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Paleobiological Implications of the Isotopic Signatures (^{13}C, ^{15}N) of Fossil Mammal Collagen in Scladina Cave (Sclayn, Belgium)

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An isotopic investigation of upper Pleistocene mammal bones and teeth from Scladina cave (Sclayn, Belgium) demonstrated the very good quality of collagen preservation. A preliminary screening of the samples used the amount of nitrogen in whole bone and dentine in order to estimate the preserved amount of collagen before starting the extraction process. The isotopic abundances of fossil specimens from still-extant species are consistent with their trophic position. Moreover, the ^{15}N isotopic abundance is higher in dentine than in bone in bears and hyenas, a phenomenon already observed in modern specimens. These results demonstrate that the isotopic compositions of samples from Scladina cave can be interpreted in ecological terms. Mammoths exhibit a high ^{15}N isotopic abundance relative to other herbivores, as was the case in Siberian and Alaskan samples. These results suggest distinctive dietary adaptations in herbivores living in the mammoth steppe. Cave bears are clearly isotopically different from coeval brown bears, suggesting an ecological separation between species, with a pure vegetarian diet for cave bear and an omnivorous diet for brown bear.

INTRODUCTION

Isotopic biogeochemistry of fossil bone and tooth collagen is a relatively new approach to paleoenvironnemental and paleodietary research on Pleistocene mammals. A number of such studies in Western Europe (Bocherens *et al.,* 1991, 1994a, 1995b; Fizet *et al.,* 1995), Siberia (Bocherens *et al.,* 1996), North America (Koch, 1991; Bocherens *et al.,* 1994b, 1995a; Matheus, 1995; Heaton, 1996), South America (Fernandez *et al.,* 1991), and Australia (Gröcke and Bocherens, 1996; Gröcke, in press) have illustrated that different species exhibited different isotopic signatures, and some of these studies have suggested the ecological basis for such isotopic differences (Bocherens *et al.,* 1994a, 1996; Fizet *et al.,* 1995; Matheus, 1995; Heaton, 1996; Gröcke, in press). Scladina Cave is an extremely rich site that yielded a mammal fauna of high taxonomical diversity, ranging in age from 40,000 to 130,000 yr old (Simonet, 1992). The purpose of this paper is to assess the preservation of biogenic isotopic composition of the collagen fraction of mammal bone and tooth from the uppermost well-defined layer of the cave (numbered 1A) and to determine the ecological basis for interspecific differences in the carbon and nitrogen isotopic compositions of mammals from other late Pleistocene sites in Eurasia.

MATERIAL

About 50 bone and tooth specimens of different mammal species collected in layer 1A of Scladina Cave have been analyzed. This cave is located 400 m southwest of the village of Sclayn (Andenne, Namur, Belgium), close to the Meuse River (Bonjean, 1996). The fauna was composed of herbivorous species, including horse (*Equus caballus*), bovines (*Bison priscus* and *Bos primigenius*), giant deer (*Megaceros giganteus*), woolly rhinoceros (*Coelodonta antiquitatis*), woolly mammoth (*Mammuthus primigenius*), omnivorous species, including brown bear (*Ursus arctos*) and cave bear (*Ursus spelaeus*), and a carnivorous species, the cave hyena (*Crocuta crocuta*). The analyzed bovine fragments could not be determined to the genus level, and could belong either to *Bos* or *Bison.* The geological age of the deposits in layer 1A seems to be contemporaneous with the mid-Wurmian interstade, about 40,000 yr old (Simonet, 1992). The habitat preferences of herbivorous species include open environments, forest, and cold climate and suggest an environment with grassland, herbaceous steppe, and some woods under a continental humid climate (Simonet, 1992).

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TABLE 1
Results of Whole Bone and Dentine Analysis and Isotopic Composition of Collagen Extracted from Herbivorous Species from Layer 1A in Scladina Cave

Analysis number	Excavation number	Species	Sample	% N in bone or dentine	Yield (mg · g^{-1})	Collagen				
						% C	% N	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
SC3900	SC87 140 (I27)	*Equus caballus*	upper tooth (D)	1.4	18.9	42.5	15.6	3.2	−21.7	5.2
SC4100	SC89 135 (G29)	*Equus caballus*	upper tooth (D)	1.7	51.7	42.6	15.8	3.1	−21.7	5.1
SC4200	SC87 126 (F25)	*Equus caballus*	upper tooth (D)	1.7	43.9	42.8	15.8	3.2	−21.9	5.0
SC4300	SC85 121	*Equus caballus*	upper tooth (D)	2.3	54.2	39.9	14.5	3.2	−21.5	4.8
SC4400	SC85 141	*Equus caballus*	upper tooth (D)	2.0	59.8	40.9	15.0	3.2	−21.6	7.0
								Av.	−21.7	5.4
								SD	0.1	0.8
SC4500	SC83 304	*Bos* or *Bison*	lower P (D)	1.3	35.5	41.7	15.2	3.2	−20.5	4.8
SC4700	SC89 94 (I28)	*Bos* or *Bison*	lower P3 (D)	1.2	32.4	42.7	15.7	3.2	−20.5	4.3
SC4800	SC86 21	*Bos* or *Bison*	lower M1 (D)	1.7	28.6	41.2	15.3	3.1	−19.9	5.3
SC4900	SC83 282	*Bos* or *Bison*	lower P3 (D)	1.7	29.7	40.8	15.0	3.2	−20.7	4.4
								Av.	−20.4	4.7
								SD	0.3	0.4
SC2300	SC81 172	*Megaloceros giganteus*	ectocuneiform	3.1	116.2	41.6	15.2	3.2	−20.2	4.0
SC2500	SC85 158	*Megaloceros giganteus*	upper tooth (D)	2.0	48.0	43.7	15.9	3.2	−20.2	5.0
SC900	SC86 131	*Coelodonta antiquitatis*	lower P2 (D)	2.8	86.7	42.6	15.5	3.2	−20.9	5.5
SC1000	SC82 303	*Coelodonta antiquitatis*	lower P2 (D)	1.8	58.5	42.1	15.4	3.2	−20.3	6.9
SC1100	SC82 246	*Coelodonta antiquitatis*	lower P2 (D)	1.8	57.5	42.2	15.2	3.2	−20.0	6.4
SC1200	SC82 210	*Coelodonta antiquitatis*	lower P2 (D)	2.5	79.4	41.2	15.0	3.2	−21.1	5.3
SC1300	SC87 129 (I26)	*Coelodonta antiquitatis*	lower P2 (D)	1.8	44.9	41.8	15.2	3.2	−20.4	7.5
SC1400	SC81 205	*Coelodonta antiquitatis*	lower P2 (D)	2.6	80.8	43.1	15.7	3.2	−20.6	5.5
								Av.	−20.6	6.2
								SD	0.4	0.8
SC600	SC83 285	*Mammuthus primigenius*	tooth (E + D)	1.4	45.1	42.6	15.5	3.2	−20.9	8.4
SC700	SC85 121	*Mammuthus primigenius*	tooth (E + D)	1.2	31.7	41.5	15.2	3.2	−21.5	9.4
SC800	SC87 31 (I26)	*Mammuthus primigenius*	tooth (E + D)	0.9	25.9	41.3	15.1	3.2	−21.6	8.3
								Av.	−21.3	8.7
								SD	0.3	0.5

Note. (D) represents teeth where dentine only has been analyzed, whereas (E + D) represents teeth analyzed with enamel and dentine together.

METHODS

Fragments were sampled by sawing or breaking small pieces from bones and teeth. These samples were then sonicated in acetone in order to remove any possible synthetic glue and, after abundant rinsing, were powdered to a particle size of less than 0.7 mm. To screen a large number of specimens for the quality of collagen preservation, a preliminary determination of the total nitrogen (N) content was performed, as suggested by Iacumin *et al.* (1996). This measurement is done using a CHN elemental analyzer Carlo Erba NA 1500. About 5 mg of accurately weighed bone or tooth powder is flash-combusted in a quartz oven and, after trapping of water and chromatographic separation of the evolved CO_2 and N_2, the amounts of N are calculated by comparison with those of a standard (tyrosine). Determination of % N is performed with a standard deviation of 0.1%.

The extraction of collagen is performed using the protocol

TABLE 2
Results of Whole Bone and Dentine Analysis and Isotopic Composition of Collagen Extracted from Omnivorous and Carnivorous Species from Layer 1A in Scladina Cave

Analysis number	Excavation number	Species	Sample	% N in bone or dentine	Yield (mg·g^{-1})	Collagen				
						% C	% N	C/N	δ^{13}C (‰)	δ^{15}N (‰)
SC3100	SC85 130	*Ursus spelaeus*	mandible	2.6	75.7	40.9	14.8	3.2	−22.5	3.7
SC3200	SC82 352	*Ursus spelaeus*	mandible	1.6	73.4	42.4	15.2	3.3	−22.1	5.7
SC3300	SC87 171 (G27)	*Ursus spelaeus*	mandible	2.3	60.1	43.3	15.8	3.2	−22.2	6.0
SC3500	SC87 103 (H25)	*Ursus spelaeus*	phalanx II	2.1	80.0	44.0	16.0	3.2	−21.8	5.1
SC3600	SC82 131	*Ursus spelaeus*	phalanx II	2.1	73.4	42.8	15.6	3.2	−21.8	3.0
SC3700	SC86 136	*Ursus spelaeus*	phalanx II	1.4	55.3	42.4	15.4	3.2	−22.0	6.1
SC3800	SC83 291	*Ursus spelaeus*	phalanx II	2.8	119.7	42.5	15.5	3.2	−22.2	5.0
								Av.	−22.1	4.9
								SD	0.2	1.1
SC2700	SC83 295	*Ursus spelaeus*	lower I3	1.6	44.1	42.5	15.5	3.2	−23.0	6.5
SC2800	SC83 283	*Ursus spelaeus*	lower I3	2.7	89.8	43.8	15.9	3.2	−22.7	5.7
SC2900	SC83 295	*Ursus spelaeus*	lower I3	1.7	46.1	42.9	15.7	3.2	−22.8	7.1
SC3000	SC83 63 bis	*Ursus spelaeus*	lower I3	2.8	95.1	43.5	15.9	3.2	−22.5	7.0
SC3150	SC85 130	*Ursus spelaeus*	lower P4	2.9	87.0	43.2	15.7	3.2	−23.3	4.5
SC3250	SC82 352	*Ursus spelaeus*	lower P4	2.3	62.3	40.3	14.7	3.2	−23.2	8.4
SC3350	SC87 171 (G27)	*Ursus spelaeus*	lower P4	2.4	77.6	43.0	15.7	3.2	−23.3	7.3
								Av.	−23.0	6.6
								SD	0.3	1.2
SC300	SC85 94	*Ursus arctos*	lower I3	2.0	62.1	39.7	14.6	3.2	−20.2	7.1
SC1800	SC85 150 (K28)	*Crocuta crocuta*	phalanx I	2.1	62.5	42.9	15.6	3.2	−20.2	8.8
SC1900	SC86 127	*Crocuta crocuta*	phalanx I	3.6	124.4	42.6	15.6	3.2	−19.6	10.1
SC2000	SC83 93	*Crocuta crocuta*	phalanx I	3.2	110.0	42.8	15.7	3.2	−19.7	8.2
SC2100	SC87 162 (G27)	*Crocuta crocuta*	phalanx I	3.4	149.9	42.9	15.6	3.2	−19.4	9.5
SC1700	SC83 93	*Crocuta crocuta*	mandible	1.3	31.9	39.9	14.6	3.2	−19.8	9.6
SC2200	SC83 93	*Crocuta crocuta*	maxillary	2.8	113.7	42.0	15.4	3.2	−19.3	9.4
								Av.	−19.7	9.3
								SD	0.3	0.6
SC1500	SC89 30 (H28)	*Crocuta crocuta*	lower P3	1.3	18.0	40.3	14.7	3.2	−19.3	10.3
SC1600	SC87 174 (F26)	*Crocuta crocuta*	lower P3	0.9	12.5	37.8	13.7	3.2	−19.3	10.1
SC1750	SC83 93	*Crocuta crocuta*	lower P3	1.7	60.4	42.2	15.4	3.2	−19.4	11.5
SC2230	SC83 93	*Crocuta crocuta*	upper C	1.2	19.6	38.6	14.0	3.2	−19.4	10.3
SC2260	SC83 93	*Crocuta crocuta*	upper P2	2.0	77.4	41.2	15.0	3.2	−19.0	10.7
								Av.	−19.3	10.6
								SD	0.1	0.5

Note. In teeth, only dentine has been analyzed.

described by Bocherens *et al.* (1991). Briefly, about 300 mg of bone powder is decalcified in 1 M HCl for 20 min at room temperature and filtered through a 5 μm filter. The insoluble residue is soaked in 0.125 N NaOH for 20 h at room temperature. The rinsed residue is heated in closed tubes at 100°C for 17 h in a 10^{-2} M HCl (pH 2) solution, in order to gelatinize the collagen. After filtration through a 5 μm filter, the filtrate containing gelatin is freeze-dried. Yield is expressed as the amount of freeze-dried gelatin relative to the dry weight of bone (in mg g^{-1}).

Determination of stable carbon and nitrogen isotope ratios was performed on a Carlo-Erba NA1500 CHN-elemental analyzer coupled to an isotopic ratio mass spectrometer (Fisons Optima). One milligram of sample in a tin capsule was flash-combusted in a quartz oven at 1020°C, and the evolved gases, swept by a helium flow, were purified successively on an oxidation, and then a reduction, furnace. After trapping of water and chromatographic separation of evolved CO_2 and N_2, the amounts of C and N were measured with a thermal conductivity detector by comparison of the integrated areas with those of a standard (alanine). For CO_2 and N_2 isotopic analysis, the gases were introduced in a VG Optima isotopic ratio mass spectrometer in a continuous helium flow and isotopic measurement was done by comparison with coinjected reference gases, calibrated against international standards. Results are calibrated against a well-known product analyzed the same way as the samples, used as an internal reference. Isotopic abundances measured this way are relative abundances: enrichment or depletion of heavy isotopic varieties (^{13}C, ^{15}N) are expressed versus international standards. The isotope ratios are expressed for carbon as δ^{13}C vs PDB (a marine carbonate) and for nitrogen as δ^{15}N vs atmospheric N_2: $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$, where X stands for ^{13}C or ^{15}N and R stands for ^{13}C/^{12}C or ^{15}N/^{14}N, respectively. The precision is 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N (Bocherens *et al.*, 1995a).

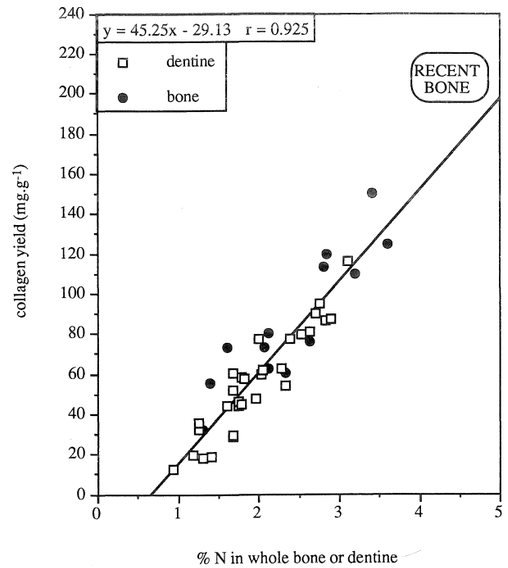

FIG. 1. Relationship between N amount in whole bone or dentine and collagen yield in samples from layer 1A in Scladina Cave (Sclayn 1A).

RESULTS

Preservation of Organic Matter

In whole bone and dentine, nitrogen concentrations range from 0.8 to 3.6% N (Tables 1 and 2). Considering an average nitrogen amount in fresh bone of 4.4% N (mean value of 36 fresh bone samples analyzed under the same conditions), the amount of nitrogen still present in the analyzed fossil bones corresponds to proportions ranging from 4.5 to 72.5% of the collagen present in fresh bone.

The extraction yields, which express the weight of extracted collagen relative to the dry weight of bone or dentine before collagen extraction, range from 12.5 to 149.9 mg g^{-1} (Tables 1 and 2). The C/N values measured on the extracted residues are all within the accepted range for fresh collagen (2.9–3.6; DeNiro, 1985). After taking into account the actual carbon and nitrogen amounts in the extracted insoluble residues (% C and % N in Tables 1 and 2), which is necessary due to the possible occurrence of mineral contaminants, the collagen amounts range from 4.6 to 72.7%, taking a value of 210 mg g^{-1} as an average for fresh bones treated similarly to the fossil samples.

There is a highly significant correlation between the amount of nitrogen in whole bone and dentine and the yield of extracted collagen ($r = 0.925$, $p < 0.01$, $n = 45$; Fig. 1). The regression line is close to the domain of fresh bone values, which indicates that the amount of collagen extracted relative to the amount of collagen actually present in the

TABLE 3
Whole Bone and Dentine Nitrogen Amounts and Isotopic Composition of Collagen Extracted from Bone and Dentine of the Same Individuals of Cave Bears and Hyenas

Analysis number	Excavation number	Species	Sample	% N in bone or dentine	Yield (mg·g^{-1})	Collagen				
						% C	% N	C/N	δ^{13}C (‰)	δ^{15}N (‰)
SC3100	SC85 130	*Ursus spelaeus*	mandible	2.6	75.7	40.9	14.8	3.2	−22.5	3.7
SC3150	SC85 130	*Ursus spelaeus*	P4	2.9	87.0	43.2	15.7	3.2	−23.3	4.5
SC3200	SC82 352	*Ursus spelaeus*	mandible	1.6	73.4	42.4	15.2	3.3	−22.1	5.7
SC3250	SC82 352	*Ursus spelaeus*	P4	2.3	62.3	40.3	14.7	3.2	−23.2	8.4
SC3300	SC87 171 (G27)	*Ursus spelaeus*	maxillary	2.3	60.1	43.3	15.8	3.2	−22.2	6.0
SC3350	SC87 171 (G27)	*Ursus spelaeus*	P4	2.4	77.6	43.0	15.7	3.2	−23.3	7.3
SC1700	SC83 93	*Crocuta crocuta*	mandible	1.3	31.9	39.9	14.6	3.2	−19.8	9.6
SC1750	SC83 93	*Crocuta crocuta*	P3	1.7	60.4	42.2	15.4	3.2	−19.4	11.5
SC2200	SC83 93	*Crocuta crocuta*	maxillary	2.8	113.7	42.0	15.4	3.2	−19.3	9.4
SC2230	SC83 93	*Crocuta crocuta*	C	1.2	19.6	38.6	14.0	3.2	−19.4	10.3
SC2260	SC83 93	*Crocuta crocuta*	P2	2.0	77.4	41.2	15.0	3.2	−19.0	10.7

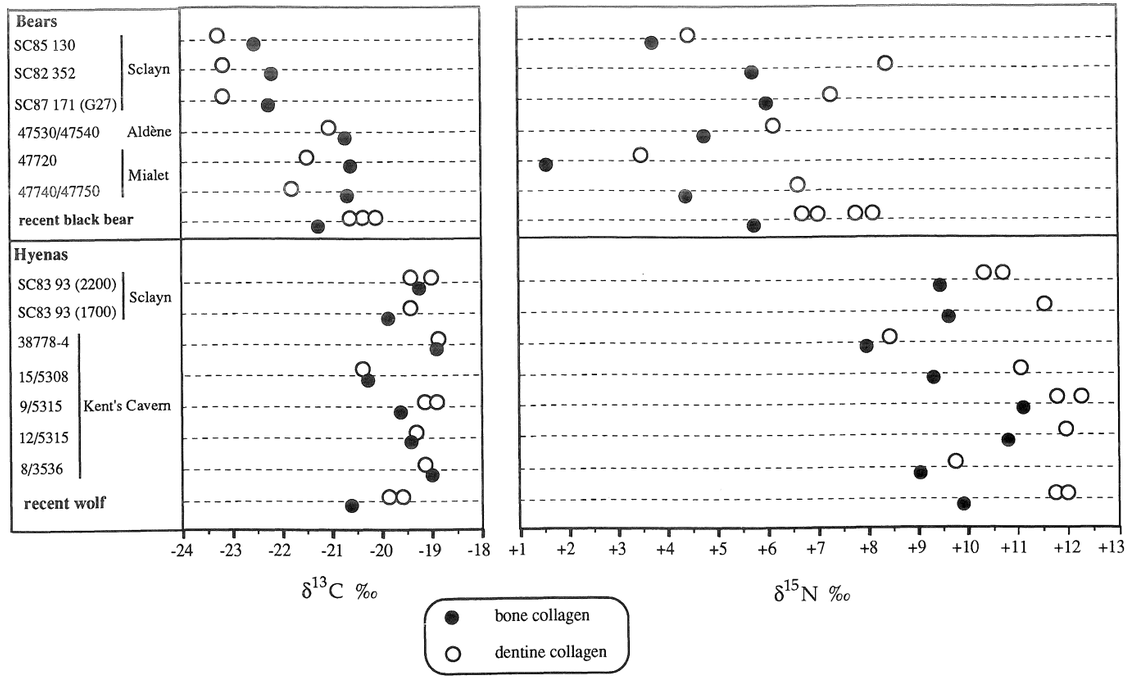

FIG. 2. Comparison of bone and dentine $\delta^{13}C$ and $\delta^{15}N$ values of individuals belonging to fossil cave bears and hyenas, and to modern black bear and wolf. Data for modern samples and Aldène, Mialet, and Kent's Cavern samples are from Bocherens *et al.* (1994a, 1995b).

bone is close to the yield in fresh bone, using the same extraction protocol. This suggests that the remaining collagen in fossil bone and dentine presents solubility properties similar to those of native collagen. These results demonstrate that the amount of nitrogen in whole fossil bone and dentine is a good proxy for extractable collagen. Since measuring the amount of nitrogen in whole bone is fast and easy with a CHN analyzer and can be performed on very small samples, this technique can be used to screen large numbers of fossil samples in order to check collagen preservation prior to sampling on a larger scale. Moreover, it can be used to select the samples with the best preserved collagen in a given site and to calculate the quantity of powder necessary to have an optimum extraction.

Isotopic Composition of Mammal Bones

Fossil collagen $\delta^{13}C$ values range from −23.3 to −18.9‰ and $\delta^{15}N$ values range from 3.0 to 11.5‰ (Tables 1 and 2). Hyenas present the highest $\delta^{13}C$ and $\delta^{15}N$ values (−20.2 to −19.0‰ and 8.2 to 11.5‰, respectively), mammoths are next (−21.6 to −20.9‰ and 8.3 to 9.4‰, respectively), and cave bears present the lowest $\delta^{13}C$ and $\delta^{15}N$ values (−23.3 to −21.8‰ and 3.0 to 8.4‰, respectively). Herbivores other than mammoths have low $\delta^{15}N$ values (4.0 to 7.0‰) and intermediate $\delta^{13}C$ values (−21.9 to −19.9‰). A more-detailed discussion of the isotopic abundances according to the trophic groups and the analyzed tissue (i.e., bone or dentine) will be presented below.

DISCUSSION

Quality of Preserved Organic Matter

The total nitrogen amount exhibits differences between bone and dentine. In bone, the average is 2.5 ± 0.7% N (n = 14) whereas in dentine, the average is 1.9 ± 0.5% N (n = 29). The higher total nitrogen content in bone appears clearly in Figure 1, and a Student's t test shows that the difference is statistically significant (α = 2.87 for ν = 41, $p < 0.01$). Thus, bone seems to preserve collagen better than dentine in layer 1A of Scladina Cave. This is quite an unexpected result since no such difference had been reported for other late Pleistocene caves, such as Marillac (Fizet *et al.,* 1995) and Kent's Cavern (Bocherens *et al.,* 1995b). Moreover, dentine could have been considered *a priori* to be more stable than bone, due to its denser structure and partial protection by cement in the root and enamel in the crown, although dentinal microtubules are long and permit invasion and removal of collagen.

In samples from layer 1A in Scladina Cave, it appears that samples with total nitrogen amounts as low as 0.9% N (sample SC800) yield collagen of an acceptable quality for isotopic analysis (C/N = 3.2). In all extracted collagen, the C/N values range from 3.1 to 3.3, which are well within the acceptable range of values (DeNiro, 1985).

By comparison with other cave sites from western Europe, such as Marillac and Kent's Cavern, it is noteworthy that all the samples analyzed in Sclayn layer 1A yielded well-preserved collagen, whereas in both other sites, some samples failed to yield collagen (Bocherens *et al.,* 1995b; Fizet *et al.,* 1995). The quantities of collagen recovered from Sclayn layer 1A specimens are, however, lower than in the case of Siberian and Alaskan specimens excavated from frozen ground (Bocherens *et al.,* 1995a, 1996).

Preservation of Collagen Isotopic Abundances

Diagenetic alteration of biogenic isotopic abundances can be detected through disruption of *in vivo* isotopic patterns,

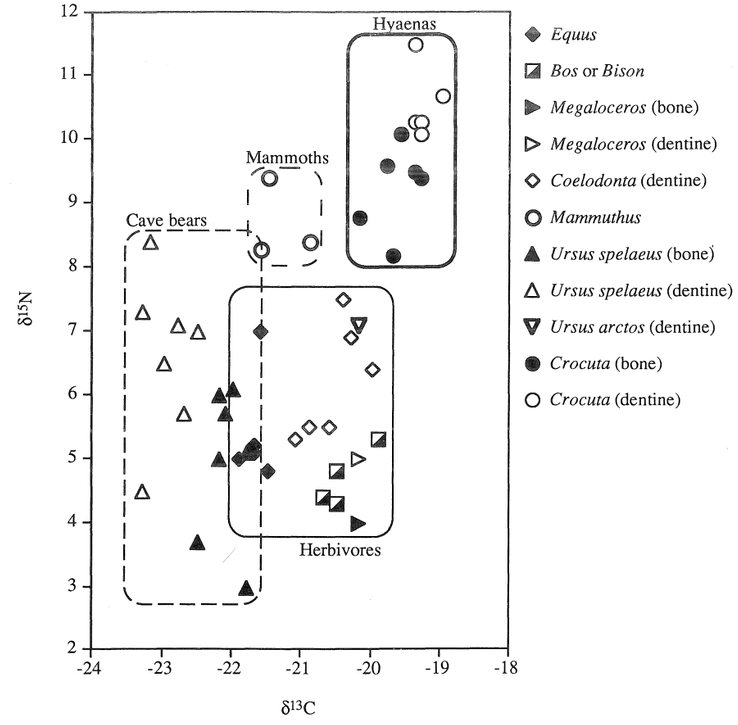

FIG. 3. Bone and dentine collagen $\delta^{13}C$ and $\delta^{15}N$ values from Sclayn 1A mammals.

TABLE 4

Results of Student's t Tests for the Different Taxa with More than Four Analyzed Individuals in Sclayn 1A, for Carbon Isotopic Composition

$\delta^{13}C$	Horse	Bovine	Rhinoceros	Cave bear	Hyena
Horse		S	S	S	S
Bovine	S		NS	S	S
Rhinoceros	S	NS		S	S
Cave bear	S	S	S		S
Hyena	S	S	S	S	

Note. S, significant; NS, not significant.

TABLE 5

Results of Student's t Tests for the Different Taxa with More than Four Analyzed Individuals in Sclayn 1A, for Nitrogen Isotopic Composition

$\delta^{13}C$	Horse	Bovine	Rhinoceros	Cave bear	Hyena
Horse		NS	NS	NS	S
Bovine	NS		NS	NS	S
Rhinoceros	NS	NS		NS	S
Cave bear	NS	NS	NS		S
Hyena	S	S	S	S	

Note. S, significant; NS, not significant.

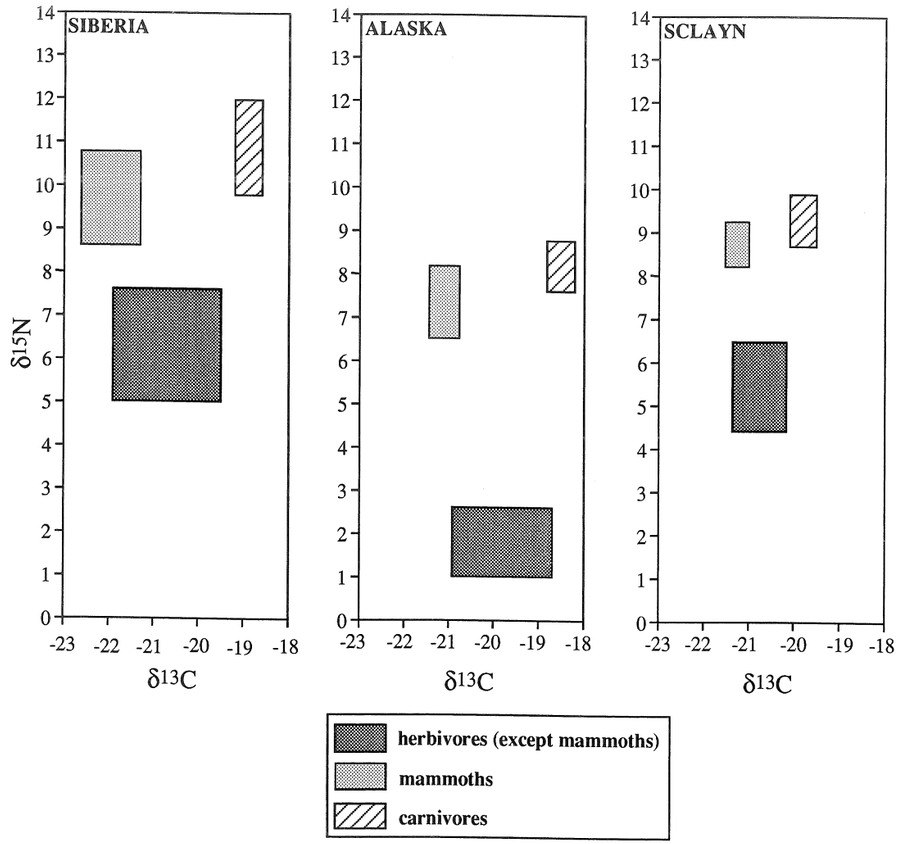

FIG. 4. Comparison of average ±1 SD for herbivores (except mammoths), mammoths, and carnivores in late Pleistocene Siberia, Alaska, and Sclayn 1A. Data for late Pleistocene Siberia and Alaska are from Bocherens *et al.* (1994b, 1995a, 1996).

such as the increase in $\delta^{15}N$ values between two successive trophic levels (DeNiro and Epstein, 1981; Minagawa and Wada, 1984), and ^{15}N enrichment in dentine relative to bone in carnivores and deer, due to the so-called suckling effect (Bocherens *et al.,* 1994a). Indeed, in a given species, milk consumed by young suckling individuals is one trophic level higher than adult diet. Since dentine is formed in part before weaning and does not remodel contrarily to bone, it is formed on a diet enriched in ^{15}N relative to the adult diet, whereas bone is formed on the diet of the few months or years before death. Thus dentine collagen is enriched in ^{15}N relative to bone collagen in adult individuals belonging to species where teeth stop their growth early after weaning, such as hyenas, bears, and some deer species (Bocherens *et al.,* 1994, 1995b; Fizet *et al.,* 1995; Bocherens and Mariotti, 1997). In the present study, in each case where collagen was extracted from bone and dentine of a given bear or hyena individual, $\delta^{15}N$ values are systematically higher in dentine than in bone (Table 3; Fig. 2). Such variations are observed in modern samples, where $\delta^{15}N$ values are higher in dentine than in bone, probably due to suckling (Bocherens *et al.,* 1994). Similar variations have been observed in specimens from the same species at other Pleistocene sites (Fig. 2). When considering a given species, such as hyena, cave bear, and giant deer, the average $\delta^{15}N$ value of dentine is systematically higher than the average $\delta^{15}N$ value of bone (Table 1). Such a phenomenon has also been previously observed in other late Pleistocene hyenas, cave bears, and reindeer (Bocherens *et al.,* 1994a, 1995b; Bocherens and Mariotti, 1997).

In the Sclayn specimens, the average $\delta^{15}N$ values of carnivore bone collagen ($\delta^{15}N = 9.3 \pm 0.6‰$, $n = 6$) are clearly higher than those of herbivore bone and dentine collagen ($\delta^{15}N = 5.0 \pm 0.8‰$, $n = 10$, including horse and bovine dentine, and deer bone values: Fig. 3). To calculate the average $\delta^{15}N$ value of herbivores, only bone collagen has been considered to reflect adult deer diet, since in this species, dentine is enriched in ^{15}N relative to bone due to the formation of this tissue early in life, when milk forms the bulk of the diet suckling isotopic signature (see above). In contrast, the $\delta^{15}N$ values of bone and tooth have been reported to be equivalent, and both correspond to adult diet in horse and bovine; thus, both tissues can be used to calculate the $\delta^{15}N$ values of adults. The difference between $\delta^{15}N$ values of herbivores and carnivores is 4.3‰ in Sclayn 1A, which is a value similar to those observed in modern ecosystems where the trophic level ^{15}N enrichment ranges from 3‰ (Schwarcz, 1991) to 5.7‰ (Ambrose and DeNiro, 1986). Similar values have been reported in late Pleistocene ecosystems from Asia and Europe (Bocherens *et al.,* 1991, 1994a, 1995b, 1996; Fizet *et al.,* 1995).

No disruption of predictable biogenic isotopic signals has been found in the studied Sclayn specimens. Thus, bone and dentine collagen preservation was excellent, and the isotopic abundances of collagen are considered to reflect those of the living animals.

Paleobiological Implications of Collagen Isotopic Abundances

The measured collagen $\delta^{13}C$ values correspond to those of animals living in a C_3-plant ecosystem and they are similar to those reported in other Pleistocene sites in western Europe (Bocherens *et al.,* 1994, 1995b; Fizet *et al.,* 1995). In herbivores, collagen $\delta^{13}C$ values range from −21.9 to −20.1‰, with a mean of $\delta^{13}C = -20.9 \pm 0.6‰$ ($n = 20$). In hyenas, the $\delta^{13}C$ values range from −20.2 to −19.0‰ with a mean of $-19.5 \pm 0.3‰$ ($n = 11$). The $\delta^{13}C$ values measured for herbivore bone collagen are lower than those measured for carnivore bone collagen (Fig. 3). Such a difference is in agreement with a small isotopic fractionation observed for carbon in trophic chains with C_3 plants (van der Merwe, 1986; Bocherens, 1997).

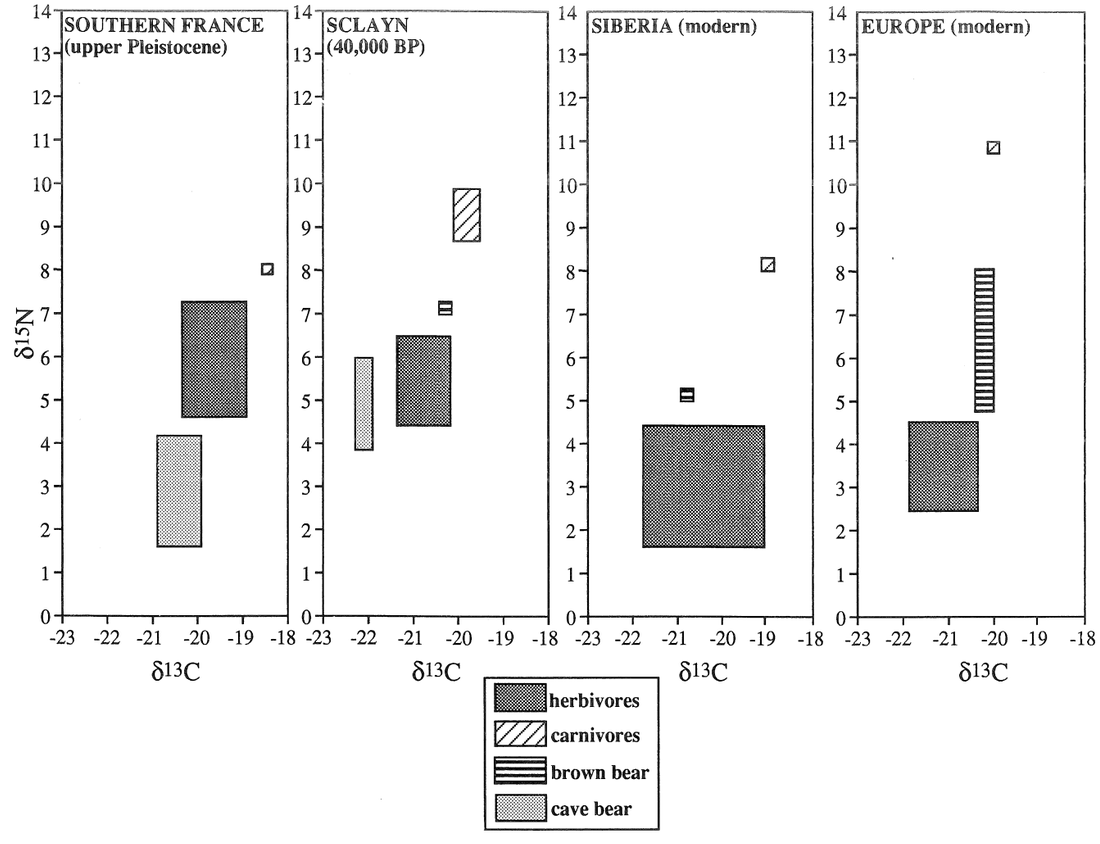

FIG. 5. Comparison of average ±1 SD for herbivores, carnivores, cave bears, and brown bears in late Pleistocene southern France and Sclayn 1A, and modern Siberia and Europe. Data for late Pleistocene southern France are from Bocherens *et al.* (1994a), data from modern Siberia are from Bocherens *et al.* (1996), and data from modern Europe are from Bocherens *et al.* (1994a).

Differences in the $\delta^{13}C$ values occur between herbivorous species. Student's t tests have been performed in order to compare isotopic values of species for which more than four samples have been analyzed (i.e., horse, bovines, and rhinoceros; Table 4). Horse collagen $\delta^{13}C$ values are significantly lower than those of other herbivorous species. Mammoths, although not numerous, also seem to have more-negative $\delta^{13}C$ values than other herbivores except horses. Significant differences are also seen in herbivore $\delta^{15}N$ values (Table 5). Bovines and deer exhibit the lowest $\delta^{15}N$ values, whereas mammoths show high $\delta^{15}N$ values, almost as high as those of hyenas. A similar pattern has been reported for Siberian and Alaskan mammoths (Bocherens *et al.,* 1996; Fig. 4) and for central Europe (Ambrose, in press) but it is the first confirmation of this difference in western European samples. The possible reasons for such a pattern in mammoths relative to other herbivores have been discussed in detail elsewhere (Bocherens *et al.,* 1996) and might be linked to differences in digestive physiology, such as ruminant versus nonruminant, or to differences in diet quality, such as low nitrogen diet versus high nitrogen diet, leading to a more important fractionation of nitrogen in mammoths.

The $\delta^{13}C$ values of cave bear bone collagen are significantly more negative than those of all the other species, herbivores or carnivores. A similar pattern occurred in other late Pleistocene sites from western Europe (Bocherens *et al.,* 1994). Such low $\delta^{13}C$ values might be due to a closed, more forested micro-habitat for this species, or to lipid storage during hibernation and subsequent recycling in the synthesis of some amino acids (Bocherens *et al.,* 1994). The $\delta^{15}N$ values of cave bear are not significantly different from those of the herbivorous species (Table 5). On the contrary, the brown bear specimen analyzed here exhibits a $\delta^{13}C$ and $\delta^{15}N$ values intermediate between those of herbivores and carnivores, which is comparable to the results obtained for modern brown bears (Fig. 5; Bocherens *et al.,* 1994a). For the first time, it appears that in a given site, both coexisting bear species present distinct isotopic results, reflecting different ecological niches: a strictly vegetarian diet for cave bear but a more omnivorous diet, with an animal component, for brown bear. It is noteworthy that in the Pleistocene fauna of Kent's Cavern, the single specimen of brown bear analyzed yielded isotopic values similar to those of the carnivorous species in the site (Bocherens *et al.,* 1995).

Comparisons can be made for some taxa between the isotopic compositions measured in Sclayn 1A samples and those of the same species and measured on the same tissue (bone or dentine) in other western European late Pleistocene sites (distribution shown on Fig. 6), i.e., Kent's Cavern (horse, woolly rhinoceros and hyena), Marillac (horse), Aldène (cave bear), and Mialet (cave bear) (Table 6). The $\delta^{13}C$ and $\delta^{15}N$ values are very similar, almost identical, between similar taxa and similar tissues from Sclayn 1A and Kent's Cavern. One apparent exception is the $\delta^{15}N$ values of horses,

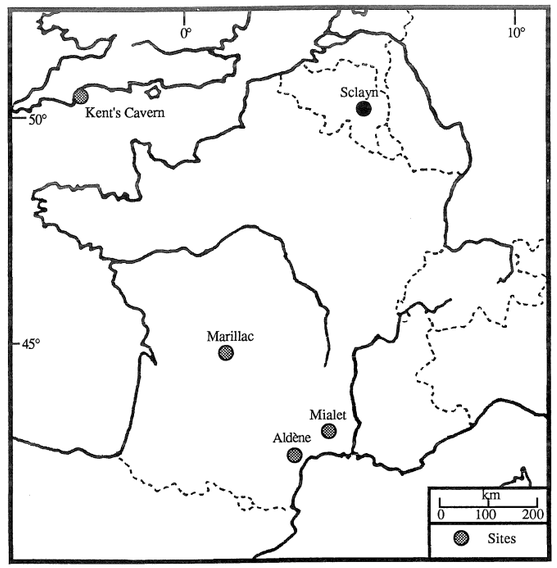

FIG. 6. Map of France and adjacent areas showing the geographic location of the sites discussed in the text.

but a Student's t test shows that the difference is not significant. Besides, horse $\delta^{13}C$ and $\delta^{15}N$ values are clearly different between Marillac on one hand and Kent's Cavern and Sclayn on the other hand. This difference is even more pronounced when values measured on samples from Marillac layer 7 are excluded from the average, since this layer has been recognized to have experienced particular climatic conditions yielding to higher ^{15}N contents in herbivores from this layer (Fizet *et al.,* 1995). In the case of cave bear, the $\delta^{13}C$ values are clearly lower in Sclayn 1A than at sites in southern France, in bone and dentine collagen, respectively; a Student's t test shows that the difference is highly significant. The $\delta^{15}N$ values appear higher in Sclayn 1A bears than in bears from southern France, but the difference is not statistically significant, at least for Aldène (Student's t test).

The comparisons with other western European late Pleistocene sites emphasize the fact that no isotopic difference could be seen between the Belgian site of Sclayn and the southern England site of Kent's Cavern. This is consistent with the fact that during the last glaciation both land masses were united by low sea level and southern England and Belgium belonged to the same vegetation and climatic unit (Frenzel, 1968; Renault-Miskovsky, 1985). Contemporaneous lithic industries show striking similarities between Belgium and Great Britain (Otte, 1974). On the other hand, southern France experienced milder climatic conditions, and the isotopic data confirm that ecological differences existed at that time. The observed trend in carbon isotopic abundances, i.e., more ^{13}C-depleted collagen in Belgium and

TABLE 6
Average ± 1 SD Isotopic Compositions of Collagen from Bone and Dentine of Different Taxa Occuring in Sclayn 1A and Other Western European Sites

Taxon	Site	Sample	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Horse (*Equus caballus*)	Kent's Cavern	bone and dentine	10	−21.4 ± 0.3	6.5 ± 1.7
	Marillac (all)	bone and dentine	25	−20.6 ± 0.6	5.1 ± 1.4
	Marillac (without layer #7)	bone and dentine	19	−20.5 ± 0.4	4.6 ± 1.1
	Marillac (layer #7)	bone	6	−21.1 ± 0.7	6.7 ± 0.8
	Sclayn 1A	dentine	5	−21.7 ± 0.1	5.4 ± 0.8
Rhinoceros (*Coelodonta antiquitatis*)	Kent's Cavern	dentine	10	−20.6 ± 0.3	6.5 ± 1.8
	Sclayn 1A	dentine	6	−20.6 ± 0.4	6.2 ± 0.8
Hyena (*Crocuta crocuta*)	Kent's Cavern	bone	5	−19.6 ± 0.7	9.7 ± 1.3
	Sclayn 1A	bone	6	−19.7 ± 0.3	9.3 ± 0.6
	Kent's Cavern	dentine	14	−19.5 ± 0.4	10.4 ± 1.4
	Sclayn 1A	dentine	5	−19.3 ± 0.1	10.6 ± 0.5
Cave bear (*Ursus spelaeus*)	Aldène	bone	3	−20.8 ± 0.4	3.1 ± 1.5
	Mialet	bone	5	−20.2 ± 0.5	2.8 ± 1.6
	Sclayn 1A	bone	7	−22.1 ± 0.2	4.9 ± 1.1
	Aldène	dentine	12	−21.5 ± 0.3	6.2 ± 2.0
	Mialet	dentine	9	−21.3 ± 0.3	4.5 ± 1.7
	Sclayn 1A	dentine	7	−23.0 ± 0.3	6.6 ± 1.2

Note. Data for Kent's Cavern are from Bocherens *et al.* (1995b), Marillac (Fizet *et al.,* 1995), Aldène and Mialet (Bocherens *et al.,* 1994a).

Great Britain relative to southern France, is consistent with the observed pattern for Holocene bone collagen, a pattern which is tentatively linked to the influence of climatic conditions on plant photosynthesis ^{13}C fractionation (van Klinken *et al.,* 1994). More detailed inter-site isotopic comparisons will most probably lead to a better understanding of the distribution of ecological conditions during the last glaciation in western Europe.

CONCLUSIONS

The present study demonstrates the good preservation of collagen in Sclayn 1A bones. All samples yielded well-preserved collagen suitable for reliable stable isotopic measurements. Preservation of the biogenic isotopic signatures is demonstrated by the measured N content and C/N ratios of extracted collagenic organic matter and the isotopic differences between herbivorous and carnivorous species and between bone and dentine collagen in specimens from carnivorous species. The isotopic results obtained for the rich Sclayn 1A mammal fauna are in complete agreement with those previously reported from contemporaneous faunas. The distinctiveness of the woolly mammoth is confirmed, as well as the ecological difference between cave bear and brown bear.

It thus appears that Sclayn Cave is a highly favorable site for paleoecological investigations based on fossil bone isotopic biogeochemistry. Further studies are in progress on material from older layers deposited under various climatic conditions and on fossil human material from the site.

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LETTER TO THE EDITOR

Comment on ''^{10}Be and ^{26}Al Evidence for Exceptionally Low Rates of Australian Bedrock Erosion and the Likely Existence of Pre-Pleistocene Landscapes'' (Bierman and Turner, 1995)

The paper published in *Quaternary Research* by Bierman and Turner (1995) is marred by inadequate attention to the literature, both systematic and regional, concerning inselbergs, bornhardts, and Eyre Peninsula. The authors' speculations are not only at odds with the field evidence and general theory but also internally inconsistent. Such inconsistencies, and especially the conflicting claims concerning dating on the basis of cosmogenic nuclide accumulations on the one hand and the denial of the possibility of dating on that basis, are confusing. These perceived shortcomings are considered here under three headings, but as the thrust of the paper under discussion concerns the antiquity of some elements of the landscape of Eyre Peninsula, South Australia, it is prudent first to consider what geologists, geomorphologists, and physicists mean by the ''age'' of a land surface.

In geological terms, the age of a stratum, or surface, refers to its date of origin. A Neocomian sandstone bed originated in early Cretaceous times, about 130–140 myr ago. Its age is not measured by a calendar or hourglass, but its relative age is deduced from its relationship to adjacent strata, and its stratigraphic age from its fossil content or from its position vis-à-vis strata or other rocks of known age. Since deposition, it has not remained in its pristine state, but it persists as a contemporary feature; yet it is referred to as Neocomian—its age of origin.

Similarly, and concerning destructional or erosional landscapes, an inselberg or a massif may be deduced to have formed in early Cretaceous times. Its age is not measured in the sense that a rate of erosion may be measured by direct comparisons over time, or to some fixed datum, but is inferred, perhaps by reference to associated deposits laid down in an adjacent basin (e.g., Campbell and Twidale, 1991). There are exceptions, but like most strata, the majority of landforms are not formed instantly, but over a period of time, and are best accorded an age range rather than an age (Twidale, 1956; King, 1962). This requirement is, however, usually met by the latitude implied in a stratigraphic age, for to label a feature or a stratum Neocomian, for instance, is to imply an absolute age within a range of rather more than 10 myr.

Commonly, the morphology of the inselberg is manifestly altered by weathering and erosion after initiation. For example, though Ayers Rock (Uluru) can be argued to be of Maastrichtian age (70 myr), and the prominent summit bevel is the etch equivalent of the original, it is equally certain that the flanks of the residual have been steepened by successive alternations of scarp-foot weathering and erosion, so that the overall morphology and relief amplitude of the inselberg have changed through time (Twidale, 1978; Harris and Twidale, 1991). Similarly, there are persuasive arguments that many of the inselbergs of northern Eyre Peninsula have increased in relief amplitude as a result of repeated lowerings of the surrounding plains (Twidale and Bourne, 1975a; Twidale, 1982a). But these processes have not eliminated the contemporary features, which closely mimic the original (Hills, 1975, p. 300). The present form is a direct descendent of the original feature which is of at least late Cretaceous age.

DO COSMOGENIC NUCLIDE ACCUMULATIONS PERMIT DATING OF LAND SURFACES?

Bierman and Turner evidently employ the terms ''age'' or ''date'' in this geological or stratigraphic sense. In the title of the paper they refer to the likely existence of pre-Pleistocene landscapes. In columns 11 and 12 of their Table 1, they list minimum ages of samples, and presumably the surfaces from which they were taken, according to abundance of different isotopes. In an earlier contribution (Bierman and Caffee, 1994) it was stated that isotope abundances imply that the surfaces sampled ''are not remnants of a Mesozoic landscape.'' Again (p. 382), they extrapolate their maximum limiting erosion rates and conclude, though in apparent denial of the previously quoted comment, that ''the form of some Australian inselbergs originated in the Tertiary or earlier . . . .'' These statements clearly imply a capacity to recognize antiquity and to evaluate dates and ages. Yet in reference to the inselbergs of Eyre Peninsula, Bierman and Turner (p. 382) also state that cosmogenic nuclide abundances ''cannot be used to assign an 'age' to these bedrock landforms.'' They assign age limits to surfaces, and claim them to be very old, but how old is old? An analogy may be drawn with exhumed surfaces like that exposed on the

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