although the authors noted the high content of detrital ^{230}Th, the ages agreed within the standard deviation, indicating that this specimen was about 15 ka old.

The results for *N. platensis* shown in Table 1 belong to 3 individuals, and offer a comparison of dates using various materials from each individual. Two individuals (UGAMS-9438 and -9439) show

Table 1 Ages of giant mammals from Brazil by different dating techniques[a] Calibration was done using IntCal09 data (Reimer et al. 2009).

			$\delta^{13}C$ (‰)			^{14}C age (yr BP)			Calibrated age (yr BP)		
Species	Lab code #	Material	Enamel	Dentine	Bone	Enamel	Dentine	Bone	Enamel	Dentine	Bone
E. laurillardi	UGAMS-9432	Apatite	n/a	–3.85	n/a	n/a	22,440 ± 50	n/a	n/a	26,640–27,690	n/a
E. laurillardi	UGAMS-9435	Apatite	n/a	0.5	n/a	n/a	15,490 ± 40	n/a	n/a	18,580–18,850	n/a
E. laurillardi	n/a[1]	Collagen	n/a	n/a	–26.9	n/a	n/a	11,340 ± 50	n/a	n/a	13,110–13,330
E. laurillardi	U series[2]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	~15,000
Wood	n/a[1]	n/a	n/a	n/a	–31.3	n/a	n/a	37,700 ± 540	n/a	n/a	41,570–43,110
N. platensis	UGAMS-9438	Apatite	–1.04	0.57	–0.35	13,980 ± 40	14,970 ± 40	14,590 ± 40	16,800–17,240	17,990–18,530	17,480–18,010
N. platensis	UGAMS-9439	Apatite	–1.86	1.07	1.43	17,910 ± 50	20,490 ± 50	18,900 ± 50	21,170–21,560	24,180–24,820	22,240–22,960
N. platensis	UGAMS-9440	Apatite	0.44	–0.21	n/a	16,150 ± 40	11,710 ± 30	n/a	18,940–19,450	13,420–13,710	n/a
N. platensis	NZA-6984[3]	n/a	n/a	dung	n/a	n/a	n/a	12,200 ± 120	13,770–14,640	n/a	n/a
T. platensis	UGAMS-9442	Apatite	–1.32	–0.8	n/a	10,730 ± 30	11,310 ± 30	n/a	12,560–12,720	13,110–13,290	n/a
T. platensis	UGAMS-9445	Apatite	–13.24	–4.83	n/a	10,970 ± 30	10,190 ± 30	n/a	12,660–12,970	11,760–12,030	n/a
T. platensis	UGAMS-9446	Apatite	–3.68	–3.28	n/a	10,050 ± 30	10,580 ± 30	n/a	11,400–11,750	12,430–12,630	n/a
T. platensis	Beta-215330[4]	Collagen	–23.5	n/a	n/a	n/a	11,380 ± 40	n/a	n/a	13,150–13,770	n/a
T. platensis	Beta-218193[4]	Collagen	–26.3	n/a	n/a	n/a	11,090 ± 40	n/a	n/a	12,900–13,180	n/a

[a]1. Rossetti et al. (2004); 2. Auler et al. (2006); 3. Steadman et al. (2005); 4. Neves et al. (2007). All other dates were measured at CAIS (lab code UGAMS).

dates that were derived from all 3 materials: bone, enamel, and dentine. One sample (UGAMS-9440) has dates derived from enamel and dentine only. Because bone and dentine are more porous than enamel, the possibility of contamination in these materials is greater than in the enamel. Two types of contamination as a result of isotopic exchange are evident in these *N. platensis* results: 1) contamination by old carbon (UGAMS-9438 and -9439) and 2) by young carbon (UGAMS-9440). The ages of enamel for *N. platensis* in 2 cases were 16,800–17,240 and 21,170–21,560 cal BP, which are younger than the ages of other materials from the same individuals. This phenomenon could be explained by isotopic exchange of the bioapatite with the surrounding carbonate material.

In the case of a *N. platensis* individual (UGAMS-9440), the enamel date is significantly older when compared to the dentine date (18,940–19,450 versus 13,420–13,710 cal BP, respectively). Such a considerable rejuvenation can only be explained by isotopic exchange with soil solutions saturated with young carbonates. The only date that we were able find in the publications for this species was an analysis of dung by Steadman et al. (2005), with a date of 13,770–14,640 cal BP. *T. platensis* is the most studied of the 3 mammalian species described in this paper, with more dates available than for the other 2 species. Three tooth samples from different individuals found in the different locations were dated by CAIS, with results included in Table 1. Dates determined for the enamel materials of both UGAMS-9442 (12,560–12,720 cal BP) and UGAMS-9446 (11,400–11,750 cal BP) were younger than the dentine dated using the same teeth: 13,110–13,290 and 12,430–12,630 cal BP, respectively. For the sample UGAMS-9445, the enamel age determined of 12,660–12,970 cal BP is slightly older than the dentine age of 11,760–12,030 cal BP.

Neves et al. (2007) dated the collagen fraction from 2 dentine samples that had been previously dated using the electron spin resonance (ESR) technique (Baffa et al. 2000). The ESR dates on enamel and dentine were 5.0 ± 1.6 and 6.7 ± 1.3 ka BP, respectively. The method described by Baffa et al. (2000) did not include any procedure for removal of the secondary carbonates that could possibly rejuvenate the results. These dates are significantly younger that the dates obtained on collagen-like material extracted from dentine by Neves et al. (2007) using AMS: 13,150–13,770 and 12,900–13,180 cal BP. However, even these older dates could be affected by contamination, such that the stable isotope ratio of the extracted organic material is significantly depleted compared to the collagen of C_3-consuming animals.

Bone and dentine are mesodermic tissue composed of bioapatite connected to an organic matrix of collagenous proteins. However, enamel is an ectodermic tissue highly mineralized and which has an organic fraction that is gradually eliminated during the maturation phase. Due to this structural difference, the porosity decreases by a factor of 40 from bone to enamel. Thus, the enamel material is more stable and has more resistance to isotopic exchange. The date on the enamel, therefore, especially in the condition of the warm and wet climate of northeastern Brazil, seems more reliable than the dates resulting from analysis of the dentine and bone phases.

CONCLUSIONS

The dating of bone, dentine, and enamel material for the tropical conditions of northeastern Brazil is a complicated task. Due to the extremely poor preservation of collagen in most of the bone samples collected from this area, it is almost impossible to get a valid date on this fraction. It is well known that the wet conditions in the caves and depressions of this region could also alter the mineral fraction of bone. Because the carbon of bioapatite can undergo isotopic exchange with the environmental carbonates, the pretreatment and removal of the secondary carbonates is necessary but not always sufficient for obtaining a valid date. In most cases, the alteration of bone samples by isotopic

exchange could result in apparently younger ages. However, in situations where bones were buried in weathered limestone containing no ^{14}C, it is possible that contamination by "dead" carbon occurs, resulting in apparently older ages. This situation was clearly demonstrated by our dating of several *N. platensis* samples.

Tooth enamel is the most resistant material to isotopic exchange; however, we cannot completely exclude the possibility of its contamination in the environment of the study area. Thus, dates derived from the bioapatite fraction of enamel should be considered approximate and not absolute.

The youngest date for the giant sloth *Eremotherium laurillardi* obtained on the bioapatite fraction is 18,580–18,850 cal BP; for *Notiomastodon platensis*, the youngest date is 16,800–17,240 cal BP; and for *Toxodon platensis*, the youngest range is 11,400–11,750 cal BP. We have demonstrated that the proper pretreatment of bone and tooth samples permits the separation of diagenetic carbonates from bioapatite, as long as the carbon in these samples has not degraded completely.

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