Int J Legal Med
DOI 10.1007/s00414-010-0521-x

TECHNICAL NOTE

Single tooth tells us the date of birth

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Received: 10 June 2010 / Accepted: 1 October 2010

Abstract The atmospheric carbon-14 (^{14}C) concentration remained relatively stable until 1955, but then rapidly increased after 1955 by nuclear bomb tests, peaked in 1963, and decreased thereafter. Recently, Spalding et al. proposed epoch-making method for determining date of birth (DOB) using the tooth enamel ^{14}C incorporated during enamel formation. However, because the ^{14}C level analyzed in one tooth gives two possible age ranges (up-slope or down-slope of the bomb curve), a variety of teeth that formed in different periods are required for estimating DOB in this method. Enamel formation in a tooth moves from the incisal (occlusal) side to the cervical side. Taking advantage of this characteristic, we have first succeeded in specifying the age range from only single tooth by measuring ^{14}C in the incisal (occlusal) and cervical regions of the enamel separately. To date, no method of determining DOB or age estimation from single tooth enamel has been made. Furthermore, this method of dividing tooth into smaller parts could be useful for producing a more accurate DOB. Our new method is a powerful tool for identification when we can use only extremely few specimens in forensic casework.

Keywords Radiocarbon · Single tooth · Age estimation · Nuclear bomb-testing effect · Enamel · Forensic odontology

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Introduction

In major disasters and accidents with a large number of victims, age estimation of cadavers is a key factor in preliminary screening during forensic identification. In childhood, age estimation can be performed accurately using morphological features especially those related to dental growth stage, including the development of crowns and roots and the state of eruption. However, morphological features vary in individuals aged more than about 20 years old, and multiple methods of age estimation are used based on a variety of age-related structures. A biochemical method is required for more accurate results. Aspartic acid racemization in dentin shows a close correlation with chronological age of the individual at the time of death (within ±4.00 years by Ogino et al., ±5.69 years by Ritz et al., and 5.4±4.2 years by Alkass et al.). However, determination of age at death based on racemization has a number of problems, given the temperature-, pH-, and moisture-dependence of amino acid racemization. In addition, at least four teeth of the same type are required to draw a calibration curve [1–5].

In 2005, Spalding et al. proposed a precise method of estimating date of birth (DOB) using carbon-14 (^{14}C) contained in enamel [6]. The ^{14}C concentration in the atmosphere remained relatively stable until 1955, but then increased with the increase in the number of aboveground nuclear bomb tests, and reached a peak in 1963 when the Test Ban Treaty was signed. The ^{14}C concentration then decreased, not primarily due to radioactive decay, but to diffusion and equilibration with other carbon reservoirs such as the ocean (Fig. 1) [7–9]. Atmospheric ^{14}C is present in carbon dioxide ($^{14}CO_2$), and $^{14}CO_2$ is incorporated into plants by photosynthesis, and into animals that feed on plants, before being taken into the human body through the food chain. In this way, the in vivo ^{14}C concentration

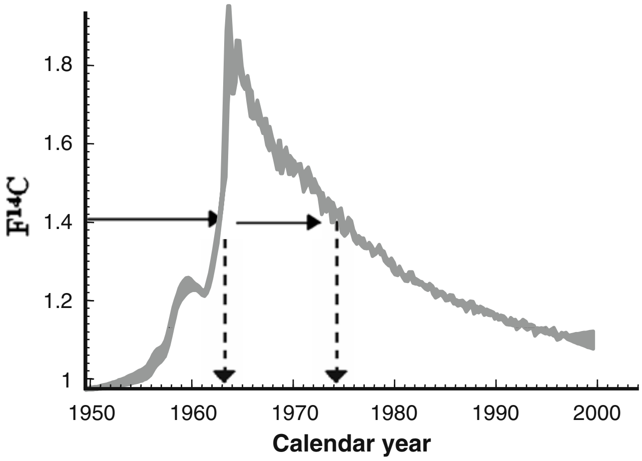

Fig. 1 Schematic bomb curve showing the problem caused by the 1963 bomb peak. The ^{14}C concentration of samples is related to the atmospheric ^{14}C concentration and gives two possible enamel formation ranges by reading off the x-axis

reflects the atmospheric ^{14}C level during the period in which an individual lives.

Enamel does not turn over after enamel formation is completed, and so the ^{14}C concentration in enamel reflects that in the atmosphere at the time of enamel formation. Therefore, the method presented by Spalding et al. [6] can be applied to determine the DOB of individuals with teeth enamel which is formed after 1955. Alkass et al. [5] also showed the precision of radiocarbon analysis with absolute error of 1.0±0.6 years. However, it is difficult to determine the DOB correctly because the bomb curve shows two possible age ranges before and after the peak concentration in 1963 (Fig. 1). There is ambiguity as to which of the two age ranges is correct, but Spalding et al. suggested that this problem can be resolved by measuring the activity in teeth formed at different times [6]. Ubelaker et al. resolved this ambiguity by comparing the ^{14}C concentrations of teeth and cortical or trabecular bone samples from a skeleton, since there is some turnover in bone [10]. Similarly, Cook et al. compared the ^{14}C concentration of enamel with that in the root collagen component of a tooth (combined dentin and cementum) to evaluate the age range [11].

Materials and methods

Tooth samples

Eight teeth were obtained from five Japanese subjects of known DOB and sex (autopsy cases of the Department of Forensic Medicine, The Jikei University School of Medicine; Table 1). The study was approved by the Ethics Committee of The Jikei University School of Medicine. Samples were anonymized in an unlinkable fashion.

Enamel preparation

Samples were physically cleaned to remove adherent tissue, cut from the root at the level of the cervical line, and then further cut at the mesiodistal line. One side of the enamel was analyzed as the crown sample. Most of the dentin was removed from the crown with a high-speed hand-held dental instrument. To remove the remaining dentin, the crown was immersed in 10N NaOH in a water bath sonicator at room temperature for 30 min, rinsed three times with double-distilled H_2O (ddH_2O), and resubmerged in 10N NaOH in the sonicator for 30 min. The sample was then washed three times with ddH_2O, resubmerged in 10N NaOH at room temperature overnight, and rinsed twice with ddH_2O in the sonicator for 10 min until all dentin and soft tissues were removed from the enamel. The enamel sample was then pretreated in 0.25 N HCl for 10 min, rinsed three times with ddH_2O in the sonicator for 10 min, and placed on a heating block at 65°C to dry overnight.

Accelerator mass spectrometry

The conversion to graphite and measurement of the graphite target with accelerator mass spectrometry were performed by Paleo Labo Co. (Toda, Japan). The enamel sample was hydrolyzed to CO_2, evacuated, heated, and acidified with orthophosphoric acid at 90°C. The evolved CO_2 was purified, trapped, and reduced to graphite. Radiocarbon concentration data are presented as $F^{14}C$ and $\Delta^{14}C$. $F^{14}C$ is defined in equation 2 of Reimer et al. [12], which was designed for reporting bomb curve data and contains a $\delta^{13}C$ fractionation correction. $\Delta^{14}C$ is calculated as: $\Delta^{14}C = 1000 \times \left\{F^{14}C \times \exp[\lambda \times (1950 - y)] - 1\right\}$, where $\lambda = 1/8{,}267$ years^{-1}, and y is the year of measurement after 1950 A.D. Calibration of ^{14}C data was undertaken using the Oxford Radiocarbon Accelerator Unit calibration

Table 1 Eight tooth enamel samples from five individuals. The date of birth is shown in decimal form

Case	Sex	Date of birth	Sample	Tooth
A	Female	1935.2	1	47
B	Male	1949.4	2	46
			3	47
C	Male	1956.0	4	46
			5	47
D	Male	1971.0	6	16
			7	17
E	Male	1981.3	8	36

A tooth number with a units digit of 6 (such as 16 or 46) is a first molar

Similarly, a tooth number with a units digit of 7 is a second molar program OxCal 4.0 with a smoothing to a resolution of 0.2 years [13, 14] and the Northern Hemisphere zone 2 (NH2) data.

Results

Analysis of two teeth to identify the side of the bomb curve showing the correct age range

To estimate DOB using the least number of teeth, we first measured the ^{14}C of enamel samples from two teeth formed during different periods. Tooth enamel formation occurs over 0–3 years of age for first molars and 2.5–8 years of age for second molars [15]. Therefore, these periods of tooth enamel formation were added to the DOB of individuals as the calculated range for enamel formation (Tables 2 and 3). $F^{14}C$ values of less than 1.0136 are shown as “pre-bomb” because the minimum value in the NH2 data is 1.0136 (in 1955) [16].

The results of ^{14}C analysis of samples 1–7 are presented in Table 2. The $F^{14}C$ values showed that sample 1 (case A) and samples 2 and 3 (case B) were formed during the pre-bomb stage because the values were less than 1.0136. By calibration of the ^{14}C data in samples 4–7, two possible date ranges were produced according to the corresponding periods before and after the 1963 peak of nuclear bomb testing (Fig. 1). The correct range was chosen by analyzing the $F^{14}C$ values of two teeth taken from each individual that had formed at different periods. On the up-slope of the bomb curve (before the 1963 peak), the $F^{14}C$ value of the first molar should be lower than that of the second molar because the first molar was formed at an earlier age. On the down-slope of the bomb curve (after the 1963 peak), the $F^{14}C$ value of the first molar should be higher than that of the second molar (Fig. 2a). In samples 4 and 5 (case C), the $F^{14}C$ value of tooth 46 (the mandibular right first molar) was lower than that of tooth 47 (the mandibular right second molar), indicating that these teeth formed before the 1963 peak. In contrast, in samples 6 and 7 (case D), the $F^{14}C$ value of tooth 16 (the maxillary right first molar) was higher than that of tooth 17 (the maxillary right second molar), indicating that these teeth formed after the 1963 peak. The individuals in cases C and D were actually born in 1956.0 and 1971.0, respectively, and the ^{14}C-estimated ranges for enamel formation in samples 4–7 were compatible with the calculated ranges for enamel formation (Table 2). In addition, the ^{14}C-estimated DOB which was calculated using the overlapped ranges for enamel formation of first and second molar each other in cases C (1953.28 to 1957.16), D (1969.70 to 1973.70) were also compatible with DOB.

Analysis of a single tooth can also identify the side of the bomb curve showing the correct age range

We next focused on process of tooth formation that starts on the incisal (occlusal) side and is completed on the cervical side. It is informative for determining which side of the peak of the bomb curve indicates the correct age range dating to analyze ^{14}C separately in each enamel region, since these regions are formed at other times. Samples 4 and 8 were divided into occlusal and cervical regions with diamond disk using high-speed hand-held dental instrument, isolated from enamel, and the results of analysis of these regions are presented in Table 3. On the up-slope of the bomb curve (before the 1963 peak), the $F^{14}C$ value of the occlusal region should be lower than that of the cervical region, because the occlusal region formed at an earlier age. On the down-slope of the bomb curve (after the 1963 peak),

Table 2 Results of ^{14}C analysis of seven tooth enamel samples from four individuals

Case	Sample	Calculated range for enamel formation[a]	$\delta^{13}C$	$\Delta^{14}C$	$F^{14}C$	^{14}C-estimated range for enamel formation[b]
A	1	1937.7 to 1943.2	−14.43±0.26	−13.6±2.8	0.9864±0.0028	pre-bomb
B	2	1949.4 to 1952.4	−16.19±0.21	1.0±2.7	1.0082±0.0027	pre-bomb
B	3	1951.9 to 1957.4	−17.23±0.14	−19.7±2.7	0.9873±0.0027	pre-bomb
C	4	1956.0 to 1959.0	−16.97±0.16	51.8±2.7	1.0593±0.0028	1956.28 to 1957.16
C	5	1958.5 to 1964.0	−17.64±0.12	207.7±3.3	1.2164±0.0033	1960.90 to 1961.30
D	6	1971.0 to 1974.0	−13.08±0.10	446.4±3.3	1.4568±0.0034	1972.70 to 1973.70
D	7	1973.5 to 1979.0	−16.79±0.11	359±2.8	1.3590±0.0028	1976.04 to 1976.66

Comparison of the $F^{14}C$ values of each set (from the same case) of first and second molars indicates the correct range; i.e., “before” or “after” the 1963 bomb peak

[a] The range for each date of birth based on the enamel formation range (first molar, 0–3 years of age; second molar, 2.5–8 years of age [15])

[b] The range estimated by the ^{14}C concentration of samples compared with the atmospheric ^{14}C concentration using the established atmospheric ^{14}C bomb curve

Table 3 Respective samples from the occlusal and cervical enamel regions of a single tooth from each of two individuals

Sample/region	Calculated range for enamel formation	$\delta^{13}C$	$\Delta^{14}C$	$F^{14}C$	^{14}C-estimated range for enamel formation
4/Occlusal	1956.0 to 1959.0	−17.46±0.13	37.2±2.5	1.0372±0.0025	1955.70 to 1956.78
4/Cervical		−16.06±0.19	55.1±2.5	1.0551±0.0025	1956.28 to 1957.10
8/Occlusal	1981.3 to 1984.3	−16.02±0.14	234.1±3.0	1.2341±0.0030	1982.82 to 1983.82
8/Cervical		−13.19±0.18	209.3±2.7	1.2093±0.0027	1984.76 to 1985.80

Comparison of the F^{14} C value of the occlusal region with that of the cervical region indicates the correct range; i.e., "before" or "after" the 1963 bomb peak

the $F^{14}C$ value of the occlusal region should be higher than that of the cervical region (Fig. 2b). In sample 4 (case C), the $F^{14}C$ value of the occlusal region was lower than that of the cervical region, indicating that sample 4 formed before the 1963 peak. In contrast, in sample 8 (case E), the $F^{14}C$ value of the occlusal region was higher than that of the cervical region, indicating that sample 8 formed after the 1963 peak. The individuals in cases C and E were born in 1956.0 and 1981.3, respectively, and the ^{14}C-estimated ranges for enamel formation in samples 4 and 8 were compatible with the calculated range for enamel formation (Table 3). In addition, the ^{14}C-estimated DOB in cases C (1953.28 to 1956.78), E (1981.76 to 1983.82) was also compatible with DOB in consideration from the error range by Alkass et. al. [5].

Discussion

^{14}C measurements for estimating DOB during the nuclear era provide two possible age ranges. Spalding et al. suggested that the correct range can be chosen by measuring the activity in a variety of teeth (especially the anterior or premolar teeth) that formed in different periods [6]. In the current study, we showed that measuring ^{14}C in first and second molars can clarify which side of the peak of the bomb curve contains the correct age range. To narrow the estimation range, we selected the first molars, since these teeth have the advantage of forming shortly after birth over a relatively short period (0–3 years of age). To determine which of the two possible ranges on the bomb curve is correct, we selected the second molars because these teeth form over a relatively long time (2.5–8 years of age) after formation of the first molars [15]. Two teeth in which the enamel formation ranges do not overlap with each other can be used to determine whether the age range is located before or after 1963.

Since little is known about the period in which ^{14}C is incorporated into the tooth and whether the rate of enamel formation is regular, we subtracted the tooth formation range each from the enamel formation range estimated from

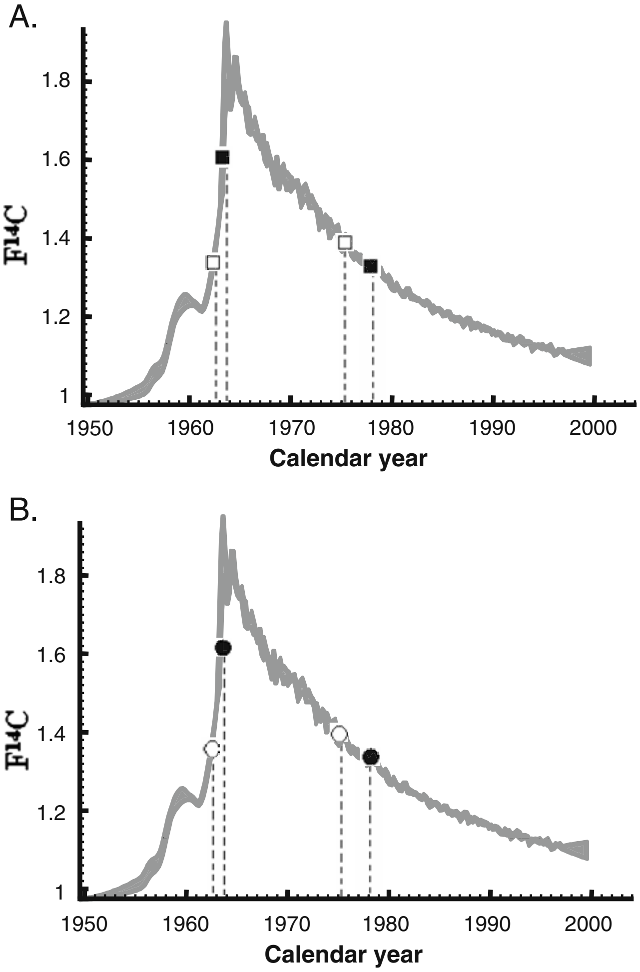

Fig. 2 Schematic bomb curves. **a** Comparison of the enamel formation ranges of two teeth from one individual. On the up-slope of the bomb curve (before the 1963 peak), the $F^{14}C$ values of second molars (*filled square*) should be higher than those of first molars (*empty square*). On the down-slope of the bomb curve (after the 1963 peak), the $F^{14}C$ values of second molars (*filled square*) should be lower than those of first molars (*empty square*). **b** Comparison of the enamel formation range in two different regions of a single tooth. On the up-slope of the bomb curve, the $F^{14}C$ value of the cervical region (*filled circle*) should be higher than that of the occlusal region (*empty circle*). On the down-slope of the bomb curve, the $F^{14}C$ value of the cervical region (*filled circle*) should be lower than that of the occlusal region (*empty circle*)

the ^{14}C concentration to calculate a DOB form the ^{14}C enamel value.

Then, we showed that analyses of two different regions of enamel from a single tooth also allowed identification of which of the two possible age ranges is correct. We paid attention to the time lag in amelogenesis from the occlusal side to the cervical side. This lag results in the ^{14}C contained in enamel in the occlusal region reflecting that in the atmosphere of an earlier time, compared to ^{14}C in the cervical region. This method enables an estimate of DOB using only a single tooth. As mentioned above, overlap of the enamel formation ranges of two teeth may lead to the wrong estimate of DOB. Since the enamel formation ranges of two regions of a tooth do not overlap with each other, the single tooth method may avoid critical mistakes. In fact, we found a significant difference (considering the F^{14}C measurement error in the laboratory) between the two regions of first molars, in which the enamel formation range should be shortest (0–3 years of age). Enamel formation in the occlusal region of a first molar begins shortly after birth, and thus the ^{14}C-estimated range for DOB calculated using the occlusal region of first molars is likely to represent DOB more accurately. Similarly, using this method should minimize the effect of the enamel formation range when estimating DOB using teeth in which this range is relatively wide, such as canines or second molars.

The ^{14}C calibration curve contains short cycle waves (wiggle) that are affected by the number of sunspots. This wiggle may be helpful for estimating DOB since it can be used to match the shape of a series of closely spaced ^{14}C dates (wiggle matching). In this way, the approach of dividing samples from a single tooth into smaller samples may produce a more accurate DOB. Actually, in case C (born in 1956.0), ^{14}C-estimated DOB by use of dividing samples (1953.28 to 1956.78) have narrower range of value than that by use of two teeth (1953.28 to 1957.16).

In conclusion, we have developed a novel system for determination of DOB. This method uses separate parts of the enamel of a single tooth to estimate DOB and may minimize errors in age estimation using teeth with a wide enamel formation range.

Acknowledgements This work was supported by Paleo Labo Co. (Toda, Japan). We thank Dr. Koichi Kobayashi for his assist with data analysis of this study.

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