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NON-AGE-RELATED VARIATIONS IN ASPARTIC ACID RACEMIZATION IN BONE FROM A RADIOCARBON-DATED LATE HOLOCENE ARCHAEOLOGICAL SITE

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ABSTRACT. Wide variations in D/L_{asp} values are exhibited in a series of bone samples of assumed similar age associated with a suite of ^{14}C determinations from stratified contexts in a late Holocene archaeological site. In this group of bone samples, major differences in D/L_{asp} ratios appear to be correlated primarily with variability in amino acid nitrogen content and Gly/Glu ratios.

INTRODUCTION

In previous studies (Ennis *et al,* 1986; Prior *et al,* 1986), we have investigated the problems of employing aspartic acid racemization (AAR) data to obtain accurate age estimates on terrestrial bone. We examined the accuracy of Pleistocene age estimates on human skeletal samples from several California localities inferred from AAR values. Earlier workers calibrated the rate of aspartic acid racemization with a ^{14}C determination on a bone sample from an assumed similar temperature regime. AAR-based age estimates were calculated assuming that time and effective environmental temperature were the only important variables (Bada & Protsch, 1973; Bada, Schroeder & Carter, 1974; Bada & Helfman, 1975).

These AAR-deduced age values subsequently proved to be as much as an order of magnitude older than ^{14}C determinations obtained on the same bone samples using AMS techniques (Taylor *et al*, 1983; Taylor, 1983; Taylor, Payen & Slota, 1984; Stafford *et al,* 1984; Taylor *et al,* 1985). The seriously anomalous AAR age estimates on California human bone materials can be traced, in part, to the calibration sample (the Laguna human skeleton) which, for reasons not yet clear, was assigned an inflated ^{14}C age (Berger *et al,* 1971, *cf* Bada *et al,* 1984). However, we have elsewhere noted (Ennis *et al,* 1986) that this fact alone is insufficient to account for the magnitude of the anomalous AAR age assignment given to the California skeletons.

In agreement with earlier workers (eg, Hare, 1974a,b; Williams & Smith, 1977; Smith, Williams & Wonnacott, 1978; Von Endt, 1979, 1980; Kessels & Dungworth, 1980; Matsu'ura & Ueta, 1980), we concluded that an important factor influencing the degree of aspartic acid racemization in bone is the chemical state of the amino acids and that comparisons of D/L aspartic acid ratios in bone for the purpose of inferring age relationships should be made only on chemically-comparable organiç fractions (Prior *et al,* 1986; *cf* Bada, 1985a,b). We note, eg, that free amino acids are known to racemize at a different rate than peptide-bound amino acids. Also, in

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studies of isoleucine in fossil shells, the rate of epimerization decreases as the relative concentration of free amino acids increases (Mitterer & Kriausakul, 1984; P E Hare, pers commun, 1988).

ARCHAEOLOGICAL CONTEXT

The Encino Village site (CA-LAn-43) is on the northward edge of the Santa Monica Mountains in the south San Fernando valley, Los Angeles, California (118° 45′W, 34° 15′N) at ca 15m above the prehistoric or "old" Los Angeles River bed which lay ca 4.5km to the north. The extent of the prehistoric cultural deposits at the time of European contact in the 16th century has been estimated at ca 6000 m^2. The site seems to have extended ca 100m along the upslope portion in a bend of a stream channel which flowed into a major tributary of the old Los Angeles River drainage system. During early historic times, various structures were built on the site which is now part of a high-density urban zone.

At CA-LAn-43, 54 2×2m or 1×2m units were excavated in 10cm arbitrary increments from the modern soil surface to culturally sterile bedrock. The average depth of these units was ca 2m with a maximum depth of 4.2m. From 94 features, 21 human (Cerreto, 1987) and 13 animal burials including 10 dogs (*Canis familiaris*) (Langenwalter, 1986) were recovered. The artifact assemblage includes >9000 chipped stone tools of which 900 are projectile points. Ca 700,000 pieces of bone and over 5000 shell artifacts (mostly beads) were recovered along with ceramics and ground stone and bone artifacts. For details of site excavation data, see Mason (1986).

RADIOCARBON DATA

Based on 50 ^{14}C age estimates on charcoal, bone and marine shell recovered in stratigraphic context, along with geoarchaeological studies and preliminary analysis of the artifacts, the temporal span of human occupation at CA-LAn-43 was documented. All of the ^{14}C values from CA-LAn-43 including detailed provenance data has been presented in Taylor *et al* (1986). The corpus of ^{14}C determinations on charcoal, bone (both human and other mammalian fauna), and marine shell samples, D/L_{asp} values obtained on bone, and obsidian hydration measurements on chemically characterized obsidian from Ca-LAn-43 constitutes one of the largest suites of chronometric data ever assembled for a single archaeological site in California.

Preliminary analysis of the archaeological data along with the indication of the ^{14}C results suggests that CA-LAn-43 was permanently occupied from ca 2000 BP to European contact. Evidence of human occupation earlier than 2000 BP is limited to a single ^{14}C value obtained on marine shell and two cogged stone artifacts. Both the marine shell sample and the artifacts were recovered from the lowest occupation levels at the site. The marine shell sample yielded a ^{14}C age of 4570 ± 80 (reservoir-corrected ^{14}C age = 3870 ± 220). In California, cogged stones are associated with the Millingstone Horizon (Wallace, 1955) which, in some areas, can date to as early as 7000–8000 BP and, in other areas, to as late as 4000 BP (Meighan, 1978).

The bone samples on which D/L_{asp} values and other biogeochemical data were obtained for this study were all excavated from a single excavation profile, Unit 8 at Ca-LAn-43. Unit 8 was excavated to a depth of 210cm in 10cm arbitrary increments. Fifteen ^{14}C determinations were obtained on charcoal recovered from all but one level (70–80cm) beginning at 50cm and continuing down to the 200–210cm level which rested on bedrock. The ^{14}C values from Unit 8, expressed in calendar years (cal BP) using calibration data presented in Stuiver and Pearson (1986) employing procedures outlined in Stuiver and Reimer (1986), are listed in the lower section of Figure 1.

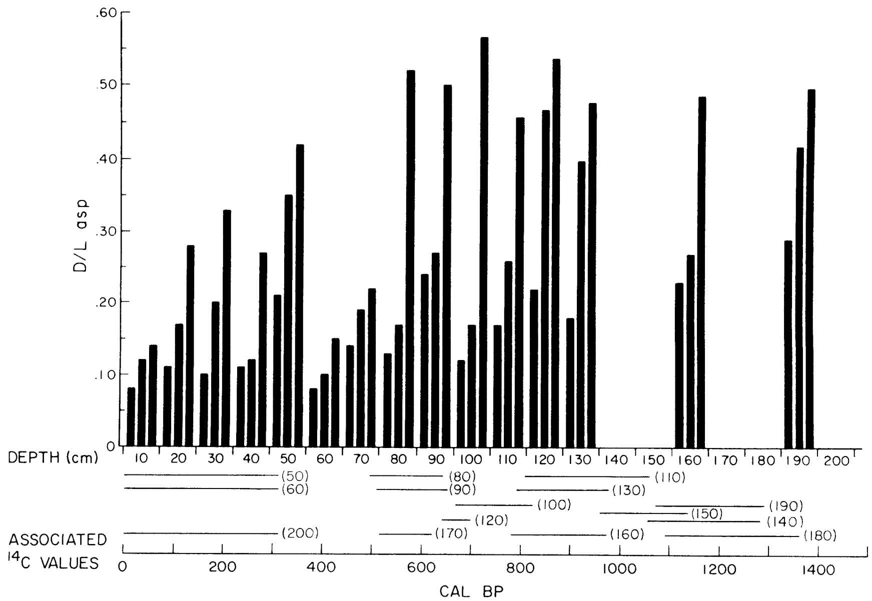

Fig 1. D/L_{asp} (upper section) and associated ^{14}C values (lower section) from 10cm stratigraphic increments in Unit 8 at the Encino Village site (CA-LAn-43). D/L_{asp} values obtained on the total hydrolysate listed by depth in profile where depth value (in cm) indicates lower value in 10cm increment (eg, 10cm indicates 10–20cm level). Unit 8 ^{14}C values obtained on charcoal and are listed in cal BP form as age ranges at ± 1 times the combined standard deviation of the sample and calibration curve using procedures outlined in Stuiver and Reimer (1986) employing calibration data of Stuiver and Pearson (1986). Levels from which ^{14}C values obtained are listed in parentheses to the right of the age ranges.

ASPARTIC ACID RACEMIZATION DATA

Aspartic acid D/L ratios were obtained on the total hydrolysate of 3 separate bone fragments from 15 of the 21 levels excavated in Unit 8 for a total of 45 D/L_{asp} measurements (Fig 1, upper section). While there appears to be a general trend for the D/L_{asp} values to increase with depth, these data reveal that, in a significant number of cases, there is a wide range in the D/L_{asp} ratios exhibited by bones from the same 10cm stratigraphic level. In making these observations, we assume that bones derived from the same stratigraphic level are of essentially similar age. While there appears to have been some stratigraphic disturbance in Unit 8, based on the distribution of the ^{14}C values, serious mixing appears only in the 170–180cm level and the bottom of the profile (200–210cm level). We interpret the presence of modern organics at the 200–210cm level in Unit 8 as resulting from post-abandonment activity of burrowing animals which have moved surface materials to the bottom of the profile.

We previously demonstrated that the analytical error of our D/L_{asp} measurements can explain only a few percent of the observed variations in D/L_{asp} values. In a prior study involving 5–6 replicate analyses of various solubility fractions (total organic, acid-soluble and acid-insoluble organics) of modern (n = 5) and fossil bone (n = 4), our average reproducibility in the D/L_{asp} values were ± 2–3%. The largest percent analytical deviation was ± 7.4% (on six measurements of the same hydrolysate). In one case, no significant difference (<0.01) in D/L_{asp} ratios was observed in five measurements of the same hydrolysate. In these experiments to examine analytical variability, one hydrolysate was prepared from each fraction from which 5–6 aliquots were taken for duplicate analysis (Prior *et al,* 1986).

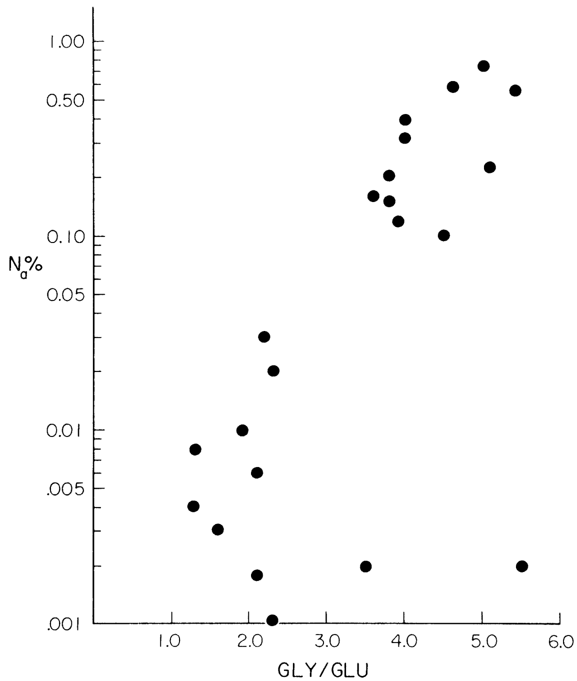

Fig. 2. Relationship of amino acid nitrogen content (N_a%) and ratio of glycine and glutamic acid (Gly/Glu) in total hydrolysate of bones from Unit 8 at CA-LAn-43

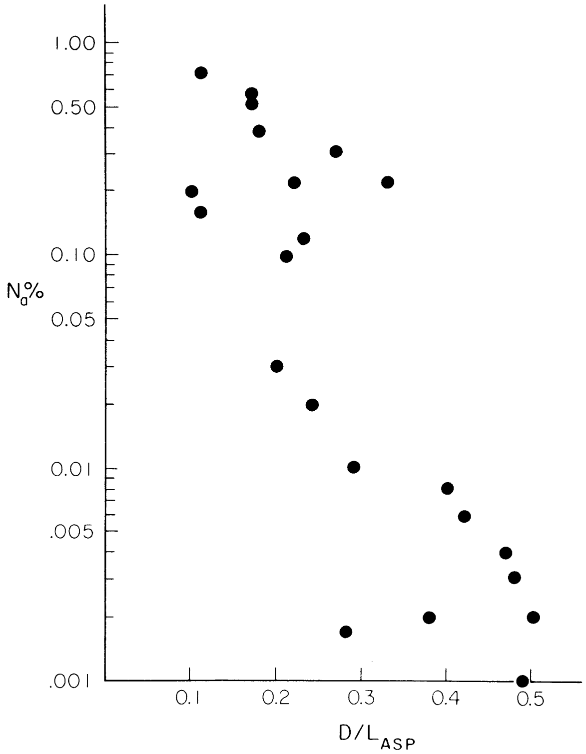

Fig 3. Relationship of amino acid nitrogen content ($N_a\%$) and D/L_{asp} in total hydrolysate of bones from Unit 8 at CA-LAn-43

DISCUSSION

The ^{14}C data indicate that the total period of time represented by the bone samples from Unit 8 does not exceed 2000 yr. The wide variation in the D/L_{asp} values exhibited by bones from the same stratigraphic levels point to the need to explain these variations in terms of one or more factors in addition to differences in chronological age of the bones. We have examined the relationship between the racemization values and two indices of the degree of preservation of the collagen structure in the bone samples – total amino acid nitrogen (N_a) content and the ratio of glycine (Gly) to glutamic acid (Glu) in 22 of the bone samples from Unit 8. (For a discussion of the distinction between conventional microanalytical Dumas or Kjeldahl nitrogen values and nitrogen values based on amino acid compositional analysis, see Ennis *et al,* 1986). Both the amino acid nitrogen and Gly/Glu ratio is inferred from amino acid composition analysis.

We use the Gly/Glu ratio as a quantitative measure of the degree of preservation of the collagen-like profile in bone rather than the hydroxy-

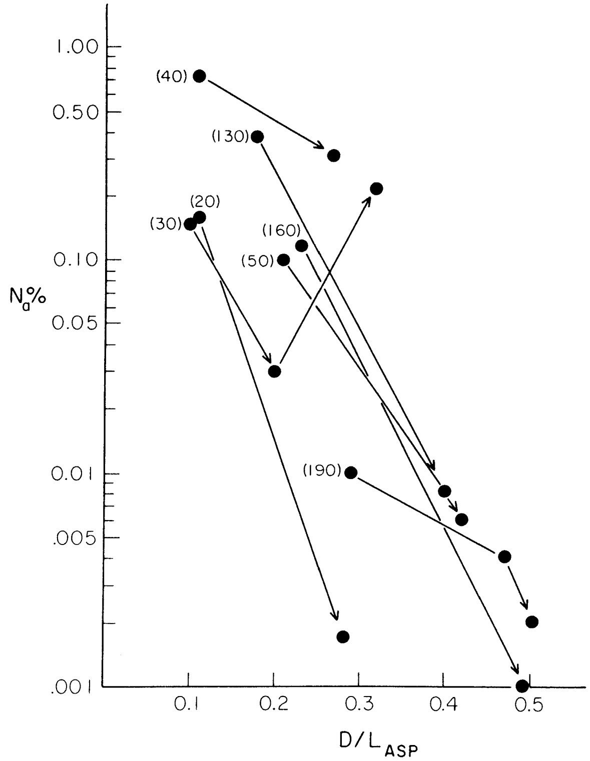

Fig 4. Relationship of amino acid nitrogen content (N$_a$%) and D/L$_{asp}$ for bones from common 10cm increments in Unit 8 at CA-LAn-43. Stratigraphic level from which bone samples obtained are listed in parentheses to the left of lowest D/L$_{asp}$ value in each set of values.

proline concentration because the HPLC system used in this study did not detect hydroxyproline. In previous studies (eg, Hare, 1969, 1979; Taylor, 1980) we showed that in fresh, modern bone which exhibits the overall amino acid pattern characteristic of mammalian collagen, the Gly/Glu ratio is ca 4 ($\pm$1). Bones in which the Gly/Glu ratio is below 2.5 are viewed as having lost their collagen-like amino acid pattern. Typically, these bones also exhibit amino acid nitrogen values below 0.1%. Figure 2 shows the relationship between N$_a$ and Gly/Glu in 22 bone samples from Unit 8. Except for two samples, Gly/Glu ratios of <2.5 are associated with samples exhibiting N$_a$ concentrations below .05%.

Figure 3 plots the relationship between the amino acid nitrogen (N$_a$) content and D/L$_{asp}$ values in the total hydrolysate of the 22 bones. As expected from previous studies, there appears to be a general trend in the relationship of D/L$_{asp}$ and amino acid nitrogen content – as nitrogen content decreases, there is a tendency for the D/L$_{asp}$ values to increase. Because Figure 3 shows the N$_a$/D/L$_{asp}$ relationship of bones in Unit 8 without regard to stratigraphic level or any possible age offset, we have plotted in Figure 4 the instances where both N_a and D/L_{asp} values were obtained on one or more samples from the same stratigraphic level. As previously noted, we assume that bones derived from the same stratigraphic level are of similar age. While there is a tendency for the D/L_{asp} values to increase slightly with depth (*cf* Fig 1), the intralevel variation in D/L_{asp} values is obviously much greater. With the exception of one sample from the 30cm level, significant increases in D/L_{asp} values are associated with reductions in N_a values.

CONCLUSIONS

Previous studies by several groups have noted that time and effective mean annual environmental temperature are not the only significant variables that must be taken into account in deriving age estimates on the basis of aspartic acid racemization values in terrestrial bone samples. We show that wide variations in D/L_{asp} values occur in samples of assumed similar age and that these variations are typically associated with indices of the degree of retention of the original collagen-like structures in bone as measured by amino acid nitrogen content and Gly/Glu ratio. These data support the view that, for the purpose of deriving temporal relationships among terrestrial bone samples, comparison of D/L_{asp} ratios should be made only on biogeochemically comparable organic fractions.

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