

FIRST ^{14}C RESULTS FROM ARCHAEOLOGICAL AND FORENSIC STUDIES AT THE VIENNA ENVIRONMENTAL RESEARCH ACCELERATOR

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ABSTRACT. ^{14}C dating with the new Vienna Environmental Research Accelerator (VERA) began with the age determination of a mummified marmot found in the Austrian Alpine region. Soft tissue and bones of the marmot were used for the investigation. For comparison, bone material from known-age samples was also processed and measured. These exercises showed that ^{14}C dating with VERA is reliable, and since that time various samples from archaeological context have been dated.

We also studied the applicability of the ^{14}C method in forensic sciences to determine the time of death of human individuals. $^{14}\text{C}/^{12}\text{C}$ measurements of samples from different organic human material (bone collagen, lipids from bone and bone marrow, hair) were performed and compared with the tropospheric “bomb peak” values to transform the measured ratios into “calibrated ages”. For specific substances with rapid turnover rates, this gives an estimate for the time of death of the individual. In our study, lipids and hair yield reasonable times of death, whereas the collagen fraction from bones, which has a relatively long turnover time, is not suitable for this purpose.

INTRODUCTION

VERA, the newly established AMS facility in Vienna, has been operational since 1996 (Kutschera *et al.* 1997). After extensive testing of the machine (Priller *et al.* 1997), ^{14}C measurements of samples with unknown ages started in autumn 1996. Since that time, the ^{14}C investigations have concentrated on two major fields of research, archaeology and forensic science.

Archaeological Applications

The first object for dating, a frozen marmot from the Pitztal glacial region in Austria, was submitted to the VERA lab in September 1996 (H. Seidler, private communication 1996). It was speculated that the marmot might date back to roughly the same period as the famous ice man “Ötzi” found in 1991 in a glacier of the Ötztal. Soft tissue and bone material of the animal was available for the age determination. The isolation of collagen or gelatin from the bone matrix requires sample preparation procedures that cannot be applied to the common ^{14}C reference materials. Therefore, two bone samples from different time periods, dated by conventional beta counting at the ^{14}C dating laboratory of the Institut für Radiumforschung und Kernphysik, and one sample dated by accelerator mass spectrometry (AMS) in Zurich, were chosen as control materials. Results on these samples established the reliability of VERA’s ^{14}C dating and consequently its capability to perform routine dating. Since that time, various samples of archaeological interest have been investigated.

Application in Forensic Sciences

An important new application of the ^{14}C AMS method is in forensic sciences, with a current court case motivating a study concerning the determination of the year of death of humans. The idea was that the ^{14}C concentration in recent organic human materials, actually at the end of the carbon uptake

time, reflects the “bomb” enhancement of ^{14}C concentration in the atmosphere, which is rapidly decreasing, presently by *ca.* 1% per year (Levin and Kromer 1997). The aim of this investigation was to find organic components of the human body with a rapid turnover time, so that measuring the $^{14}\text{C}/^{12}\text{C}$ ratios of their organic fractions and comparison of the derived values with the atmospheric “bomb” peak would lead to an accurate estimate of the time of death of the human individual. Different turnover times for bone collagen of only a few months up to many years can be found in the literature. Many studies applying isotope labeling techniques focusing on the investigation of common laboratory animals (rats and mice) determined short turnover times, but inference from short-lived animals to humans seems not to be valid (*e.g.*, Knese 1979). A study of stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of collagen isolated from ribs of human remains originating from various historical burial sites in Canada led to the conclusion that the collagen turnover may be >20 yr and that the turnover rate depends on the biological age of the individuals and is slower for older individuals (Katzenberg 1993).

Because of these divergent estimates of the turnover rates of human collagen from bones, materials with expected shorter turnover rates were also examined. Lipids from the bones and the bone marrow, and hair samples of some excavated or recently deceased individuals with known biological age and time of death were used. In addition, bone material and hair were analyzed from two ~85-yr-old women—subjects of the current court case mentioned above—with unknown time of death.

METHODS

Sample Preparation

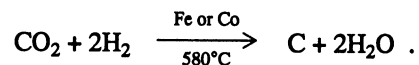
In most cases the samples were first cleaned in an ultrasonic bath to remove adherent particles. The so-called ABA (acid-base-acid, sometimes also referred as “AAA”, acid-alkaline-acid) method was applied as a further pretreatment for sample materials such as charcoal, textiles and peat, (*see, e.g.*, Bonani *et al.* 1992).

The isolation method of gelatin referred to in the literature as “crude gelatin” was applied to both forensic and archaeological bone samples (Law and Hedges 1989). In the case of the forensic samples, traces of lipids not removed during the alkaline step of the procedure will be present in the gelatin fraction. However, this amount should be negligible since lipids degrade faster than collagen as a consequence of general decomposition. For example, the lipid concentration in the bone sample of a 30-yr-old man, deceased in 1995 and recently excavated, was only ~0.2% (w/w), whereas the collagen content of recent bone samples is in the range of 20% (w/w).

Lipids from the bones and the bone marrow were extracted with distilled (residue-free) acetone. After removal of the solvent, the residue was kept in a rotavapor at 90°C (pressure: 14 mmHg) for 2 h. Hempseed oil harvested in 1995 was used as a check for a possible contribution of dead carbon from acetone to the lipid fraction. Therefore we also processed one oil sample treated with acetone in the same way as the bones, along with one untreated sample.

An amount of 10 mg pretreated sample material was transferred to a quartz tube containing 1 g CuO. Silver wire was added as a binder for sulphur and halogens and the tubes were evacuated and sealed with a glassblowing torch. For complete combustion of the sample carbon to CO_2 , the sealed samples were heated in a muffle furnace at 900°C for 2 h.

The catalytic reduction of CO_2 to elemental carbon was adopted from Vogel *et al.* (1984) according to the reaction:



This “graphitization” technique is now a standard method used by many AMS ^{14}C laboratories and was also chosen for the VERA lab.

An amount of the catalyst-carbon mixture corresponding to ~1 mg carbon was pressed into the 1-mm holes of the aluminum target holders with a recess of 0.5 mm. From one graphitization process up to three targets can be produced.

During the start-up phase of our AMS facility and for our first age determinations, the target materials were produced using Co as catalyst. The graphitization reaction was completed after 6–7 h. This time could be decreased to 3–4 h by changing the catalyst from Co to Fe. Since no deterioration of the accelerator beam quality was detected after this change, all further targets were produced with the iron catalyst.

For all age determinations, the IAEA standards C-6 (ANU-sucrose) and C-3 (cellulose) were used as reference materials. Dead carbon from graphite rods was selected as machine and chemistry blanks.

AMS Measurements

Two to three replicates (targets) were used for all samples, including IAEA C-3 and C-6 standards and both chemistry and machine blanks. They were distributed uniformly in the 40-position target wheel of the MC-SNICS sputter ion source of VERA. The targets gave $^{12}\text{C}^-$ currents in the range of 15 to 25 μA at relatively cold running conditions of the ion source. Sequential isotope injection was used with cycle times of 120 ms. Typical time periods were 0.5, 1.5 and 98 ms for ^{12}C , ^{13}C and ^{14}C , respectively, plus some waiting time. During the time of $^{14}\text{C}^-$ injection, $^{12}\text{C}^-$ and $^{13}\text{C}^-$ ($+^{12}\text{CH}^-$) currents were measured in the low-energy offset Faraday cups. The tandem accelerator was operated at 2.7 MV terminal voltage, with Ar gas stripping and with generating voltmeter plus capacitive pick-off voltage control. At the high-energy analyzing magnet, $^{13}\text{C}^{3+}$ and $^{12}\text{C}^{3+}$ currents were measured in the respective offset Faraday cups. A quasi-continuous monitoring of the accelerator transmission was possible through the $^{12}\text{C}^{3+}/^{12}\text{C}^-$ ratio (ca. 47%). For ^{14}C detection, the high-energy ion beam was further cleaned up with the Wien filter and $^{14}\text{C}^{3+}$ ions were counted with a solid-state surface barrier detector. After careful tune-up of the entire AMS system, $^{14}\text{C}^{3+}/^{12}\text{C}^{3+}$, $^{14}\text{C}^{3+}/^{13}\text{C}^{3+}$ and $^{13}\text{C}^{3+}/^{12}\text{C}^{3+}$ ratios for the 40 targets were measured in a fully software-controlled mode. Typically, each target was measured 5 to 7 times with 3 min per individual measurement. The precision (stand. dev. from mean) achieved for the measured $^{14}\text{C}/^{12}\text{C}$ ratios of modern samples was <0.5% by averaging the measured values of three targets.

Details of the machine performance and longtime characteristics are described in Rom *et al.* (1998), where it is also shown that a precision of 0.5% can be reached for single targets.

Age Determination

The ^{14}C content in percent Modern Carbon (pMC) and the ^{14}C ages of the investigated samples were calculated from the $^{14}\text{C}/^{12}\text{C}$ and the $^{13}\text{C}/^{12}\text{C}$ ratios measured with the AMS system as described in detail in Priller *et al.* (1997). For background correction, the $^{14}\text{C}/^{12}\text{C}$ ratio of the chemistry blank was subtracted from the $^{14}\text{C}/^{12}\text{C}$ ratios of both the standards and the samples.

The $\delta^{13}\text{C}$ values of the samples were determined from the $^{13}\text{C}/^{12}\text{C}$ ratios measured with the AMS machine and referenced to the value of the C-3 cellulose standard. Currently, this leads to uncertainties of at least $\pm 1\%$, which causes a non-negligible contribution to the calculated ^{14}C values. In the near future, a stable isotope mass spectrometer will be added to the VERA lab, allowing for $\delta^{13}\text{C}$ measurements (from CO_2) with much higher precision. Although this will undoubtedly help in characterizing the sample material, these offline-measured $\delta^{13}\text{C}$ values can only be used reliably for overall fractionation corrections, if no differences of fractionation occur after the CO_2 production.

The ^{14}C ages of the samples were transformed into calibrated ages with the computer program OxCal (Bronk Ramsey 1995) using the calibration curves from Stuiver, Long and Kra (1993). For the recent samples the atmospheric bomb peak values measured at Schauinsland and Vermunt by Levin and Kromer (1997) and Levin *et al.* (1985) were added to the calibration curve and “calibrated ages” were determined with a modified version of the OxCal program.

RESULTS AND DISCUSSION

Archaeological Applications

The ^{14}C dating results of selected archaeological samples are shown in Table 1. An improvement of both $\delta^{13}\text{C}$ and ^{14}C measurements is apparent for VERA sample numbers greater than 6. This is due to a corrective measure applied to the injector magnet (Rom *et al.* 1998). The ages of a mammoth and a human bone sample dated with the conventional ^{14}C method (E. Pak, private communication 1996) and the age of a mummified cat (mean value of bone and soft tissue), previously dated by the AMS lab in Zurich (G. Bonani, private communication 1993), are listed together with the ages derived by VERA. The ages of the cat samples and the human bone agree very well. A small discrepancy between the AMS method and conventional dating was found for the mammoth sample, which is not yet understood. One possible explanation may be that the age determination of old sample material is very sensitive to the background correction, which is large for both methods. Further cross-checks between the conventional ^{14}C method and the AMS method on old samples are planned to clarify this problem.

The marmot turned out to originate from the time period of enhanced atmospheric ^{14}C levels due to atmospheric nuclear testing. $^{14}\text{CO}_2$ started to rise after 1950, with a maximum of almost 200 pMC in the early sixties. After the Nuclear Test Ban Treaty in 1963, $^{14}\text{CO}_2$ decreased steadily by the exchange of atmospheric CO_2 with other reservoirs (Levin and Kromer 1997). This seems to be reflected by the higher ^{14}C content of the marmot bone compared to that of the tissue sample. As discussed below in the forensic studies, the time lag can be interpreted as a consequence of the slower turnover rates of bone collagen compared to those of soft tissue. Therefore, from the two time periods obtained by using the “bomb peak” as “calibration curve” (compare Fig. 1), we consider the period of 1975–1977 to be the more likely time of death of the marmot. For the cat samples, no difference was found between the ^{14}C values of bone and tissue. This is consistent with the results of anatomic investigations, which found that the biological age of the cat was only a few months at death (Weisgram *et al.* 1996).

The wood sample (Tumpfen, KBT-3) was correlated with a landslide event, and from the sediment above an age of the sample of >3000 yr was estimated by the sample submitter (G. Patzelt, private communication 1997). The age determined with the ^{14}C method is in agreement with this estimate. The charcoal samples (Spiegelmahd, SM-2 and Leiersalm, LA-5) were suspected to originate from man-made forest fires, and the ages of the samples were important for dating early settlements in

TABLE 1. ¹⁴C Dating Results of Selected Archaeological Samples

Lab code (VERA-)	Sample (sample identification)	δ ¹³ C (‰)	¹⁴ C concentr. (pMC)	¹⁴ C age (yr BP)	Calibrated age*
0001	Marmot bone	-30.1 ± 3.0	137.8 ± 1.1	>Modern	1962 AD (0.04), 1973–1976 AD (0.96)
0002	Marmot tissue	-29.9 ± 2.5	134.3 ± 0.9	>Modern	1962 AD (0.05), 1976–1979 AD (0.95)
0003	Cat bone	-19.6 ± 4.7	122.9 ± 1.3	>Modern	1958–1961 AD (0.42), 1981–1985 AD (0.58)
0004	Cat tissue	-27.7 ± 7.4	122.9 ± 2.4	>Modern	1958–1962 AD (0.36), 1982–1984 AD (0.64)
		-23.7 ± 0.7	123.7 ± 0.5	>Modern†	1959–1962 AD, 1980–1983 AD†
0005	Human bone	-23.0 ± 6.4	80.8 ± 1.4	1715 ± 145	0 AD–650 AD (1.00)
		-20 ± 2	81.9 ± 0.5	1600 ± 50‡	380–600 AD (1.00)
0006	Mammoth bone	-22.8 ± 4.3	2.98 ± 0.10	28,250 ± 250	
		-20 ± 2	3.89 ± 0.23	26,100 ± 450‡	
0009	Wood (Tumpen, KBT-3)	-27.3 ± 0.8	68.92 ± 0.33	2991 ± 39	1380–1340 BC (0.05), 1320–1060 BC (0.95)
0013	Charcoal (Spiegelmahd, SM2)	-23.6 ± 0.9	64.3 ± 0.47	3507 ± 47	1949–1680 BC (1.00)
0016	Charcoal (Leiersalm, LA-5)	-27.9 ± 1.1	49.16 ± 0.24	5704 ± 39	4680–4640 BC (0.10), 4620–4460 BC (0.90)
0008	Antler (China, Han Dynasty)	-17.3 ± 1.4	76.2 ± 0.52	2182 ± 55	380–60 BC (1.00)
0019	Linen (Eastern Europe)	-25.5 ± 0.8	97.9 ± 0.35	171 ± 34	
0036	Wood (Tusch, spruce, outer tree rings, 70–80 yr)	-24.2 ± 2.3	69.49 ± 0.47	2924 ± 54	1270–930 BC (1.00)
0037	Wood (Tusch, spruce, middle tree rings, 36–45 yr)	-23.3 ± 1.4	69.36 ± 0.33	2939 ± 38	1260–1010 BC (1.00)
0038	Wood (Tusch, spruce, inner tree rings, 1–8 yr)	-24.4 ± 1.1	69.30 ± 0.30	2946 ± 35	1260–1020 BC (1.00)
	Chemistry blank	-30.2 ± 0.8	0.31 ± 0.03	46,400 +850/-760	
	Machine blank	-29.2 ± 0.9	0.05 ± 0.01	61,000 +1800/-1500	

*Values correspond to 2σ confidence levels, probability for the time range is given in parentheses.

†Mean value of tissue and bone determined by AMS measurement at Zurich (ETH-10325/6; G. Bonani, private communication 1993).

‡Results from the conventional radiocarbon laboratory of our institute (E. Pak, private communication 1996). The δ¹³C values are taken from Stuiver and Polach 1977.

Alpine highlands. The determined ages of both samples also confirm the time periods expected by the sample submitter (G. Patzelt, private communication 1997).

The ¹⁴C result on an antler sample (part of a sculpture) from a Viennese gallery for ancient art confirmed that the object originates from a time period belonging to the Han Dynasty (206 BC–AD 220). A second sample from art trade—a linen with lithography of possible significance for ancient art in Eastern Europe—turned out to be modern.

The other three samples (Tusch) listed in Table 1 are tree rings from different parts of a spruce log cross-section from the Hallstatt region (F. E. Barth and P. Stadler, private communication 1997). The

age of the spruce is related to the beginning of salt mining in this famous cultural region. These samples may allow wiggle matching to achieve a tighter time resolution.

Results of typical blank measurements are listed in Table 1. For the chemistry blanks, values of ~ 0.3 pMC, corresponding to a ^{14}C age of ~ 46 ka BP, were achieved for our 3 mg carbon batches (used for three targets). The values of the machine blanks—pure carbon mounted directly into the target holders of the ion source—were ~ 0.05 pMC, which is equivalent to a ^{14}C age of ~ 60 ka BP. This indicates that cross-contamination in the ion source is virtually nonexistent.

Application in Forensic Sciences

The ^{14}C results of the forensic samples investigated up to now are shown in Table 2. It can be seen from this table that without a model describing the turnover rates of bone collagen as a function of the biological age of humans, an estimate for the time of death is not possible based on the determined ^{14}C values of gelatin. This is most striking in the case of the 30-yr-old male person deceased in 1995: without modeling, the ^{14}C value of the gelatin misleadingly suggests a time of death between 1973 and 1975 (see Fig. 1). The second value of 1962 can be ruled out because at this time the man was not yet born. Our results from bone collagen confirm the findings of a study by Jull, Kalin and Burns (1995). From the lipids extracted from the bone of the same individual a time of death in the period of 1990 to 1994 can be determined. (Again, the second time period derived from

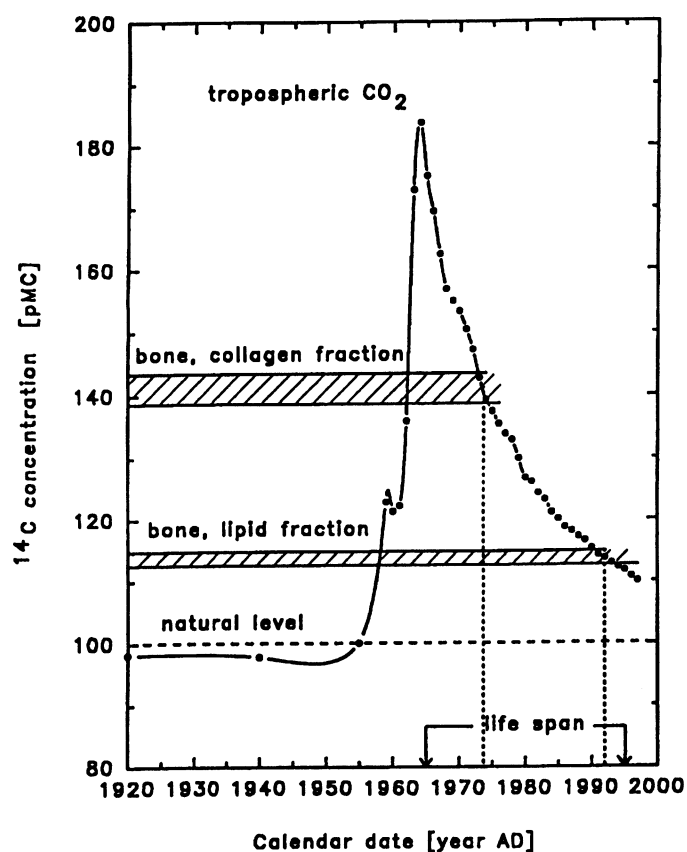


Fig 1. Annual averages of tropospheric ^{14}C concentrations measured during the “bomb peak” period at Vermont and Schauinsland (Levin *et al.* 1985; Levin and Kromer 1997) extended to 1920 by data from the ^{14}C calibration curve of Stuiver *et al.* (1993). The 2σ ranges for the collagen and the lipid fraction of a long bone from a 30-yr-old man are indicated (death date 1995). “Calibrated ages” from the measured ^{14}C values falling within the lifespan of the individual are also shown.

TABLE 2. ^{14}C Dating Results of Forensic Samples

Lab code (VERA-)	Individual	Year of death (AD)	Sample composition	$\delta^{13}\text{C}$ (‰)	^{14}C concentr. (pMC)	Calibrated age* (AD)
0020	30-yr-old male	1995	Long bone, collagen	-16.1 ± 3.8	141.12 ± 1.20	1962 (0.04) 1973–1975 (0.96)
0076	30-yr-old male	1995	Long bone, lipid fraction	-22.0 ± 0.9	113.73 ± 0.55	1957–1958 (0.07) 1990–1994 (0.93)
0021	Newborn baby	1994/1995	Long bone, collagen	-14.2 ± 2.6	112.17 ± 0.88	1957–1958 (0.09) 1991–1997 (0.91)
0023	Newborn baby	1994/1995	Hair	-21.5 ± 2.1	112.89 ± 0.63	1957–1958 (0.08) 1991–1995 (0.92)
0024	~85-yr-old female	1985 (?)	Long bone, collagen	-23.6 ± 0.6	118.22 ± 0.74	1958–1959 (0.09) 1985–1989 (0.91)
0026	~85-yr-old female	1985 (?)	Hair	-16.1 ± 2.1	120.61 ± 0.68	1958–1961 (0.36) 1983–1986 (0.64)
0027	~85-yr-old female	1986 (?)	Long bone, collagen	-19.2 ± 2.9	112.31 ± 0.89	1957–1958 (0.09) 1991–1997 (0.91)
0028	~85-yr-old female	1986 (?)	Rib, collagen	-18.0 ± 2.7	123.68 ± 0.84	1959–1961 (0.35) 1981–1984 (0.65)
0029	~85-yr-old female	1986 (?)	Hair	-16.9 ± 2.4	120.51 ± 0.72	1958–1961 (0.35) 1983–1986 (0.65)
0030	~20-yr-old male	1943/45	Long bone, collagen	-15.1 ± 2.8	97.24 ± 0.65	1910–1954 (0.16) 1630–1880 (0.77) 1520–1580 (0.07)
0083	46-yr-old female	1997	Long bone, lipid fraction	-20.0 ± 0.7	113.98 ± 1.32	1957–1958 (0.11) 1989–1996 (0.89)
0082	46-yr-old female	1997	Lipids from bone marrow	-21.4 ± 3.1	113.85 ± 0.86	1957–1958 (0.10) 1990–1995 (0.90)
0084	46-yr-old female	1997	Hair	-25.8 ± 1.0	113.92 ± 0.77	1957–1958 (0.10) 1990–1994 (0.90)
0080	73-yr-old male	1997	Long bone, lipid fraction	-25.5 ± 2.6	114.53 ± 0.77	1957–1958 (0.11) 1989–1993 (0.89)
0079	73-yr-old male	1997	Lipids from bone marrow	-18.8 ± 2.4	113.30 ± 0.75	1957–1958 (0.09) 1990–1995 (0.91)
0081	73-yr-old male	1997	Hair	-23.2 ± 1.7	112.49 ± 0.84	1957–1958 (0.10) 1991–1996 (0.90)
0077			Hempseed oil 1995, untreated	-28.6 ± 2.6	111.69 ± 1.18	1957–1958 (0.11) 1991–1997 (0.89)
0078			Hempseed oil 1995, acetone treated	-28.0 ± 3.6	111.48 ± 0.93	1957–1958 (0.11) 1992–1997 (0.89)

*Values correspond to 2σ confidence levels; probability for the time range is given in parentheses.

the bomb peak can be ruled out for the same reason as given above.) From these results it can be concluded that the turnover rate of lipids in bones is rapid compared to the turnover rate in collagen. Clearly, the ^{14}C values of lipid samples lead to a more reliable estimate of the time of death. The fast exchange of carbon could also be verified for the lipids extracted from the bone marrow and for the hair samples. This can be seen from the results of the samples from a 46-yr-old woman and a 73-yr-old man also listed in Table 2. As expected, no difference between the bone collagen and the hair sample from the newborn baby could be found. The ^{14}C value determined for a soldier from the Second World War was in the range of the pre-bomb values.

The two hempseed oil samples at the bottom of Table 2 show no difference in ^{14}C values, confirming the negligible contribution of the acetone treatment of the lipid samples (see “Sample Preparation” above).

The results of the two old women with unknown date of death also show that the turnover rate of collagen in bone is very slow. Of the two time periods indicated by ^{14}C measurement, the one *ca.* 1958–1960 may be the more likely time period, reflecting slow turnover, whereas the second, very recent, periods can be ruled out, because these would indicate extremely short turnover times even in very old individuals. From the ^{14}C values of the hair samples, the time of death of the two ladies can be estimated to lie between 1983 and 1986. This value may be biased by the shelf life of the foodstuff comprising the diet of the individuals, but the error due to the uptake of “older carbon” with food should not exceed one year. The alternative periods derived for the hair samples (*ca.* 1960) can be ruled out because of the relatively short hair style of the women (corresponding to a short hair lifetime).

From our results it can be concluded that for the determination of the time of death of humans—often an important question in forensic investigations—application of the ^{14}C method yields good estimates, provided that lipids from bones or from bone marrow are available. In cases of advanced decomposition, where lipids are already degraded, hair is a good alternative for such investigations.

CONCLUSION

^{14}C measurements at VERA gave valuable results in the most common application of the ^{14}C method—the age determination of archaeological samples—and in the relatively new application in forensic sciences. Besides routine dating, investigations in the forensic field will be continued with the aim of establishing a model for the turnover rates of different organic materials of human remains, especially of bone collagen.

Future ^{14}C projects will deal with carbon target preparation and measurements of sub-milligram samples from both atmospheric ^{14}CO studies initiated by C. Brenninkmeijer (see, *e.g.*, Brenninkmeijer 1993) and from aerosol samples in cooperation with L. Currie, where first results are already presented by Weissenböck *et al.* (1998).

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