[RADIOCARBON, VOL 22, No. 2, 1980, P 537-544]

RADIOCARBON DATING OF BONE APATITE USING THERMAL RELEASE OF CO_2

HERBERT HAAS* and JOHN BANEWICZ**

Southern Methodist University, Dallas, Texas

ABSTRACT. Extraction of carbon from bone hydroxy apatite as CO_2 by heating in an oxygen atmosphere is an alternative method to hydrolysis of the bone. Heating in specific steps allows separation of CO_2 fractions from different sources, including weakened or sound bone material and secondary deposits. Pretreatments to remove most secondary carbonate and much of the collagen are necessary. Thermogravimetric (weight loss) curves and CO_2 release patterns during heating show that the temperature interval for collection of the most reliable CO_2 sample for dating purposes lies between 800 and 950°C. Age dates run on such samples support this conclusion.

INTRODUCTION

The inorganic phase of bone belongs to the apatite family, specifically hydroxyapatite with the basic formula $Ca_{10}(PO_4)_6(OH)_2$ (LeGeros and others, 1970) where PO_4^{3-} ions are partly replaced by CO_3^{2-} and F^-. It has also been established that the carbonate ion in biological apatites is present in the apatite lattice and not in a separate phase such as calcite. A very common contaminant in fossil bone is secondary calcium carbonate, *ie,* calcite. This mineralogic difference between hydroxyapatite carbon in the bone sample and postdepositional carbon in the form of calcite should make it possible to separate the two sources. An additional difficulty which may be encountered lies in the postmortem exchange of carbon in the hydroxyapatite. Evidence for this is given by *in vivo* studies on animals demonstrating rapid turnover of bone carbonate (Buchanan and Nakao, 1952) and in numerous incorrect ^{14}C dates based on bone apatite.

Radiocarbon dating procedures

The common procedure of dating the mineral phase in bone begins with leaching the ground sample with acetic acid or triammonium citrate to remove secondary calcite. Carbon dioxide from the hydroxyapatite is evolved in slow hydrolysis, usually performed in three steps. It is assumed that early reactions remove residual calcite and weakened bone structure with exchanged carbon content. The last carbon dioxide fraction is most likely to yield the most reliable age date (Haynes, 1968; Hassan, 1976). Satisfactory results from this dating process can be expected only with the assumption of simultaneous exposure of all surfaces of the internal bone structure to the acid solutions utilized. In reality, leaching out of all secondary calcite is not feasible, especially when much of the available space within the bone structure, such as the haversian canals and cavities, formerly occupied by collagen, are filled by the secondary mineral.

Another important consideration is the carbon exchange which takes place along surfaces of the interlocking individual apatite crystals. The size of these crystals has been estimated to be less than 1000 Å (Hassan,

* Institute for the Study of Earth and Man
** Department of Chemistry

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

Termine, and Haynes, 1977; Eanes, 1973). Obviously, hydrolysis by layers along the surface of individual crystals is not possible; instead, the reaction proceeds along the surfaces created in the grinding process.

The limits of the hydrolysis are, thus, clearly demonstrated. Any new technique promising more accurate age dates must be as independent as possible of geometrical properties of the sample. This condition seems to be fulfilled with the thermal release of carbon dioxide. An analogy can be made between techniques used in potassium-argon dating and the present suggested method for extraction of carbon from biological apatite. In the former, argon is sometimes extracted from the sample in stepwise heating (Dalrymple and Lanphere, 1971) and the reliable age date obtained from the last fractions. An important assumption for successful application of this method to biological hydroxyapatite is that the carbon sites in the apatite that has undergone post-burial changes can be activated with release of the carbon more readily than the unchanged apatite. This means that the carbon content of the sample that may have been partially exchanged should be released at a lower temperature than the unaffected original carbon content. It is also important that any secondary carbonate minerals release their carbon content in a temperature interval that does not entirely coincide with the activation temperature of the desired apatite-carbon fraction.

Thermogravimetric studies

The nature of carbon release during slow heating in a stream of oxygen for all substances likely to be present in bone needed to be known. We investigated fresh bone and fossil bone apatite, collagen, and a carbonate (tufa) similar to the secondary carbonate expected to occur in fossil bone. For this purpose the following experimental setup was utilized:

— A thermal balance[1] in which the samples are heated and their weight changes recorded.

— A sampling device for the effluent gases from the thermal balance, linked to

— a gas chromatograph[2] for separation of the various components of the effluent gases

— a mass spectrometer[2] for quantitative analyses of the different gas species, in particular, H_2O, SO_2 and, most important, CO_2.

For the present study, the weight loss curves recorded in the thermal balance and the CO_2 release patterns are of primary concern. With these, the following four parameters, which are the most important in the application of the proposed method, were studied:

1) rate of temperature increase of the sample,

2) most suitable size fraction of crushed bone,

[1] Dupont 950 Thermogravimetric Analyzer connected to Dupont 900 Differential Thermal Analyzer.

[2] Combined gas chromatograph and mass spectrometer, Dupont Dimaspec 321 GC/MS.

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

3) optimal pretreatment methods of bone to remove contaminants without introducing new sources of error,
4) experimental behavior of a variety of bone samples, ranging from fresh bone to fossil bones of contrasting provenience.

Most of the experiments were carried out with fossil Bison bone from the Hudson Meng site, Sioux County, NW Nebraska. The bone is relatively well-preserved with adequate collagen content to permit radiocarbon dating. Only long bones were used. These were washed, crushed, and ground, sized to metric sizes sieve fractions no. 18, 35, 60, 100, (1.00, 0.50, 0.25, 0.15mm) and "fines". Each size fraction was individually treated with acetic acid for removal of secondary carbonate and with hydrazine for removal of organic matter. Before each step, small samples were retained for making x-ray diffraction patterns and experiments

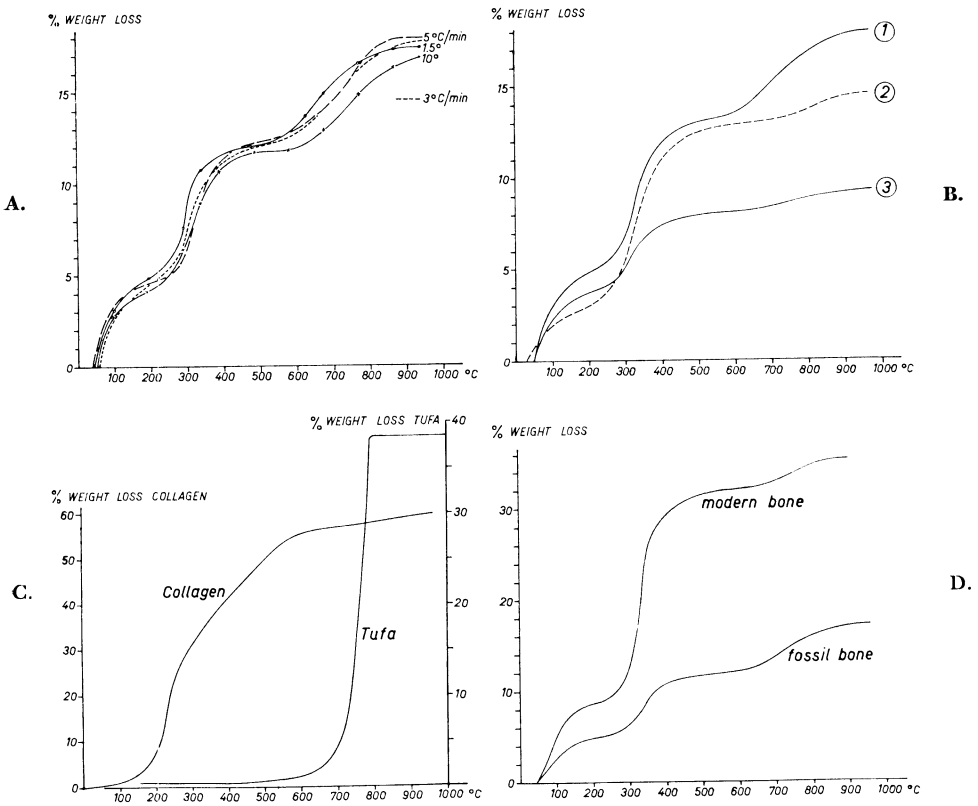

Fig 1. Weight loss in % of initial weight during slow heating of bone apatite and related substances.

A. Weight loss observed during heating rates of 1.5, 3, 5, and 10°C per minute.

B. Weight loss for untreated bone (1), pretreated with acetic acid for removal of secondary carbonate (2), pretreated with hydrazine for removal of organic fraction (collagen) in bone (3).

C. Weight loss and combustion of collagen and thermal breakdown of calcium carbonate (tufa).

D. Difference between weight loss curve of modern bone and fossil bone.

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

with the thermal balance, in which each run was performed with samples ranging from 30 to about 100mg. Most samples were powdered in a mortar prior to runs in the thermal balance. Figure 1A shows weight loss as a function of temperature increase for the above sample material. Major weight changes occur in temperature intervals:

0-225°C	loss of water
225-500°C	combustion of organic materials
500-1000°C	mineralogical changes

The experiment was repeated with four rates of temperature increase: 1.5° C/min, 3° C/min, 5° C/min, and 10° C/min.

All rates produce essentially the same pattern. However, as expected, at the slowest rate, major weight changes occur at lower temperatures and weight loss lags behind for the fastest speed. The difference is small; isothermal runs have shown that, after a fast heating to a fixed temperature, the sample undergoes 85 percent of its final weight loss at that temperature in less than 1 minute, and 99 percent, in less than 15 minutes. This observation differs from results of a study on carbonate-fluorapatite (francolite) of sedimentary origin (Matthews and Nathan, 1977) in which substantial weight losses were observed after 100 hours of heating at ca 600°C. The much more porous structure of bone material is probably responsible for its faster response to heating.

The effect of various pretreatment techniques on the bone material is shown in figure 1B. Curve 1 was obtained from an untreated sample: curve 2 shows the flattening of the peak above 550° of a sample from which secondary carbonate was removed with acetic acid. The difference below 250° can be attributed to varying moisture contents of the powdered samples.

Curve 3 shows the effect of rigorous hydrazine treatments and alcohol washes. The procedures recommended by Termine and others (1973) and by Hassan (1976) were followed. Weight loss attributed to combustion of organic material was reduced to approximately half the initial amount. Reduction of weight loss in the high temperature region may be explained by the physical removal of remaining secondary carbonate after the protecting collagen was dissolved.

Thermal curves for collagen and carbonate (tufa) are shown in figure 1C. Collagen was obtained from an archaeological dating project on human skulls. Moisture loss and combustion process below 600°C are distinct. Continuous weight loss above 600°C may be due to residual inorganic chemicals used in the extraction and purification of the collagen (hydrochloric acid, sodium hydroxide). Tufa is a close equivalent to secondary carbonate in fossil bone. At 800°C, weight loss ceases abruptly; an isothermal run confirmed this result by reaching final weight loss in 7 minutes.

The two curves, in figure 1D, show that the difference in thermal weight loss between untreated modern bone (elephant) and untreated fossil bone (Hudson Meng bison) lies mainly below 600°C and is attrib-

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

uted to combustion of much higher organic content in modern bone. Above this temperature, weight loss of fossil bone is 40 percent larger, which can be explained by presence of secondary carbonate.

Weight losses above 600° are, at least partially, due to CO_2 release, as will be demonstrated below. Consequently, the studies presented in Figure 1 indicate that a small sample of CO_2 gas can be collected above 800° which stems mainly from original bone apatite. Thus, it should yield the most reliable age date obtainable with this material.

Measurements on thermally released CO_2

A sweep gas (oxygen) was passed over the bone sample during heating experiments in the thermogravimetric analyzer. Effluent gases from the bone sample were carried by the sweep gas and sampled at approximately 20°C intervals. These gas samples were injected into the gas chromatograph, linked directly to a mass spectrometer. The mass 44 peak was monitored and a quantitative CO_2 release pattern obtained over the total temperature range of 50 to 1000°C. The measurements were calibrated to show milligrams of CO_2 per liter of oxygen sweep gas. The bone sample size was about 100mg.

Figure 2A shows the release pattern of an untreated sample. The major peak at 350°C coincides with the collagen combustion. The release reaches a minimum near 600°. Between 700° and 750°C, the secondary carbonate breakdown reaches its peak. A slow continuous release above 800° is attributed to bone-apatite breakdown. This is supported by the curve in figure 2B, which shows CO_2 release after the sample was treated with acetic acid for secondary carbonate removal. The curve has a maximum around 850°C and shows a slow release over the temperature range from 600° to 950°C. In the lower half of this range, the part of the apatite structure that was weakened during burial is apparently breaking down, since almost no CO_2 release is observed around 600° for a modern bone control sample.

Figure 2C traces the CO_2 release pattern after hydrazine treatments. This curve supports conclusions drawn from thermal weight loss data, namely, that hydrazine treatment removes only part of the organic material and some of the mineral substance.

Bone dating results and conclusions

The method presented here consists of pretreating a sufficiently large bone sample, *ie*, 600g or more, with acetic acid and hydrazine, heating it slowly in oxygen to 600°C to permit combustion of organics, then raising the temperature rapidly to 800°C. The thermal inertia of the large system allows sufficient time for breakdown of secondary carbonate. Actual sample CO_2 collection takes place during a third temperature rise to 950°C.

A series of thermally released CO_2 samples collected from different size fractions of crushed bone and at different temperature intervals was dated prior to the present study. The results are shown on the bottom

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

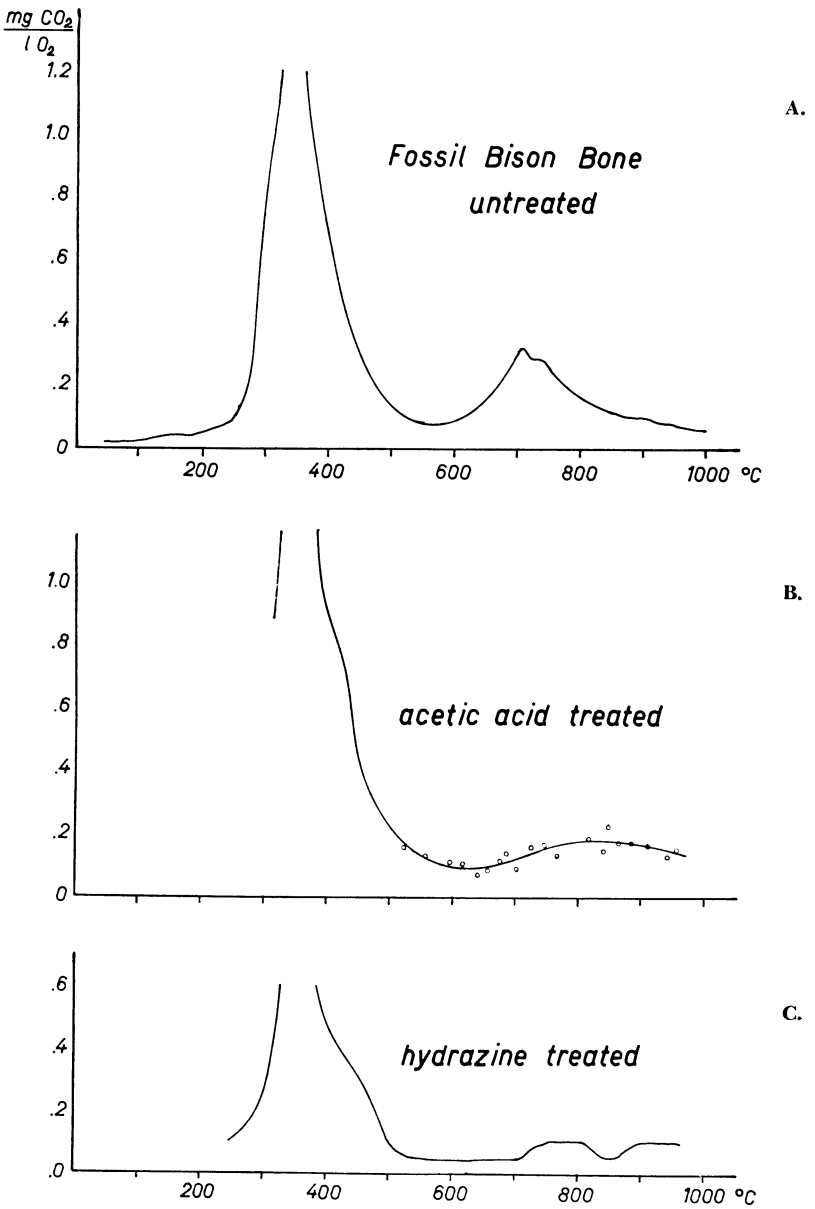

Fig 2. Release of carbon dioxide during slow heating of fossil bone apatite. CO_2 measured in mg per liter of oxygen sweepgas.

A. Untreated bone sample.

B. Bone sample treated with acetic acid for removal of secondary carbonate.

C. After additional treatment with hydrazine for removal of organic fraction (collagen).

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

TABLE 1

Fraction dated	Date (yr BP)	SMU date no.
Acid-hydrolization of bone		
Secondary carbonate		
1st fraction	115.3% modern	49
2nd fraction	109.8% modern	50
Apatite		
1st fraction	8320 ± 100 BP	51
2nd fraction	8990 ± 190 BP	52
Collagen	9380 ± 100 BP	103
Impurities, filtered from collagen	8560 ± 270	100
Charcoal, collected with bone	9820 ± 160	224
Thermally released CO_2 on no. 35 sieve fraction of bone		
Collected at 650°C	7970 ± 490	525
Collected at 720°C	8270 ± 280	523
Collected at 820°C	9670 ± 660	527
Collected at 920°C	7240 ± 620	529

of table 1 and generally support the conclusions drawn from our present work. The dates, SMU 523-529, in table 1, are based on isothermal heating periods for each step up to 12 hours. This limits direct comparison to the present study, which uses shorter run times. We do not presently have an explanation for the unexpectedly young age of the 920°C heating step. However, contamination from an external source added to the small sample of 0.5L CO_2 could be the cause.

An important difference from the dates, SMU-49-103 (top part of table 1), should be noted. The secondary carbonate, leached with acetic acid, is shown to be a contaminant of recent origin with strong potential for shifting age dates toward a too-recent age. Collagen is close to the true age, best expressed by the charcoal age, 9820 ± 160 (SMU-224). Apatite ages show an admixture of residual modern carbonate and follow the predicted age trend for a series of fractional hydrolizations.

Conclusions from the present research must now be tested on several series of radiocarbon dates on fossil bone samples selected for variable environments of burial and states of preservation. Again, effluent gas will be monitored, $\delta^{13}C/^{12}C$ ratios measured, and additional tests for H_2O, SO_2, hydrazine and alcohol remnants (at low temperatures) will furnish data for refining pretreatment and actual dating procedures.

ACKNOWLEDGMENTS

We thank Paul Larson and Rick Rizos for their dedicated support of the laboratory investigation. Much helpful advice and experimental bone material were given to us by C Vance Haynes. This work was made possible by National Science Foundation Grant BNS 7826499.

REFERENCES

Buchanan, L D and Nakao, Akira, 1952, Turnover of bone carbonate: Jour Biol Chem, v 198, p 245-257.

Dalrymple, G B and Lanphere, M A, 1971, $^{40}Ar/^{39}Ar$ technique of K–Ar dating: A comparison with the conventional technique: Earth Planetary Sci Letters, v 12, p 300-308.

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press

Eanes, E D, 1973, Eleven x-ray diffractions of vertebrate hard tissue, *in* Zipkins, Isadore, ed, Biological mineralization: New York, John Wiley & Sons, Inc, p 227-256.
Hassan, A A, 1976, Geochemical and mineralogical studies on bone material and their implications for radiocarbon dating: PhD dissert, Southern Methodist Univ, Univ microfilms, Ann Arbor, Michigan.
Hassan, A A, Termine, J D, and Haynes, C V, Jr, 1977, Mineralogical studies on bone apatite and their implications for radiocarbon dating: Radiocarbon, v 19, p 364-374.
Haynes, C V, 1968, Radiocarbon: Analysis of inorganic carbon of fossil bone and enamel: Science, v 161, p 687-688.
LeGeros, R Z, LeGeros, J P, Trautz, O R, and Klein, Edward, 1970, Spectral properties of carbonate in carbonate-containing apatites: New York, Plenum Press, p 3-23.
Matthews, Alan and Nathan, Yaacov, 1977, The decarbonation of carbonate-fluorapatite (francolite): Am Mineralogist, v 62, p 565-573.
Termine, J D, Eanes, E D, Greenfield, D J, Nylen, M V, and Harper, R A, 1973, Hydrazine deproteinated bone mineral physical and chemical properties: Calcified Tissue Research, v 12, p 73-90.

DISCUSSION

Berger: The difference in radiocarbon ages of thermally released CO_2 from bone may perhaps be due to isotopic fractionation. If $^{13}C/^{12}C$ ratios were measured this point could be cleared up.

Haas: $\delta^{13}C$ measurement on most CO_2 fractions have been made and no trend has been observed. More measurements will be included in the application stage of this research. In general, fractionation is less likely to occur at these high temperatures, high activation energies tend to minimize kinetic effects.

https://doi.org/10.1017/S0033822200009863 Published online by Cambridge University Press