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DATING BONES NEAR THE LIMIT OF THE RADIOCARBON DATING METHOD: STUDY CASE MAMMOTH FROM NIEDERWENINGEN, ZH SWITZERLAND

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ABSTRACT. Preparation of bone material for radiocarbon dating is still a subject of investigation. In the past, the most problematic ages appeared to be the very old bones, i.e. those with ages close to the limit of the dating method. Development of preparative methods requires sufficient amounts of bone material as well as the possibility of verification of the ages. In the peat section at Niederweningen, ZH Switzerland, numerous bones of mammoth and other animals were found in the late 19th century. The first accelerator mass spectrometry (AMS) radiocarbon ages of those bones from 1890/1891 excavations placed the age between 33,000 and 35,000 BP. The excavations in 2003/2004 provided additional material for ^{14}C dating. An age of 45,870 ± 1080 BP was obtained on base (NaOH step) cleaned gelatin from mammoth bone, which was very close to the age of 45,430 ± 1020 BP obtained for the peat layer that buried the mammoths. The ^{14}C age of gelatin cleaned using the ultrafiltration method obtained in this study, 45,720 ± 710 BP, is in a very good agreement with the previously obtained results. Moreover, the study shows that 3 pretreatment methods (base+Longin, Longin+ultrafiltration, and base+Longin+ultrafiltration) give ages consistent with each other and with the age of the peat section.

INTRODUCTION

Problems with radiocarbon ages >40,000 are often thought to result from contamination by modern carbon since this age corresponds to about 7 ^{14}C half-lives and only ~0.8% of the initial amount is left, and the addition of small amounts of modern carbon can significantly change ^{14}C ages.

Bones, teeth, and ivory belong to material that is frequently dated by the ^{14}C method. During the last 60 yr, numerous bones of animals, humans, and the last Neanderthals have been dated. Moreover, artifacts made of ivory and turtle shell provide organic material suitable for dating. Archaeologists have always valued this possibility, although the literature reports frequent discordant ages obtained on charcoal and bone from the same stratigraphic levels. Apparently, ages obtained on bones tend to be younger than those obtained on charcoal, which implies contamination with younger carbon.

One possible source of younger carbon is post-depositional incorporation of humic substances into the bone material either due to humification processes occurring in the bone and/or due to an interaction with the burial environment (van Klinken and Hedges 1995). Humic acids migrating with groundwater might attach to the porous bone structure and build cross-links within the collagen. Therefore, the main focus of the treatment methods for ^{14}C dating is removing such contamination. The carbonates precipitated at the surface of the bone are removed in the acid step of dissolving the mineral fraction.

Although bone mineral is partly made of carbonate-apatite that contains carbon suitable for dating and numerous studies have shown a very successful dating of mineral part of the bone (e.g. Saliège et al. 1995), separation of the organic fraction is at the moment the prevailing method for dating bone material and ivory. Dried, defatted fresh bones contain about 20% collagen. The degree of decomposition and the amount of protein remnants are important factors in isotopic studies of bone material. These can be estimated by C/N ratio, collagen content, C content, or amino acid composition (van Klinken 1999 and references therein). The organic fraction is often described as "collagen"

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or protein remnants, i.e. the remainder after the dissolution of the mineral part. Longin (1971) proposed separation of the acid-soluble gelatin fraction. An additional cleaning of "collagen" with a base step, such as NaOH, before extracting the gelatin fraction is used to dissolve and remove humic substances (Arslanov and Svezhentsev 1993; Piotrowska and Goslar 2002). Other methods involve removal of the light humic fraction (<30 kD) using ultrafiltration (Brown et al. 1988) or cleaning protein remnants in ion-exchange columns (Law and Hedges 1989; van Klinken et al. 1994) showed that a separation of collagen-specific peptides obtained from collagenase cleavage yields organic material that can be separated using chromatography (HPLC) and then AMS ^{14}C dated. Nelson (1991) used a reaction of the dye nihydrin and α amino acids to obtain carbon dioxide from gelatin, which was then applied by Piotrowska and Goslar (2002) and Tisnerat-Laborde et al. (2003).

The last 50 kyr of the history of the Late Pleistocene woolly mammoth (*Mammuthus primigenius*) in Eurasia and North America can be reconstructed using the ^{14}C dating of mammoth remains. Dating of numerous mammoth sites showed that during the Last Glacial Maximum (LGM), woolly mammoth crossed the land bridge of Beringia to join the American mammoth (*Mammuthus columbi*) and then went extinct very rapidly at the Pleistocene/Holocene boundary in the area. An isolated population survived in the Alaskan Beringia until the early Holocene as shown by bones found on St. Paul Island and dated to 7000 BP (Guthrie 2004). In Europe, a southward expansion has been documented by numerous bones and artifacts left by *Homo sapiens*, who coexisted with mammoths for thousands of years. The most southward presence of mammoth, reaching the Crimea and the Caucasus, lasted until 20,000 yr ago (Arslanov et al. 1998). From that time, mammoth began to gradually retreat to the northern regions of Arctic Siberia. Bones as young as 3700 BP, which were found on Wrangel Island, document the last Holocene refugia of mammoth (Kuzmin et al. 2003; Kuzmin and Orlova 2004).

In Switzerland, the woolly mammoth survived until the Late Glacial (Hünermann 1985; Aubry et al. 2005). The mammoths found in the peat bog of Niederweningen appear to be the oldest of ^{14}C-dated mammoth remains in Switzerland (Hajdas et al. 2007).

The main methodological aspect addressed in this study is the reliability of ^{14}C ages of very old mammoth bones from the Niederweningen site. Wood, plant macrofossils, and peat provide excellent material for ^{14}C dating, which are here used to crosscheck ^{14}C ages of bones.

THE MAMMOTH SITE

Bones of at least 5 mammoths, a mammoth calf and fossils of other fauna buried in a peat section underneath a gravel and silt layer were discovered in 1890 during railway construction work at the Niederweningen site, near Zürich (Lang 1892). Sediment cores recovered at 2 locations near the original mammoth find showed that the contact of the peat layer with the overlaying lake sediments showed numerous deformations (Schlüchter 1988). The excavations in 2003/2004 confirmed this picture of a very complicated stratigraphy with the surface of the peat being deformed (Furrer et al. 2007). Three peat layers have been documented at the Niederweningen site. The uppermost peat horizon that was found in the 2003 excavations (Furrer et al. 2007) contained numerous fragments of wood and macrofossils. The ^{14}C ages obtained on wood and cone from this layer (upper peat) resulted in ages older than 40,000 BP (Hajdas et al. 2007). The middle peat layer, which buried the mammoths, is correlated with the 1890/1891 "mammoth pit." The third, lowermost peat is older than 50,000 yr and could not be dated using ^{14}C dating. Bones, wood, macrofossils, and peat provided material for dating of this section (Hajdas et al. 2007). Multidisciplinary studies focused on reconstructions of the environment and the climate in which the mammoths lived (Drescher-Schneider et al. 2007; Furrer et al. 2007; Preusser and Degering 2007; Tütken et al. 2007).

METHODS

The first results of ^{14}C dating of the Niederweningen mammoth (Schlüchter 1994) were obtained on the "collagen" fraction (COL), i.e. on the organic matter remaining after treatment with acid, i.e. dissolution of the mineral part of the bone (Figure 1). The new finds of 2003/2004 were treated in the same way, but the results obtained on the "collagen" fraction were inconsistent (Hajdas et al. 2007).

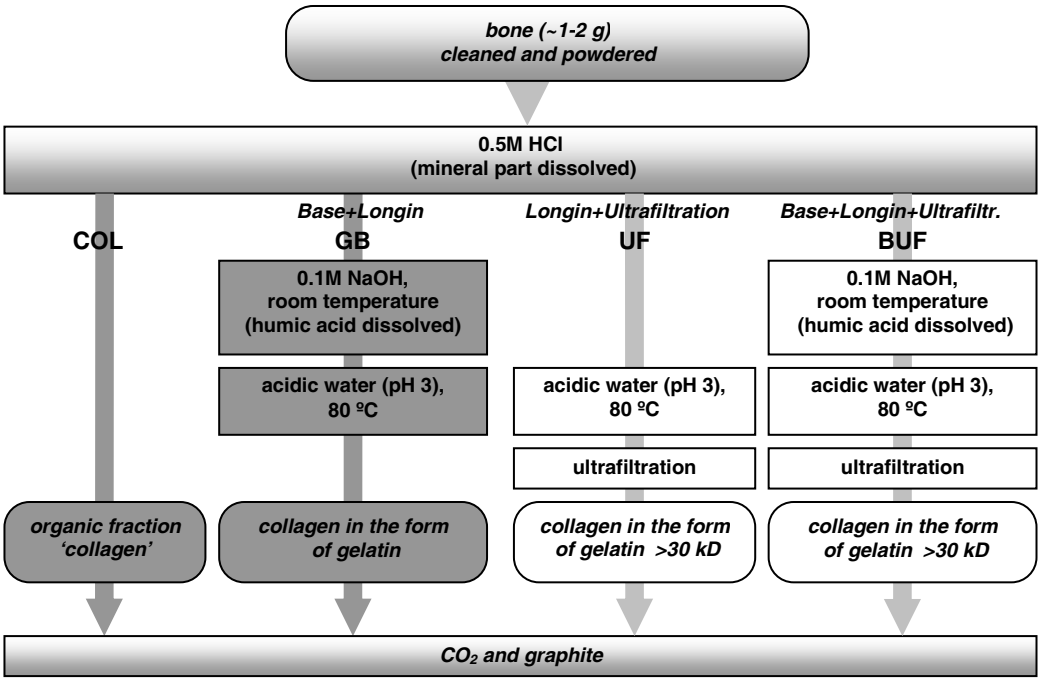

Figure 1 Schematic diagram of the chemical preparation of the dated bones. The gray color marks steps of preparation methods applied in previous studies (Hajdas et al. 2007), while the white color indicates steps of methods used in this study.

An improved preparation technique was applied for the separation of carbon from bone material and compared to the dating of the "collagen" fraction. Bone material (~2 g) was cleaned in an ultrasonic bath, dried, crushed, and pulverized. The pulverized samples were treated with acid (0.5M HCl at room temperature for several hours) to remove the mineral part of the bone.

In order to compare ages of gelatin cleaned using different methods, demineralized samples were divided into 2 parts. The first part was prepared following the procedure of Arslanov and Svezhentsev (1993): a base wash was applied (0.1M NaOH, room temperature, 30 min) and in the last step gelatin was obtained by dissolution in 0.01M HCl at 80 °C (Figure 1) for 24 or longer. This fraction is described as the GB fraction (gelatin treated with base).

The base-cleaned gelatin (GB) fraction was then cleaned using ultrafiltration (Brown et al. 1988; Higham et al. 2006). Gelatin was placed in Millipore Amicon Ultra-15 ultrafiltration tubes, precleaned following the protocol of Brock et al. (2007) and centrifuged at 4000 rpm for >20 min to collect the heavy-molecule fraction (>30 kD). This fraction is described as BUF (base+ultrafiltration).

In order to assess the effect of the ultrafiltration treatment on the age of the bone, the gelatin from the second part of the original "collagen" fraction was extracted without base treatment. This fraction is called the (ultrafiltration) UF fraction.

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After preparation of each part of the sample, the freeze-dried material was placed in precooked quartz tubes together with silver and CuO, evacuated, torch-sealed. and combusted at 950 °C. The graphitization procedure at the ETH laboratory applied to these samples is described by Hajdas et al. (2004).

The ^{14}C ages of mammoth bones obtained before 2007 (Hajdas et al. 2007) are based on measurements of $^{14}C/^{12}C$ ratios performed using the 6MV Van der Graaf tandem accelerator at the ETH/PSI AMS facility (Bonani et al. 1987). Results of this study were measured using the new 200 kV, MICADAS system (Synal et al. 2007) in January and March 2008. All ^{14}C ages are calculated following the procedure outlined by Stuiver and Polach (1977).

RESULTS AND DISCUSSION

The ages of mammoth bone A/V 4430 (ETH-28092) obtained in this study confirm results obtained using the method of Arslanov and Svezhentsev (1993). The ^{14}C ages of 40,910 ± 830 and 37,910 ± 520 BP were obtained for the "collagen" fraction (COL) of the mammoth bone sample A/V 4430 (ETH-28092) (Hajdas et al. 2007). The new analyses, which involved the Longin method of separation gelatin and additional treatment with base (GB), ultrafiltration (UF), and combined base+ultrafiltration (BUF), are consistently older than the collagen fraction. The mean value calculated from the 4 measurements on A/V 4430 bone is 45,720 ± 710 BP and is very close to the age of the middle peat of 45,430 ± 1020 BP, in which this bone was found (Hajdas et al. 2007). The results presented in Table 1 show that ^{14}C ages obtained for samples prepared with ultrafiltration are older than values obtained previously for the organic collagen fraction (COL) and similar to the age for the GB fraction. It confirms the influence of young humic acids on the ^{14}C dating results for the COL fraction. Moreover, a very good agreement was found between dating results for parts of sample prepared with and without additional base treatment (UF and BUF). A concordance of all 4 ages obtained in this study ($\chi^2_{red} = 0.73$) allows calculation of the weighted mean ^{14}C age, which is equal to 45,720 ± 710 BP. It should be emphasized that this age is also consistent with the ^{14}C age of the peat section and with the age gained from measurement of the GB fraction, i.e. samples prepared using the base-cleaned gelatin. The latter suggests that the ultrafiltration procedure may be not necessary for the removal of contamination by humic acids and can easily be replaced by the method introduced by Arslanov and Svezhentsev (1993).

Table 1 Results of ^{14}C dating obtained for sample A/V 4430 (bone of the mammoth from Niederweningen found in 2003) in previous and present studies.

Sample code & lab nr	Meas. date	Fraction	^{14}C age (BP)	^{14}C age BP weighted mean		$\delta^{13}C$ (‰)
A/V 4430						
ETH-28092						
Previous results (Hajdas et.al. 2007)						
	2003	COL	40,910 ± 830	—	—	–24.2 ± 1.2
	2004	COL	37,910 ± 520	—	—	–19.8 ± 1.2
	2005	GB	45,870 ± 1080	—	—	–19.3 ± 1.2
Results from this study						
	Jan 2008	UF	46,760 ± 1470	45,600 ± 1000	**45,720 ± 710**	–23.7 ± 1.2
	Mar 2008	UF	44,605 ± 1365			–14.9 ± 1.2
	Jan 2008	BUF	46,765 ± 1370	45,830 ± 1010		–21.5 ± 1.2
	Mar 2008	BUF	44,720 ± 1500			–17.9 ± 1.2

In this study, another 2 samples of mammoth rib bone A/V 4580 (ETH-33628) and fragments of mammoth tusk A/V 4500 (ETH-33630) were also dated. These samples were collected from a site excavated in 2004, located at the western end of the first 1890/1891 Niederweningen mammoth site

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(Furrer et al. 2007). The samples were treated the same way as the sample A/V 4430 (Figure 1), but each fraction of those samples (UF and BUF fraction) was measured only once (Table 2). Also, these results show a very good agreement between ^{14}C ages for the UF and BUF fraction.

Table 2 Results of ^{14}C dating obtained for samples A/V 4500 and A/V 4580 (mammoth tusk and rib bone, respectively, found in 2004 at the west end of the first Niederweningen mammoth pit 1890/91).

Sample code & lab nr	Meas. date	Fraction	^{14}C age (BP)	^{14}C age (BP) weighted mean	$\delta^{13}C$ (‰)
A/V 4500 ETH-33630	Jan 2008	UF	39,385 ± 615	39,240 ± 430	–20.8 ± 1.2
		COL	39,100 ± 590		–20.9 ± 1.2
A/V 4580 ETH-30071		COL	41,730 ± 730	—	—
Previous results (Hajdas et al. 2007)					
A/V 4580 ETH-33628	Jan 2008	UF	39,990 ± 585	39,560 ± 400	–20.9 ± 1.2
	Jan 2008	BUF	39,190 ± 540		–20.5 ± 1.2

The ^{14}C age obtained previously on the collagen fraction (COL) of mammoth rib bone A/V 4580 (ETH-30071) found in 2004 in a layer with reworked peat fragments was 41,730 ± 730 BP (Hajdas et al. 2007). In the present study, the gelatin BUF and UF fraction obtained on an additional sample (ETH-33628) resulted in ages of 39,200 ± 540 and 40,000 ± 580 BP, respectively. The reason for this younger age of 39,560 ± 400 BP (mean value) of the cleaned gelatin might be the removal of old humic acid contaminating the bone in the peat section. However, such contamination would require a substantial amount (more than 25%) of very old (dead) carbon to be present in the collagen fraction. Additional analysis should clarify this point.

The chronology of the Niederweningen site relies on the ages of bones, peat, and wood (Hajdas et al. 2007). The consistent ^{14}C ages of the A/V 4430 bone sample presented in this paper show that a similar approach is required for the final correlation of the 1890/91 mammoth pit excavations and the middle peat layer. This was based on geologic (geometric) interpretations and the presence of mammoth bones. However, young ages were obtained on the A/V 4580 rib bone sample and the horse tooth and skull, which were found in the 1890/1891 mammoth pit (Hajdas et al. 2007).

CONCLUSIONS

^{14}C ages of mammoth bones from Niederweningen were measured on 3 bones and gelatin precleaned using 2 methods (base and UF). There is a very good agreement between ^{14}C ages obtained for parts of samples prepared using ultrafiltration with and without base treatment, as shown by the ages of the UF, BUF, and BG fractions of gelatin extracted from the bone sample A/V 4430. These ages are in very good agreement and significantly older than the age obtained on the so-called COL fraction. Moreover, the ^{14}C age of the precleaned gelatin is in agreement with the ^{14}C age of the peat section in which the mammoth's remains were found.

The bone sample A/V 4580 found in 2004 near the old 1890/1891 mammoth pit location shows slightly younger ages of UF and BUF gelatin compared to the COL fraction.

In summary, our study shows that gelatin treated either by base or UF returns coherent and reliable ages for very old bones. This concordance suggests that the base treatment of collagen results in sufficiently clean gelatin and can be an alternative to the ultrafiltration cleaning step.

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