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Stable isotope abundances (^{13}C, ^{15}N) in collagen and soft tissues from Pleistocene mammals from Yakutia: Implications for the palaeobiology of the Mammoth Steppe

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Abstract

A suite of skeletons and frozen carcasses of upper Pleistocene mammals from Yakutia (Sakha Republic, Russia) has been analyzed for their stable isotopic abundances in carbon and nitrogen. Results from bone collagen and soft tissues have been compared. The samples studied belong to herbivorous (mammoths, woolly rhinoceros, horse, bison, muskox) and carnivorous (wolf, lion) species. Bone collagen of herbivorous and carnivorous modern mammals from the same region have been analyzed for comparison.

The bone samples exhibit a very good preservation of collagen. The isotopic enrichment between herbivorous and carnivorous species is similar in modern and Pleistocene specimens, except for the mammoths, which show more negative $\delta^{13}C$ values and higher $\delta^{15}N$ values of collagen relative to other herbivorous species. A similar isotopic pattern can be seen in upper Pleistocene mammoths from Alaska, and this pattern suggests a paleobiological significance.

Keywords: C-13; collagen; *Mammuthus primigenius*; N-15; Pleistocene; Siberia

1. Introduction

During the late Pleistocene, a large area covering middle and southern Europe, northern Asia, and Alaska was the domain of the so-called "mammoth steppe fauna" (Guthrie, 1982,1990). This landscape was boarded to the northwest and to the east by icecaps, and was characterized by a rather homogenous faunal assemblage dominated by *Mammuthus*, *Bison* and *Equus* (Guthrie, 1982). The species diversity of this fauna and the gigantism of its elements reminds more of the African savannah than of today's arctic tundra. The reasons permitting the development of such a particular environment have generated much investigation for more than a century (e.g. Howorth, 1880).

The species diversity of the herbivore fauna has been interpreted in terms of diet specialization of each species towards one short segment of a large spectrum of available plant material (Bliss and Richards, 1982; Guthrie, 1982; Martin, 1982;

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Vereshchagin and Baryshnikov, 1982). Due to the excellent state of organic matter preservation in the arctic environment, it is possible to investigate the isotopic specificity of this fauna, based on the stable isotope abundances of carbon and nitrogen in fossil organic matter.

1.1. Isotopic abundances as ecological markers in C_3-plant food webs

The following review will focus on C_3-plant food webs. Indeed, since C_4-plants have a greater efficiency of CO_2 uptake under limited amounts of water and high summer temperatures (Berry, 1975), they are not present in Siberia and Alaska (Teeri and Stowe, 1976). In the perspective of using isotopic signals in fossil bones, we will focus on the record of environmental information in collagen, a protein potentially resistant to diagenetic alteration.

In collagen, the $\delta^{13}C$ values[1] are enriched around +5‰ relative to the average body for large herbivores and carnivores (Vogel, 1978; Vogel et al., 1990). There is a slight enrichment in ^{13}C between herbivore and carnivore collagen, up to 2‰, at each trophic step (Van der Merwe, 1989; Lee-Thorp et al., 1989). However, this difference in collagen $\delta^{13}C$ values between herbivores and carnivores seems to be observed only in ecosystems where all the plants have a C_3 photosynthetic pathway (Van der Merwe, 1989). Some variations in $\delta^{13}C$ values can be seen in pure C_3-plant food webs, for instance a significant depletion in ^{13}C is measured in plants and animals living under close-canopied forest, due to the mixing of atmospheric CO_2 with low $\delta^{13}C$ CO_2 produced from decaying litter and soil organic matter (Medina and Minchin, 1980; Schlesser and Jayasekera, 1985; Van der Merwe and Medina, 1991).

A clear enrichment in ^{15}N has been shown to exist between herbivore and carnivore collagen. This enrichment is reported to range in terrestrial ecosystems from 2.8 (Schwarcz, 1991) to 5.7‰ (Ambrose and DeNiro, 1986) with an average value between 3 and 4‰ (Schoeninger and DeNiro, 1984; Schoeninger, 1985; Sealy et al., 1987). Due to suckling, an enrichment in ^{15}N is observed in young mammals. Indeed, milk consumed during the nursing period is one trophic level higher than adult diet, and leads to an enrichment in ^{15}N of collagen of nursing animals relative to adults (Fogel et al., 1989). Since dentine starts to form during nursing, continues to accumulate new tissue after weaning, and is not remodelled afterwards, it records a mixed isotopic signal from nursing and adult period. On the contrary, bone remodels even when the growth is over, and the isotopic signal measured in bone collagen corresponds to the material accumulated some time before death (Castanet and Ricqlès, 1987). As a result, the $\delta^{15}N$ values of dentin collagen are higher than those of bone collagen from the same individual, when teeth stop their growth shortly after weaning (Bocherens et al., 1994a,1995b). Climate aridity and dietary stress increases also nitrogen isotopic abundances at each trophic level (Ambrose and DeNiro, 1986; Heaton et al., 1986; Sealy et al., 1987).

1.2. Assessment of the preservation of isotopic signals in fossil samples

Before drawing paleoecological conclusions from the isotopic composition of the organic phase of fossil vertebrate phosphatic tissues, it is essential to assess the preservation of the biogenic isotopic signals. Indeed, these messages are susceptible to suffer diagenetic alteration during the fossilization processes. Several methods are available to identify alteration of collagen (the dominant protein in bones and teeth), including analysis of C/N ratios and amino acid composition (DeNiro, 1985; Ambrose, 1990; Tuross et al., 1988; DeNiro and Weiner, 1988; Bocherens et al., 1991a,b; Fizet et al., 1995). However, minor alterations of the amino acid composition for the a collagen-like organic extract may lead to important shifts of the isotopic abundances, especially in the case of the nitrogen (Grupe et al., 1993). Consequently, alteration of these isotopic signals can be assessed by examining the extent to which expected biological

[1]The delta value for each isotope is calculated as $\delta^{E}X=[(R_{sample}/R_{standard})-1]\times 1000$, where $\delta^{E}X=\delta^{13}C$ or $\delta^{15}N$, and $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. The standards are PDB for carbon and AIR for nitrogen.

carbon and nitrogen isotope patterns are disrupted in fossils. This can be done by comparing the carbon isotopic abundances of fossil herbivores and carnivores with modern equivalents, and by comparing the nitrogen isotopic abundances of herbivores to those of carnivores in a given paleoecosystem (Bocherens, 1995).

Moreover, within one given individual, there is an enrichment between dentine and bone collagen in species with definite tooth growth, such as carnivores and deer, but not in species with continuous tooth growth, such as horses (Bocherens et al., 1994a). This predictable signal can be used to test the preservation of nitrogen isotopic compositions in the fossil collagen samples (Bocherens, 1995; Bocherens et al., 1994a, 1995a,b).

1.3. Previous studies on Pleistocene fauna

A number of previous studies used carbon and nitrogen isotopic abundances in bones and teeth of Pleistocene mammals from Beringia to reconstruct paleodiets and paleoenvironments. Beringia is a paleogeographic unit including northeastern Asia and Alaska, together with the landbridge that connected them during the Pleistocene, when the sea-level was lower than today. This region provided numerous frozen carcasses (Guthrie, 1990), from which biomolecules could be extracted and studied (Barnhart et al., 1980; Goodman et al., 1980). Carbon isotopic abundances in mummified soft tissues of mammoths and other herbivorous mammals from Alaska have been measured in order to test the occurrence of C_4 grasses in Pleistocene Alaska (Bombin and Muehlenbachs, 1985). Moreover, carbon and nitrogen isotopic abundances in collagen of mammoths from Alaska (Bocherens et al., 1994b), and of other mammals (moose *Alces alces*, reindeer *Rangifer tarandus*, lion *Felis leo*, giant short-faced bear *Arctodus simus*) from Alaska (Bocherens et al., 1995a) have been measured. In addition, a few isotopic values for mammoth collagen have been presented along with those of other species in Eastern Europe (Ambrose, 1992) and in North America (Koch, 1991). However, none of these previous studies considered at the same time a large selection of different species in a given environment.

Also a number of studies have focused on the isotopic biogeochemistry of the upper Pleistocene mammal fauna of western Europe, which belongs to the same biogeographical unit as that from Beringia. In that case, only bones and teeth are available for study (Bocherens et al., 1990, 1991a,b,1994a,1995b; Fizet et al., 1995). The results of these studies can be compared to those of the better preserved samples from Beringia.

2. Material and techniques

2.1. Material

Soft tissue and bone samples from mammoths, woolly rhinoceros, horses, bison, muskox, wolf, lion and dog have been collected during the exhibition "Mammouths de Sibérie et de Bourgogne", which was held in the Museum of Natural History of Autun from April 23 to September 26, 1994. The specimens were on loan from the Museum of Mammoth in Yakutsk (Sakha Republic). The samples were handled with gloves to avoid gross

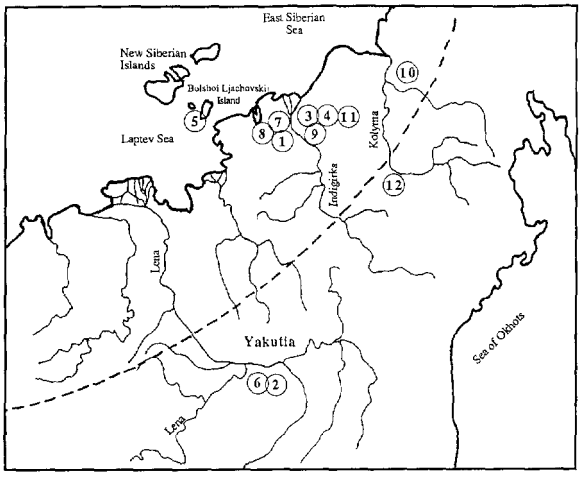

Fig. 1. Map of Eastern Siberia showing the location of the sites of sampling. *1*= #94000–#94500; *2*= #94800; *3*= #94900, #95000, #95700 and #95800; *4*= #95200 and #95300; *5*= #95500 and #95600; *6*= #95900 and #96000; *7*=#96100; *8*= #96200 and #96300; *9*= #96400; *10*= #96500; *11*= #96600; *12*= #101300–103000.

Table 1
List of analyzed specimens, with indication of geographical origin, anatomical part studied, and geological age

No.	Species	Origine	Analyzed samples	Age (yr B.P.)
94000–94500	mammoth (*Mammuthus primigenius*)	Kien-Urech river, Omoloj river basin (Northern Yakutia)	humerus, radius, rib, "muscle" from humerus, radius and thoracic cage	30,000
94800	mammoth (*Mammuthus primigenius*)	Singnigese-Urech river (Central Yakutia)	scapula	15,000
94900, 95000	mammoth (*Mammuthus primigenius*)	East of Tchokourdakh, Indiguizka river basin	vertebra and adhering soft tissue	upper Pleistocene
95100	mammoth (*Mammuthus primigenius*)	Kien-Ajaan river, Indigirka river basin (northern Yakutia)	skull	upper Pleistocene
95200, 95300	mammoth (*Mammuthus primigenius*)	East of Tchokourdakh, Indiguizka river basin	pelvis and adhering soft tissue	upper Pleistocene
95500, 95600	mammoth (*Mammuthus primigenius*)	Bolshoj Ljachovskij island	skull and molar	50,000
102100	mammoth (*Mammuthus primigenius*)	Yakutia	undetermined bone fragment	upper Pleistocene
95700, 95800	mammoth (*Mammuthus primigenius*)	Yakutia	scapula and soft tissue adhering to rib (2 months old specimen)	upper Pleistocene
95900, 96000	rhinoceros (*Cœlodonta antiquitatis*)	Tchouraptcha (central Yakutia)	fibula and soft tissue adhering to tibia	20,000
96100	horse (*Equus caballus*)	Dukarskoje lake (northern Yakutia)	skin from the neck	26,000
96200, 96300	horse (*Equus caballus*)	Maksounuocha river (northern Yakutia)	mandible and adhering soft tissue	25,000
10200	horse (*Equus caballus*)	Yakutia	undetermined bone fragment	upper Pleistocene
101900	bison (*Bison priscus*)	Yakutia	undetermined bone fragment	upper Pleistocene
102200	muskox (*Ovibos moschatus*)	Yakutia	undetermined bone fragment	upper Pleistocene
96400	wolf (*Canis lupus*)	Shandrin river (Yakutia)	skull	upper Pleistocene
96500	lion (*Felis leo*)	Kolyma river (Yakutia)	skull	upper Pleistocene
96600	dog (*Canis familiaris*)	Alezeja river (northern Yakutia)	skull	upper Pleistocene
101300	reindeer (*Rangifer tarandus*)	Zyrjanka area (Yakutia)	undetermined bone fragment	recent
102300	reindeer (*Rangifer tarandus*)	Yakutia	undetermined bone fragment	recent
102800	reindeer (*Rangifer tarandus*)	Yakutia	undetermined bone fragment	recent
102900	reindeer (*Rangifer tarandus*)	Yakutia	undetermined bone fragment	recent
103000	reindeer (*Rangifer tarandus*)	Yakutia	undetermined bone fragment	recent
101400	moose (*Alces alces*)	Central Yakutia	vertebral disk	recent

Table 1 (*continued*)

No.	Species	Origine	Analyzed samples	Age (yr B.P.)
102400	moose (*Alces alces*)	Yakutia	undetermined bone fragment	recent
102700	mountain sheep (*Ovis ammon*)	Yakutia	undetermined bone fragment	recent
101600	hare (*Lepus timidus*)	Kolyma river (Yakutia)	mandible	recent
101500	bear (*Ursus arctos*)	Kolyma river (Yakutia)	undetermined bone fragment	recent
102500	polar fox (*Alopex lagopus*)	Yakutia	undetermined bone fragment	recent
102600	red fox (*Vulpes vulpes*)	Yakutia	undetermined bone fragment	recent
101700	wolf (*Canis lupus*)	Kolyma river (Yakutia)	mandible	recent

contamination, but bare hand handling of the specimens very likely took place previously to the exhibition. Soft tissues consist of fibrous brown material (most likely muscle remnants), skin, and other dry membraneous material that was still adhering to some of the bones. All the samples come from different locations in Yakutia (Fig. 1), and their age range from around 50,000 to 10,000 yr B.P. (Lazarev, 1985; Table 1). The occurrence of a dog in the upper Pleistocene is intriguing and dating is in process in order to check the age of this important specimen.

As a modern comparison material, fresh bones of herbivorous and carnivorous species from the same areas than the mammoths in Yakutia have been analyzed (Table 1). The specimens come from individuals killed recently by local hunters. Herbivorous species such as reindeer, moose, mountain sheep and hare have been obtained. Brown bear, polar fox and red fox are the omnivorous species. Polar fox eats mainly rodents except on coastal areas, red fox also consumes principally rodents, but also fruits, insects and eggs (Wooding, 1982). Wolf is the carnivorous species.

2.2. Techniques

Soft tissue samples have been sonicated in distilled water and then freeze-dried. The extraction of collagenic organic matter from bone and dentine has been performed using the protocol described in Bocherens et al. (1991a), starting with about 15–200 mg of modern bone powder or 40–500 mg of fossil bone powder. After decalcification by HCl and soaking into NaOH, the rinsed residue was gelatinized and freeze-dried. As a comparison, three samples have been also treated with EDTA in order to extract collagen, according to Tuross et al. (1988), with modifications. Chuncks of bone (about 150–540 mg) were demineralized with 0.5 *M* EDTA, buffered at pH = 7.2, at room temperature. The insoluble residues were washed 15 times with distilled water and freeze-dried. Extraction yield is expressed in mg g^{-1} as the ratio of the freeze-dried organic matter on dry weight of the bone sample. Total C and N content of each organic matter sample were accurately measured by CHN elemental analyzer, with a standard-deviation of 0.2% for C and 0.1% for N. The biochemical purity of collagen has been routinely checked by the measurement of the quantity of carbon and nitrogen in the extracted organic matter, and the C/N value. Using the yield of insoluble residue and the percent of nitrogen in this residue, it is possible to calculate the amount of nitrogen per gramme of bone.

Isotopic abundances have been measured either by a modified Dumas combustion (Bocherens et al., 1991a), or by a combustion on a CHN elemental analyzer connected to a mass spectrometer (Bocherens et al., 1995a). In the modified Dumas combustion, carbon and nitrogen isotope compositions were measured on CO_2 and N_2 obtained by combustion of collagen in a sealed quartz tube with CuO. The evolved CO_2 and N_2

are purified and analysed on an isotopic mass spectrometer (Finnigan Delta E or VG Sira 9). In the second method, collagen and soft tissue samples were analysed for their carbon and nitrogen isotopic composition on a Carlo-Erba NA1500 CHN-elemental analyzer connected to an isotopic ratio mass spectrometer (Fisons Sira 10 for CO_2 and Fisons Optima for N_2). In both cases, the sample was combusted in a quartz oven, 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 CO_2 and N_2, the amounts of C and N were measured. For CO_2 isotopic analysis, the gas was cryogenetically trapped and purified, and then introduced into the isotopic ratio mass spectrometer through a dual inlet for comparison with a reference gas. For N_2 isotopic analysis, the gases were introduced into the isotopic ratio mass spectrometer in a continuous helium flow and isotopic measurement was done on N_2 by comparison with a coinjected reference gas calibrated against international standard. Results are calibrated against a well-known product analysed the same way than 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 measured vs international standards. Analytical precision was better than 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$ values. The difference between the isotopic values measured by the two different techniques is less than 0.3‰ for $\delta^{13}C$ values, and less than 0.1‰ for $\delta^{15}N$ values. The isotopic values shown in Tables 2 and 3 have been measured either way, or they represent the average value of duplicate analyses using both techniques on the same samples.

Table 2
Results of collagen extraction and isotopic ratio measurements of modern bones from Siberia

No.	Species	Sample	Extraction technique of collagen	Initial weight (mg)	Final weight (mg)	Yield (mg g^{-1})	Wt % of collagen		mg N g^{-1}	C/N	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
							% C	%N				
101300	reindeer	undetermined bone	HCl			n.d.	42.8	15.0	n.d.	2.9	−19.6	1.8
102300	reindeer	undetermined bone	HCl	135	24	178	40.2	14.7	26	3.2	−20.2	3.8
102800	reindeer	undetermined bone	HCl	104	23	224	43.1	15.6	35	3.2	−19.4	1.8
102900	reindeer	undetermined bone	HCl	17	2.5	147	41.1	15.2	22	3.2	−20.2	1.8
103000	reindeer	undetermined bone	HCl	36	6	163	38.4	13.6	22	3.3	−19.5	2.2
101400	moose	vertebral disk	HCl	201	64	318	43.2	14.7	47	2.9	−23.4	6.1
102400	moose	undetermined bone	HCl	205	40	195	42.7	15.9	31	3.1	−21.5	2.5
102700	mountain sheep	undetermined bone	HCl	118	27	227	42.8	15.3	35	3.3	−19.0	3.8
101600	hare	mandible	HCl	202	31	152	40.9	14.8	23	2.8	−24.5	4.8
101500	bear	undetermined bone	HCl	201	47	233	42.6	15.2	36	2.8	−20.8	5.1
102500	polar fox	undetermined bone	HCl	15	1.7	113	37.2	14.0	16	3.1	−22.4	9.9
102600	red fox	undetermined bone	HCl	87	18	203	41.1	15.4	31	3.1	−21.4	9.4
101700	wolf	mandible	HCl			n.d.	43.2	15.1	n.d.	2.9	−18.9	8.2
								Average	30±8			

Table 3
Results of collagen extraction and isotopic ratio measurements of modern and fossil samples from Siberia. The values in italic have been discarded since the C/N values indicate alteration or contamination of the sample

No.	Species	Sample	Extraction technique of collagen	Initial weight (mg)	Final weight (mg)	Yield (mg g^{-1})	Wt % of collagen		mg N g^{-1}	C/N	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
							% C	% N				
94000	mammoth	"muscle" (humerus)					49.0	8.9		6.4	*−26.1*	*10.5*
94500	mammoth	"muscle" (humerus)					45.2	15.3		3.4	−22.8	10.7
94200	mammoth	humerus	HCl	228	47	206	39.9	15.3	31	3.0	−22.4	9.1
94100	mammoth	"muscle" (thoracic cage)					37.0	11.8		3.6	−23.4	10.6
94300	mammoth	rib	HCl	64	9	141	39.9	12.3	17	3.8	*−25.1*	*11.1*
94400	mammoth	radius	HCl	277	41	148	43.1	15.5	23	3.3	−22.8	9.0
94800	mammoth	scapula	HCl	109	12	112	37.5	14.6	16	3.0	−20.7	8.1
94900	mammoth	soft tissue (vertebra)					44.7	15.1		3.5	−21.8	11.0
95000	mammoth	vertebra	HCl	98	17	168	41.9	15.3	26	3.2	−21.8	10.8
95100	mammoth	skull	EDTA	148	34	230	49.5	18.1	42	3.2	−22.9	8.8
95100	mammoth	skull	HCl	179	39	218	41.2	15.6	34	3.1	−22.7	8.8
95200	mammoth	soft tissue (pelvis)					42.8	14.9		3.3	−21.5	11.7
95300	mammoth	pelvis	HCl	86	15	171	43.3	15.6	27	3.2	−21.5	10.4
95500	mammoth	molar	EDTA	541	124	229	48.4	18.5	42	3.1	−21.9	10.0
95500	mammoth	molar	HCl	555	120	216	45.7	16.2	35	3.3	−22.3	10.2
95600	mammoth	skull	EDTA	229	54	234	47.1	18.0	42	3.1	−22.0	9.1
95600	mammoth	skull	HCl	352	72	204	43.2	15.8	32	3.2	−22.2	9.2
102100	mammoth	undetermined bone	HCl	201	44	220	42.1	15.5	34	3.2	−21.6	11.4
95700	mammoth	soft tissue (rib)					43.0	14.0		3.6	−22.5	7.3
95800	mammoth	scapula	HCl	39	7	184	39.6	15.1	28	3.1	−21.3	7.2
95900	rhinoceros	soft tissue (tibia)					42.6	14.9		3.3	−20.9	7.5
96000	rhinoceros	fibula	HCl	137	19	136	40.5	15.0	20	3.2	−20.6	8.4
96100	horse	skin (neck)					30.8	8.3		4.3	*−24.4*	*10.1*
96200	horse	soft tissue (mandible)					43.6	15.4		3.3	−21.9	5.5
96300	horse	mandible	HCl	61	10	169	38.5	14.3	24	3.1	−21.1	4.3
102000	horse	undetermined bone	HCl	202	62	304	42.3	15.2	46	3.2	−22.7	6.8
101900	bison	undetermined bone	HCl	204	38	185	41.9	15.5	29	3.2	−19.3	6.3
102200	muskox	undetermined bone	HCl	205	57	276	42.2	15.5	43	3.2	−19.9	5.8
96400	wolf	skull	HCl	42	12	282	38.4	15.0	42	3.0	−20.1	12.0
96500	lion	skull	HCl	141	39	275	35.8	13.8	38	3.0	−19.6	9.8
96600	dog	skull	HCl	85	21	250	37.8	14.8	37	3.0	−22.8	13.7

3. Results

Yields in collagen range from 110 to 320 mg g^{-1} for modern bones, and from 112 to 304 mg g^{-1} in fossil bones. The lowest yield are obtained in modern samples with the smallest quantities, which is probably partly due to proportionally more important losses during the extraction pro-

cess. The C/N ratios of the collagen extracted from the fossil bones and teeth are all within the acceptable range (3.0–3.6: DeNiro, 1985), except for the rib no. 94300, with a C/N value of 3.8. This value is higher than the maximum value accepted for unaltered collagen (DeNiro, 1985), and suggest that this sample was altered or contaminated. Its isotopic values have thus been discarded in the following discussion. The amount of nitrogen in the insoluble residues from fossil bones are as high or even higher than those of modern bones, indicating that for the majority of the fossil samples, almost all the collagen present in the fresh bone has been preserved.

$\delta^{13}C$ values of collagen range from −23.4 to −18.9‰ in modern samples (Table 2 and Fig. 2) and from −23.1 to −19.7‰ in fossil samples (Table 3 and Fig. 2). In mammoths, $\delta^{13}C$ values of collagen are in the most negative range (−23.1 to −21.4‰), and they are usually higher for the other herbivorous species (−20.8‰ for the woolly rhinoceros, −22.7 and −21.2‰ for the horse, −19.3‰ for the bison, and −19.9‰ for the muskox) and for the carnivores (−20.1 and −19.6‰), except for the dog (−22.9‰).

The $\delta^{15}N$ values of collagen range from 1.8 to 6.1‰ in modern herbivores (Table 2 and Fig. 2). They range from 7.2 to 11.4‰ in mammoths, and from 4.3 to 8.4‰ in other herbivorous species (Table 3 and Fig. 2). One of the horses presents higher $\delta^{15}N$ values for its soft tissues (10.1‰ for sample no. 96100). The $\delta^{15}N$ value of the collagen of the modern wolf bone is 8.2‰, and 9.4‰ and 9.9‰ in foxes. The $\delta^{15}N$ values of the fossil carnivores range from 9.8 to 13.7‰, with the highest value for the dog.

The C/N ratios of the soft tissues samples range from 3.3 to 6.4 (Table 3). $\delta^{13}C$ values of soft tissue samples range from −26.1 to −20.9‰ (Table 3). The lowest $\delta^{13}C$ values are seen in the soft tissue samples with the highest C/N values, whereas the $\delta^{13}C$ values of the soft tissue with collagen-like C/N values are very similar to those measured on the collagen extracted from the nearby bone. The $\delta^{15}N$ values of the soft tissues are close to those of the corresponding bone collagen when both have been measured, the difference being in most cases less than 1.5‰.

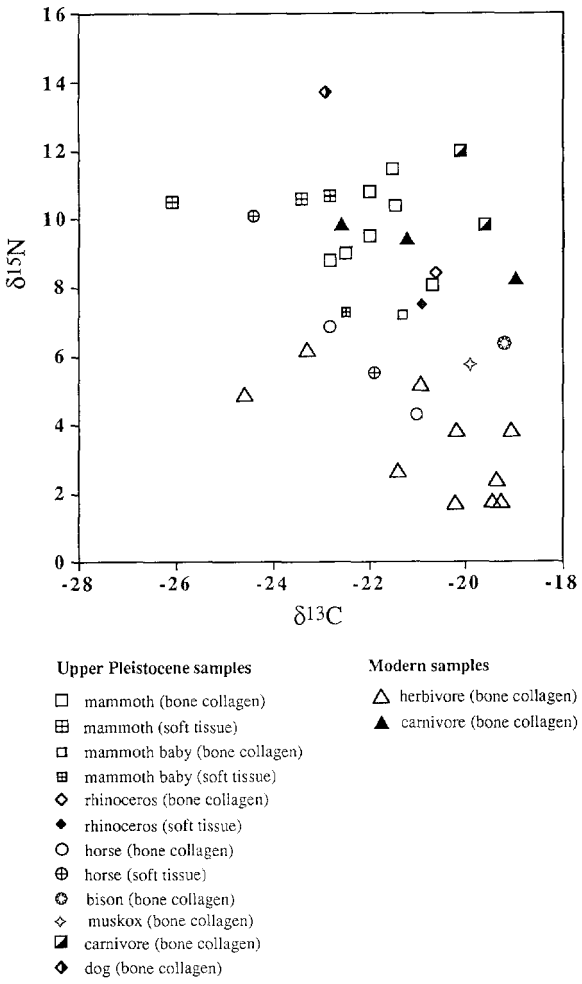

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ values of soft tissue and bone collagen from Upper Pleistocene samples and of bone collagen from modern samples from Yakutia.

4. Discussion

4.1. Preservation of the isotopic signals in bone collagen

The quantities and quality of the collagenous organic matter retrieved from the fossil bones are very comparable to those extracted from upper Pleistocene mammal bones from Alaska (Bocherens et al., 1994b,1995a). These amounts are very high regarding the age of the samples. The C/N ratios are within the acceptable range of values (DeNiro, 1985), except for the sample no.

94300, which is considered altered and its isotopic values are not considered in the following discussion.

A comparison of the two collagen extraction techniques used (EDTA vs HCl) shows slightly higher yields for the EDTA technique. The percent of carbon and nitrogen in the extracted residue are also higher when the extraction has been performed with EDTA (Table 3). However, the C/N values and the isotopic abundances in carbon and nitrogen are within the standard-deviation of the analysis technique, indicating that for such well-preserved samples, the HCl extraction technique is acceptable.

The isotopic values of still extant species are similar to those of modern equivalents, especially the difference in $\delta^{15}N$ between herbivores and carnivores. The high $\delta^{15}N$ values of the mammoths deserve more detailled discussion in a further coming paragraph.

4.2. *Isotopic values in soft tissues relative to bone collagen*

The carbon isotopic values in soft tissues are similar to those of bone collagen in most cases, except when the C/N values of the soft tissues are higher than those of collagen, such as in sample no. 94000 and no. 96100. This is particularly clear in the case of the humerus of the mammoth no. 94000, where two "muscle" fragments sampled near the humerus give clearly different values: the sample with a C/N value of 6.4 has a $\delta^{13}C$ value of $-26.1‰$ whereas the sample with a C/N value of 3.4 has a $\delta^{13}C$ value of $-22.8‰$, almost identical to the $\delta^{13}C$ value of collagen extracted from the humerus itself ($\delta^{13}C = -22.4‰$ with C/N = 3.0). In other frozen carcasses from Siberia, histological and biochemical investigations revealed that most of the remaining material of the soft tissues was formed of collagen (Barnhart et al., 1980; Goodman et al., 1980). Thus, C/N values higher than 3.6 probably reflect the occurrence of compounds such as carbohydrates and lipids, both known to be more depleted in ^{13}C than collagen in a given tissue (DeNiro and Epstein, 1978). This may explain the lower $\delta^{13}C$ values of such samples relative to the collagen extracted from bone nearby, and the similar $\delta^{13}C$ values in bone collagen and soft tissues whenever both had similar C/N values. The use of the $\delta^{13}C$ values of whole preserved soft tissues is thus rendered difficult due to the differential preservation of the different categories of biomolecules with different isotopic fractionation for carbon relative to the diet. A way to avoid this complication would be to extract specific biomolecules from these soft tissues. Alternatively, lighter $\delta^{13}C$ values on samples with C/N values higher than 3.6 may also be due to an extraneous contamination by humic compounds. Because this possibility cannot be ruled out and would possibly add some contaminant nitrogen, the $\delta^{15}N$ values measured on these samples (no. 94000 and no. 96100) will be considered dubious as well and will not be discussed as reflecting biogenic isotopic signals.

The comparison of the $\delta^{15}N$ values of bone collagen and soft tissues is interesting since the $\delta^{15}N$ values are similar in the different proteins of an animal body, provided that they have been synthesized on a source of nitrogen with similar $\delta^{15}N$ values. In the fossil samples studied here, small differences in the $\delta^{15}N$ values occur between soft tissues and nearby bone collagen (Table 3). These differences could be due to alteration of the nitrogen isotopic values caused by decay and bacterial growth sometimes observed on the soft tissues (Farrand, 1961). Conversely, they could be caused by the fact that the nitrogen isotopic composition varied through time, and that bone collagen had a much slower turn-over than soft tissue proteins (Tieszen et al., 1983), thus both kinds of isotopic records do not correspond to the same period of time during the individual life. If the second assumption is correct, this would indicate that when the $\delta^{15}N$ values of soft tissues are higher than those of bone collagen (such as for samples no. 94000, 95200, 96200), the diet during the few months prior to the death of the animals was ^{15}N-enriched relative to the average diet of the last years prior to death. In the case of the baby mammoth no. 95700, there was no difference in $\delta^{15}N$ values between soft tissue and bone collagen, which is not surprising since proteins in both tissues were formed during the early part of the animal life. In the case of the rhinoceros no. 95900,

the diet during the few months prior to the death of the animal was ^{15}N-depleted relative to the average diet of the last years prior to death, since the $\delta^{15}N$ value of soft tissue is lower than that of bone collagen. The partial results presented here are not sufficient to prove or disprove seasonal variations of the isotopic composition of the diet of the extinct species of Beringia, but a more detailed comparison of the isotopic composition of soft tissues of frozen carcasses may prove to be a good way to test this hypothesis.

4.3. Palaeobiological significance of isotopic values of bone collagen

As pointed out earlier in this article, the $\delta^{13}C$ and $\delta^{15}N$ values are different in mammoth collagen than in other herbivorous species. This is even more striking when extending the comparison to the isotopic abundances of mammoth bone collagen published in Bocherens et al. (1994b) with those of other herbivorous species from the same area published in Bocherens et al. (1995a). The $\delta^{15}N$ values of mammoths are comparable to those of contemporaneous carnivores, in Siberia as well as in Alaska, but their $\delta^{13}C$ values are clearly lower than those of carnivores (Fig. 3). On the contrary, herbivores other than mammoth exhibit $\delta^{15}N$ values much lower than those of contemporaneous carnivores, more similar to those measured in modern mammals from these areas (Bocherens et al., 1994b, 1995a). Preliminary results suggest that the same pattern was present in Alberta (Bocherens et al., 1994b). Ambrose (1992) presented a similar isotopic differences between mammoths, other herbivores and carnivores, for samples from the upper Pleistocene of Czechoslovakia. Mammoths presented also higher $\delta^{15}N$ values than mastodons in a Pleistocene site of North America (Koch, 1991).

This pattern seems thus rather widespread and leads to questions about the isotopic fractionation between different herbivores according to their digestive strategy. Is there a relationship between $\delta^{15}N$ values in herbivores and the kind of fermentation process used by the herbivorous mammals? Two main kinds of digestive physiology are found in large herbivorous mammals. In short, ruminant

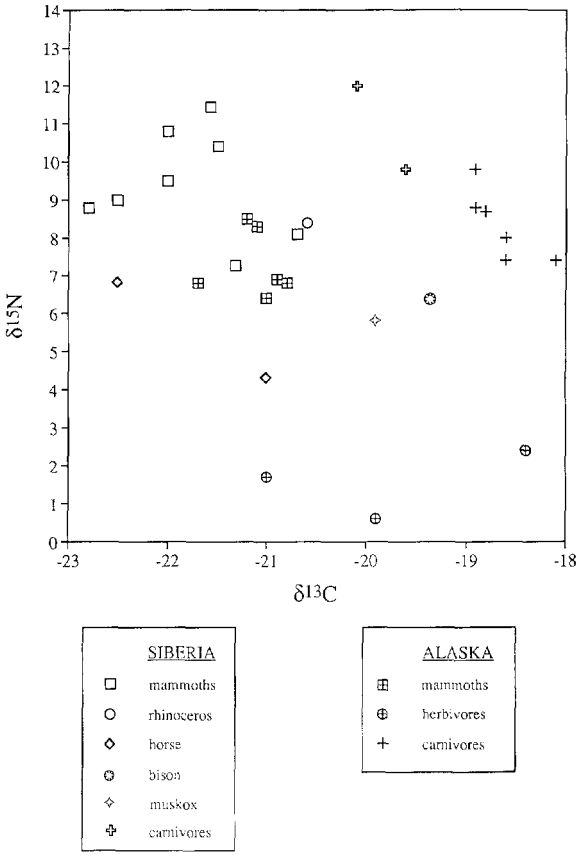

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen from Upper Pleistocene samples from Siberia and Alaska. Isotopic values of Alaskan mammoths are from Bocherens et al. (1994b). Isotopic values from Alaskan herbivores and carnivores are from Bocherens et al. (1995a).

herbivores (foregut fermenters), such as reindeer, moose, and deer, have a protein-rich diet and digest microbes, whereas non-ruminant herbivores (hindgut fermenters), such as mammoth, woolly rhinoceros, horse, have a low-protein diet, which they process in large amounts, and do not digest microbes (Stevens, 1988; Payne, 1990). The ruminant herbivores have rather low $\delta^{15}N$ values whereas the non-ruminant herbivores, especially mammoth, present high $\delta^{15}N$ values (Fig. 3). It is known that the abundance in dietary nitrogen is an important variable for understanding the nitrogen isotopic abundances in herbivorous mammals, although no relationship between low protein and high $\delta^{15}N$ values has been unambiguously demon-

strated (Ambrose, 1991). Ruminants select plant material with higher protein levels than non-ruminants do, since they are able to recycle some of their body nitrogen thanks to the rumen flora (Janis, 1975). The metabolic pathways of nitrogen recycling and amino acid synthesis are different in ruminant and non-ruminant mammals (Stevens, 1988). Since most of the enrichment in ^{15}N occurs in non-essential amino acids (Gaebler et al., 1966), which are synthesized by the animal, it is conceivable that these different pathways in ruminant and non-ruminant mammals lead to a more important enrichment in ^{15}N in non-ruminant than in ruminant mammals. A comparison of the individual $\delta^{15}N$ values measured on isolated amino acids from ruminant and non-ruminant mammal collagen may allow to test this hypothesis. In African savannas, ruminants present usually higher $\delta^{15}N$ values than non-ruminants, which is the reverse of what is observed in the arctic palaeoecosystem. Nevertheless, in that case, the ruminants are also non-obligate drinkers, whereas the non-ruminant species are obligate drinkers. The maximal urinary osmolality in response to water and heat stress is an other key factor for nitrogen isotopic abundances in herbivores (Ambrose, 1991). Thus, the interference of the physiological strategy used to deal with water and heat stress in African ecosystems may complicate the comparison with the pattern of stable isotope abundances for nitrogen in large herbivores in the arctic area.

In any case, it seems that in both ecosystems, the nitrogen isotopic fractionation is not solely under the influence of trophic levels, and the herbivores present a large range of $\delta^{15}N$ values, overlapping with the range of $\delta^{15}N$ values of carnivores in the case of the arctic samples. The distinction between hindgut herbivores and carnivores can be done by using the collagen carbon isotopic values in this case. The carnivores clearly present higher $\delta^{13}C$ values than most of the herbivores, and especially than mammoths (Fig. 3). Taken together, $\delta^{13}C$ and $\delta^{15}N$ values distinguish unambiguously the herbivorous from the carnivorous taxa. Moreover, the spacing values between the $\delta^{13}C$ values of collagen and carbonate hydroxylapatite of mammoths are clearly typical of herbivores (around 8–10‰: Bocherens et al., 1994b), and very different from those of carnivores (around 4‰: Lee-Thorp et al., 1989; Bocherens and Mariotti, 1992).

The rather low $\delta^{13}C$ values of mammoth collagen are surprizing, since they are lower than those of the other herbivorous species. It is difficult to interpret these low $\delta^{13}C$ values as a marker of forest-dwelling for mammoths, since morphological characters and stomach contents found in frozen carcasses indicate clearly that mammoths were grazing animals living in an open environment (Kubiak, 1982). Tree bark and twigs were part of their diet, though a small one, especially during the winter (Olivier, 1982; Vereshchagin and Baryshnikov, 1982), and may explain partly the lower $\delta^{13}C$ values. An other particularity of mammoths which may have influenced their $\delta^{13}C$ values is their ability to accumulate fat deposits in order to survive the winter food shortage (Kubiak, 1982; Olivier, 1982). Fat is known to be ^{13}C-depleted and thus the use of fat could have led to more negative $\delta^{13}C$ values in mammoths than in other herbivores.

Whatever the reasons for these high $\delta^{15}N$ values in mammoth collagen, it must be taken into account when using the difference between $\delta^{15}N$ values of known herbivorous and carnivorous species in order to test the preservation of isotopic abundances of collagen extracted from fossil bones as recommended by Bocherens (1995). Moreover, the use of $\delta^{15}N$ values of mammoth collagen in order to infer aridity may be misleading if compared to the $\delta^{15}N$ values of modern herbivores which belong to different species. Consequently, the conclusions published by Bocherens et al. (1994b) are to be reconsidered in view of the results of the present study. Due to the previously unrecognized differences in $\delta^{15}N$ values in herbivorous species, only comparison of $\delta^{15}N$ values measured on samples of one given species living in different places or at different times can be used to give clues about the changes in aridity conditions.

The isotopic values of the two-months old baby mammoth no. 95800 are interesting. Especially the $\delta^{15}N$ values were expected to be higher than those of adult mammoths due to suckling of the baby, being thus one trophic level higher than its

mother. On the contrary, the $\delta^{15}N$ values of the baby mammoth are not higher than those of the adults, and they are even slightly lower. There is some variation in the $\delta^{15}N$ values of adult mammoths and we do not have the actual $\delta^{15}N$ values of this baby's mother, but they were probably in the range of the other samples. It seems thus that the baby mammoth did not exhibit lower $\delta^{15}N$ values than the adults, this may be due to its very young age. In a study of the variations of $\delta^{15}N$ values in human infants, no difference in $\delta^{15}N$ values were observed between the mothers and their children for individuals younger than two-months old (Fogel et al., 1989).

The isotopic values of the dog sample from Siberia are very peculiar. The $\delta^{13}C$ value is more negative than those of wild carnivores, and the $\delta^{15}N$ values are significantly higher (Table 3 and Fig. 2). According to the nitrogen isotopic abundances, it seems that the wild carnivores did not eat mammoth flesh on a regular basis; such a hypothesis is not impossible for the dog. These isotopic values seem to indicate a diet different from that of wild carnivores, although at this stage it is difficult to interpret these values in terms of dietary intake for the dog. Nonetheless, this example points out the potential interest of stable isotopic studies in order to investigate the influence of domestication during its early stages.

5. Conclusion

The isotopic study of the extremely well-preserved mammal specimens from Siberia may be used as a reference for comparison with the more widespread, but less well preserved material from Western Europe. The best material for isotopic studies seems to be bone collagen from carcasses and specimens preserved in frozen grounds. In these samples several dozens of thousand years old, collagen is extremely well-preserved, and the chemical identification of this protein is a guarantee for the absence of isotopic variations due to differential contribution of various biomolecules with different isotopic discrimination relative to the food.

Some isotopic specificity seems to exist between the different species of herbivores constituting the mammoth steppe fauna, but the reasons for these differences are still difficult to assess. Nevertheless, they emphasize the necessity of a better knowledge of recent fauna of large mammals in temperate and arctic ecosystems. With the developing field of isotopic biogeochemistry applied to upper Pleistocene mammals, it is hoped that a framework will emerge, allowing a good understanding of the structure of the ecosystems that do not exist today any more. These ecosystems sustained the Middle and Upper Paleolithic cultures, and the reasons for the extinction of large herbivores at the end of the upper Pleistocene are still hotly debated. An isotopic approach may be a good way to test some hypotheses and to help to bring some new elements into the debate.

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References

Ambrose, S.H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J. Archaeol. Sci., 17: 431–451.

Ambrose, S.H., 1991. Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. J. Archaeol. Sci., 18: 293–317.

Ambrose, S.H., 1992. Purification and characterization of bone collagen of European Upper Pleistocene mammals for stable isotopic analysis. In: 28th Int. Symp. Archaeometry, Univ. Calif., Los Angeles, 23 March, 1992.

Ambrose, S.H. and DeNiro, M.J., 1986. The isotopic ecology of East African mammals. Oecologia, 69: 395–406.

Barnhart, M.I., Barmatoski, S.P., Goodman, M., Romero-Herrera, A.E., Lande, M.A., Birk, D.E., Shoshani, J., Prychodko, W., Lerman, E.J. and Mikhelson, V.M., 1980. Tissue vestiges of an ancient Magadan mammoth calf. Scan. Electr. Microsc., 2: 163–170.

Berry, J.A., 1975. Adaptation of photosynthetic processes to stress. Science, 188: 644–650.

Bliss, L.C. and Richards, J.H., 1982. Present-day arctic vegetation and ecosystems as a predictive tool for the arctic-steppe

mammoth biome. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. Academic Press, pp. 241–257.

Bocherens, H., 1995. Assessment of the preservation of isotopic signals (^{13}C, ^{15}N) in Pleistocene bones and teeth from Western European localities. Terra Nova (Abstr. suppl., 1), 7, p. 237.

Bocherens, H. and Mariotti, A., 1992. Biogéochimie isotopique du carbone dans les os et les dents de mammifères actuels et fossiles de zones froides et tempérées. C.R. Acad. Sci. Paris, 315: 1147–1153.

Bocherens, H., Fizet, M. and Mariotti, A., 1990. Mise en évidence du régime alimentaire végétarien de l'ours des cavernes (*Ursus spelaeus*) par la biogéochimie isotopique (^{13}C, ^{15}N) du collagène fossile. C.R. Acad. Sci. Paris, 311: 1279–1284.

Bocherens, H., Fizet, M., Mariotti, A., Lange-Badré, B., Vandermeersch, B., Borel, J.P. and Bellon, G., 1991a. Isotopic Biogeochemistry (^{13}C, ^{15}N) of fossil vertebrate collagen: implications for the study of fossil food web including Neandertal Man. J. Hum. Evol., 20: 481–492.

Bocherens, H., Fizet, M., Mariotti, A., Billiou, D., Bellon, G., Borel, J.P. and Simone, S., 1991b. Biogéochimie isotopique (^{13}C, ^{15}N, ^{18}O) et paléoécologie des ours Pléistocènes de la grotte d'Aldène. Bull. Mus. Anthropol. Préhist. Monaco, 34: 29–49.

Bocherens, H., Fizet, M. and Mariotti, A., 1994a. Diet, physiology and ecology of fossil mammals as inferred from stable carbon and nitrogen isotope biogeochemistry: implications for Pleistocene bears. Palaeogeogr. Palaeoclimatol. Palaeoecol., 107: 213–225.

Bocherens, H., Fizet, M., Mariotti, A., Gangloff, R.A. and Burns, J.A., 1994b. Contribution of isotopic biogeochemistry (^{13}C, ^{15}N, ^{18}O) to the paleoecology of mammoths (*Mammuthus primigenius*). Hist. Biol., 7: 187–202.

Bocherens, H., Emslie, S.D., Billiou, D. and Mariotti, A., 1995a. Stable isotopes (^{13}C, ^{15}N) and paleodiet of the giant short-faced bear (*Arctodus simus*). C. R. Acad. Sci. Paris, 320: 779–784.

Bocherens, H., Fogel, M.L., Tuross, N. and Zeder, M., 1995b. Trophic structure and climatic information from isotopic signatures in a Pleistocene cave fauna of Southern England. J. Archaeol. Sci., 22: 237–340..

Bombin, M. and Muehlenbachs, K., 1985. 13C/12C ratios of Pleistocene mummified remains from Beringia. Quat. Res., 23: 123–129.

Castanet, J. and Ricqlès, A. de, 1987. Sur la relativité de la notion d'ostéones primaires et secondaires et de tissus osseux primaire et secondaire en général. Ann. Sci. Nat., Zool. Paris, 8: 103–109.

DeNiro, M.J., 1985. Post-mortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature, 317: 806–809.

DeNiro, M.J. and Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, 42: 495–506.

DeNiro, M.J. and Weiner, S., 1988. Chemical, enzymatic and spectroscopic characterization of "collagen" and other organic fractions from prehistoric bones. Geochim. Cosmochim. Acta, 52: 2197–2206.

Farrand, W.R., 1961. Frozen mammoths and modern geology. Science, 133: 729–755.

Fizet, M., Mariotti, M., Bocherens, H., Lange-Badré, B., Vandermeersch, B., Borel, J.P. and Bellon, G., 1995. Effect of diet, physiology and climate on carbon and nitrogen stable isotopes of collagen in a late Pleistocene anthropic paleoecosystem (France, Charente, Marillac). J. Archaeol. Sci., 22: 67–79.

Fogel, M.L., Tuross, N. and Owsley, D.W., 1989. Nitrogen isotope tracers of human lactation in modern and archeological populations. Annu. Rep. Dir. Geophys. Lab. Carnegie Inst. Washington, 1988–1989, pp. 111–117.

Gaebler, O.H., Choitz, H.C., Vitti, T.G. and Vukmirovich, R., 1966. Isotope effects in metabolim of ^{14}N and ^{15}N from unlabeled dietary proteins. Can. J. Biochem., 44: 1249–1257.

Goodman, M., Birk, D.E., Romero-Herrera, A.E., Lande, M.A., Dene, H. and Barnhart, M.I., 1980. Collagen preservation in soft tissue from the Magadan mammoth. FEBS Lett., 114(1): 30–34.

Grupe, G., Dreses-Werringloer, U. and Parsche, F., 1993. Initial stages of bone decomposition: Causes and consequences. In: J.B. Lambert and G. Grupe (Editors), Prehistoric Human Bone, Archaeology at the Molecular Level. Springer, Berlin, pp. 257–274.

Guthrie, R.D., 1982. Mammals of the mammoth steppe as paleoenvironmental indicators. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. Academic Press, New York, pp. 307–326.

Guthrie, R.D., 1990. Frozen fauna of the Mammoth Steppe. Univ. Chicago Press, 323 pp.

Heaton, T.H.E., Vogel, J.C., Chevallerie, G.V.L. and Collett, G., 1986. Climatic influence on the isotopic composition of bone nitrogen. Nature, 322: 822–824.

Howorth, H.H., 1880. The mammoth in Siberia. Geol. Mag., 7: 550–561.

Janis, C., 1975. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. Evolution, 30: 757–774.

Koch, P.L., 1991. The isotopic ecology of Pleistocene proboscideans. J. Vertebr. Paleontol., 11: A40.

Kubiak, H., 1982. Morphological characters of the mammoth: an adaptation to the arctic-steppe environment. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. Academic Press, New York, pp. 281–289.

Lazarev, P.A., 1985. Finds of Mammoth Fauna remains in the area of lower Indigirka Northern Yakutia. Curr. Res. Pleistocene, 2.

Lee-Thorp, J.A., Sealy, J.C. and Van der Merwe, N.J., 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. J. Archaeol. Sci., 16: 585–599.

Martin, P.J., 1982. Digestive and grazing strategies of animals in the arctic steppe. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. Academic Press, New York, pp. 259–266.

Medina, E. and Minchin, P., 1980. Stratification of $\delta^{13}C$ values of leaves in Amazonian Rain Forests. Oecologia (Berlin), 45: 377–378.

Olivier, R.C.D., 1982. Ecology and behavior of living elephants: Bases for assumptions concerning the extinct woolly mammoths. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. New York, Academic Press, pp. 291–305.

Payne, W.J.A., 1990. An Introduction to Animal Husbandry in the Tropics. Longman, Singapore, 4th Ed., 881 pp.

Schlesser, G.H. and Jayasekera, R., 1985. $\delta^{13}C$-variations of leaves in forests as an indication of reassimilated CO_2 to from the soil. Oecologia, 65: 536–542.

Schoeninger, M.J., 1985. Trophic level effects on $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in bone collagen and strontium levels in bone mineral. J. Hum. Evol., 14: 515–525.

Schoeninger, M.J. and DeNiro, M.J., 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim. Cosmochim. Acta, 48: 625–639.

Schwarcz, H.P., 1991. Some theoretical aspects of isotope paleodiet studies. J. Archaeol. Sci., 18: 261–275.

Sealy, J.C., Van der Merwe, N.J., Lee-Thorp, J.A. and Lanham, J.L., 1987. Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. Geochim. Cosmochim. Acta, 51: 2707–2717.

Stevens, C.E., 1988. Comparative physiology of the vertebrate digestive system. Cambridge Univ. Press, 300 pp.

Teeri, J.A. and Stowe, L.G., 1976. Climatic patterns and the distribution of C4 grasses in North America. Oecologia (Berlin), 23: 1–12.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. and Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia (Berlin), 57: 32–37.

Tuross, N., Fogel, M.L.F. and Hare, P.E., 1988. Variability in the preservation of the isotopic composition of collagen from fossil bone. Geochim. Cosmochim. Acta, 52: 929–935.

Van der Merwe, N.J., 1989. Natural variation in ^{13}C concentration and its effect on environmental reconstruction using $^{13}C/^{12}C$ ratios in animal bones. In: T.D. Price (Editor), The Chemistry of Prehistoric Human Bone. Cambridge Univ. Press, pp. 105–125.

Van der Merwe, N.J. and Medina, E., 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. J. Archaeol. Sci., 18: 249–259.

Vereshchagin, N.K. and Baryshnikov, G.F., 1982. Paleoecology of the mammoth fauna in the Eurasian arctic. In: D.M. Hopkins et al. (Editors), Paleoecology of Beringia. Academic Press, New York, pp. 267–279.

Vogel, J.C., 1978. Isotopic assessment of the dietary habits of ungulates. S. Afr. J. Sci., 74: 298–301.

Vogel, J.C., Talma, A.S., Hall-Martin, A.J. and Viljoen, P.J., 1990. Carbon and nitrogen isotopes in elephants. S. Afr. J. Sci., 86: 147–150.

Wooding, G.H., 1982. Les mammifères sauvages du Canada. Broquet, 272 pp.