[RADIOCARBON, VOL 28, NO. 2A, 1986, P 539–546]

USE OF AMS ^{14}C ANALYSIS IN THE STUDY OF PROBLEMS IN ASPARTIC ACID RACEMIZATION-DEDUCED AGE ESTIMATES ON BONE

PAUL ENNIS*, E A NOLTMANN*, P E HARE**,
P J SLOTA, Jr, L A PAYEN, C A PRIOR, and R E TAYLOR†

ABSTRACT. Major discordances between AMS ^{14}C- and aspartic acid racemization (AAR)-deduced age estimates on bone samples have led to an examination of factors other than time and temperature that can fundamentally influence the degree of racemization observed in fossil bone. Our studies support previous suggestions that for many bone samples the chemical state of amino acids must be routinely considered if AAR-deduced age estimates are to be used to make meaningful chronologic inferences.

INTRODUCTION

The amino acid racemization (AAR) method of dating bone is based on the fact that amino acids present in the proteins of most living organisms are composed only of the L-enantiomer form. Over time, however, L-amino acids undergo slow racemization producing the corresponding D-amino acids. Fossil bone, along with a wide spectrum of other fossil materials, has been found to contain both L- and D-amino acids with the D/L ratios typically increasing with the diagenetic age of a sample. The fundamental studies of amino acids in fossil materials were initiated by Abelson (1954) and continued by Hare (1969, 1974a,b) who found bone to be less than an ideal material with which to work because of its porosity and the potential effects of leaching by ground water.

Beginning in the early 1970s, Bada and co-workers renewed interest in the AAR method particularly applied to bone. Bada proposed that the method had several advantages over the ^{14}C method including significantly smaller sample size requirements and an extended age range (Bada & Protsch, 1973). Due in part to the difficulty in establishing the temperature dependency of isoleucine, Bada turned to the use of aspartic acid. Among other things, this amino acid has one of the fastest rates of racemization of any of the stable amino acids. At 20°C in bone, the "half-life" (*ie,* the time it takes the L- to D-ratio to reach 0.33) of aspartic acid is ca 15,000 years (Bada & Schroeder, 1975).

CALIBRATION METHOD

Since racemization is a chemical reaction, a fundamental factor influencing the racemization rate is environmental temperature. Bada and Protsch (1973) described a "calibration method" of evaluating the *in situ* average temperature to which a bone had been exposed by measuring the degree of racemization of aspartic acid in the total hydrolysate of a *known age*—usually ^{14}C dated—bone. They suggested that this known age bone could serve as a calibration of the average temperature experienced by all bone samples from the same site or region. Using this aspartic acid D/L ratio and the known age of the calibration sample, an apparent *in situ* first

* Department of Biochemistry, University of California, Riverside 92521
** Geophysical Laboratory, Carnegie Institute of Washington 20008
† Radiocarbon Laboratory, University of California, Riverside 92521

order rate constant (k_{asp}) could be calculated. An AAR-deduced age could then be calculated for other bones from the same temperature regime using this calculated k_{asp} value and the relationship expressed in equation 1:

$$t(yrs) = \frac{\ln\left(\frac{1 + D/L}{1 - D/L}\right) - \ln\left(\frac{1 + D/L}{1 - D/L}\right)_{t=0}}{2k_{asp}} \quad (1)$$

where the D/L ratio in the first term is that of a bone sample of unknown age and the D/L ratio in the second term (t = 0) is that of a modern bone carried through the same analytical procedure as the bone sample to be dated. Table 1, part A illustrates the method employed using the first two published aspartic acid AAR age estimates on bone.

Up to 1981, Bada and co-workers had published aspartic acid AAR data which in 20 instances permitted a direct comparison between the AAR-deduced age of a bone sample using the calibration procedure and a finite ^{14}C age determination (references in Bada, 1985). Almost all of these samples were from Old World archaeologic or paleontologic contexts. With one major exception, the differences between the ^{14}C- and racemization-deduced ages ranged from ca 100 to 5000 years with an average difference of ca 1500 years. Because of this reasonable concordance and the assertion that temperatures calculated using the k_{asp} values compare favorably with current average air temperatures of the sites from which the samples were derived, Bada argued that for non-contaminated bone samples

TABLE 1
Calculation of amino acid racemization age estimates on bone by the Bada/Protsch calibration method

Site	Level/ description provenience	^{14}C age (yr BP)	D/L asp	k_{asp} (yr^{-1})	AAR age (yr BP)
	A. *Illustration of method* (Bada & Protsch, 1973)				
Olduvai Gorge	Naisiusiu Beds	17,500 ± 1000	0.32	1.48×10^{-5}	Calibration
Olduvai Gorge	Ndutu Beds	—	0.72	1.48×10^{-5}	56,000 ± 3500
Lake Eyasi	Eyasi I Hominid	—	ca 0.5	1.48×10^{-5}	Ca 34,000
	B. *New World example* (Bada, Schroeder & Carter, 1974)				
Laguna	—	17,150 ± 1470	0.25	1.08×10^{-5}	Calibration
Del Mar	—	—	0.53	1.08×10^{-5}	48,000
Los Angeles	—	23,600	0.35	1.08×10^{-5}	26,000
	C. *Revised example*				
Laguna	—	5100 ± 500*	0.25	3.64×10^{-5}	Calibration†
Del Mar	—	(5400 ± 120)*	0.53	3.64×10^{-5}	14,300
Los Angeles	—	(3560 ± 220)**	0.35	3.64×10^{-5}	8100

* Bada *et al,* 1984
** Taylor *et al,* 1985
† Bada (1985a) has suggested a k_{asp} value of $6.0 \pm 2 \times 10^{-5}$ yr^{-1} for skeletons from this area in which the preservation of the amino acids is poor and as 1.5×10^{-5} yr^{-1} where the preservation is good to excellent.

which have not been exposed to anamolous heating, the only two critical variables that affect amino acid racemization in bone were time and environmental temperature (Bada, 1974).

AMS ^{14}C COMPARISONS

While AAR-deduced ages on bone from a number of Old World localities were apparently acceptable, the application of the AAR method to New World human skeletal samples yielded several disputed age assignments. Most notable was an aspartic acid AAR age of ca 70,000 years assigned to a morphologically fully modern human skeleton from Sunnyvale, California (Bada & Helfman, 1975). Another controversial age estimate involved that assigned to the Del Mar skeleton from the San Diego region of California (Bada, Schroeder & Carter, 1974). Table 1, part B illustrates the Bada/Protsch method of deriving these AAR-deduced ages (Bada & Protsch, 1973). The calibration value for the Del Mar example was based on a ^{14}C analysis by conventional decay counting of the organic fraction of a skeleton from Laguna Beach, California. Using the original Laguna calibration value, both the Del Mar and several other skeletons, including the Los Angeles (Baldwin Hills) human skeleton, were assigned a Pleistocene age. In the case of the Los Angeles human skeleton, a ^{14}C value by decay counting on an amino acid fraction was obtained which initially supported the AAR-deduced age estimate (Berger *et al,* 1971).

With the advent of AMS ^{14}C analysis, a re-evaluation of the accuracy of AAR age estimates was made by direct dating of various organic fractions of the relevant bones (Taylor, 1983; Taylor *et al,* 1983; Taylor, Payen & Slota, 1984; Stafford *et al,* 1984; Bada *et al,* 1984; Taylor *et al,* 1985). In each case, it was determined that the original AAR-deduced age was significantly in excess of that indicated by the AMS ^{14}C value—by as much as an order of magnitude in some cases. As a result of these AMS ^{14}C measurements, there is now no directly dated human skeleton from North America with well-established ^{14}C ages in excess of 11,000 yr BP. This includes the Laguna skeleton which had originally been used as the calibration sample to infer the AAR age of the Del Mar and Los Angeles skeletons. The revised (AMS) ^{14}C age of the Laguna skeleton is ca 5000 ^{14}C years and the Los Angeles skeleton ca 3500 ^{14}C years. Assuming that the revised AMS ^{14}C age for the Laguna sample is correct and calculating a revised k_{asp} value in the same manner as originally suggested by Bada and Protsch (1973), the revised AAR-inferred ages of both the Del Mar and Los Angeles skeletons using the relationship expressed in equation 1 still appear to be somewhat anomalous (Table 1, part C).

AMINO ACID BIOGEOCHEMISTRY

The major revisions in the previous AAR age assignments based on the AMS ^{14}C values support a view previously expressed by Hare (1974) that some of the original AAR dates on bone were in error by as much as an order of magnitude. Over the last decade, several researchers including Bender (1974), Hare (1974a,b), Williams and Smith (1977), Smith, Williams and Wonnacott (1978), Kessels and Dungworth (1980), Von Endt

https://doi.org/10.1017/S0033822200007694

(1979, 1980) and Matsu'ura and Ueta (1980) have offered alternative views of factors which might be responsible for the anomalous AAR age estimates for bone. Some of their concerns include: 1) the influence of the chemical state of the amino acids on racemization kinetics, 2) the improbability of the requirement that a calibration sample and a sample to be dated have experienced essentially identical temperature histories, 3) the effects of bacterial and other types of contamination, 4) the actual D/L ratio at time zero in the racemization process in different bones, and 5) homogeneity of D/L ratios in fossil bone from the same skeleton or from skeletons with identical age and temperature histories. Our studies to date have been focused on documenting variations in D/L aspartic acid ratios observed in single bone samples and in bone samples of similar age (as measured by their decay counting and AMS ^{14}C values) and temperature history which exhibit variations in their degree of organic preservation as measured by nitrogen and amino acid composition values (*cf* Kessels & Dungworth, 1980).

EXPERIMENTAL DATA

In light of previous reports of inconsistent interlaboratory comparisons of D/L ratios on presumed duplicate samples and the importance of quantifying the reproducibility and accuracy of our work-up procedures for D/L aspartic acid measurements, we examined our analytic and instrumental variability while measuring D/L variability in different organic fractions from the same bone sample. Following upon the observations of Matsu'ura and Ueta (1980), we have been particularly interested in examining D/L aspartic acid ratios in the total, soluble, and insoluble fractions of both modern and fossil bone. Based on data summarized in Table 2, we have determined that our overall *analytic* and *instrumental* variability is typically ca 3% for modern bone and ca 2% for Pleistocene bone. For details of these experiments, see Prior *et al* (in press). With these figures in mind, we have determined that our modern bone exhibits a D/L asp variability of ca 10% between the three organic fractions, while our fossil bone reflects a 60% variation between the soluble and insoluble fractions.

It is important to note that the nitrogen values in Table 2 have been determined from amino acid composition data. Table 3 illustrates the differences between nitrogen (and carbon) values obtained on duplicate sam-

TABLE 2
D/L_{asp} variability in modern and fossil bone samples

	Modern		Fossil*	
	N(%)**	D/L_{asp}†	N(%)	D/L_{asp}†
Total	3.3	.07 ± .003 (5)	.007	.38 ± .009 (4)
Soluble	0.3	.08 ± .002 (5)	.003	.45 ± .012 (4)
Insoluble	2.5	.07 ± .003 (5)	.004	.12 ± .006 (4)

* *Bison occidentalis*, 12 Mile Creek, Kansas, 10,435 ± 260 ^{14}C yr (GX-5812 A; apatite); 10,245 ± 335 ^{14}C yr (GX-5812, gelatin). Ref: Rogers & Martin (1984)
** Nitrogen content determined from amino acid composition data
† Figures in parentheses indicate number of replicate analyses

TABLE 3

Comparisons of carbon and nitrogen composition (Kjeldahl) and amino acid analysis in duplicate samples of the total organic fraction of bone of different ages

Bone sample*	Kjeldahl technique			Amino acid analysis**		
	C(%)	N(%)	C/N	C(%)	N(%)	C/N
Modern	5.83	2.05	2.84	8.4	3.3	2.54
Haverty†	1.40	0.33	4.24	0.87	0.38	2.29
Boomplaas‡	0.62	0.08	7.75	0.0034	0.0012	2.83

* Inorganic carbon removed by 0.5 N HCl treatment until pH < 2

** Nitrogen and carbon concentration calculated based on nitrogen and carbon composition of amino acids measured in samples using the following expression (molecular weight of amino acid) (% N_2 or C in that amino acid) (1×10^{-9} n/nm) (1000 mgs/gm) (no. of nmoles of particular amino acid in sample) corrected for proline which is not measured by technique employed

† Approximate age = 5000 yr

‡ Approximate age = 60,000–80,000 yr

ples of three bones differing in age and nitrogen content by about three orders of magnitude by a standard microanalytic technique (Kjeldahl) and that based on the nitrogen (and carbon) composition of amino acids contained in the sample. For the modern bone sample, the amino acid composition method reports a nitrogen value ca 60% higher than the Kjeldahl method, while for the Haverty bone with a nitrogen content of <1%, the analyses are essentially identical within experimental error. In the Pleistocene age bone, however, the differences become pronounced—the amino acid analyses measuring more than an order of magnitude less than the microanalytic technique.

Table 4 reports measurements on the total D/L aspartic acid ratios exhibited on bones from six human skeletons recovered from the Haverty (Angeles Mesa) site, Los Angeles County, California (Prior *et al,* in press).

TABLE 4

Haverty (Angeles Mesa), Los Angeles skeletal samples: Variations in D/L aspartic acid ratios in bones of similar age and temperature history (Prior *et al,* in press)

Haverty individual	N* (%)	N** (%)	D/L_{asp}† (total)	Apparent AAR age‡	
				1.08×10^{-5} (yr^{-1})	3.64×10^{-5} (yr^{-1})
1§	1.98	1.1	0.12 ± .006 (6)	4700 ± 600	1400 ± 200
5	.33	0.38	0.30 ± .006 (5)	22,000 ± 600	6600 ± 200
4#	.10	0.024	0.32 ± .014 (6)	24,000 ± 1500	7200 ± 500
6	.07	0.0024	0.34 ± .005 (5)	26,000 ± 500	7800 ± 200
2	.04	0.0022	0.48 ± .012 (5)	41,000 ± 1500	12,500 ± 500
3	.13	0.0022	0.49 ± .004 (5)	43,000 ± 500	12,800 ± 200

* Kjeldahl technique

** Amino acid composition technique

† Figures in parentheses indicate number of replicate analyses

‡ Errors reflecting analytic precision only

§ 5350 ± 150 (UCR – 1349A)
5280 ± 180 (UCR – 1349D)

\# 7900 ± 1440 (UCLA – 1924A)
5200 ± 400 (GX – 1140)
4050 ± 100 (UCR – 1568A)

Both the report of the proveniences (S Brooks, R H Brooks & G Kennedy, pers commun) as well as ^{14}C determinations on two of the skeletons support the view that these skeletons were buried at the same time and, thus, would have similar ages as well as similar temperature histories. That they do not have similar preservation histories is suggested by the nitrogen content of the bones obtained both by the Kjeldahl and amino acid composition techniques. Interestingly, the D/L_{asp} values on the total amino acid fraction of the bones range from 0.12 to 0.49 and there appears to be an inverse correlation between the decreasing nitrogen content and the increasing D/L_{asp} values. Using the "old" k_{asp} value for coastal southern California as employed by Bada, Schroeder and Carter (1974; Table 1, part B), the AAR-inferred ages range over almost an order of magnitude. Using the revised k_{asp} values for the same region (Table 1, part C) the range in AAR ages is reduced to ca 10,000 years.

For the Harverty samples, the degree of organic preservation as measured by the organic nitrogen correlates with variability in D/L_{asp} values exhibited. It would seem that meaningful comparisons of D/L_{asp} ratios, particularly those of a calibration sample and a sample to be dated, must be made on chemically comparable proteinaceous materials. Bada (1985a, footnote 1; 1985b, p 261) has recently agreed that this factor must be considered in deriving AAR-inferred age estimates on bone. He suggests that groundwater leaching of the bone as one probable factor in contributing to the differences in the extent of collagen preservation in the series of California prehistoric skeletons for which the anomalous AAR age determinations had been obtained. It might be noted that each of the D/L_{asp} values reported in Table 4 is based on at least five replications of the analyses. In two cases (Harverty individuals 1 and 4), the range in values was a few percent in excess of what we typically observe. We would suggest that the range in analytic precision in D/L_{asp} measurements should be routinely reported when using them to make chronologic inferences. It should also be noted that the errors assigned to the apparent AAR ages reflect analytic precision only and does not reflect any environmental temperature variation effects.

CONCLUSIONS

Based on these data, several general conclusions can be offered: 1) AMS ^{14}C analysis of various organic fractions of bone have been instrumental in supporting a view that time and environmental temperature are not the only major factors influencing the D/L_{asp} ratios observed in fossil bone; 2) an important factor influencing the degree of racemization in bone is the chemical state of the amino acids in the proteinaceous material comprising the bone which, in part, can be reflected in the amino acid nitrogen content of the bone; 3) valid comparisons of D/L ratios in bone made for the purpose of deriving age estimates should be made on chemically comparable organic fractions; 4) if age estimates on the basis of D/L ratios are to be made, analytic precision based on replicate assays should be reported.

ACKNOWLEDGMENTS

This research has been supported by NSF Grants BNS-8211804 and BNS-8408192 (Anthropology Program) with additional funding from the

Center for Social and Behavioral Science Research, University of California, Riverside. The collaboration of R Berger, S Brooks, R H Brooks, D McJunkin and G Kennedy is very much appreciated. This is contribution 85/12 of the Institute of Geophysics and Planetary Physics, University of California, Riverside.

References

Abelson, P H, 1954, Amino acids in fossils: Science, v 119, p 576.
Bada, J L, 1974, Reply to M L Bender: Nature, v 252, p 379–381.
———— 1985a, Aspartic acid racemization ages of California paleoindian skeletons: Am Antiquity, v 50, p 645–647.
———— 1985b, Amino acid racemization dating of fossil bones: Ann Rev Earth Planetary Sci, v 13, p 241–268.
Bada, J L, Gillespie, R, Gowlett, A J and Hedges, R E M, 1984, Accelerator mass spectrometry radiocarbon ages of amino acid extracts from Californian Paleo Indian skeletons: Nature, v 312, p 442–444.
Bada, J L and Helfman, P M, 1975, Amino acid racemization dating of fossil bones: World Archaeology, v 7, p 160–173.
Bada, J L and Protsch, R, 1973, Racemization reaction of aspartic acid and its use in dating fossil bones: Natl Acad Sci Proc, v 70, p 1331–1334.
Bada, J L and Schroeder, R A, 1975, Amino acid racemization reactions and their geochemical implications: Naturwissenschaften, v 62, p 71–79.
Bada, J L, Schroeder, R and Carter, G F, 1974, New evidence for the antiquity of man in North America deduced from aspartic acid racemization: Science, v 184, p 791–793.
Bender, M L, 1974, Reliability of amino acid racemization dating and paleotemperature analysis on bones: Nature, v 252, p 378–379.
Berger, R, Protsch, R, Reynolds, R, Rozaire, R and Sackett, J R, 1971, New radiocarbon dates based on bone collagen of California Paleo Indians: Univ California Archaeol Research Fac Contr, v 12, p 43–49.
Hare, P E, 1969, Geochemistry of proteins, peptides, and amino acids, *in* Eglinton G and Murphy, M T J, eds, Organic geochemistry: methods, and results: New York, Springer-Verlag, p 438–463.
———— 1974a, Amino acid dating—a history and an evaluation: MASCA Newsletter, v 10, p 4–7.
———— 1974b, Amino acid dating of bone—the influence of water: Carnegie Inst Washington Yearbook, v 73, p 576–581.
Kessels, H J and Dungworth, G, 1980, Necessity of reporting amino acid compositions of fossil bones where racemization analysis are used for geochronological applications: inhomogeneities of D/L amino acids in fossil bones, *in* Hare, P E, Hoering, T C, and King, K, Jr, eds, Biogeochemistry of amino acids: New York, John Wiley & Sons, p 527–542.
Matsu'ura, S and Ueta, N, 1980, Fraction dependent variation of aspartic acid racemization age of fossil bone: Nature, v 286, p 883–884.
Prior, C A, Ennis, P J, Noltmann, E A, Hare, P E and Taylor, R E, in press, Variations in D/L aspartic acid ratios in bones of similar age and temperature history: Internatl Archaeometry conf, 24th, Proc: Washington, Smithsonian Inst Press.
Rogers, R K and Martin, L D, 1984, The 12 Mile Creek site: a reinvestigation: Am Antiquity, v 49, p 757–764.
Smith, G G, Williams, K M and Wonnacott, D M, 1978, Factors affecting the rate of racemization of amino acids and their significance to geochronology: Jour Organic Chemistry, v 43, p 1–5.
Stafford, T W, Jr, Jull, A J T, Zabel, T H, Donahue, D J, Duhamel, R C, Brendel, K, Haynes, C V, Brischoff, J L, Payen, L A and Taylor, R E, 1984, Holocene age of the Yuha burial: direct radiocarbon determinations by accelerator mass spectrometry: Nature, v 308, p 446–447.
Taylor, R E, 1983, Non-concordance of radiocarbon and amino acid racemization deduced age estimates on human bone, *in* Stuiver M and Kra, R S, eds, Internatl ^{14}C conf, 11th, Proc: Radiocarbon, v 25, no. 2, p 647–654.
Taylor, R E, Payen, L A, Gerow, B, Donahue, D J, Zabel, T H, Jull, A J T, and Damon, P E, 1983, Middle Holocene age of the Sunnyvale human skeleton: Science, v 220, p 1271–1273.
Taylor, R E, Payen, L A, Prior, C A, Slota, P J, Gillespie, R, Gowlett, J A J, Hedges, R E M, Jull A J T, Zabel, T H, Donahue, D J and Berger, R, 1985, Major revisions in the Pleistocene age assignments for North American human skeletons by C-14 accelerator mass spectrometry: none older than 11,000 C-14 years BP: Am Antiquity, v 50, p 136–140.
Taylor, R E, Payen, L A and Slota, P J, Jr, 1984, Impact of AMS ^{14}C determinations on consid-

erations of the antiquity of *Homo sapiens* in the Western Hemisphere: Nuclear Instruments & Methods, v 233, p 312–317.

Von Endt, D W, 1979, Techniques of amino dating, *in* Humphrey, R and Standford, D, eds, Pre-Llano cultures of the Americas: Washington, Anthropol Soc Washington, p 71–100.

———————— 1980, Protein hydrolysis and amino acid racemization in sized bone, *in* Hare, P E, Hoering T C and King, K Jr, eds, Biogeochemistry and amino acids: New York, John Wiley & Sons, p 297–304.

Williams, K M and Smith, G G, 1977, A critical evaluation of the application of amino acid racemization to geochronology and geothermometry: Origins of Life, v 8, p 91–144.