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RADIOCARBON DATING OF PLEISTOCENE BONE: TOWARD CRITERIA FOR THE SELECTION OF SAMPLES

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ABSTRACT. Amino acid composition data and stable isotope ratios (^{14}N, D, and ^{13}C) are being evaluated as sources of information to indicate the presence of non-indigenous organics in bone samples intended for ^{14}C analyses. The study is being conducted in the context of the planned measurement of Pleistocene bone samples by a high energy mass spectrometric ^{14}C detection system.

A list of the problems associated with ^{14}C dating of bone begins with the fact that bone tissue is primarily composed of inorganic constituents. While there are variations between different species as well as between different bones and bone structures within the same animal, generally speaking, the inorganic fraction constitutes about 70 to 80 percent of fresh, dry compact bone (Herring, 1972). Using the most abundant fraction in early ^{14}C analyses was reasonable, but it was quickly found that determinations on whole bone samples, which includes an inorganic fraction, usually yielded discordant values (Libby, 1955, p 45; Olson, ms, p 61-65). Experiments using the apatite component in bone have also yielded inconsistent results. Under certain conditions, ^{14}C determinations on this fraction seem to yield accurate values (Haynes, 1968). However, geochemical and mineralogic studies revealed a number of mechanisms that can significantly alter the carbon isotope values in apatite structures (Hassan, ms; Hassan, Termine, and Haynes, 1977). Such obstacles may not completely exclude use for dating bone as other researchers are reporting more encouraging results (Haas and Banewicz, 1980).

The heretofore questionable reliability of most ^{14}C determinations on inorganic components of bone has led this laboratory to concentrate on one or more of the organic fractions (Taylor, 1978; Taylor and Slota, 1979). In modern, dry, fat-free bone, over 90 percent of organics exist in the form of protein collagen with the remaining non-collagenous proteins composed of a complex assortment of, as yet, incompletely characterized organic substances (Hare, 1979). In living bone, collagen is deposited in a dense framework of laminated fibers with a highly specific physical structure (Miller, 1972). In the case of bone derived from most geologic or archaeologic contexts, it appears that one is not, strictly speaking, dealing with unaltered collagen (Tuross and Hare, 1978). Thus, it is probably more appropriate to use the term, "collagen-derived," when referring to this particular organic component. Emphasis should also be placed on the danger of using the terms "collagen" or "collagen-derived" simply as synonyms for the acid soluble, insoluble, or undissolved fractions (Olsson and others, 1974, p 172). It should not be automatically assumed that any of these preparations, especially in Pleistocene age bone, will necessarily contain only, or even primarily, collagen-derived organics.

Several procedures have been employed to prepare various organic fractions of bone for ^{14}C analyses. Probably the most extensive published corpus of data on the relative merits of a number of chemical pretreatment approaches is available as a result of the studies of Ingrid Olsson and her collaborators (Olsson, 1974; Olsson and others, 1974; El Daoushy, Olsson, and Oro, 1978). All chemical pretreatments assume an initial physical examination of the external surface and fracture zones to insure the removal of preservatives, micro-organisms, and humic materials, rootlets, and other non-bone organic fragments (*cf* Hassan, ms; Hassan and Ortner, 1977). Chemical processing involves, initially, the elimination of inorganic carbonates. Both EDTA and HCl have been used for this purpose (eg, Berger, Horney, and Libby, 1964; Olsson and others, 1974). However, fear of contamination with "old" carbon from the EDTA treatment has been expressed (Hassan, ms, p 98; Olsson, 1974, p 174). Such a problem can apparently be minimized or eliminated with sufficient washing (El Daoushy, Olsson, and Oro, 1978, p 213-214). Further preparations have included conversion to gelatin (eg, Sinex and Faris, 1959; Longin, 1971; Protsch, ms), treatment with NaOH to remove humates and other base soluble fractions (eg, Haynes, 1967; Olsson and others, 1974), separation of total amino acids or a single amino acid (eg, Ho, Marcus, and Berger, 1969; Taylor and Slota, 1979) and a combination of these methods (eg, Håkansson, 1976).

In every case, the goal of any pretreatment procedure is to isolate one or more "uncontaminated" organic fraction(s) which is (are) unambiguously indigenous to the original bone sample. Final conclusions concerning the reliability of such values are generally based on the degree of concordance in ^{14}C determinations on different organic fractions of the same sample and/or a comparison of the bone-derived ^{14}C value(s) with those on less problematic samples, such as charcoal assumed to be in direct association with the bone(s). There are a number of situations, especially with Pleistocene samples, in which comparative values cannot be obtained, either because of sample size limitations and/or lack of associated organics. In such cases, other strategies must be developed.

One approach is to develop specific geochemical indices to permit the characterization of autochthonous organics in bone samples and, thus, discriminate between indigenous organics and "contaminants" presumed to contain organic compounds of varying ^{14}C activity that can be applied even when sample size is limited. The geochemical theory behind this study is that organics not indigenous to a bone will manifest distinctive amino-acid composition and stable isotope patterns. The ultimate goal is to establish a quantitative or semi-quantitative criterion to determine the suitability of individual bone samples for ^{14}C analyses. This paper will present initial preliminary data primarily focusing on sample preparation problems in the determination of amino acid composition and stable isotope characterization measurements on modern, Holocene, and Pleistocene bone samples. It is primarily a progress report to permit comparisons with similar studies being conducted at other

laboratories. The context of this particular study is the development of high energy mass spectrometric methods of ^{14}C analyses with samples of 10 to 50mg of carbon with expectations of obtaining ^{14}C ages up to a little over 100,000 years (Gove, 1978). Our projected measurements will be obtained on the Lawrence Berkeley cyclotron direct counting system being readied for routine operation by a group headed by Richard Muller and Terry S Mast (Muller, 1979; Stephenson, Mast, and Muller, 1979; *cf* Gove, 1978). Although the maximum indicated age value potentially attainable with the direct measurement approach exceeds 10^5 years (Muller, 1977; Muller, Stephenson, and Mast, 1978), Table 1 provides data only to 75,000 years because of present uncertainties concerning the operating characteristics of the Berkeley cyclotron system operating with an external ion source (T S Mast, personal commun). Comparable ^{14}C mass spectrometer measurements are expected also to be available at the University of Arizona facility (P E Damon, personal commun).

The utilization of a direct-counting technique will permit ^{14}C measurements on multiple organic extracts from Pleistocene bone samples and associated samples in which previous sample yield and/or indicated sample age make it extremely difficult or impossible to obtain meaningful ^{14}C values with conventional decay counting. In addition to the significant reduction in sample size, an important practical consideration of using the direct counting technique includes significantly shortened counting times when retaining meaningful statistical errors (Stuiver, 1978).

With the extension of the ^{14}C time scale and almost an order of magnitude reduction in sample size, it is even more important that the magnitude of various "contamination" effects, particularly acute for Pleistocene age materials, be considered. Figure 1 represents a summary of the relationship between the actual and apparent ages in samples to which have been added varying percentages of contemporary carbon. This plot particularly illustrates the effects on the final age value of the addition of ppm levels of modern carbon for samples in the 50,000 to 100,000 years range. For a 75,000 year sample, for example, a 100ppm addition of modern carbon results in approximately a 5000-year error in the final

TABLE 1
Expected counting parameters of Berkeley direct counting ^{14}C system with external ion source*

Sample age (yr)	0 (modern)	10,000	15,000	25,000	50,000	75,000
Total counts	25,000	7400	4100	1200	60	3
Counting error (yr)	—	± 100	± 130	± 240	± 1100	± 5000

* Estimates supplied by T S Mast, Lawrence Berkeley Lab. Calculations assume counting times of 100 min, overall efficiency of 10^{-5}, background of less than 1 count in a 100 min counting interval, and sample size of 50mg of carbon. Counting error based on statistics only.

measured age. A more probable situation would be the addition of carbon differing in age from the original sample of up to several tens of thousands of years. Olsson (1974, p 313) has provided a plot of the effects of introducing varying amounts of sample materials with age differences of up to 25,000 years between sample and contaminant. For example, she shows that errors in the range of 800 to 1600 years can result from the addition of from 1 to 2 percent of younger materials with a 20,000-year difference in age. Extrapolating her data also shows that a 5 percent addition of a 75,000-year-old fraction to a sample with an actual age of 10^5 years would reduce the resultant age value by about 5700 years. Parenthetically, it might be noted that in this time range, contamination with ^{14}C of infinite age would pose a much less serious problem. Each percent of addition of "dead" ^{14}C would add approximately 80 years to the final result.

In order to develop independent "contamination indices" for bone, it is necessary to be able to distinguish between the effects of diagenetic processes as opposed to the introduction of non-autochthonous organics at an appropriate level of discrimination. Several studies have investigated the possibility of using amino acid composition as a means of characterizing indigenous organics in bone samples (Hassan, ms; Hassan and Hare, 1978). The "collagen pattern" in modern mammalian bone is characterized by a high glycine (GLY) content, relatively high proline (PRO) content, and the presence of hydroxyproline (HPR) and hydroxylyxine (HYL). Except for a limited distribution in some plant

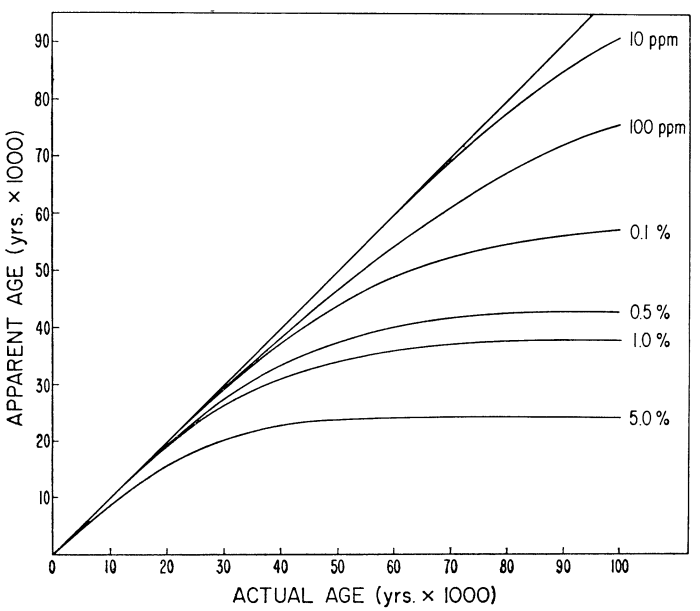

Fig 1. Actual and apparent ages in samples to which have been added varying percentages of contemporary carbon.

extracts, hydroxyproline has been found only in the hydrolyses products of collagen and its diagenetic derivitives. By contrast, the amino acid signature of the non-collagenous protein fraction in modern bone contains no HPR or HLY and has relatively less GLY and PRO and more glutamic acid (GLU) and aspartic acid (ASP). The amino acid composition of modern bone reflects both the collagen and non-collagen proteins, but the overall bone amino acid signature resembles collagen since that is the dominant fraction (Hare, 1979).

Table 2 lists the nitrogen and amino acid composition of a series of modern, archaeologic and paleontologic bone samples of varying age. The modern sample exhibits high GLY/GLU and PRO/GLU as well as characteristic HPR and HLY concentrations. Interestingly, this same pattern is present in the 80,000-year-old bone sample even though the organic composition as measured by the nitrogen content has decreased by 50 percent. All except two of the archaeologic samples exhibit a collagen pattern. By contrast, the amino acid data on the XIIth dynasty Egyptian and CA-Imp-109 bones reflect a non-collagen profile. Some workers have suggested the absence of the collagen signature indicates the presence of contamination (Wyckoff, 1972). Others, however, have suggested that, in cases where the organic content is extremely low (below 0.4 to 0.1 percent nitrogen), the amino acid pattern may reflect the indigenous non-collagenous protein residue rather than the presence of contamination (Hare, 1979).

As a means of providing analytical data to complement the amino acid composition measurements, we are examining stable isotope values on bone samples to determine the feasibility of using such data to monitor the transport of organics from the surrounding soil matrix into bone. Our study involves the examination of hydrogen, nitrogen, as well as carbon isotopic patterns. Because of unresolved gas purity problems which makes the interpretation of the hydrogen isotope values difficult (G Rau, personal commun), tables 3 and 4 present only the nitrogen and carbon isotope values obtained to date. The principal purpose of the initial measurements on contemporary bone (table 3) is to examine the effects of species, environmental, and dietary variability on isotope ratios and evaluate problems in sample pretreatment methods. The purpose of the measurements on archaeologic and paleontologic samples (table 4) is to identify diagenetic processes affecting isotopic composition.

Table 3 lists the analytical data on modern mammalian bone samples from natural and captive (zoo) environments. Samples of bone from herbivorous, omnivorous, and carnivorous mammals have been included. Bones from the non-captive animals were collected in their native habitats and were maintained at temperatures below 0°C during storage. Bones from the captive or zoo environment were maintained below 0°C between the death of the animal and the defleshing process. Bones were defleshed by a method which does not involve the use of any solvent or other chemical agent which might add extraneous carbon.

TABLE 2

Nitrogen content and amino acid composition of dated bone samples*

Locality	Bone type	Approximate age (yr BP)	Nitrogen content (%)	HPR (residues/1000)	HLY (residues/1000)	GLY/GLU	PRO/GLU
	bovine**	modern	4.0	100	4	4.23	1.39
CA-SMA-77 (burial)	human***	2560 ± 150 (UCR-958)†	1.7	—	—	4.04	—
Egypt, XXVth dyn (burial)	human**	ca 2500‡	3.7	99	nd	4.30	1.36
CA-SMA-77 (burial)	human***	3060 ± 150 (UCR-960)†	0.4	—	—	4.43	—
CA-SMA-77 (burial)	human***	3200 ± 170 (UCR-954)†	0.2	—	—	3.82	—
Egypt, XIIth dyn (burial)	human**	ca 4000‡	0.2	63	nd	1.60	0.44
CA-Im-109 (burial)	human**	4990 ± 250 (GX- ?)§	0.03	nd	nd	0.89	0.11
San Diego, CA (burial)	human**	ca 6000†	0.3	90	2	4.07	1.34
Wailes Bluff, Maryland	dolphin**	ca 80,000¶	2.0	97	2	4.65	1.69

* Whole bone samples were dissolved and heated in 6N HCl (150°C for 10 min) to hydrolyze the proteins to free amino acids.

** Data taken from Hare, 1979.

*** P E Hare (personal commun). Samples supplied to R E Taylor by B Gerow, Stanford University.

† Age assignment based on associated charcoal and/or marine shell ^{14}C determinations.

‡ Age assignment based on historic association.

§ Age assignment based on apatite ^{14}C determination on bone sample.

¶ Age assignment based on geologic criteria (Pleistocene terrace).

nd = not detected.

The ratios listed in table 3, not in parentheses, were obtained on samples prepared according to procedures outlined in Berger, Horney, and Libby (1964, p 999). Tests performed on these samples, following the apparent completion of the decalcification and hydrolyses process, disclosed the presence of residual inorganic carbonates in amounts exceeding, in some cases, one percent (*cf* Olsson and others, 1974, p 172). To determine the effect of incomplete elimination of the inorganic carbonate on the isotopic ratios, a number of duplicate samples were prepared in a manner to insure the complete removal of the carbonate fraction.

This procedure involved the complete dissolution of the bone matrix in 12N concentrated HCl for at least a 12-hour period. The solution was then cooled to liquid nitrogen temperatures and connected to a high vacuum system. The volatiles were allowed to distill until the sample reached room temperature. This took, on the average, approximately 8 hours. The reaction mixture was then heated in 100°C water bath for no longer than 10 minutes to facilitate the removal of the volatiles and excess HCl. During this time, there was no indication of sublimation of any part of the sample. The reaction product that remained could easily be broken into a fine powder. The results of measurements of the isotopic ratios of these samples are listed in parentheses in tables 3 and 4.

For modern bone, the presence of relatively small amounts of inorganic carbonates does not seem to effect the $\delta^{15}N$ and $\delta^{13}C$ values by more than 1‰. The effect on older archaeologic or paleontologic bone (table 4) seems to be variable. In two cases there is even a significant

TABLE 3

Stable isotope determinations on contemporary mammalian bone

Species	Source*	Diet**	^{15}N*** (‰)	^{13}C† (‰)
Bos taurus (Domestic bull)	N	H	+3.19 (+3.19)	−19.17 (−18.16)
Box taurus (Domestic cow)	N	H	+5.24	−21.39
Equus caballus (Domestic horse)	N	H	+8.78 (+9.06)	−21.97 (−21.05)
Axis axis (Axis deer)	C	H	—	−19.69
Litocranius walleri (Gerinuk)	C	H	—	−19.37
Cavia patagonia (Patagonian Cavy)	C	H	+5.06	−17.07
Lagothrix lagothrica (Wooley monkey)	N	O	—	−16.37
Urocyon cinereoargenceus (Eared fox)	N	C	+5.94	—
Otocyon magalotis (Long-cared fox)	N	C(I)	+11.37	−21.54

* N = non-captive environment; C = captive (zoo) environment
** H = herbivore, O = omnivore, C = carnivore, C(I) = carnivore (insectivore)
*** Expressed with respect to AIR, errors on values ±0.2‰
† Expressed with respect to PBD, errors on values ±0.2‰

variation in the $\delta^{13}C$ values. To maximize the value of the stable isotope data, it seems imperative that rigorous procedures be employed that insure the total removal of the inorganic carbonate fraction during the processing of the samples and to avoid fractionation effects in sample preparation.

Six of the 9 bone samples listed in table 3 are herbivorous mammals, equally divided between those from non-captive and zoo environments. A major species effect on the isotopic values does not seem to be present, but the present unavailability of values on several samples makes it difficult to address this question. Whether the somewhat anomalous $\delta^{13}C$ value for *Cavia patagonia* reflects the presence of inorganic carbonates in the sample or the C_4 plants included in the dietary supplement provided this animal in captivity must await further study (*cf* DeNiro and Epstein, 1978). Interpretation of the data from the omnivorous and carnivorous animals are also difficult due to several seemingly anomalous isotopic ratios. Whether these reflect variations in isotopic composition due to dietary or environmental effects or can be attributed to other factors is currently under study.

Table 4 lists stable isotope measurements on both acid insoluble as well as total amino acid fractions obtained from archaeologic and

TABLE 4

Stable isotope measurements on archaeologic and paleontologic samples

Sample no.	Site	Age (yr BP)	^{15}N† (AI)	^{15}N† (AA)	^{13}C‡ (AI)	^{13}C‡ (AA)
		Archaeologic samples				
—	Gatecliff Shelter, Nevada	(1000±90)*	+6.41	+2.37	−17.69	−18.89
—	Gatecliff Shelter, Nevada	(ca 7000)**	−3.83	+2.30	−17.70	−19.53
UCR-474***	Riverdale, CA	290±100	+3.20	−41.60	−16.86	−18.74
UCR-195	CA-Yol-159 (CA)	940±150	+10.15 (+4.10)	—	−8.87 (−19.69)	—
UCR-118 A	CA-Mad-159	1690±100	—	−11.54	−21.07	−20.23
UCR-118 O	CA-Mad-106	2000±80	+8.31	+14.11	−18.66	−30.23
UCR-144	Ca-SJo-145	2500±200	+9.34	−67.89	−20.55	−20.22
UCR-450 B	CA-SJo-112	2835±140	+5.33	—	−20.37	—
UCR-450 C	CA-SJo-112	2960±140	+7.58	+11.42	−21.22	−19.63
		Paleontologic samples				
UCR-643	Samuel Cave, CA	17,880±900	—	—	−13.08 (−22.14)	—
UCR-381	Potter Creek Cave, CA	8250±330	+3.94	—	−7.37 (−22.14)	—
UCR-331	Silver Creek, Utah	18,150±1000	−7.84 (−7.66)	— —	−18.62 (−19.65)	— —

* Gak-3608, on associated charcoal.
** Sample immediately above Mazama ash.
*** All UCR ^{14}C values on acid insoluble fractions.
† Expressed with respect to AIR, errors on values 0.2‰.
‡ Expressed with respect to PBD, errors on values ±0.2‰.

paleontologic samples. The ratios listed under "AI" (=acid insoluble) in table 4, not in parentheses, were prepared according to the procedures outlined in Berger, Horney, and Libby (1964, p 999). Those values in parentheses were prepared in the manner listed previously to insure the complete removal of the inorganic carbonates. Those values listed under "AA" (=amino acid) were obtained on a total amino acid fraction prepared according to procedures outlined in Bada and others (1979). Archaeologic samples were derived from both a single stratigraphic profile (Gatecliff Shelter, Nevada) and a suite of late Holocene bone samples from a series of sites located within a 100km radius. The variability in isotopic values introduced as a result of variation in sample preparation procedures is also apparent in these values. This is especially evident for samples UCR-195 and UCR-643. Also, the difficulty in interpreting the stable isotope values on the amino acid fractions have led to the suggestion that these values may have been affected by residual amounts of acetic acid remaining in the samples. Thus, no statement concerning their significance can be offered at this time. There is only a hint that there has been a shift in ^{15}N values with age when the Holocene values are compared with oldest archaeologic (oldest Gatecliff) and paleontologic sample (UCR-331). The isotopic values reported here provide primarily a basis for emphasizing that specific procedures used in the preparation of the organic fractions for specific isotopic analyses must avoid effects that will produce fractionation in the sample gases. Studies to deal with these problems are currently under way.

With the anticipated employment of direct ^{14}C counting methods utilizing significantly reduced sample size requirements, it becomes crucial to develop methods to insure that sample fractions being dated are appropriately characterized. The preparation of bone samples presents particularly difficult challenges. A consideration of the potential effect of both modern contamination and contamination with organics of significantly younger age provides a framework within which sample preparation strategies can be developed. In any calculation of this kind, it is important to consider the expected measurement uncertainties anticipated in the accelerator ^{14}C systems.

For example, if the expected measurement error for a 75,000-year-old sample is anticipated to be on the order of $\pm$ 5000 years (table 1), then the maximum level of modern contamination which would not significantly affect the final results would be on the order of 100ppm. Other researchers are projecting lower measurement uncertainties, *ie,* $\pm$ 500 years for a 60,000-year sample (Purser, 1978, p 19). At this level, modern contamination on the order of 10ppm is equivalent to about a 500-year reduction in age. However, there is a higher probability that contaminating organics will be much closer in age to the original sample age. In such cases, the resultant dilution effect cannot be determined unless the actual age of the original sample, the age of the contaminant, and the percentage contribution of the contaminants are all known. This is, in practice, usually difficult, if not impossible, to quantitatively determine.

The closest approach would be to obtain ^{14}C determinations on different fractions of the same sample. The ability to obtain meaningful ^{14}C values on different organic fractions and inorganic and organic extracts from bone samples made possible by the development of accelerator technology should greatly facilitate confidence in the integrity of ^{14}C determinations on bone of Pleistocene age. The development of geochemical criteria, such as provided primarily by amino acid composition and perhaps by stable isotope data, to identify seriously contaminated samples, will make more efficient the selection process for Pleistocene bone samples intended for ^{14}C analyses by direct counting.

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