IS TOOTH ENAMEL CARBONATE A SUITABLE MATERIAL FOR RADIOCARBON DATING?

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ABSTRACT. We present the results of 18 radiocarbon determinations on the carbonate fraction of tooth enamel prepared from nine separate teeth. The known ages of the teeth vary from 11 to >200 ka BP, and are from three sites. The sample preparation procedures varied somewhat, but were broadly based on procedures found to give satisfactory results on carbon stable isotope measurements. All the ^{14}C dates obtained are too young, by an equivalent contamination of *ca.* 6% Modern (pMC). This value is fairly consistent despite variations in sample preparation. We discuss the implications for using enamel as a possible alternative to bone when insufficient collagen is available.

INTRODUCTION

We report here work aimed at evaluating the potential of tooth enamel carbonate for radiocarbon dating. The motivation and rationale for doing this are as follows: 1) carbonate can be recovered from tooth enamel in depositional contexts in which bone collagen does not survive, so that its ^{14}C measurement could permit the direct dating of otherwise undatable (by ^{14}C) faunal remains; 2) a substantial history exists of ^{14}C dating carbonate from *bone* apatite, which gives some encouragement. This is greatly strengthened by more recent work on stable carbon isotope ($\delta^{13}C$) measurements on the carbonate fraction of *tooth* enamel, which we discuss more fully below. Also, studies of diagenetic changes in bone are beginning to elucidate the chemistry by which carbonate ions are exchanged or incorporated, and to improve methods for their removal.

Enamel differs from bone and dentine in possessing a greatly reduced organic molecular content (<2%), a much lower porosity and surface area, and a greater degree of crystallinity. Biological apatites resemble the mineral dahllite, with substitutions in the PO_4 and Ca positions. Carbonate ions are both substituted in the former position and are also adsorbed on surfaces and at hydration layers (Legeros *et al.* 1969; Brown and Chow 1976; Rey *et al.* 1991). This structure is diagenetically more stable in enamel than in bone. Enamel typically contains 0.5–1% by weight of carbon as carbonate. Collecting enamel samples in quantities of up to 100 mg is feasible, and ^{14}C dating is only possible using accelerator mass spectrometry (AMS). The pretreatment methods necessary to prevent diagenetically altered carbonate from being measured can be based on previous work developed for stable isotope measurements. The two types of isotopic measurements, $\delta^{14}C$ and $\delta^{13}C$, are, of course, sensitive in different ways to alteration of the original carbon isotope composition by incorporation of exogenous carbonate.

PREVIOUS RESEARCH

Radiocarbon Dating

Bone contains a few percent of carbon as carbonate, and this has been used for ^{14}C dating, although the reliability of the method has not been generally accepted, despite long-term efforts (Tamers and Pearson 1965; Haynes 1968; Hassan, Termine and Haynes 1977; Sullivan and Krueger 1981; Saliege, Person and Paris 1995). Some chemical treatment to remove exogenous carbonate is necessary—usually limited acid hydrolysis is used. Another approach uses CO_2 fractions differentially

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liberated over an increasing temperature range, the least exchangeable carbonate fraction being released last (Haas and Banewicz 1980). Obtaining a ^{14}C age in agreement with the expected age is not always a sufficient criterion that exogenous carbon has been removed, however, because it may have a similar ^{14}C age. This is not unlikely for material in the age range of 2 to 8 ka BP, but is generally much less likely for material >20 ka BP. To our knowledge, no previous AMS measurements have been made on tooth enamel carbonate; a comparison of a bone carbonate date with other (amino acid) fractions is given in Stafford *et al.* (1991).

Dietary Studies

The $\delta^{13}C$ value of bone and enamel carbonate contains different information from that of collagen carbon. Further, such biological information is potentially available from Lower Pleistocene material, because carbonate is still present. Thus, the field is being actively researched (see Lee-Thorp and van der Merwe (1991) for further references). For ^{13}C, the difference in isotopic composition between endogenous and exogenous material is seldom >10‰, and a 10% mixing of exogenous material should be just detectable in favorable cases. Studies on archaeological faunal material (Lee-Thorp and van der Merwe 1991; Bocherens *et al.* 1995) suggest that if the enamel is carefully pretreated, the original isotopic composition is sufficiently preserved for the recovery of the original composition. This is in contrast to work on bone, for which scientists still disagree on the ability to recover original signals (Sullivan and Krueger 1981, 1983; Schoeninger and DeNiro 1983; Lee-Thorp and van der Merwe 1987; Koch *et al.* 1995).

Diagenesis and Pretreatment

Both carbonate exchange and the additional adsorption and/or deposition of carbonate ions are expected to occur. One frequently observes deposition of carbonate as calcite, which decomposes faster than dahllite-type apatites when exposed to weak acids. Carbonate exchange is a more serious problem. Krueger recently conducted an interesting experiment (1991), in which he showed that "properly cleaned" *bone* apatite did not retain significant quantities of exchanged carbonate when immersed for 2 yr in 10 atom % ^{13}C carbonate, or for 11 ka in seawater of 10‰ difference in $\delta^{13}C$. Proper cleaning, in this case, meant periodic evacuation in 1N acetic acid for 48 hr. Some controversy has been generated by the effects of different pretreatment methods (Lee-Thorp and van der Merwe 1991) since inappropriate methods may cause artifacts. Acetic acid treatment may induce isotopic exchange of carbonate oxygen (Koch *et al.* 1995), while prolonged immersion in strong acetic acid has been shown to stimulate the formation of brushite ($HCaPO_4 \cdot 2H_2O$)(Lee-Thorp and van der Merwe 1991). Most workers now recommend a bleaching treatment to remove organic carbon followed by treatment with 1N or 0.1N acetic acid.

However, the success of any particular pretreatment method at this stage tells us relatively little about the diagenetic processes responsible for incorporating exogenous carbonate. There is little direct evidence for the rather vague models of diagenetic alteration, and such basic questions as what proportion of structural carbonate is potentially exhangeable have no answer at present. The issue has important implications, not only for the studies referred to here, but for example, for any potential extension to the ^{14}C dating of carbonate in bone, where even a small degree of diagenetic alteration may be more serious.

THIS STUDY

Material

We selected material from three sites; two calcitic cave systems (Kent's Cavern in England and Equus Cave, South Africa) and a generally anoxic, silty, fluvial deposit on limestone (Stanton Har-

court, England). These sites yielded convenient megafauna, were suitably old (in order to maximize the sensitivity to modern contamination) and are the subject of related studies (*e.g.*, electron spin resonance (ESR) dating of enamels at Stanton Harcourt, diagenetic studies at Kent's Cavern, dating and stable isotope studies at Equus Cave). Details concerning the material are in Table 1.

Pretreatment

Pretreatment varied slightly depending on the site. Samples from Stanton Harcourt were prepared at the Radiocarbon Accelerator Unit, Oxford, Kent's Cavern at the Conservation and Analytical Laboratory of the Smithsonian Institution, and Equus Cave at the Department of Archaeology at Capetown, the latter two by methods developed for stable isotope studies. All three laboratories confirmed through AMS measurement that their acetic acid (HAc) contained no ^{14}C, and that CO_2 liberated from geological (Tertiary) calcite was at least 50 ka BP.

Stanton Harcourt

The expected age of the large number of mammoths found at this site was from 200–250 ka, so the enamel should have contained no detectable ^{14}C. We made two independent consecutive preparations. Enamel was extracted by hand and broken into 1-mm fragments. These were treated with dil (0.5 M) HCl for 20 sec, followed by 1N NaOH (20 sec), then washed and crushed to *ca.* 50–100 μ. The fine powder was treated with 10% H_2O_2 overnight, followed by 1M HAc in a vacuum system, which allowed us to collect any evolved CO_2 for 2 hr. In fact, we were unable to collect enough CO_2 for subsequent measurement. After removal of the HAc, followed by washing in acidifed water, the evolution of CO_2 from the enamel by the action of phosphoric acid was collected in two stages, on the assumption that the earlier stage was likely to contain more diagenetically altered material. (The results do not bear out this assumption.)

Kent's Cavern

We selected teeth from herbivores and carnivores and used them to provide control dates from dentine. Enamel was ground to 0.5 mm, bleached in 2% "Clorox" overnight, rinsed 5 times with water, then treated either with 1 N acetic acid or 1 N HCl; the HAc treatment being overnight, the HCl for 5 min; CO_2 is collected from phosophoric acid treatment. The ages found for the collagen fraction agreed with archaeological expectation, with the possible exception of OxA-4831, which is somewhat younger (Table 1). The date on the breccia is from the carbonate fraction, and indicates the likely ^{14}C age of the main source of exchangeable carbonate ions.

Equus Cave

Enamel from two samples was prepared by overnight bleaching with 2% NaOCl, followed by several days in 1 N HAc, before the CO_2 liberated by H_3PO_4 was collected. Equus Cave[4] is not well dated, and the age of each sample is not exactly known.

Results

We present our findings (as ^{14}C dates) in Table 1.We include a column in which we have calculated the percent of equivalent modern carbon required as contaminant to reconcile the enamel carbonate date with the expected date.

[4]P5264 is from Square 20I, depth 52–60 cm and underlay a bone dated (from collagen) to 7840 ± 80 BP (Pta-2495), and overlay an ostrich eggshell dated to 11870 ± 105 BP (AA-5826). P5265 is from Square 24 J, depth 180–187 cm. It is above, but in the same general stratum as an ostrich eggshell dated to 27,330 ± 340 BP (AA-5827).

TABLE 1. Results of ^{14}C Dating Tooth Enamel Carbonate

ORAU ID	Site	Sample ID	Species	Prep*	OxA-no.	Date	Error	% ^{14}C	Exp. % C†	Difference
P5939	Stanton Harcourt		Mammoth	HAc-HP1	5097	19,560	340	8.98	0	8.98
			Mammoth	HAc-HP1	5098	22,780	380	6.08	0	6.08
			Mammoth	HAc-HP2	5099	18,100	260	10.74	0	10.74
			Mammoth	HAc-HP2	5100	19,560	260	8.98	0	8.98
P4097	Kent's Cavern	25	Rhino	Colla	3403	39,630	1420	0.72	0.5–1	
P4099		25.1	Rhino	HP	4271	16,250	200	13.23	0.5–1	12.51
		25.5	Rhino	HP	4275	18,540	200	9.95	0.5–1	9.23
		25.3	Rhino	HCl-HP	4273	24,570	310	4.70	0.5–1	3.97
		25.4	Rhino	HAc-HP	4274	19,760	200	8.54	0.5–1	7.82
P6064	Kent's Cavern	4/3470	Rhino	Colla	4829	40,600	1700	0.64	0.5–1	
P6058		4/3470	Rhino	HCl-HP	4823	17,530	140	11.28	0.5–1	10.64
P6056		4/3470	Rhino	HAc-HP	4821	25,400	280	4.23	0.5–1	3.60
P6065	Kent's Cavern	9/5315	Hyena	Colla	4830	39,400	1500	0.74	0.5–1	
P6061		9/5315	Hyena	HCl-HP	4826	8390	120	35.19	0.5–1	34.50
P6066	Kent's Cavern	2/3536	Horse	Colla	4831	27,640	400	3.20	0.5–1	
P6062		2/3536	Horse	HCl-HP	4827	10,920	100	25.68	0.5–1	22.40
P6059		2/3536	Horse	HAc-HP	4824	21,040	240	7.29	0.5–1	4.04
P6057	Kent's Cavern	9/3478	Horse	HCl-HP	4822	14,810	130	15.82	0.5–1	15.00
P6063	Kent's Cavern	6/3536	Hyena	HP	4828	17,390	140	11.48	0.5–1	10.50
P4100	Kent's Cavern	KC25	Breccia	HCl	4272	5360	80	51.31	50	
P6060		RP4	Shark	HCl-HP	4825	32,300	550	1.79	0	1.79
P5264	Equus Cave	UCT 2979	Quagga	HAc-HP	4276	9830	120	29.41	25–28	3.00
P5265	Equus Cave	UCT 2985	Megalotragus	HAc-HP	4277	20,760	220	7.54	4.0–5.0	3.00

*HP = phosphoric acid in first (HP1 or HP) or second (HP2) etch; HAc = acetic acid pre-etch; HCl = 1 N HCl and pre-etch; HP alone = no pre-etch; Colla = extracted collagen

†Percentage of carbon calculated for the expected date

DISCUSSION

All the enamel dates are, as expected, somewhat too young. If only the main pretreatment method, treatment with acetic acid, is considered, the equivalent modern contamination level is fairly consistent at 6.5 ± 2.8% (for n = 9). This might be fortuitous, and further work might find less consistency, but given the different burial environments and laboratory procedures involved, we are inclined to interpret the results as showing that tooth enamel contains a moderately consistent fraction of comparatively recently exchanged or adsorbed carbonate that is resistant to removal by acetic acid. Much the same residual fraction seems to apply to the 200 ka sample, the 20–40 ka samples and the 11 ka sample, implying that the incorporation of younger ^{14}C is recent (in comparison to the length of time of burial). That is to say, a relatively small amount of "modern" carbon is more likely as contaminant than a larger amount of older carbon. This would be consistent with the general results from stable isotope analysis, where incorporation of <10% environmental carbon would not be easily detected.

The results show no effect ascribable to the time for which the samples have been exposed to the atmosphere away from their burial context. (Kent's Cavern samples have been stored in a museum for >70 yr, Stanton Harcourt samples for 3 yr.) The results from HCl treatment are somewhat puzzling. HCl was used as an alternative to HAc because it appeared to cause less exchange in the oxygen isotopes of enamel carbonate. The initial result with Kent's Cavern (OxA-4273) was encouraging. However, all subsequent results on other material show the HCl treatment to be less successful than HAc in removing exogenous carbon. Presumably, the dissolution of hydroxyapatite by HCl is less discriminating. It is also worth pointing out that, although acetic acid treatment has clearly improved the date (where comparison is made with untreated material), the improvement is only

about a factor of two (*ca.* 1 half-life). That is, the enamel does not seem to have incorporated particularly large amounts of "modern" carbon in the first place. One picture of this is that the carbonate remaining after acetic acid treatment contains a small proportion that remains in comparatively rapid equilibration with exogenous bicarbonate.

The Prospect for the Accurate ^{14}C Dating of Enamel Carbonate

The results reported here indicate an ambivalent prospect. On the one hand, the contamination has been reduced to a low and fairly consistent level; on the other hand, the very consistency obtained argues that it will be difficult to develop significantly improved methods to remove additional exogenous carbonate. If the present consistency (*i.e.*, standard deviation in residual "modern" carbon) were taken as a random dating error, then an age limit of *ca.* 24 ka BP would apply. This would have some, if limited, value. However, enamel is a valuable archaeological resource and is only abundant in megafaunal remains. For example, human enamel would be available for dating only in crucial cases, where reliability of dating is essential. Further improvements in removal of exogenous carbonate are likely to depend on improvements in understanding the processes of diagenesis alteration of enamel—research that is proceeding on a fairly broad front (*e.g.*, in ESR dating (Rink and Schwarcz 1995), as well as in the recovery of dietary signals).

Other Studies

In terms of the contribution to other studies, these results tend to support the reliability of stable isotope determinations that use similar pretreatment methods. Although they do not distinguish decisively between the incorporation of major quantities of "older" carbon or minor quantities of "younger" carbon, they do suggest the latter to be more likely, and to be at a level that would not seriously compromise the $\delta^{13}C$ measurements. They also suggest that, by the same argument, the extent of post-depositional structural alteration is small, so the accumulated ESR signal is not thereby greatly altered. Thus, this tends to support the reliability of the ESR methodology and suggests a possible test for ESR samples.

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References

Bocherens, H., Fogel, M. L., Tuross, N. and Zeder, M. 1995 Trophic structure and climatic information from isotopic signatures in Pleistocene cave fauna from Southern England. *Journal of Archaeological Science* 22(2): 327–340.

Brown, W. E. and Chow, L. C. 1976 Chemical properties of bone minerals. *Annual Review of Materials Science* 6: 213–250.

Haas, H. and Banewicz, J. 1980 Radiocarbon dating of bone apatite using thermal release of CO_2. *In* Stuiver, M. and Kra, R. S., eds., Proceedings of the 10th International ^{14}C Conference. *Radiocarbon* 22(2): 537–544.

Hassan, A. A., Termine, J. D. and Haynes, V. C., Jr. 1977 Mineralogical studies on bone apatite and their implications for radiocarbon dating. *Radiocarbon* 19(3): 364–374.

Haynes, V. 1968 Radiocarbon: Analysis of inorganic carbon of fossil bone and enamel. *Science* 161: 687–688.

Koch, P. L., Tuross, N. and Fogel, M. L. 1995 Isotopic preservation of calcified tissue during early diagenesis. *Geochimica Cosmochimica Acta*, in press.

Kreuger, H. W. 1991 Exchange of carbon with biological apatite. *Journal of Archaeological Science* 18: 355–361.

Lee-Thorp, J. A. (ms.) 1989 Stable Carbon Isotopes in Deep Time. Ph.D. dissertation, University of Cape Town, South Africa.

Lee-Thorp, J. A. and van der Merwe, N. J. 1991 Aspects of the chemistry of modern and fossil biological apa-

tites. *Journal of Archaeological Science* 18: 343–354.

Legeros, R. Z., Legeros, J. P., Trautz, O. and Klein, E. 1969 Two types of carbonate substitution in apatite structure. *Experientia* 24: 5–7.

Rey, C., Renugo, G., Palakrishnan, V., Shimuzu, M., Collins, B. and Glimcher, M. J. 1991 A resolution-enhanced fourier transform infra red study of the environment of the CO_3^{2-} ion in the mineral phase of enamel during its formation and maturation. *Calcified Tissue International* 49: 259–268.

Rink, W. J. and Schwarcz, H. P. 1995 Tests for diagenesis in tooth enamel: ESR dating signals and carbonate contents. *Journal of Archaeological Science* 22(2): 301–312.

Saliège, J.-F., Person, A. and Paris, F. 1995 Preservation of the $^{13}C/^{12}C$ original ratio and ^{14}C dating of the mineral fraction of human bones from Saharan tombs, Niger. *Journal of Archaeological Science* 22(2): 301–302.

Schoeninger, M. J. and DeNiro, M. J. 1983 Reply to: "Carbon isotope ratios of bone apatite and animal diet reconstruction". *Nature* 301: 177–178.

Stafford, T. W., Hare, P. E., Currie, L., Jull, A. J. T. and Donahue, D. J. 1991 Accelerator radiocarbon dating at the molecular level. *Journal of Archaeological Science* 18: 35-72.

Sullivan, C. Y. and Krueger, H. W. 1981 Carbon isotope analysis of separate chemical phases in modern and fossil bone. *Nature* 292: 333–335.

Sullivan, C. H. and Krueger, H. W. 1983 Carbon isotope ratios of bone apatite and animal diet reconstruction. *Nature* 301: 177.

Tamers, M. A. and Pearson, F. J. 1965 Validity of radiocarbon dates on bone. *Nature* 208: 1053–1055.