solution at 125.76 gauss (Bruker WP-500). Spectral parameters were chosen to achieve a digital resolution of 0.33 hertz (Hz); the natural resolution (width at half-height) was 0.6 Hz. Carbon type was determined by spin-polarization transfer experiments. Both synthetic and natural **2** and a 1:1 mixture of both gave signals (δ scale, in parts per million) of 19.680 (CH_3), 19.748 (CH_3), 19.777 (CH_3), 19.797 (CH_3), 24.356 (CH_2), 24.452 (CH_2), 24.473 (CH_2), 29.494 (CH), 32.789 (CH), 32.796 (CH), 33.045 (CH), 34.297 (CH_2), 37.301 (CH_2), 37.373 (CH_2), 37.396 (CH_2), 37.473 (CH_2), 37.542 (CH_2), 39.944 (CH_2), and 61.268 (CH_2). The intensity of the peak at 37.396 ppm suggests that it arises from the resonance of two carbons.

35. A 1:1 mixture of (3*R*)- and (3*S*)-dihydrophytol in $CDCl_3$ showed 28 signals at 125.76 gauss. The two peaks at 32.858 ppm and 37.515 ppm appear to result from the overlap of one resonance common to two isomers and a resonance unique to one isomer. If it is assumed that these two peaks do arise from such overlap, the following 30 resonances may be assigned (δ scale, in parts per million): 19.681 and 19.746, 19.774 and 19.847, 19.823, 22.701, 22.797, 24.436 and 24.443, 24.535 and 24.557, 24.873, 28.046, 29.566 and 29.586, 32.858, 32.858 and 32.871, 37.354, 37.389 and 37.451, 37.515 and 37.529, 37.560 and 37.570, 39.437, 40.017 and 40.102, and 61.281. A single number refers to a peak that results from the coincident resonance of corresponding carbons in the isomers, and a pair of numbers refers to two peaks, each approximately half the height of the taller ones, that result from the separate resonances of corresponding carbons.
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38. Supported by U.S. Public Health Service grants AI15027 and GM21328. We thank P. Connolly, A. Paul, and R. Nunlist for assistance with the NMR spectra; T. Rosen for assistance with the optical rotations; and L. Daniels for providing *M. thermoautotrophicum*. This is part 33 in a series of publications on acyclic stereoselection; for part 32, see K. Takai and C. H. Heathcock, *J. Org. Chem.*, in press.

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Chronology of Guitarrero Cave, Peru

Abstract. *Dating by accelerator mass spectrometry of wooden artifacts, cord, and charcoal samples from Guitarrero Cave, Peru, supports the antiquity of South America's earliest textiles and other perishable remains. The new dates are consistent with those obtained from disintegration counters and leave little doubt about the integrity of the lower Preceramic layers and their early cultivars. Reevaluation of the mode of deposition suggests that most of the remains resulted from short-term use of the cave in the eighth millennium B.C., with a possible brief human visit as early as 12,560 years ago.*

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At Guitarrero Cave, in a high Peruvian mountain valley, unusually dry conditions permitted recovery of a wide range of artifacts, including textiles, wood, bone, and domesticated plants (*1*), that were previously unknown for such an early period in South America (10,000 years ago). However, conventional radiocarbon age determinations made by three laboratories (1968 to 1973) left the Guitarrero stratigraphy and chronology unresolved (*2–4*). Accelerator mass spectrometry (AMS) makes possible the direct dating of minute samples of rare organic artifacts and cultivars, removing all question of their association with charcoal samples. Our analysis shows that the principal use of Guitarrero Cave, from which most of the remains resulted, occurred between 9,500 and 10,000 years ago.

The new dates were obtained by means of a tandem electrostatic accelerator as described (*5*). All samples were treated with acid to remove carbonates and with alkali to remove humic acids. Cellulose was extracted from wood and textiles by bleaching them with sodium chlorite. Excellent preservation allowed the use of small samples; for example, 60 to 70 mg of cord yielded 15 to 20 mg of purified carbon for combustion.

Charcoal from woody plants is the traditionally perferred material for radiocarbon dating. The Guitarrero charcoal samples, presumably from dispersed hearths, yielded a straightforward chronology when they were first tested by Geochron (Table 1). Samples GX 1778 and GX 1779 were out of apparent stratigraphic order, but their dates were within 2 standard deviations of counting error. Only the date of 12,560 years ago, in precedence of generally accepted dates for North American artifacts, was controversial. Dates determined from a verification series of Complex I charcoal conducted at the Smithsonian Institution laboratory were considerably younger than GX 1859 and equal to or younger than GX 1779 (Table 1). These results were consistent with both the previously determined dates and the stratigraphy of Complex I. The dates on Complex I, all internally consistent, were weighed equally when the site was described in 1980 (*1*).

The Smithsonian dates on Complex II, in grid square B1/A2 (Fig. 1), form a consistent series of five determinations (Table 1). However, the Smithsonian date on Complex IIa in grid square B1/A2 (9,580 years ago) is nearly 1,000 years more recent than the Geochron date of 10,535 years ago, whereas the Smithsonian date on Complex IIa in grid square C6 is essentially identical to the two Geochron dates. The Smithsonian date on Complex IIe overlaps the Geochron dates at 2 SD of counting error. As with the Geochron dates, there was only one stratigraphic inconsistency—that between the dates for samples SI 1502 and SI 1499 and those for Complex I. Because the relation between Complex IIa in grid square C6 and in B1/A2, which is more than 6 m distant (see Fig. 1), is based on interpolation, the discrepancy in apparent age is not extraordinary.

Despite the long chronology based on the Geochron dates (12,560 to 7,575 years ago), the shorter Smithsonian series (10,240 to 8,175 years ago), and Lynch's attempt to reconcile the dates (*1*), Vescelius (*4*) proposed as few as two brief occupations, one 10,000 years ago and another around 7,900 years ago, each lasting for perhaps a single generation. Mixture of charcoal from the two brief Preceramic occupations and intrusion of modern organics from the Christian era, when the cave was reused, would explain all discordant dates. The new dates from Oxford support the proposal of a brief occupation rather than steady use over several millennia, but they do not show contamination of Complex II with modern artifacts. Further, they do not support Patterson's reorganization of the stratigraphy nor his assumption of technological progression from unifacial to bifacial industries (*3*).

The Oxford dates from grid squares B1/A2 and B2N1/2 are all on charcoal and are uniform from top to bottom. The pooled mean age of these seven samples is 9,425 ± 55 years, all dates being effectively the same as judged from the procedures of Ward and Wilson to determine the mean, test statistic, and variance (*6*). The agreement with the five Smithsonian results on Complex I and the lower part of Complex II is excellent. Only sample SI 1501 is seriously divergent. However, as with Geochron sample GX 7575, SI 1501 might be contaminated with Ceramic age charcoal from mixed Complex IV. Both of these conventional dates came from samples composed of several pieces of charcoal, one of which might have been recent.

The accelerator dates support the antiquity of the Guitarrero artifacts (*1*). Moreover, a wood dowel from a Preceramic context in grid square B6 that

could not be placed within Complex II had the same age as the other wooden artifacts (Table 2). An age of 10,000 years (OxA 108) on a wood dowel from Complex III strengthens the interpretation that Complex III consists of restratified material from Complex II that has been minimally contaminated by the modern remains from badly mixed Complex IV (*1*). A fresh-looking scrap of leather from Complex III was dated to test the possibility of mixture in this area. If most of the charcoal at Guitarrero came from the early Preceramic occupation (or occupations), it would not be surprising that mixed samples from restratified Complexes III and IV were dominated by the earlier charcoal to yield "mixed" dates (Table 2). In this interpretation, uppermost Complex IIe might be reassigned to minimally mixed Complex III. A Complex IV firedrill hearth (S1 1504 and OxA 110) represents the fairly recent but prehistoric component of Complex IV.

The accelerator dates are a few hundred years older for the wood and tex-

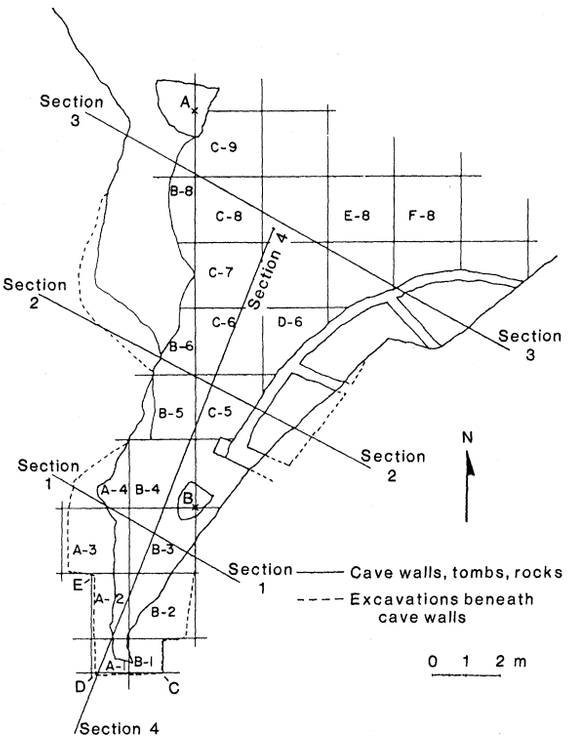

Fig. 1. Map of Guitarrero Cave and its excavation grid (Peruvian site number PAn 14-102), showing horizontal relation of grid squares referred to in the text and tables.

Table 1. Location of radiocarbon-dated samples in stratigraphic Complexes I and II. The correct relative depth is shown between grid squares B1/A2 and B2 N1/2 and between grid squares C5 and C6. The relation between grid squares B2 N1/2 and C5 is interpolated across a 3-m gap in the strata. Abbreviations: GX, Geochron samples; SI, Smithsonian Institution samples; OxA, Oxford accelerator samples.

Complex	Age (years before present ± standard deviation on counting error)			
	B1/A2	B2 N1/2	C5	C6
IIe	*Unit 18* 7,575 ± 200 (GX 1860) 8,175 ± 95 (SI 1501) 9,600 ± 130 (OxA 193)			*Unit 146* 7,680 ± 280 (GX 1861)
IId				*Unit 150* 10,180 ± 130 (OxA 195)*
IIc	*Unit 20* 8,910 ± 90 (SI 1500)		*Unit 122*† 9,980 ± 120 (OxA 196)	
IIb			*Unit 123* 9,930 ± 300 (OxA 104)‡	
IIa	*Unit 22* 10,535 ± 290 (GX 1778) 9,580 ± 135 (SI 1499) 9,430 ± 150 (OxA 194)			*Unit 159* 10,475 ± 300 (GX 1780) 10,240 ± 110 (SI 1502) 10,340 ± 130 (OxA 197)†
I		*Unit 59* 9,140 ± 90 (SI 1497)		
	Unit 26 9,660 ± 150 (SI 1498) 9,520 ± 150 (OxA 181)	Unit 60 9,280 ± 150 (OxA 182)		
	Unit 28 9,790 ± 240 (GX 1779)	*Unit 62* 9,475 ± 130 (SI 1496) 9,340 ± 150 (OxA 183)		
		Unit 63 12,560 ± 360 (GX 1859) 9,400 ± 150 (OxA 184)		
		Unit 64 9,350 ± 150 (OxA 185)		

* Bipointed wood dowel. † Cord. ‡Wood batten.

Table 2. Radiocarbon samples from stratigraphic Complexes P, III, and IV. Complex P is an unmixed Preceramic context that cannot be correlated directly to the subdivisions of Complexes I and II. Complex III overlay Complex II, contained no potsherds, but showed some other signs of possible mixture with later remains. Complex IV was an obvious mixture of Preceramic material, later prehistoric artifacts, and modern contaminants; its restratified levels lay above Complexes P, II, and III.

Complex	Grid square	Unit	Sample	Date (years before present ± SD)	Material dated
IV	B2 N1/2	47	SI 1504	2,315 ± 125	Wood firedrill hearth
	B2 N1/2	47	OxA 110	2,150 ± 150	Wood firedrill hearth
	B2 N1/2	47/48	SI 1503	8,225 ± 90	Pooled charcoal
III (?)	B6	2	GX 1451	6,610 ± 160	Pooled charcoal
III	B3	82	RL 112	7,730 ± 150	Pooled charcoal
	B3	82	OxA 198	0 ± 100	Leather scrap
	B2 S1/2	35	OxA 108	10,000 ± 200	Wood dowel
P	B6	133	OxA 109	9,860 ± 200	Wood dowel

tiles than for the charcoal of Complex II. Curation alone does not seem to account for the difference. We applied a combining procedure to test which dates might be considered effectively the same (*6*). The 13 Oxford dates older than 9,000 years ago have a pooled mean age of 9,693 years, but the distribution is not homogeneous (*6*). However, separately, the wood samples have a pooled mean age of 10,050 ± 90 years, and the dates are effectively equal (*6*). The pooled mean age of the two cord samples is 10,150 ± 90 years, which is consistent with the age of the combined series of wood and cords (10,100 ± 65 years). This figure contrasts with the pooled mean age of 9,425 ± 55 years on the seven Oxford charcoal samples, which are homogeneous (*6, 7*).

Bones of cervids surely entered the site by human agency and were unlikely to be intrusive from younger levels. However, these artifacts lacked sufficient collagen to provide hydroxyproline for AMS dating. Maize cobs, bean pods, fruits of *Solanum hispidum,* tubers tentatively identified as *oca* and *ullucu,* and a pepper fruit from Complex II have not yet become available for destructive analysis. Maize, which was found only in Complexes III and IV, may be less than 2,000 or 3,000 years old, but beans and pods from units 122, 123, and 150 should have ages equivalent to those for the wood and cord samples (~10,000 years). Large seed size, which correlates with loss of the hard seed coat cuticle that is impenetrable to the South American bruchid weevil, should have increased rapidly once Preceramic gatherers and planters learned to eliminate weevils by warming beans in heated sand (*8*). Heating plant foods in earth adjacent to hearths is a common practice in low-technology societies and would explain the inclusion of beans in the Complex II deposits.

Few sites of such small size and limited complexity have been so thoroughly dated, with each new set of results suggesting major revisions to the chronological framework and history of human use. It now appears that most of the archaeological remains resulted from use of the cave between 9,000 and 10,000 years ago, an interpretation that is supported by at least 16 highly concordant age determinations from several laboratories. Given the variety of materials dated and the known preferential incorporation of carbon isotopes by some organisms, the main interval of occupation may have been only 500 years, or conceivably only a generation or less.

Redating of the Guitarrero charcoal shows that the first result of 12,560 years (GX 1859) on Complex I is isolated, along with the four determinations of 10,240 to 10,535 years on materials from units 22 and 159 (GX 1778, GX 1780, SI 1502, and OxA 197). If these dates represent earlier episodes of human use of the cave, the occupants 9,000 or 10,000 years ago must have churned and redeposited their trash. (Mixing, intrusions, and redepositions that postdated the Preceramic occupation were recognized during excavation and account for most of the deposits labeled Complex II?, III, and IV.) Except for the piece of cord directly dated in sample OxA 197 (10,340 years), we cannot attribute any specific artifacts to earlier short visits to Guitarrero cave (*10*).

Some archeologists will disregard GX 1859 entirely, even though massive contamination by dead carbon (more than 30 percent) would be required to produce a radiocarbon date of 12,500 years from a sample 10,000 years old. Nevertheless, Rick (*9*) calls attention to five Peruvian charcoal-based dates falling between 11,800 and 12,795 years ago. Although all are problematic, at Guitarrero, Pachamachay, Quirihuac, and Telarmachay the oldest dates are separated by a gap of at least 2,000 years before the next younger dates (*10*). To Rick's cluster of problematic dates, we add the similarly isolated dates from Los Toldos in Argentina (12,600 years) and El Abra in Colombia (12,400 years); these are rock shelter sites where the dated charcoal can be reasonably presumed to be from campfires (*11*). A number of dates on charcoal and ivory at the open-air Monte Verde site in Chile may also show human activity between 12,000 and 13,000 years ago (*12*).

There is no intrinsic reason to doubt the 12,560-year date at Guitarrero Cave, nor do we suspect secular variations in the production of ^{14}C that would have been great enough to bring that and similarly aged samples close (in real calendrical age) to the well-dated occupation about 10,000 years ago. However, the AMS dates indicate a primary period of human use that probably did not exceed 500 to 1000 years.

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$$A_p = \left(\sum_1^n A_i/S_i^2\right) \Big/ \left(\sum_1^n 1/S_i^2\right)$$

The test statistic is then calculated from

$$T = \sum_1^n (A_i - A_p)^2/S_i^2$$

which has a χ^2 distribution with $n - 1$ degrees of freedom under the null hypothesis

$$H_0: R_1 = \ldots = R_1 = \ldots = R_n$$

If the dates are judged not to be significantly different (at probability level 0.05), then the determinations can be combined, and A_p has a variance given by

$$V(A_p) = \left(\sum_1^n 1/S_i^2\right)^{-1}$$

$V(A_p)$ is the variance of the mean of the group of n observations and not the variance of the group of determinations.
7. From the 13 Oxford dates alone, J.A.J.G. suggests that Guitarrero was occupied briefly about 10,000 years ago and again about 9,400 years ago. T.F.L. thinks it more likely that the segregation of assays on burned and unburned material has to do with some unexplained systematic error. The Smithsonian dates older than 9,000 years have a pooled mean that lacks homogeneity at the 5 percent level. The five dates can be reduced to three groups as follows (in years before present): (i) SI 1497, 9,140 ± 90; (ii) SI

1496, 9,475 ± 130; SI 1499, 9,580 ± 135; and SI 1498, 9,660 ± 150; and (iii) SI 1502, 10,240 ± 110. The pooled mean age for the second group is 9,560 ± 80 years. Oxford dates on charcoal from units 26 and 62 are also compatible at the 5 percent level. If the Geochron dates are introduced, without regard to the stratigraphy and with all dates segregated to homogeneity at the 5 percent level, four discrete occupations may have occurred earlier than 9,000 years ago. The first and the fourth would be represented by single determinations of 12,560 ± 360 years (GX 1859) and 9,140 ± 90 years (SI 1497). The second occupation would be represented by the pooled mean age of two Geochron assays on charcoal (10,510 ± 210 years), one Smithsonian charcoal age (10,240 ± 110 years), and the pooled mean age of six Oxford assays on cordage and wood artifacts (10,100 ± 65 years). A third occupation would be represented by a Geochron date of 9,790 ± 240 years ago), a pooled mean of three Smithsonian dates (9,560 ± 80 years ago), and the pooled mean of seven Oxford dates (9,425 ± 55 years ago), all on charcoal. As judged from the Oxford dates, the four discrete occupations would have occurred about 12,200, 10,100, 9,400, and 9,000 years ago. Andean artifact typology is not exact enough to support this proposal.

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13. We thank R. Stuckenrath, H. Krueger, and C. Tucek for services and advice on radiocarbon dating; E. S. Wing for selecting and forwarding faunal samples; A. D. Bowles, J. F. Foreman, M. J. Humm, E. Hendy, B. Phillips, and B. Donnell for technical assistance; J. Rick for permission to cite his unpublished paper; and T. P. Volman for help with statistics. Supported in part by NSF grants GS-1969, GS-2399, and BNS-8418815. Work at the Oxford Radiocarbon Accelerator Unit was supported in part by the Science and Engineering Research Council.

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A Macrophage Factor Inhibits Adipocyte Gene Expression: An in Vitro Model of Cachexia

Abstract. *Certain infections and malignancies in mammals cause the development of a condition known as cachexia in which the animal continues to lose weight, often while consuming an adequate diet. When macrophages are stimulated with an endotoxin, they produce a factor or factors, termed cachectin, that inhibits the activity of fat-producing (lipogenic) enzymes in cultured adipocytes. This effect may reflect one of the physiological bases for cachexia. In the present study, clones of complementary DNA from genes whose expression is increased during the differentiation of adipocytes were used to study the molecular basis of cachectin's actions. In the presence of cachectin, the expression of the corresponding genes was reversibly and specifically inhibited. Furthermore, when mature adipocytes were exposed to cachectin, the messenger RNA's of those genes diminished and rapidly approached the levels present before differentiation.*

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The development of a chronic catabolic state is a hallmark of certain infections and malignancies. The weight loss that accompanies this condition is termed cachexia, and is associated with the mobilization of triglycerides from adipose tissue, a process that often persists in spite of adequate caloric intake. A factor or factors (termed cachectin) produced by endotoxin-stimulated macrophages in vitro dramatically decreases the synthesis and activity of key lipogenic enzymes of cultured 3T3L1 adipocytes (*1*, *2*). In this study, we used the stable adipogenic cell line TA1 to examine the mechanism of inhibition of lipogenic enzyme activity.

When cultured in monolayers, TA1 adipocytes develop a typical adipocyte morphology approximately 3 days after reaching confluence (*1*). As this differentiation occurs, several genes are expressed whose activity is evident only when differentiation has been initiated (*3*) (clones 1, 20, 28, 47). These messenger RNA's (mRNA's) are expressed largely or completely as a result of transcriptional activation of the corresponding genes (*4*). To assess the influence of cachectin on the coordinate induction of these adipose genes, we added cachectin to preconfluent TA1 cells and to TA1 cells on the day they reached confluence. Total RNA isolated from these and control cells 6 days after they reached confluence was probed with radiolabeled cDNA's (complementary DNA's) of genes whose expression was observed in adipocytes, but not in preadipocytes. Treatment with cachectin prevented the accumulation of adipose-inducible mRNA's (Fig. 1). Lipid accumulation was also completely inhibited by cachectin. Cultures of TA1 cells treated with cachectin were maintained for as long as 23 days without the appearance of neutral lipid, as detected by staining with oil red O. However, on removal of cachectin from the media, adipocyte morphology returned as did the expression of adipose inducible genes (for example, see clone 1 in Fig. 2).

The effects of cachectin are not due to endotoxin itself, since control supernatants from RAW 264 cells to which endotoxin has been added do not inhibit lipid accumulation or the production of lipogenic enzymes (*1*, *2*). Furthermore, cachectin does not generally affect gene expression; for example, the level of β-actin mRNA is unaffected by cachectin (Fig. 1). In addition, cachectin treatment of preadipocyte cultures does not affect cell growth or viability. In experiments similar in design to those in Fig. 1, cellular proliferation was determined by cell counting and [^{3}H]thymidine incorporation. Control and cachectin-treated

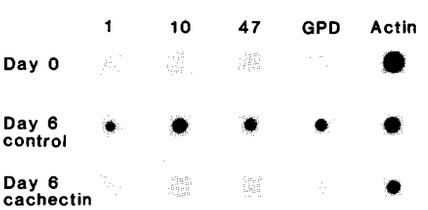

Fig. 1. TA1 cells, a stable adipogenic cell line derived from 5-azacytidine treatment of 10T1/2C18 cells (*3*, *10*, *11*), were grown in Eagle's basal medium supplemented with 10 percent heat-inactivated fetal calf serum. Dexamethasone ($10^{-6}M$) was present in the medium for the first 3 days after the cells reached confluence, and bovine insulin (5 μg/ml) for the first 6 days after confluence. Conditioned medium from the macrophage cell line RAW 264 treated with endotoxin (24 hours at 10 μg/ml in serum-free medium) was first added to preadipocyte cultures 2 days before they reached confluence at a concentration of 10 μl/ml, which inhibits 90 percent of lipoprotein lipase activity in cultured adipocytes. Cell cultures were resupplemented with hormones at day 0 (confluence) and day 3. Cells were harvested at day 6. Total RNA was isolated by the method of Chirgwin *et al.* (*12*), and applied to nitrocellulose in a dot blot apparatus (BRL). Nick-translated cDNA clones of genes whose expression is seen only in differentiated TA1 adipocytes (clones 1, 10, and 47, and glycerophosphate dehydrogenase) (*3*), as well as a β-actin cDNA clone were used to probe these filters under hybridization conditions previously described (*1*). Filters were washed, then exposed to XAR5 film at −70° with an intensifying screen.