RADIOCARBON, Vol 52, Nr 1, 2010, p 157–163 © 2010 by the Arizona Board of Regents on behalf of the University of Arizona

AMS ^{14}C DATING OF HUMAN BONES USING SEQUENTIAL PYROLYSIS AND COMBUSTION OF COLLAGEN

Hong Wang[1] • Stanley H Ambrose[2] • Kristin M Hedman[3] • Thomas E Emerson[3]

ABSTRACT. The Radiocarbon Dating Laboratory at the University of Illinois has been using the pyrolysis-combustion technique to separate pyrolysis-volatile (Py-V) or low molecular weight and pyrolysis-residue (Py-R) or high molecular weight compounds for ^{14}C dating of organic remains since 2003. We have applied this method to human collagen dating to examine the ^{14}C age difference between low and high molecular weight organic compounds. Results show that both fractions of late prehistoric period human bones from Illinois archaeological sites yield identical ^{14}C dates but that Py-V or low molecular weight fractions of Archaic period human bones appear to be slightly contaminated. In this case, Py-V components or low molecular weight collagen fraction yield older ^{14}C dates, which could result from contamination from old organic-rich sediments. The pyrolysis-combustion technique provides an economical alternative method to date bones that have not been satisfactorily dated using conventional purification techniques.

INTRODUCTION

A small number of radiocarbon dating laboratories perform high-resolution bone collagen dating. ^{14}C dating of bone collagen is challenging because its preservation is influenced by many environmental factors, including temperature, moisture, pH, and microbial activity (van Klinken 1999; Collins et al. 2002). Bone organic matter could be altered physically and chemically through absorbing dissolved organic carbon and "fusing" humic substances with collagen compounds through humification (Maillard reaction) processes (van Klinken and Hedges 1995). These contaminants could cause ^{14}C dates of bone collagen to be either too young or too old depending on variations of local environment and soil chemistry. To completely remove molecular contaminants is cumbersome and expensive (Stafford et al. 1988; van Klinken et al. 1994; Tripp et al. 2006).

The most common protocol to extract collagen is the method described by Longin (1971). In this protocol, collagen is defined as a gelatin-like, acid insoluble residue after weak acid decalcification. Theoretically, this protocol produces total bone organic matter that includes untwisted triple-helical gelatin-like collagen molecules. However, humic substances absorbed by bone cannot be completely removed through weak acid treatment. An improved protocol adds a weak base solution (0.1–0.125M NaOH) to remove humic acids, and then uses near-boiling (95 °C) weak acid (pH 3) to solubulize and filter bone collagen (Ambrose 1990). Although this protocol provides collagen that generally yields more accurate ^{14}C dates, NaOH decreases collagen yields (Minami et al. 2004), and does not completely remove humic contaminants (Brown et al. 1988; Stafford et al. 1988; van Klinken and Hedges 1995; van Klinken 1999; Bronk Ramsey et al. 2004). Refined molecular compound-specific methods of purification of collagen have been developed, including ninhydrin derivatization (Nelson 1991; Tisnerat-Laborde et al. 2003), ion-exchange techniques (Hedges et al. 1989), the isolation of single amino acids (usually hydroxyproline) (Stafford et al. 1987, 1988, 1991; van Klinken and Mook 1990; Tripp et al. 2006), collagenase digestion (DeNiro and Weiner 1988; van Klinken et al. 1994), isolation of tripeptides (van Klinken et al. 1994), and ultrafiltration (Brown et al. 1988; Higham et al. 2006). Among these methods, ultrafiltration is currently the most popular protocol (Higham et al. 2006). Ultrafiltration separates high molecular weight (HMW) collagen compounds (>30 kD; 1 kD = 1000 atomic mass units) from low molecular weight (LMW) fractions

[1]Illinois State Geological Survey, Institute of Natural Resource Sustainability, University of Illinois at Urbana-Champaign, Champaign, Illinois 61820, USA.
[2]Department of Anthropology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.
[3]Illinois State Archeological Survey, Institute of Natural Resource Sustainability, University of Illinois at Urbana-Champaign, Champaign, Illinois 61820, USA.

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

(<30 kD). The HMW fraction includes undegraded alpha chains of bone collagen, while LMW compounds include degraded collagen fragments and soil-derived mobile and more refractory organic compounds. It is believed that the HMW fraction provides ideal ^{14}C dates for bone dating. However, one problem with this protocol is that the regenerated-cellulose membrane of the ultrafilter needs glycerol to keep it moist, and glycerol is made of petroleum products that contain dead or old organic carbon, which is hard to remove (Bronk Ramsey et al. 2004).

Since 2003, we have applied the technique of high-temperature (800 °C) pyrolysis-combustion technique to separate pyrolysis-volatile (Py-V) or LMW compounds and pyrolysis-residue (Py-R) or HMW compounds for ^{14}C dating of wood, peat, cave sediments, and soil organic matter (Wang et al. 2003). In this paper, we further apply this technique to human bone collagen to examine the significance of age differences between LMW and HMW fractions of bone collagen. The Py-V fraction includes degraded collagen molecules that could link to soil humates. The Py-V fraction of collagen may contain compounds that induce errors in collagen dates. During high-temperature pyrolysis, these compounds are broken and detached from aromatic carbon rings, and brought to a combustion system by an inert carrier gas (Ar) for oxidation. The high-temperature Py-V fraction should contain the LMW compounds, although the exact molecular weights are not known. The high-temperature Py-R fraction is mostly carbon black or graphitic-like material, which is derived mainly from carbon-carbon bonded aromatic hydrocarbon molecules. The Py-R fraction is equivalent to HMW collagen compounds. We believe the Py-R fractions reflect the most degradation-resistant compounds and yield more valid ^{14}C dates for ^{14}C dating.

METHODS

Pyrolysis is carried out in the Radiocarbon Dating Laboratory at the Institute of Natural Resources Sustainability of University of Illinois at Champaign using a pyrolysis-combustion system that consists of inner and outer quartz tubes (Figure 1), as described in Wang et al. (2003). The inner tube is purged with Ar during the anoxic pyrolysis step, and with O_2 during the combustion phase, while the outer tube is continuously purged with O_2. The flow of the Ar and O_2 gases is controlled by 2 needle valves and monitored with 2 flow meters.

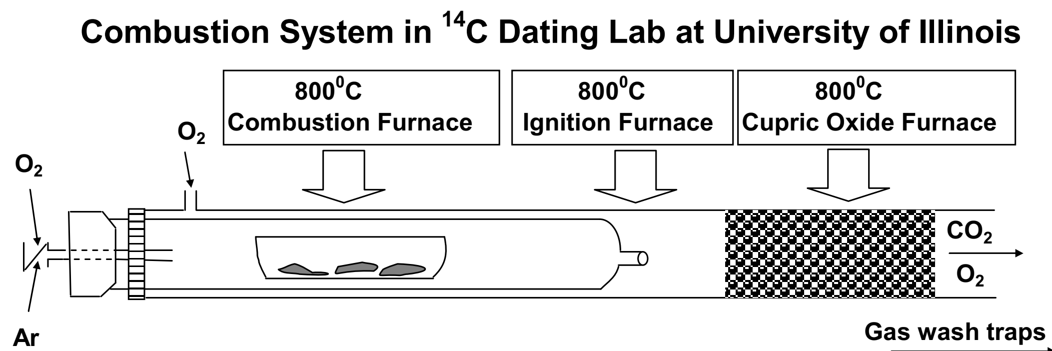

Figure 1 Schematic diagram of pyrolysis-combustion system (Wang et al. 2003). The inner and outer quartz tubes are connected using a size reducing Cajon Ultra-Torr fitting. The outer tube is always purged with O_2, while inner tube is purged with either Ar gas for anoxic pyrolysis or O_2 gas for oxidation. The collagen sample is placed in the quartz boat in the inner tube.

Pyrolysis was completed at 800 °C for 30 min. The volatile or LMW compounds released from collagen were carried by Ar gas into the outer tube through an orifice at the end of inner tube, where they were oxidized at 800 °C to produce CO_2. The CO_2 passed through a cupric oxide furnace at

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

800 °C and several gas wash traps for further oxidation and purification (Figure 1). The purified CO_2 was then collected cryogenically for AMS ^{14}C analysis. After Ar pyrolysis, the inner tube was filled with O_2 for oxidizing carbon in the pyrolysis residue of the collagen to produce a second CO_2 fraction for separate AMS ^{14}C analysis. Both purified CO_2 fractions were submitted to the Keck Carbon Cycle AMS Laboratory of the University of California-Irvine for AMS ^{14}C analysis using the hydrogen-iron reduction method (Southon 2007).

Early Upper Pleistocene bones of more than 50 fossil Leporidae (rabbit and hare) specimens from Natural Trap Cave, Wyoming (Gilbert et al. 1980; Wang and Martin 1993) were used for collagen ^{14}C background testing, while human bones collected from 4 Illinois sites were used to examine the pyrolysis method. Collagen was extracted using the protocol of Ambrose and Norr (1993; Ambrose 1990). Bones were first crushed gently with a clean mortar and pestle. About 1–3 g of sieved bone granules of >0.3 mm and <0.8 mm diameter were placed in a Pyrex® coarse frit filter funnel on annealed quartz wool with 50 mL 0.2M HCl. The funnel was drained and fresh HCl was replaced twice daily for up to 3 days. Demineralization was complete when bubbling stopped and translucent isomorphs appeared. The sample was rinsed to neutrality with distilled water. About 50 mL of 0.125M NaOH was added to funnel and rinsed to neutrality after 20 hr. About 50 mL 10^{-3} M HCl (pH 3) was added to the filter funnel, which was placed in a drying oven at 95 °C for 6 hr. The hot hydrolyzed collagen solution was drained into a vacuum flask and then transferred into a 20-mL scintillation vial. The vial was placed in an oven at 70 °C to condense the solution to <2 mL. The condensate was frozen and then freeze-dried for 48 hr.

BONE COLLAGEN RADIOCARBON BACKGROUND TESTING

The Early Upper Pleistocene fossil Leporidae bones were obtained from excavations in Natural Trap Cave, Wyoming, in 1985 by the University of Kansas, Museum of Natural History (Wang and Martin 1993). The sedimentary stratum of the fauna is underlain by 3 volcanic ash layers, and the uppermost ash has been dated by the fission track method to 110,000 yr BP (Gilbert et al. 1980). This Pleistocene faunal assemblage has bones with excellent preservation due to rapid sedimentation, absence of plant growth, and protection from direct exposure to sun, precipitation, and temperature extremes. Bones from this stratum have undetectable levels of original ^{14}C, so they are used for evaluating background ^{14}C levels and contamination during collagen purification in laboratories that submit collagen samples for dating to the University of California-Irvine AMS Laboratory. We obtained relatively high collagen concentrations (6.5%), and the gelatinized freeze-dried filtrate had a light amber color. This collagen contains 37.87% organic carbon by weight (wt% C), and its atomic C:N ratio is 3.29 (Table 1), which is close to the stoichiometric C:N ratio of 3.21 of modern collagen (Ambrose 1993). Because this cave did not support significant plant growth, the sedimentary matrix would not have substantial amounts of humified organic matter. Therefore, we did not expect significant age differences between the Py-V and Py-R fractions, and we analyzed only the bulk collagen and Py-R fractions. After removing the Py-V fraction of a few aliquots of Leporidae collagen, charred collagen (Py-R) and bulk collagen were placed into quartz tubes for sealed quartz tube combustion at 800 °C with Cu, CuO granules, and Ag foil. If the assumption of no age difference is correct, then bulk collagen of this fossil Leporidae can also be used as our lab background for routine AMS ^{14}C dating services. The results clearly demonstrate there is little age difference ($<2\ \sigma$) between the total collagen and the Py-R fractions of the Leporidae collagen (Table 1).

The less negative $\delta^{13}C$ value of total collagen suggests that the $\delta^{13}C$ value of the volatile fraction is heavier than that of the residue fraction (Table 1), which is consistent with our previous results on wood and soil organic matter experiments (Wang et al. 2003).

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

Table 1 AMS ^{14}C assays of background of bone collagen of Natural Trap Cave Leporidae. All analyses are replicates of bones that yielded 6.5% collagen. $\delta^{13}C$ is expressed relative to VPDB; wt% C and wt% N are is the percentage of carbon and nitrogen in collagen measured using a Carlo-Erba NC 2500 elemental analyzer interfaced with a Finnegan MAT-252 isotope ratio mass spectrometer. The C:N ratio is the atomic ratio of carbon to nitrogen in collagen, T. coll. is total collagen, and Py-R coll. is pyrolysis-resistant collagen.

^{14}C yr BP T. coll. ± σ	^{14}C yr BP Py-R coll. ± σ	wt% C	wt% N	Atomic C:N	$\delta^{13}C$ (‰) T. collagen	$\delta^{13}C$ (‰) Py-R coll.
42,110 ± 200	42,530 ± 280	37.87	13.44	3.29	–18.9	–21.0
42,680 ± 180	42,400 ± 170					
43,280 ± 160	—					
44,140 ± 190	44,270 ± 160					
45,090 ± 220	—					

HUMAN BONES FROM LATE PREHISTORIC SITES IN ILLINOIS

Four human bone samples were collected at the late prehistoric Hoxie Farm site, Cook County, Illinois, for collagen dating (Bluhm et al. 1990). They were collected from different units likely representing different occupations. Bones of a 12- to 15-yr-old female (CI#382) and a young adult female (CI#383) yielded 2.6% and 3.63% collagen (wt%), and 36% and 29% carbon by weight (wt% C), with atomic C:N ratios of 3.27 and 3.26, respectively. Bone from an older adult male (CI#384) yielded 2.8% collagen with 38% carbon by weight and an atomic C:N ratio of 3.30. Bone from an adult of undetermined sex (CI#388) yielded 2.6% collagen, with 33% carbon, and a C:N ratio of 3.31. All other indicators suggest that although these human bone specimens have lost a substantial proportion of the original collagen; what remains has retained its molecular integrity (Dobberstein et al. 2009). The ^{14}C ages of Py-V or LMW and Py-R or HMW fractions of the individual specimen show statistically identical results (Table 2). It appears that post-burial chemical alteration of collagen by contaminants of a different age is minimal at this late prehistoric site in Illinois.

HUMAN BONES FROM AN EARLY WOODLAND SITE

Two adult human bone specimens (CI#391; CI#392) were collected from different units at the Early Woodland Brugger Mound site, Jo Daviess County, Illinois (Philip Millhouse, personal communication, 2008) for collagen dating. The 2 bone specimens yielded 11.9% and 10.7% collagen, which yielded 38% and 29% carbon by weight with atomic C:N ratios of 3.21 and 3.26, respectively. The ^{14}C ages of the Py-V or LMW and Py-R fractions again do not show any statistical age difference, and the results were considered identical (Table 2). No significant post-burial chemical alteration by contaminants that differed in age from the collagen occurred to the human bone collagen sampled at this Woodland site.

HUMAN BONES FROM ARCHAIC SITES

Human bone samples were collected from 2 Archaic sites, Andrew Farm Gully and Tree Row. Adult (>45 yr) human bone fragments (CI#381) were collected from Andrew's Farm Gully (AFG) in Adams County, Illinois (40°8′55″N, 91°24′36″W) (Nolan and Fishel 2009:440). The burial is situated on colluvial slope along the erosion bank between 2 branches of a creek inside the Mississippi Bluff. The burial site was excavated in 1985, and the bone was sampled in 2004.

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

Table 2 AMS ^{14}C assays of bone collagen from Prehistoric, Woodland, and Archaic sites, Illinois. Weight % of collagen (wt% coll.) is the amount of collagen extracted as a percentage of the starting bone weight.

Sample#	wt% coll.	Collagen description[a]	wt% C	Atomic C:N	δ^{13}C ‰	Death age	^{14}C yr BP Py-V coll. ± σ	^{14}C yr BP Py-R coll. ± σ
Late prehistoric site, Hoxie Farm, Cook County, Illinois								
CI-382	2.60	sticky d. amber	36	3.27	−17.2	12–15 yr	370 ± 60 (ISGS A0926)	400 ± 60 (ISGS A0927)
CI-383	3.63	sticky d. amber	29	3.26	−16.6	25–30 yr	540 ± 65 (ISGS A0928)	530 ± 65 (ISGS A0929)
CI-384	2.80	crystalline d. amber	38	3.30	−16.3	>45 yr	510 ± 65 (ISGS A0930)	520 ± 70 (ISGS A0931)
CI-388	2.60	crystalline l. amber	33	3.31	−14.0	35–45 yr	790 ± 67 (ISGS A0934)	730 ± 67 (ISGS A0935)
Early Woodland site, Brugger Mound, Jo Daviess County, Illinois								
CI-391	11.9	crystalline d. amber	38	3.21	−21.0	Adult	2340 ± 95 (ISGS A0936)	2310 ± 95 (ISGS A0937)
CI-392	10.7	crystalline d. amber	29	3.26	−20.7	Adult	2310 ± 95 (ISGS A0938)	2290 ± 95 (ISGS A0939)
Archaic sites, Andrew's Farm Gully, Adams County and Tree Row, Fulton County, Illinois								
CI-381 AFG	2.58	flaky l. amber	37	3.2	−20.9	>45	8440 ± 150 (ISGS A0924)	7950 ± 150 (ISGS A0925)
CI-394 Tr. Row	N/A	flaky l. amber	26	3.16	−17.1	Adult	5050 ± 125 (ISGS A0940)	4760 ± 120 (ISGS A0941)

[a]d–dark; l–light.

About 100 human burials were excavated from the Archaic period Tree Row mortuary site in Fulton County, Illinois (40°26′00″N, 89°58′20″W) (Nolan and Fishel 2009:450–4). Collagen was extracted in 1995 from Sk 45 (CI#394), an adult (possible) female, interred as part of a multiple burial containing 4 flexed individuals (Sk 45, 46, 50, 51). The burial was deep, and the feature fill was dark relative to surrounding soil.

The Andrew Farm Gully specimen (CI#381) was light amber in color and yielded 2.58% collagen with 37% carbon by weight and a C:N ratio of 3.20. The Tree Row specimen (CI#394) was also light amber in color, and was 26% carbon by weight with an atomic C:N ratio of 3.16 (Table 2). The Py-V fractions of collagen CI#381 and CI#394 are dated 8400 ± 150 (ISGS A0924) and 5050 ± 125 (ISGS A0940) ^{14}C yr BP, whereas the Py-R fractions of collagen CI#381 and CI#394 are dated 7950 ± 150 (ISGS A0925) and 4760 ± 120 (ISGS A0941) ^{14}C yr BP (Table 2). The age differences are >3.3 standard deviations (SD) in CI#381 and 2.4 SD in CI#394. Results indicate that the ^{14}C ages of Py-V collagen compounds are systematically older than their Py-R collagen compounds. The ^{14}C ages of Py-R are the most congruent with the presumed ages of the samples based on their known archaeological associations. This suggests that old organic carbon derived from ancient soils at these Archaic burial sites could have contaminated the low molecular weight collagen compounds, resulting in unreasonably old ^{14}C ages.

DISCUSSION AND CONCLUSIONS

High-temperature Py-V and Py-R compounds of bone collagen are likely to represent predominantly low and high molecular weight collagen compounds, respectively. Because the Py-R fraction resembles the graphitic-like carbon in bone collagen, which is the most resistant organic constituent in bones, it should yield the most reliable ^{14}C dates for bone age dating. The preliminary results show that Py-V and Py-R fractions yield identical ^{14}C dates for recent prehistoric and earlier Wood-

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

land, but slightly different ^{14}C dates for Archaic human bones. These results suggest that in relatively recent archaeological burial sites, human bones are not chemically altered or contaminated by mobile soil organic compounds that differ in ^{14}C content from the collagen.

At the older Archaic sites, the thermally volatile fraction of human bone collagen is apparently contaminated by older, probably humic contaminants. An older age for contaminants seems counterintuitive if one assumes that the humic contaminants that combine with collagen came mainly from soils that formed after burial of the bones. However, because these bones were obtained from human burials, they are likely to have been interred well below the penecontemporary soil surface in soils that were formed before burial. Therefore, the skeletons would have been exposed to older soil organic carbon compounds than bones that were buried by natural sedimentation processes. Experiments by van Klinken and Hedges (1995) show that collagen absorbs humic acids very rapidly, becoming saturated within 4 days. Therefore, intentionally buried human bones would most likely absorb contaminants that were mainly controlled by the age of the labile organic matter in the soil horizon in which they were buried.

Further research is needed to determine if intentionally buried bones differ in age from those of naturally buried bones. Compound-specific analysis (Stafford et al. 1988; Tripp et al. 2006) of collagen in different burial contexts from the same occupation layers should show younger ages for low molecular weight compounds in naturally versus intentionally buried bones. Experiments like those conducted by van Klinken and Hedges (1995) using humic acids that differ in age from the bones should also be performed. If pyrolysis treatment of experimentally contaminated collagen recovers the known age of the collagen then dating of Py-R compounds may provide a simple alternative method to eliminate young or old organic contaminants for ^{14}C dating of bone collagen.

ACKNOWLEDGMENTS

We thank the Keck Carbon Cycle AMS Laboratory of the University of California at Irvine for AMS ^{14}C analysis. We particularly thank Dr John Southon for valuable suggests and comments regarding AMS ^{14}C sample preparation from our lab. We also thank Keith C Hackley, Donald A Keefer, who reviewed the manuscript, and the comments and suggestions made by Dr Nadine Tisnerat-Laborde, which improved the final version of the manuscript. We also thank Dr Larry Martin, who provided fossil Leporidae bones for collagen ^{14}C background studies.

REFERENCES

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science* 17(4):431–51.

Ambrose SH, Norr L. 1993. Isotopic composition of dietary protein and energy versus bone collagen and apatite: purified diet growth experiments. In: Lambert J, Grupe G, editors. *Molecular Archaeology of Prehistoric Human Bone*. Berlin: Springer. p 1–37.

Ambrose SH. 1993. Isotopic analysis: methodological and interpretive considerations. In: Sandford MK, editor. *Investigations of Ancient Human Tissue: Chemical Analyses in Anthropology*. New York: Gordon and Breach Scientific. p 59–130.

Bluhm E, O'Brien P, Wenner DJ. 1990. Hoxie Farm and Huber: two Upper Mississippian archaeological sites in Cook County, Illinois. In: Brown JA, O'Brien P, editors. *At the Edge of Prehistory: Huber Phase Archaeology in the Chicago Area*. Kampsville: Center for American Archaeology. p 1–190.

Bronk Ramsey C, Higham TFG, Bowles A, Hedges REM. 2004. Improvements to the pretreatment of bone at Oxford. *Radiocarbon* 46(1):155–63.

Brown TA, Nelson DE, Vogel JS, Southon JR. 1988. Improved collagen extraction by modified Longin method. *Radiocarbon* 30(2):171–7.

Collins MJ, Nielsen-Marsh CM, Hiller J, Smith CI, Roberts JP, Prigodich RV, Wess TJ, Csapò J, Millard AR, Turner-Walker G. 2002. The survival of organic matter in bone: a review. *Archaeometry* 44(3):383–94.

DeNiro MJ, Weiner S. 1988. Use of collagenase to purify collagen from prehistoric bones for stable isotopic analysis. *Geochimica et Cosmochimica Acta* 52(10): 2425–31.

Dobberstein RC, Collins MJ, Craig OE, Taylor G, Penk-

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press

man KEH, Ritz-Timme S. 2009. Archaeological collagen: Why worry about collagen diagenesis? *Archaeological and Anthropological Sciences* 1(1):31–42.

Gilbert BM, Pearsall D, Boellstorff J. 1980. Post-Sangamon record of volcanism and climatic change at Natural Trap Cave, Wyoming. In: Abstracts and Program, 6th Biennial Meeting, American Quaternary Association. p 26.

Hedges REM, Law IA, Bronk Ramsey C, Housley RA. 1989. The Oxford accelerator mass spectrometry facility: technical developments in routine dating. *Archaeometry* 31(2):99–113.

Higham TFG, Jacobi RM, Bronk Ramsey C. 2006. AMS radiocarbon dating of ancient bone using ultrafiltration. *Radiocarbon* 48(2):179–95.

Longin R. 1971. New method of collagen extraction for radiocarbon dating. *Nature* 230(5292):241–2.

Minami M, Muto H, Nakamura T. 2004. Chemical techniques to extract organic fractions from fossil bones for accurate ^{14}C dating. *Nuclear Instrumental Methods in Physics Research B* 223–224:302–7.

Nelson DE. 1991. A new method for carbon isotopic analysis of protein. *Science* 251(4993):552–4.

Nolan DJ, Fishel L. 2009. Archaic cultural variation and lifeways in west-central Illinois. In: Emerson TE, McElrath DL, Fortier AC, editors. *Archaic Societies: Diversity and Complexity Across the Midcontinent*. Albany: State University of New York Press. p 401–90.

Southon JR. 2007. Graphite reactor memory—where is it from and how to minimize it? *Nuclear Instruments and Methods in Physics Research B* 259(1):288–92.

Stafford Jr TW, Jull AJT, Brendel K, Duhamel RC, Donahue DJ. 1987. Study of bone radiocarbon dating accuracy at the University of Arizona NSF accelerator facility for radioisotope analysis. *Radiocarbon* 29(1):24–44.

Stafford TW, Brendel K, Duhamel RC. 1988. Radiocarbon, ^{13}C, and ^{15}N analysis of fossil bone: removal of humates with XAD-2 resin. *Geochimica et Cosmochimica Acta* 52(8):2257–67.

Stafford TW, Hare PE, Currie L, Jull AJT, Donahue DJ. 1991. Accelerator radiocarbon dating at the molecular level. *Journal of Archaeological Science* 18(1):35–72.

Tisnerat-Laborde N, Valladas H, Kaltnecker E, Arnold M. 2003. AMS radiocarbon dating of bones at LSCE. *Radiocarbon* 45(3):409–19.

Tripp JA, McCullagh JSO, Hedges REM. 2006. Preparative separation of underivatized amino acids for compound-specific stable isotope analysis and radiocarbon dating of hydrolyzed bone collage. *Journal of Separation Science* 29(1):41–8.

van Klinken G. 1999. Bone quality indicators for paleodietary and radiocarbon measurements. *Journal of Archaeological Science* 26(6):687–95.

van Klinken GJ, Hedges REM. 1995. Experiments on collagen-humic interactions: speed of humic uptake, and effects of diverse chemical treatments. *Journal of Archaeological Science* 22(2):263–70.

van Klinken GJ, Mook WG. 1990. Preparative high-performance liquid chromatographic separation of individual amino acids derived from fossil bone. *Radiocarbon* 32(2):155–64.

van Klinken GJ, Bowles AD, Hedges REM. 1994. Radiocarbon dating of peptides isolated from contaminated fossil bone collagen by collagenase digestion by reversed-phase chromatography. *Geochimica et Cosmochimica Acta* 58(11):2543–51.

Wang H, Hackley KC, Panno SV, Coleman DD, Liu JCL, Brown J. 2003. Pyrolysis-combustion ^{14}C dating of soil organic matter. *Quaternary Research* 60(3):348–55.

Wang X, Martin LD. 1993. Natural Trap Cave. *National Geographic Research and Exploration* 9:422–35.

https://doi.org/10.1017/S0033822200045100 Published online by Cambridge University Press