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Concordance of Collagen-Based Radiocarbon and Aspartic-Acid Racemization Ages

(radiocarbon dating/fossil bones)

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ABSTRACT **By determining the extent of racemization of aspartic acid in a well-dated bone, it is possible to calculate the *in situ* first-order rate constant for the interconversion of the L and D enantiomers of aspartic acid. Collagen-based radiocarbon-dated bones are shown to be suitable samples for use in "calibrating" the racemization reaction. Once the aspartic-acid racemization reaction has been "calibrated" for a site, the reaction can be used to date other bones from the deposit. Ages deduced by this method are in good agreement with radiocarbon ages. These results provide evidence that the aspartic-acid racemization reaction is an important chronological tool for dating bones either too old or too small for radiocarbon dating. As an example of the potential application of the technique for dating fossil man, a piece of Rhodesian Man from Broken Hill, Zambia, was analyzed and tentatively assigned an age of about 110,000 years.**

The amino acids commonly found in living organisms consist mainly of the L-isomers. However, fossil materials have been found to contain D-amino acids, and the proportion of D-amino acids to L-amino acids increases with the age of the fossil (1–9). The reaction responsible for this conversion is termed racemization. Each amino acid (with the exception of glycine) undergoes this process, some much faster than others. For example, in bone the racemization half-life (time required for D:L ratio to reach 0.333) at 20° for aspartic acid is about 15,000 years (9); at the same temperature the reaction for isoleucine has a half-life in excess of 100,000 years (8).

The amino-acid racemization reaction has important applications in anthropology and geochronology. Recent studies have shown that the reaction can be used to date deep-sea sediments (4–6) and fossil bones (7–9). Because of the much slower reaction rate, the racemization reaction can be used to date fossil materials too old for radiocarbon dating. Unfortunately, one limitation of this method is that the racemization reaction is temperature dependent. Thus, in order to date materials using the degree of racemization, some estimate of the temperature history of the region where the fossil was found must be available.

Recently it has been shown (9) that by determining the extent of racemization of aspartic acid in a bone which has been dated by radiocarbon it is possible to calculate the *in situ* first-order rate constant for interconversion of the L-isomers and D-isomers of aspartic acid. Once this "calibration" has been carried out for a site, the racemization reaction can be used to date other bones from the area which are either too old or too small for radiocarbon dating. This "calibration" procedure eliminates the need for evaluating the temperature history of a bone before it can be dated using the amino-acid racemization reaction. The only assumption required, in using this approach, is that the average temperature experienced by the "calibration" sample is representative of the average temperature experienced by other samples from the deposit.

Many radiocarbon dates suitable for calibrating the aspartic-acid racemization reaction have been derived from collagen. However, it is important to show the dependability of collagen dates by comparison with measurements made on charcoal or other organic materials.

In this study we establish the dependability of radiocarbon collagen dates and then show the equivalence of collagen and aspartic-acid racemization ages.

Comparison of charcoal (or other organic materials) and collagen radiocarbon dates

It is desirable to determine the radiocarbon age of bone directly in order to avoid association problems often found in archaeology. Many times, materials found together are not really contemporaneous, and this can lead to fallacious interpretations.

In the course of the last few years, the basic collagen dating method (10) involving the isolation of bone protein by mild acid treatment has been developed. First it was recognized that sodium hydroxide treatment of raw collagen was necessary in order to remove humic-acid contaminants. Furthermore, bones contaminated with natural oil residues were made suitable for age dating by applying a chromatographic separation procedure which isolates only amino acids native to bone (11). In addition, Longin refined the purification of collagen by converting it to gelatine, which results in a much cleaner product (12). For ultimate dependability, a combination of these methods was applied by Protsch to ensure maximum purity of the collagen (13).

Generally, the collagen content of bones decreases with age or unfavorable environmental conditions to such low levels that considerable quantities of bone may be necessary for accurate dating (Table 2). Age estimates based on the radiocarbon content of the inorganic portion of bone, notably carbonate, have been shown to be unreliable due to exchange processes involving ground water carbonates of different specific activity (14).

A number of representative comparisons of radiocarbon ages on collagen and charcoal or other organic materials are listed in Table 1.

Comparison of collagen and aspartic-acid racemization dates

It is important to test the reliability of dates deduced from the aspartic-acid racemization reaction. Therefore, collagen-

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TABLE 1. *Comparison of radiocarbon dates*

Sample	Laboratory no.	Age and material	Collagen date
Human bone plus attached shell bracelets, West Mexico	UCLA-1424A	1830 ± 55 shell	
	UCLA-1424B		1720 ± 130
Indian mummy, Chimney Cave, Nevada	UCLA-689		2500 ± 80 years
	UCLA-690	2510 ± 80 skin	
	UCLA-692	2590 ± 80 cedar mat	
Gazelle of Mentohotep, XI Dynasty, Egypt	UCLA-1696	Historic date: 2030 B.C.	2050 B.C.
Cemetery, Sakkara, IV Dynasty, Egypt	UCLA-1708A		3845 ± 60
	UCLA-1208	3980 ± 60 flax cloth	
	UCLA-1389A	4000 ± 60 grass	
Anzabegovo, Yugoslavia	UCLA-1705A	6950 ± 80 charcoal	
	UCLA-1705C		6700 ± 80
Matjes River, South Africa Layer B	GrN-5887	7050 ± 45 shell	
	UCLA-1746D-2		7380 ± 120
Matjes River, South Africa Layer C	GrN-5872	9580 ± 85 charcoal	
	UCLA-1746B-2		9230 ± 160
	GrN-5886	9450 ± 55 shell	
Matjes River, South Africa Layer D	GrN-5871	10030 ± 55 charcoal	
	UCLA-1746C		10100 ± 190
	GrN-5061	9780 ± 60 shell	
	UCLA-1746A		10120 ± 200
	L-336G	10500 ± 400 charcoal	
La Brea Tar Pits, Pit 3, 3.6 m depth, California	UCLA-1292C		14500 ± 190
	LJ-55	14400 ± 300 wood	
	Y-354B/—355B	14640 ± 115 wood	
Olduvai Gorge, Mid-Naisiusiu Bed, Tanzania	UCLA-1695		17550 ± 1000
	L-?	17000 ± ? ostrich shell	
Fish Hoek, 2.4–2.6 m, South Africa	UCLA-1235	35000 ± 2400 charcoal	
	UCLA-1744		35630 ± 2500
Florisbad, South Africa	UCLA-1745B		38680 ± 2000
	UCLA-1745C	38550 ± 3800 wood	
Border Cave, South Africa	Pta-424	35700 ± 1100 charcoal	
	UCLA-1754C		32400 ± 2500
	Pta-433	36100 ± 900 charcoal	
	UCLA-1754D		34800 ± 2500

based radiocarbon-dated bones from several sites were analyzed for the extent of racemization of aspartic acid. The D:L aspartic-acid ratios in the fossil bones were determined, using the procedures described by Bada and Protsch (9). One bone from each site was chosen as a "calibration" sample. Based on this "calibration," other bones from the site were dated and these ages compared with the collagen-derived radiocarbon dates. The results of these correlations are shown in Table 2.

Discussion

It is evident from Table 1 that there is good correspondence between the radiocarbon ages deduced from the charcoal or other organic materials and collagen. These results demonstrate the reliability of collagen-based radiocarbon dates.

Table 2 shows that there is excellent agreement between ages deduced from collagen-based radiocarbon and aspartic-acid racemization. So far, aspartic-acid racemization does not approach the accuracy of radiocarbon determinations for derivation of ages. However, the aspartic-acid racemization method has two advantages over collagen radiocarbon dating in that it requires only gram amounts of bone and analyses can be completed within a few days. By using expendable faunal material to "calibrate" the racemization reaction for a site, only a few grams of valuable hominid material are needed for aspartic-acid racemization dating. For example, we have analyzed a small piece (about 1 g) of human femur from Abri Jumeau, Souzac, Dordogne, France. The femur was the only human material found at the site and was too small for radiocarbon dating. The D:L aspartic-acid ratio was found to be 0.123 and, using the Abri Pataud as a calibration site, this ratio corresponds to an age of about 16,000 years. This age is in good agreement with the associated cultural material (Magdalenian III/IV) and its relative estimated age (16). These results show that even in the time interval datable by radiocarbon, the aspartic-acid racemization reaction should provide an important chronological tool in paleoanthropology.

Moreover, aspartic-acid dating exceeds the range of radiocarbon. In fact, the racemization reactions of aspartic acid and other amino acids, such as alanine, which racemize at slower rates (17), may provide a link between radiocarbon and potassium-argon ages. Once a site has been calibrated, it should be possible to directly date [an A.1 date according to Oakley's (18) terminology] samples thought to be too old for radio-

TABLE 2. *Comparison of aspartic-acid racemization and collagen-based radiocarbon ages*

Sample	Radiocarbon laboratory number	Amount (g) used for age determination: Aspartic acid[a]	Amount (g) used for age determination: Radiocarbon	Ratio of D:L aspartic acid	Aspartic acid age[b] (yr)	Collagen radiocarbon age (yr)
Modern bovine bone	—	—	—	0.07	—	—
Naivasha, Kenya	UCLA 1741[c]	10	205	0.153	$k = 7.74 \times 10^{-6}$ yr^{-1}	10,850 ± 330
Elmenteita, Kenya (15 km from Naivasha)	UCLA 1757	10	210	0.134	8,400	7,410 ± 160
Sarab, Iran	UCLA 1714A[c]	6	480	0.155	$k = 1.14 \times 10^{-5}$ yr^{-1}	7,620 ± 70
Asiab, Iran (located close to Sarab)	UCLA 1714C	6.2	392	0.167	8,600	8,700 ± 100
Matjes River, South Africa	UCLA 1746A[c]	5	382	0.153	$k = 8.15 \times 10^{-6}$ yr^{-1}	10,120 ± 200
	UCLA 1746D	5	590	0.140	8,000	7,380 ± 120
Mumbwa, Zambia	UCLA 1750[c]	10	256	0.166	$k = 4.96 \times 10^{-6}$ yr^{-1}	19,780 ± 130
	UCLA 1750B	10	242	0.158	18,300	18,000 ± 370
Szeleta Cave, Hungary	GrN 5130[c]	7.7	—	0.141	$k = 2.19 \times 10^{-6}$ yr^{-1}	32,620 ± 400
	GrN 6058	8	—	0.157	40,000	43,000 ± 1100
Abri Pataud, Les Eyzies, Dordogne, France	GrN 4721[c]	8.5	—	0.148	$k = 3.41 \times 10^{-6}$ yr^{-1}	23,010 ± 170
	GrN 4719	7.3	—	0.178	32,100	33,260 ± 425
Palagawra, Iraq	UCLA 1714D[c]	7	521	0.370	$k = 2.34 \times 10^{-5}$ yr^{-1}	13,600 ± 460
	UCLA 1703A	5.5	462	0.402	15,200	14,350 ± 280
Muleta Cave, Mallorca, Spain	UCLA 1704D[c]	10	1128	0.273	$k = 1.25 \times 10^{-5}$ yr^{-1}	16,850 ± 200
	UCLA 1704E	10	1420	0.293	18,600	18,980 ± 200
	UCLA 1704A	10	1281	0.455	33,700	28,600 ± 600
Olduvai Gorge, Tanzania	UCLA 1695[c]	10	412	0.32	$k = 1.48 \times 10^{-5}$ yr^{-1}	17,550 ± 1000
	Lower Naisiusiu Beds[d]	8	—	0.57	39,000	—
	L-?[e]	10	—	0.72	56,000	>29,000
Murray Springs, Arizona	A-905A,B[c]	6.7	—	0.33	$k = 4.84 \times 10^{-5}$ yr^{-1}	5,640 ± 160[f]
	A-805	8.4	—	0.52	10,500 yrs	11,230 ± 340[f]

[a] This is the amount of bone which was hydrolyzed in 6 M HCl. In general, only a small portion of the isolated aspartic acid was used for the enantiomeric determination.

[b] Ages or k_{asp} value of "calibration" sample calculated from the equation [taken from ref. (9)].

$$\text{Age (yrs)} = \left(\ln\left[\frac{1 + \text{D:L}}{1 - \text{D:L}}\right] - 0.14\right) \Big/ 2 \cdot k_{asp}$$

where D:L is the aspartic-acid enantiomeric ratio in the bone.

[c] Sample used for site calibration.

[d] Sample collected by J.L.B. July, 1973, from stratigraphic section which underlies middle Naisiusiu Beds, from where UCLA 1695 was obtained.

[e] Sample from upper Ndutu Beds, a stratigraphic section which is older than the Naisiusiu Beds (9, 15). Radiocarbon age given in ref. 15.

[f] Radiocarbon ages are not on bone collagen but, rather, on associated charcoal or other organic materials.

carbon dating. An illustration of this application is the date of 56,000 years for a bone from the upper Ndutu Beds in Olduvai Gorge (see Table 2 and ref. 9). Another example is a preliminary date we have obtained for Rhodesian Man from Broken Hill, Zambia. Analysis of a 6.5-g piece (British Museum fragment E793) yielded D:L aspartic acid = 0.55. Using the Mumbwa§ site as a calibration for Broken Hill gives an age of about 110,000 years. This is the first direct indication of the age of this important hominid fossil, although there has been much speculation based on the associated cultural and faunal materials (ref. 20 and references therein). Our preliminary results clearly demonstrate the great antiquity of this hominid. A more detailed discussion of the archeological and anthropological implications of the Broken Hill results will be given elsewhere.

§ Actually, Mumbwa is located about 150 km from Broken Hill, but the present-day climates of the two locations are identical (19), so the calibration for the two sites should be the same.

There are some precautions which should be mentioned concerning the amino-acid racemization dating technique. Unlike radiocarbon dating, which uses an immutable radioactive decay process, racemization dating uses a chemical change which inherently is temperature dependent. Thus, it is important that the average temperature experienced by the "calibration" sample is representative of the average temperature experienced by the other bones which are to be dated using this calibration. In this respect, a bone of postglacial age would not be a suitable "calibration" sample for use in dating glacial age bones. For example, a bone from Muleta Cave with a radiocarbon age of 8,570 ± 350 years (UCLA 1704C) and a D:L aspartic-acid ratio of 0.214 gives a k_{asp} value of 1.72×10^{-5} year^{-1}. If this sample were used as a "calibration" sample for Muleta Cave, the age of UCLA 1704E would be 13,500 years, which is much younger than both the radiocarbon age and the age derived using UCLA 1704D as the calibration sample. This difference arises because UCLA 1704C has been exposed to only postglacial conditions, while UCLA 1704D has had nearly equal exposure to both glacial and postglacial temperatures.

It is important to note that using UCLA 1704D as a "calibration" sample for Muleta Cave gives an age for UCLA 1704A in reasonable agreement with the radiocarbon age of this bone. This result suggests that bones with ages in the region of about 15,000 to about 20,000 years can be used to date samples which are much older. Evidently the average temperature experienced by a bone about 15,000 to about 20,000 years old is similar to the average temperature experienced by older bones. This is not surprising, since oxygen isotopic evidence (21, 22) and pollen profiles (23) indicate that glacial conditions prevailed over the earth from about 9,000 to 75,000 years ago, with some partially warm intervals occurring between 30,000 to 35,000 and 42,000 to 48,000 years ago.

There is another important aspect to amino-acid racemization dating which must not be overlooked. Since the amino-acid racemization reaction is temperature sensitive, bones which have been heated will produce spurious results. For example, bones of hominid 4 from Gamble's Cave II in East Africa yielded a collagen radiocarbon date of 8210 ± 260 years (UCLA 1756). The extent of racemization of aspartic acid gave an unrealistically high age in the tens of thousands of years using as a "calibration" Naivasha, a site which is only a few kilometers away. Close inspection of the Gamble's Cave bone revealed traces of charcoal, indicating that the bone had been burnt. This exposure to elevated temperatures produced a high degree of racemization. Additional work (J. L. Bada, in preparation) with burnt bones has shown that they possess a distinctive amino-acid pattern and that such suspect bones can be easily distinguished.

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