Reports

New Evidence for the Antiquity of Man in North America Deduced from Aspartic Acid Racemization

Abstract. *Ages of several California Paleo-Indian skeletons have been deduced from the extent of aspartic acid racemization. These dates suggest that man was present in North America at least 50,000 years before the present.*

The antiquity of man in the New World is still a subject which is debated by paleoanthropologists. In general, most evidence to date is interpreted as indicating that man has only recently populated the New World (*1*, *2*). This expansion is thought to have occurred within the last ~20,000 years, by migration across the Bering Sea land bridge at a time during the last Ice Age when the sea level was lower. There are opinions, however, that man may have been present as long ago as 100,000 years B.P. (before the present) (*3*) or perhaps even earlier (*4*). The evidence for the latter case consists of artifacts and other indications of the presence of man, but no actual hominid fossils that support this proposition have been found and dated.

One of the difficulties is the scarcity of well-dated New World hominid fossils (*5*). Although there have been a fairly large number of fragmentary hominid fossils found in North America, most of these are available in insufficient amounts for radiocarbon analysis and therefore have never been directly dated.

Recent evidence has shown that the amino acid racemization reaction can be used, with certain limitations, to estimate the age of fossil bones (*6*–*8*). Only L-amino acids are usually found in the proteins of living organisms, but over long periods of geological time these L-amino acids undergo slow racemization, producing the nonprotein D-amino acids. The proportion of D- to L-amino acids in a fossil steadily increases with time. Thus, by determining the extent of racemization in a fossil bone, its age can be estimated. Each amino acid, with the exception of glycine, undergoes racemization. However, some amino acids racemize much faster than others (*9*). In the age range of ~5,000 to ~100,000 years, the racemization of aspartic acid is the most useful reaction (*7*, *8*). Relative to radiocarbon dating, much smaller quantities are required in the racemization dating method, and, moreover, the range of applicability exceeds that of radiocarbon dating. We report here ages deduced for several California hominid fossils by using the aspartic acid racemization reaction. These ages suggest that man was present in the New World earlier than was previously estimated.

The fossil man samples used in this study were generously provided by the San Diego Museum of Man and the Los Angeles County Museum of Natural History. The Los Angeles samples were analyzed because they had already been dated by radiocarbon; in one case only a minimum age had been deduced. The site descriptions and radiocarbon dates for the Los Angeles samples have been given elsewhere (*10*).

The San Diego samples were selected by one of us (G.F.C.) because of their potentially great antiquity, estimated from geomorphological evidence. The samples were taken from a series of skeletal remains collected between 1920 and 1935 by M. J. Rogers for the San Diego Museum of Man; the specimens had been stored at the Museum since collection. The archeological descriptions of the sites, made when the samples were excavated, are given in the field notes of Rogers, which are now part of the records of the San Diego Museum of Man.

The samples we analyzed came from two sites located near La Jolla. The first site, San Diego Museum (SDM) site W-2, was located at La Jolla Shores, approximately three-fourths of a mile (about 1.2 km) south of Scripps. In 1926 a steam shovel working on a development project unearthed several human remains. Rogers, who worked at the site periodically over a series of months, described the site (*11*) as "a large thin-bedded shell midden capping an estuary sand." Sample SDM 18402 was found 1.5 feet (about 0.46 m) below ground level in a red packed sand directly underlying the midden; it consisted of a partial skeleton in what

Table 1. California Paleo-Indian samples (SDM, San Diego Museum).

Sample	Description and location	D/L aspartic acid	Collagen-based carbon-14 age (years)
Laguna skull	Skull and long bones found at Laguna Beach in 1933 (*10*)	.25	17,150 ± 1,470 (UCLA 1233A)
Los Angeles Man	Skull fragment found north of Baldwin Hills in 1936 (*10*)	.35	> 23,600 (UCLA 1430)
SDM 18402	Long bones from burial at base of shell midden at SDM site W-2	.16	5,000 to 7,500 *
SDM 16755	Human rib and miscellaneous fragments found in excavated fill at site W-2 in 1926. Olivella beads cemented to ribs	.36	
SDM 16742	Human frontal found in white sands at site W-2 in 1926	.50	
SDM 16704	Human skull and lower mandible, long bones, and scapula fragments found in lower midden at SDM site W-34-A in 1929	.53	

* Carbon-14 age range for similar shell middens in southern California and Baja California (*12*).

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appeared to be a flexed burial position. Radiocarbon dates determined on shell from similar middens in southern California and Baja California have given ages ranging from ~5000 to 7500 years (*12*). Sample SDM 16742 was recovered from 5 to 6 feet below ground level at the base of a white sand stratum underlying the red sand; it consisted of a human frontal. Sample SDM 16755, consisting of several human ribs and other miscellaneous fragments, was found in a gray-white sand and was not found in situ but, rather, was recovered from the lagoon fill excavated from the midden by the steam shovel. An olivella shell bead was cemented to one of the ribs, and the remains appeared to be part of a burial (*11*). Both SDM 16742 and SDM 16755 were recovered from stratigraphically older horizons than was SDM 18402; Rogers (*11*) suggested that these samples could be of great antiquity.

The second site, SDM W-34, was located between the towns of Del Mar and Solana Beach, at the northwest point of the San Dieguito river slough. The site consisted of an upper (W-34) and lower (W-34-A) midden; the latter had been largely destroyed by erosion and tidal action. The upper midden was thought to be similar to the other southern California middens which date between 5000 and 7500 years. Rogers suggested (*11*) that the lower midden might represent an occupation comparable in age to the remains found in the white sands (that is, SDM 16742) at SDM W-2. Sample SDM 16704 consisted of a skull, mandible, and ribs which were found eroding out of the sea cliff at the base of the lower midden; the remaining parts of the skeleton were thought to have fallen into the sea. The physical characteristics of this partial skeleton have been published elsewhere (*13*).

The bones were carefully cleaned and then analyzed, with the use of the procedures described previously (*7*). The sample descriptions and locations, measured D/L aspartic acid ratios, and radiocarbon ages, when available, are summarized in Table 1.

Contamination of the samples with modern amino acids seems to be negligible. Analysis of the Los Angeles Man and SDM 16742 samples indicated that the extent of racemization of amino acids follows the pattern aspartic acid > alanine > isoleucine, which is the sequence predicted for bones which have not been contaminated with modern amino acids (*9*).

Table 2. Ages of California Paleo-Indians deduced by aspartic acid racemization. The Laguna skull was used to "calibrate" aspartic acid racemization reaction: $k_{asp} = 1.08 \times 10^{-5}$ yr^{-1}.

Sample	Aspartic acid age (years)
Los Angeles Man	26,000*
SDM 18402	~6,000†
SDM 16755	28,000
SDM 16742	44,000
SDM 16704	48,000

* Carbon-14 age > 23,600 years (UCLA 1430).
† Dated with the corrected k_{asp} value of 1.5×10^{-5} yr^{-1}. Middens similar to that in which this sample was found range in age from 5,000 to 7,500 years (*12*).

Ages deduced from the extent of racemization of aspartic acid are calculated from the equation [see (*7*) for derivation]

$$\text{Age (years)} = \frac{\ln \left\{ \frac{1 + \text{D/L}}{1 - \text{D/L}} \right\} - 0.14}{(2)\ (k_{asp})} \quad (1)$$

where D/L is the aspartic acid enantiomeric ratio in the bone and k_{asp} is the first-order rate constant for interconversion of the L and D enantiomers of aspartic acid. In order to use Eq. 1, it is necessary to evaluate k_{asp} at the average temperature to which the bones have been exposed since their deposition. Recent results (*7*, *8*) have demonstrated that this can be accomplished by determining the extent of aspartic acid racemization in a collagen-based radiocarbon dated bone and then calculating the in situ k_{asp} value. Once this "calibration" has been carried out for a region, the reaction can then be used to estimate the age of other bones from the same general area. Bones with ages in the region of ~15,000 to ~20,000 years are the best calibration samples and can be used to date bones which are thought to be much older (*8*). Ages deduced with this calibration technique have been shown to be in good agreement with radiocarbon dates determined on the same samples (*8*).

The Laguna skull was used as a "calibration" sample for the southern California coastal region. Since the present-day climates of all the sites are nearly identical (*14*), this calibration should be applicable to the other samples shown in Table 1, with the exception of SDM 18402. Using the Laguna "calibration" to date SDM 18402 gives an age of ~8500 years, which indicates that this sample has been exposed to only postglacial temperatures. The Laguna calibration sample, on the other hand, has had a nearly equal exposure to both glacial and postglacial conditions. Schroeder and Bada (*15*) have shown that for the Mediterranean island of Mallorca,

$$\frac{k_{asp}\ \text{(samples with ages} < 10{,}000\ \text{years)}}{k_{asp}\ \text{(samples with ages} > 15{,}000\ \text{years)}} = 1.4$$

Assuming that this equation is also valid for southern California, it gives a k_{asp} value, applicable for dating SDM 18402, of 1.5×10^{-5} yr^{-1}. The ages estimated for all the samples are summarized in Table 2.

The main factor which could cause the ages in Table 2 to be in error would be that the Laguna calibration is incorrect (*16*, *17*). However, this seems unlikely, since the age of Los Angeles Man deduced by aspartic acid racemization is consistent with the radiocarbon analysis of this sample. Also, the general age of SDM 18402 is compatible with the expected age, although this correlation should be considered less reliable. A value of $k_{asp} = 1.08 \times 10^{-5}$ yr^{-1} is consistent with other calibration k_{asp} values (*8*) determined in other locations which have a mean average temperature similar to that of the southern California coastal area (that is, Mallorca, Spain, and Sarab, Iran).

As a further check on the Laguna calibration we analyzed a piece of a dwarf mammoth bone from Santa Rosa Island. The mammoth skeleton consisted of numerous large charred and uncharred bones. Radiocarbon analysis (*18*) of some of the charred bones yielded an age of 29,700 ± 3,000 years (L-290R). We obtained a small piece (~ 5 g) of one of the large uncharred bones (*16*) which had been recently dated at 30,400 ± 2,500 years (UCLA 1898), with the collagen-based radiocarbon technique. Analysis of this sample yielded D/L aspartic acid = 0.28.

Unfortunately, the Laguna calibration is not directly applicable to Santa Rosa Island, since the average temperatures on the southern California Channel Islands are somewhat lower than those of the coastal environments; we will assume a similar situation existed in the past. Thus, the Laguna calibration had to be corrected to take into account this temperature difference.

The average mean annual temperature on San Miguel Island (located ~ 15 km northwest of Santa Rosa) is 13.7°C, compared with 16.1°C at the general location (Newport Beach) where the Laguna skull was found (*19*). With an Arrhenius activation energy (E_a) of 33.4 kcal mole^{-1} (*9*), the calculated value for k_{asp} at 13.7°C is 6.6×10^{-6} yr^{-1}. Substituting this value and the measured D/L aspartic acid ratio into Eq. 1 gives an age of 33,000 years, which is in good agreement with the radiocarbon ages deduced for the mammoth skeleton. We feel that this correlation provides additional strong evidence that the ages deduced for the California Paleo-Indian samples are accurate.

The ages given in Table 2 are the oldest direct dates determined so far for any New World hominids. They suggest that man had populated the New World substantially earlier than ~15,000 to ~25,000 years B.P., the last time the Bering Sea land bridge existed (*20*, *21*). Sea level was lower at earlier times (*20*, *21*), with a level low enough for the land bridge formation to occur perhaps ~70,000 years B.P. (*21*). It would appear, based on the limited number of dates we have obtained, that man might have migrated to the New World during this time.

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22. The samples from San Diego were provided by S. Rogers and J. Moriarty, those from Los Angeles by C. Rozaire and R. Berger, and the Santa Rosa Island mammoth bone was provided by R. Berger and P. Orr; we express our gratitude to these individuals for providing the samples. We thank Linda and J.C.W. for encouragement. This work was supported by NSF grant GA-40804. R.A.S. is a Scripps Industrial Associates predoctoral fellow.

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Radioreceptor Assay of Human Chorionic Gonadotropin: Detection of Early Pregnancy

Abstract. *A rapid, sensitive, and specific radioreceptor assay for the determination of human chorionic gonadotropin and luteinizing hormone in plasma is described. Plasma membranes of bovine corpora lutea of early pregnancy, which bind biologically active labeled human chorionic gonadotropin, have been used as receptor. Pregnancy could be detected by assaying the gonadotropin in plasma samples obtained from day 6 to 8 after conception.*

It is well established that the target tissue receptors bind biologically active molecules and that hormone-receptor interaction is of high affinity and specificity (*1*). The specific receptors in target tissues have permitted the measurements of hormones in biological fluids in radioreceptor assays in vitro based on the principle of competitive protein binding methods (*2*). The plasma membranes of bovine corpus luteum exhibit a specific binding ability for bovine LH (*3*, *4*), human LH and hCG (*5*). The receptor is specific for hCG and LH without apparent species specificity; FSH, TSH, hGH, and PRL (*3*) do not bind to the hCG-LH receptor. We now report a radioreceptor assay for quantitative determination of hCG and LH in plasma, with the use of the plasma membrane of bovine corpus luteum of pregnancy as receptor. The assay procedure can be used in the detection of pregnancy in humans as early as 6 days after conception.

Fresh bovine corpora lutea were homogenized in chilled 0.01*M* tris-HCl buffer, *p*H 7.8, containing 1 m*M* $MgCl_2$ and 1 m*M* dithiothreitol and centrifuged at 480*g* for 10 minutes (Sorvall refrigerated centrifuge). The supernatant was again centrifuged at 10,000*g*, and the pellet was purified by zonal centrifugation in sucrose density gradient (*6*) (Beckman ultracentrifuge, model L3-50) to obtain plasma membranes in high yield. Highly purified hCG, containing 12,000 international units per milligram (*7*), was labeled with ^{125}I (Cambridge Nuclear, Cambridge, Mass.), as follows (*8*). A solution of 2 mc of ^{125}I in 20 μl of 0.1*M* sodium acetate buffer, *p*H 6.0, was mixed with 25 μg of hCG. Lactoperoxidase (Sigma RZ = 0.78) (50 ng in 20 μl of buffer) and 200 ng of H_2O_2 in 10 μl of water were added. Then three 100-ng portions of H_2O_2 were added at 5-minute intervals. At the end of 20 minutes the reaction was stopped by the addition of 0.5 ml of 0.15*M* NaCl containing 1 percent bovine serum albumin (BSA), *p*H 7.0. The labeled hormone was then separated from free iodine by gel filtration on a column (1 by 30 cm) of Sephadex G-100 equilibrated with 0.15*M* NaCl containing 1 percent BSA, *p*H 7.0. The labeled hCG in the unretarded fraction showed specific activity in the range of 40 to 50 μc/μg and a biological activity of 8923 I.U./mg (95 percent confidence limits of 5,826 to 12,250 I.U.) (*5*).

Our subjects were individuals who