Reports

Radiocarbon Dating of Petroleum-Impregnated Bone from Tar Pits at Rancho La Brea, California

Abstract. *A liquid-chromatography method has been developed for the separation of amino acids with the same specific activity in radiocarbon from bones impregnated with isotopically dead petroleum compounds found in the La Brea tar pits. This technique permits the application of radiocarbon dating to such bone assemblages.*

A liquid-chromatography method is reported for the separation of purified amino acids derived from collagen from petroleum-impregnated bone found in the Rancho La Brea tar pits of Los Angeles, California. These deposits have been described by Howard and by Stock (*1*). Dates obtained for bone collagen are close to dates for wood (*2*) for the same stratigraphic levels. Several specimens dated from pit 3 at Rancho La Brea give the first clear evidence for superpositional age differences between different depths, which had been suspected for this deposit (*2*). Other pits discussed by Howard (*1, 2*) and Marcus (*3*) are also being dated.

Seventeen available radiocarbon dates from wood and leaf samples (*4*) nearly exhaust the supply of plant material for which pit and depth data are available. These dates established the approximate range of activity of the asphalt traps in prehistoric times from periods older than 40,000 to 4450 years ago, in addition to the historic accounts of the last century. They now permit an assessment of differences in ages between the separate traps. Tens of thousands of bones of large and small mammals and birds are available for dating in the Los Angeles County Museum for Natural History. These bones are distributed throughout the deposits. Accurate data on the depth and locality are available for each specimen (*1, 3*). Extensive bone dating was not possible before a method was devised for the removal of the impregnating petroleum. Bone dating of major deposits at Rancho La Brea will establish the range of activity of each tar trap and the degree of temporal superposition within deposits.

Bone is now routinely dated from its collagen content by the methods of Berger *et al.* (*5*). Dating of the carbonate fraction has proved less reliable (*6*) and was not attempted for La Brea fossils. The petroleum impregnation which gives the La Brea fossil bones their characteristic color (coffee to black) was apparently an ideal preservative for bone collagen. The percentage dry weight of collagen in La Brea bone may be from one-sixth to four-fifths of that of recent bone (*7*).

Ho's procedure (*7*) for the microanalysis of collagen amino acids in fossils was modified to permit the preparation of approximately 5 g of carbon from each specimen in the form of isotopically pure amino acids. This amount was required to fill the radiocarbon counter chamber (UCLA) and to allow for losses in laboratory manipulations (*5*). Dry modern bone contains about 25 percent collagen, whereas older bone usually has a lesser collagen content (*5, 7*). For this reason and because of the unknown percentage of petroleum, 70 to 100 g of dry dense bone was used for each sample. In fact, dense bone was the most suitable sample material for amino acid analysis of La Brea fossils.

Each sample consisted of a bone approximately 9 cm long sawed from the dense shaft of a broken or damaged femur of the saber-toothed cat, *Smilodon californicus,* the second most abundant species of large mammal from the locality (*3*). Several hundred suitable femora are available and two or three 9-cm samples may be prepared from one specimen. Eleven samples have been dated by the method given below (*8*).

For initial purification each bone sample was washed with petroleum ether, and matrix and cancellous bone were scraped off. The bone segment was broken into chips, washed again in ether, and ground so as to pass through a 60-mesh screen. The bone powder was repeatedly washed in petroleum ether until the solution was clear and then it was air-dried. At this point bone powder still contained considerable petroleum contamination.

The sample was then decalcified in excess 1*N* HCl, and the collagen-petroleum residue was washed with distilled water until neutral. Subsequently, the sample was hydrolyzed in 6*N* HCl under reflux for 24 hours and the hydrolyzate was concentrated by evaporation to 50 ml. Several grams of tarry residue remained in the Büchner funnel after filtration of the hydrolyzate.

Separation by chromatography was accomplished in a column (5 cm in diameter) containing Dowex 50-WX8 resin charged with 4*N* HCl. The filtered hydrolyzate was passed into the column at a rate of 30 ml per hour; the resin was then washed repeatedly with distilled water to remove all petroleum. The column was eluted with an excess of 5*N* NH_4OH at a flow rate of 30 to 60 ml per hour in order to free the sorbed amino acids; about 1 liter of yellowish effluent was collected. The

Table 1. Dates for bone and wood from pit 3. References are in parentheses.

Depth (m)	Material	Age (years)	Laboratory sample No.
2.1	Bone	12,650 ± 160	UCLA-1292B (*11*)
3.5	Bone	14,400 ± 2100	UCLA-1292E (*11*)
3.6	Bone	14,500 ± 190	UCLA-1292C (*11*)
3.6	Wood*	14,640 ± 115	Y-354B (*5*)†
			Y-355B (*5*)†
3.6	Wood*	14,400 ± 300	LJ-55 (*6*)‡
6.7	Bone	20,500 ± 900	UCLA-1292J (*11*)§
		21,400 ± 560	UCLA-1292A (*11*)§
7.9	Bone	19,300 ± 395	UCLA-1292K (*11*)

* Wood from tree rooted at a depth of 3.6 m from surface (*2*). † Date is average of two analyses of different portions of same tree trunk dated at the Yale University radiocarbon laboratory. ‡ Sample dated at the La Jolla radiocarbon laboratory. § UCLA-1292A and UCLA-1292J were dates on the same bone; UCLA-1292J included treatment with 0.1*N* NaOH prior to hydrolysis of collagen.

effluent, containing only the desired amino acids, was evaporated to dryness. After this pure fraction was converted to highly purified CO_2, it was dated in a proportional counter to within an accuracy of at least one standard deviation (*9*).

Analysis by gas chromatography of hexane extract of a portion of the purified acids showed no petroleum contamination. Amino acid analysis of small samples gave results consistent with those of Ho (*7*) for the same species. Each sample required 3 to 4 days for completion of the separation process. The dates for bone samples from pit 3 and the previously obtained dates for wood from pit 3 are given in Table 1.

This method may be used to date bone from several other Brea deposits in California (*10*) and Peru (*11*). It may also prove satisfactory for the removal of contamination due to preservatives and humic acids. One further refinement is possible which will guarantee that all of the dated carbon is derived from bone collagen. Hydroxyproline is a major constituent of collagen and is rarely present in other proteins (*12*). Purified hydroxyproline may be separated chromatographically from the other amino acids present for dating. About 10 percent of bone collagen is hydroxyproline; therefore a sample ten times as large as that used in this study would be required to obtain sufficient carbon for dating (*7*). Such large samples are available from single bones at La Brea. Sufficient agreement of the dates for bone and wood and the repeatability of dating of the same specimen have indicated that this additional refinement was not required for dating La Brea bone.

In general, our procedure allows for greater utilization of one of the richest collections of mammals in the world and permits, for example, studies on morphological changes within the same animal species with time during the Late Pleistocene.

T. Y. Ho

Basin Geology Department, Esso Production Research Company, Houston, Texas

Leslie F. Marcus

Department of Biology, Queens College, Flushing, New York

Rainer Berger

Institute of Geophysics and Planetary Physics and *Department of Anthropology, University of California, Los Angeles 90024*

References and Notes

1. H. Howard, *Los Angeles County Mus. Contrib. Sci. No. 58* (1962); C. Stock, *Los Angeles County Mus. Contrib. Sci. No. 20* (1958).
2. H. Howard, *Science* **131**, 712 (1960).
3. L. F. Marcus, *Los Angeles County Mus. Contrib. Sci. No. 38* (1960).
4. R. Berger and W. F. Libby, *Radiocarbon* **8**, 467 (1966); E. S. Deevey, L. J. Gralenski, V. Hoffren, *ibid.* **1**, 144 (1959); C. L. Hubbs, G. S. Bien, H. E. Suess, *ibid.* **2**, 197 (1960).
5. R. Berger, A. G. Horney, W. F. Libby, *Science* **144**, 999 (1964).
6. M. A. Tamers and F. J. Pearson, Jr., *Nature* **208**, 1053 (1965).
7. T. Y. Ho, *Proc. Nat. Acad. Sci. U.S.* **54**, 26 (1965); *Comp. Biochem. Physiol.* **18**, 353 (1966); *Biochim. Biophys. Acta* **133**, 568 1967).
8. R. Berger and W. F. Libby, *Radiocarbon* **10**, 402 (1968).
9. UCLA date lists in *Radiocarbon* describe the C^{14} counting system.
10. McKittrick and Carpinteria localities; see C. Stock, *Los Angeles County Mus. Contrib. Sci. No. 20* (1958).
11. Talara, Peru; see R. H. H. Lemon and C. S. Churcher, *Amer. J. Sci.* **259**, 410 (1961).
12. This was also suggested independently by H. Scharpenseel, University of Bonn, at the 6th International Conference on Radiocarbon and Tritium Dating, Pullman, Washington, 1965.
13. We thank R. Reynolds for competent assistance, T. Downs for permission to analyze bones, and W. F. Libby for encouragement. Supported by NSF grants GA-628 and GB-5119. Publication No. 714 of the Institute of Geophysics and Planetary Physics, University of California, Los Angeles.

16 December 1968

Fatty Alcohols (Normal and Isoprenoid) in Sediments

Abstract. *Normal long-chain alcohols were isolated from Recent marine sediments from several environments. The isoprenoid alcohol dihydrophytol, which is thought to originate from phytol, the side chain of chlorophyll, by hydrogenation in the reducing environment of the sediment, was also present in most of the samples. Both the normal and isoprenoid alcohols were found in the Green River shale (Eocene). Geochemical implications are considered.*

Biochemicals and recognizable degradation products of biochemicals, although present only in trace amounts in sediments, are in most cases the only source of data on the chemical composition of ancient organisms. It is useful to study the organic compounds contained in Recent sediments and to note biological sources for various compounds and some of the chemical transformations that specific biochemicals undergo in geologically short periods of time (*1*). We now report the finding of normal and isoprenoid fatty alcohols in sediments from several environments. Although the fatty acids (*2*, *3*) and corresponding long-chain hydrocarbons (*4*) have been found in marine sediments, the finding of alcohols has not been reported.

Results of analyses on fatty alcohols in samples from three different Recent environments and three ancient sediments are presented in Table 1. The sample of Miocene age is from an undescribed outcrop in the Philippine Islands. The sample of shale from the Green River (Eocene) is from the U.S. Bureau of Mines oil-shale mine near Rifle, Colorado. The Eagleford shale (Upper Cretaceous) is from an outcrop near Austin, Texas (*5*). Baffin Bay is a hypersaline arm of the Laguna Madre located about 30 miles (48 km) south of Corpus Christi, Texas. The core from the Gulf of Mexico was taken in 25 m of water off Port Aransas, Texas. The sample from the San Nicholas Basin is a grab sample taken off the coast of southern California (*5*). The fatty-acid composition of some of these samples has been reported (*1*, *2*, *6*). All of the Recent samples of sediment were frozen soon after the time of collection and were kept frozen until used.

The following analytical procedure yields a sample suitable for gas-chromatographic analysis, but it does not exclude any type of long-chain alcohol. The sample was treated with dilute hydrochloric acid to remove carbonate and was washed with water to remove inorganic salts. After filtration, the moist mud was treated with methanol for 20 minutes while being stirred and subjected to ultrasonic vibrations. The methanol extract was recovered by filtration. The mud was again extracted with chloroform while being stirred and subjected to ultrasonic vibrations. The methanol and chloroform filtrates were combined and dried on a rotary evaporator. The organic residue was taken up in chloroform and washed with 1*N* hydrochloric acid to remove any last traces of inorganic salts. The chloroform was then dried, and the residue was weighed. The residue was saponified with 0.5*N* potassium hydroxide in methanol for 1 hour on a steam bath. The nonsaponifiable fraction containing the hydrocarbons, alcohols, and sterols was then separated from the saponifiable fatty-acid fraction by extraction of the alkaline solution with benzene. The nonsaponifiable benzene fraction was dried under a