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A PRELIMINARY ASSESSMENT OF AGE AT DEATH DETERMINATION USING THE NUCLEAR WEAPONS TESTING ^{14}C ACTIVITY OF DENTINE AND ENAMEL

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ABSTRACT. Calibration (using CALIBomb) of radiocarbon measurements made on the enamel of human teeth from people born during the nuclear era typically produce 2 possible age ranges that potentially reflect the period of tooth formation. These ranges correspond to periods before and after the 1963 atmospheric ^{14}C maximum. Further measurements made on the collagen component of the combined dentine and cementum from the roots of the same teeth enable the appropriate age range to be selected. Using this range and the formation times for individual teeth, we estimated the year of birth of the individuals and compared these to the known dates of birth. The results were relatively accurate and confirmed those of a previous study by another research group. The present study demonstrates that it is possible to produce a good estimate of the year of birth from a single tooth.

INTRODUCTION

One of the principal roles of a forensic anthropologist is to establish the identity of deceased individuals whose remains are decomposed, mutilated, burned, or otherwise compromised. Biological identity comprises 4 major criteria of identity and includes the determination of the sex, age at death, stature, and race of the deceased. The determination of age at death is a particularly important component of this preliminary profile, as an accurate evaluation will serve to eliminate possible missing persons from the police inquiry or serve to highlight those that might require additional investigation. The accurate determination of age at death is absolutely critical when working with juvenile remains, and age at death is a biological determinant that is closely related to chronological age, ensuring that the predictive capacity can achieve forensic levels of reliability (Reventlid et al. 1996; Scheuer and Black 2000). However, age determination from adults is less accurate, and in the post-40-yr age range, anthropologists may only be able to offer limited statements, such as "mature adult," depending upon which skeletal parameters are available for examination (Foti et al. 2001). In adults, many different procedures involving the examination of skeletal characteristics have been proposed and these include suture closure, pubic symphyseal morphology, and changes in the sternal rib end or the auricular surface of the ilium. Unfortunately, most suffer from methodological bias and complex variability in the skeletal aging process (Schmitt et al. 2002), as many changes in skeletal form are influenced not only by the chronological age of the individual but also by a number of internal and external factors, such as nutrition, disease, genetic influences, etc. Even the best skeletal-based methods can be somewhat limited to the identification of rather broad age groupings (Corsini et al. 2005), which is contrary to the requirements of the forensic anthropologist.

Age determination from the teeth is most accurate in the juvenile population, where the relationship between tooth mineralization, eruption, emergence and deciduous tooth loss are well documented. Further, the relationship between dental and chronological age is closer than that between dental and skeletal age, which is thought to have arisen from the tooth developing in a relatively protected environment over a more restricted time period.

Teeth offer an attractive source of information for the forensic anthropologist with regards to age determination due to their survivability. Dentition will survive both heat and chemical degradation

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more readily than bone and are therefore found more frequently in a condition that will potentially assist identification.

In 1975, Helfman and Bada proposed that racemization of aspartic acid residues in tooth enamel protein could be used to deduce the age at death. Their technique is based on the following facts:

1. Tooth enamel is formed at well-characterized times during childhood.
2. Mature tooth enamel is almost entirely composed of calcium phosphate (hydroxyapatite-like structure) containing small carbonate and protein components (<1%) that are laid down during formation. Because enamel lacks a cell structure and is therefore not living tissue, it neither repairs nor remodels itself (Mays 1999).
3. In protein synthesis, humans use exclusively the L-forms of amino acids; however, there is a slow conversion to the D-form with time.

Aspartic acid has a relatively fast rate of racemization compared to other amino acids, but none of the D-form is found in tissues that undergo rapid protein turnover. In contrast, relatively high percentages of the D-form are found in tissues where the protein is synthesized at an early stage in human development and is not subject to subsequent turnover. The following year, the same authors established a close correlation between the ages of dentine in teeth of individuals and the extent of aspartic acid racemization (Helfman and Bada 1976). Of course, the extent of aspartic acid racemization in dentine (and enamel) depends on the age of the material, and this cannot be equated directly to the age of the individual as the time (in relation to birth) and duration of dentine (and enamel) synthesis in the various teeth have to be taken into account. Overall, they concluded that dentine could be used in the same manner as enamel to estimate the age of teeth and that, in many respects, racemization analysis of dentine should be more reliable as dentine accounts for a much greater proportion by weight of a tooth, and the percent composition of protein in dentine is ×100 that in enamel. Therefore, contamination should be much less of an issue. Also, enamel is subject to surface alterations whereas dentine is protected by the enamel layer. The analytical advantages of dentine were reflected in much improved correlation coefficients over those for enamel. Subsequently, Ogino et al. (1985) concluded that age at death determination by aspartic acid racemization in dentine was correct to within ±4 yr. Ritz et al. (1990) re-assessed the technique as proposed by Ogino et al. (1985) and observed almost identical regression equations relating racemization to age of dentine. Their standard error on the estimate of age at death was somewhat larger at 5.69 yr; however, they concluded that this could be reduced by optimizing the analytical procedure. Ohtani et al. (1995) have also studied aspartic acid racemization in cementum and concluded that the cementum remains stable throughout an individual's life and that accurate age estimation of age at death by this technique was possible. Figure 1 illustrates the various component tissues of a tooth.

While the racemization technique has proved popular, a number of problems have been identified:

- The synthesis of crown dentine in permanent teeth requires a period of up to 6.5 yr, and during this process, the dentine is built up in layers such that the outermost material will be several years older than the innermost. On this basis, Ritz et al. (1990) concluded that dentine samples would have to be taken from a defined layer to obtain optimum results.
- Ohtani et al. (2005) measured aspartic acid racemization in the enamel of different teeth from a single individual. They proposed that everything else being equal, the teeth that form earliest should have the greatest degree of racemization. Contrary to expectation, they observed that racemization was greater in molars than incisors. They proposed that this may be due to a higher environmental temperature in the molar region.

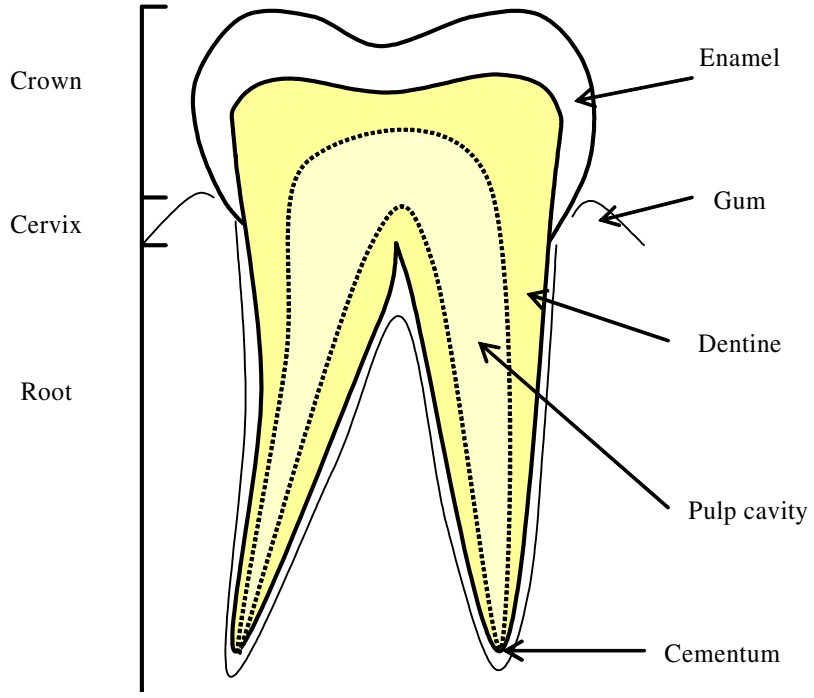

Figure 1 Various component tissues of a tooth

- The racemization of amino acids is temperature-dependent and the rate decreases with decreasing temperature. Thus, if a corpse remains outdoors in cool conditions, post-mortem aspartic acid racemization in dentine will proceed much more slowly than it would in vivo. Ogino et al. (1985) state that a storage period of 10 yr at 15 °C would result in an error of 0.2 yr employing their methodology. However, the technique is subject to much more uncertainty where a body has lain in warm conditions or has been heated to high temperature, as would be the case for fire victims.

In 2005, a method was proposed that has the potential to provide more precise determinations of age at death in adults who were born during the nuclear era or directly prior to this time (up to ~55 yr age) by determining the "bomb" radiocarbon activity in the carbonate component of tooth enamel (Spalding et al. 2005). The authors state that the ^{14}C activity of the carbonate component is a reflection of the ^{14}C activity in the atmosphere during the time of enamel formation, and because the activity in the atmosphere has been changing relatively rapidly over the past 50 yr or so, this provides the ability to assign the measured ^{14}C activity to a specific year (or years). In cases where there is ambiguity (i.e. does the ^{14}C activity reflect a year when the activity was rising to the peak in 1963 or a year post-1963 when the activity was declining?), this ambiguity can be resolved by measuring the activity in teeth that form at different times after birth. Ubelaker et al. (2006) adopted a different approach and measured the ^{14}C in crown enamel to establish the 2 age ranges and ^{14}C in cortical and trabecular bone samples from the same skeleton to establish whether the age range was pre- or post-1963. Figure 2 illustrates the Northern Hemisphere atmospheric ^{14}C activity from 1950 to 2003.

From their study, Spalding et al. (2005) concluded that their method gave "a remarkably precise estimate of age for 22 individuals." Their results are somewhat surprising, as early research in our lab-

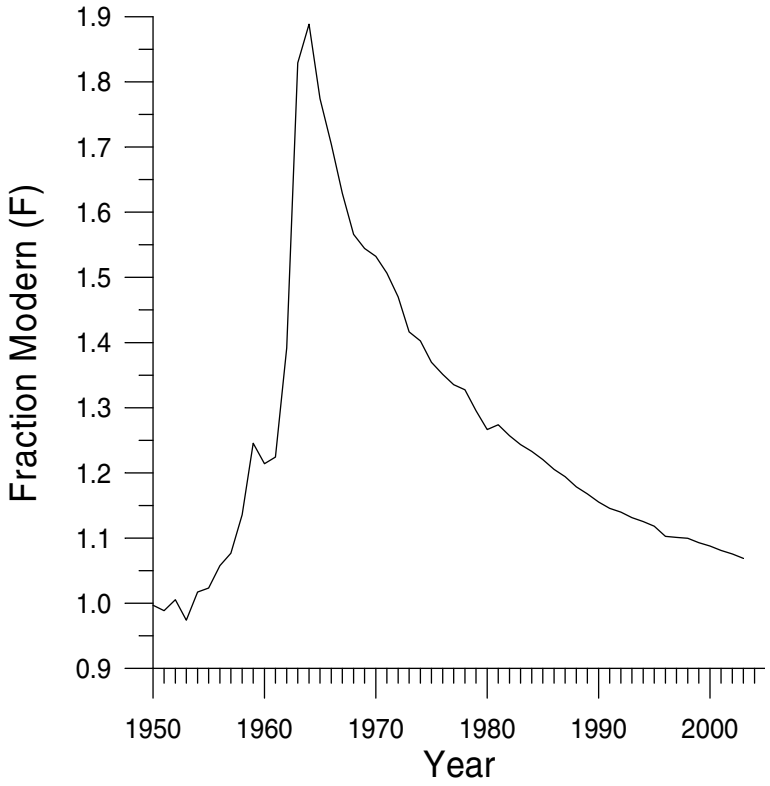

Figure 2 Northern Hemisphere atmospheric ^{14}C activity from 1950 to 2003

oratory had shown that ^{14}C activities in human tissues were unlikely to mirror atmospheric trends exactly (Harkness and Walton 1969, 1972; Stenhouse and Baxter 1977), and of course this should apply equally to tooth enamel. This assumption of mirroring atmospheric levels is perfectly valid for ^{14}C dating of archaeological remains, but on the very short time scales that Spalding et al. (2005) are dealing with, particularly in a situation of ^{14}C disequilibrium, the above research would predict that activities in humans should be lagged significantly and smoothed by diet (due to the turnover time in meat, the marine reservoir age effect in fish, etc.) and of course by the residence time in blood. However, since the carbon in the enamel is laid down at a very young age when children are actively growing and carbon turnover is rapid, this may be minimizing the lag effect. Furthermore, if the population from which Spalding et al. (2005) obtained their samples has a relatively small meat and fish component in the diet, this would minimize any dietary effects on ^{14}C activity. Thus, it is conceivable that the ^{14}C activity in enamel could, under these circumstances, mirror atmospheric levels quite closely. While their data are quite conclusive, factors such as diet could lead to anomalous results, and if the analysis involved teeth that are formed at a later stage in development, e.g. third molars (wisdom teeth), which form at around 10–13 yr of age, then change in diet could be a significant factor.

We have adopted a different approach to estimating age at death and, in the first instance, selected both the enamel and the dentine components of the teeth for analysis. The dentine was selected for the following reasons: 1) dentine accounts for a much greater proportion by weight of a tooth and has a much greater carbon content than enamel (it comprises ~25% collagen), and so contamination

and sample size should be much less of an issue; 2) the racemization work has effectively demonstrated no carbon turnover in this tissue; and 3) collagen has the potential, through stable isotope analyses (^{13}C and ^{15}N), to provide dietary information that could prove useful in identifying individuals with diets that might influence the ^{14}C activity in teeth such that they are not a reflection of the atmospheric activity during the time of tooth formation.

METHODS

Eight teeth from individuals of known date of birth, sex, and ethnic origin were provided by colleagues in the dental profession. These were physically cleaned to remove adhering tissue, and then the crown was separated from the root at the cervix (see Figure 1).

Enamel Preparation

Enamel was isolated using a modified version of the method described by Spalding et al. (2005). Briefly, the crowns were immersed in beakers containing 10M NaOH, which were then placed in a sonic bath at 50 °C. The NaOH was replaced approximately every 24 hr, and the non-enamel tissue was removed by blunt dissection. Once the dentine and other tissues had been completely removed (after ~3 weeks), the enamel samples were washed several times in 0.25M HCl followed by several washes/rinses in reverse osmosis water. The samples were then hydrolyzed at ~80 °C in small reaction vessels using orthophosphoric acid. The evolved CO_2 was cryogenically purified and 1-mL volumes graphitized according to the method of Slota et al. (1987). The remaining CO_2 was used for ^{13}C measurement using a VG Sira 10 isotope ratio mass spectrometer.

Collagen Preparation

Dentine consists of ~75% inorganic material (mainly hydroxyapatite), while the remaining 25% is mainly collagen (Mays 1999). Cementum has a similar structure to bone with 70% mineral material (again mainly hydroxyapatite) and 30% organic (mainly collagen) (Mays 1999). The entire roots were immersed in 1M HCl for ~48 hr to de-mineralize them. The roots were then washed in reverse osmosis water and placed in beakers containing ~50 mL of reverse osmosis water and heated for ~3 hr at 80 °C to dissolve the collagen. The samples were then filtered through GF/A paper and heated to reduce the volume to ~5 mL, at which point they were freeze-dried. Sub-samples of the collagen were combusted at 850 °C in sealed quartz tubes using copper oxide as the oxidant and a silver foil to mop up halide contaminants. Again, the evolved CO_2 was cryogenically purified and 2-mL volumes graphitized according to the method of Slota et al. (1987). A further sub-sample was again taken for ^{13}C measurement. It should be noted here that the collagen was derived from all of the dentine contained in the root, together with collagen contained within the cementum. While it has been demonstrated that the amino acid racemization technique works for cementum, Ohtani et al. (1995) state that it is likely that cementum is deposited throughout life and so there will be a contribution of carbon that is of post-tooth formation.

The ^{14}C data are presented as fraction modern (F), referred to as $F^{14}C$ by Reimer et al. (2004):

$$F = \frac{A_{SN}}{A_{ON}}$$

where A_{SN} is the normalized sample activity and A_{ON} is the normalized oxalic acid activity. Calibration of the ^{14}C data was undertaken using the Queen's University of Belfast calibration program CALIBomb (www.calib.org) and the Northern Hemisphere Zone 1 data set, apart from when the F

values were less than ~1.1 (year 1999 at the recent end of the bomb peak) as the data do not go beyond this date. For values less than ~1.1, the "Levin" data set was used (Levin and Kromer 2004).

RESULTS

The results of the ^{14}C analyses are presented in Table 1. The calibration range is the date range calculated by CALIBomb using the duration of tooth formation as the lifespan of the sample to smooth the data set. From this range, we subtracted the average tooth formation time to produce a date of birth range and compared the results to the known dates of birth. It can be observed that the results obtained from analysis of the enamel generally give 2 possible date ranges corresponding to periods before and after the 1963 peak in activity, one of which is a reasonably accurate reflection of the actual date of birth. In the case of the collagen, the results are generally a poorer match to the actual date of birth. However, several points arise from this: 1) for those teeth that formed after the 1963 maximum in atmospheric ^{14}C activity, i.e. between the 1970s and early 1990s, the ^{14}C activity in the collagen component of the root is lower than the activity in the enamel; 2) for the one example of a tooth formed prior to 1963, the activity in the root is higher; and 3) for the single, recently-formed sample (2000), the ^{14}C activities of the enamel and collagen are within error.

Table 1a Fraction modern ^{14}C activities in tooth enamel from 8 individual teeth, calibrated ranges for the average time of crown formation, and dates of birth of the 8 individuals.

Tooth	Fraction modern (F)	Error (1 σ)	Calibration range for crown formation	Crown formation time (yr age)	Calculated date of birth range	Actual date of birth
Deciduous Molar	1.1005	0.0047	Dec '56 to Sept '57 or Nov '95 to Jun '97	0	Dec '56 to Sept '57 or Nov '95 to Jun '97	Feb '95
Wisdom Tooth	1.2542	0.005	Dec '60 to Jul '61 or Sept '80 to Nov '82	9.5–12.4	Jan '50 to Jul '50 or Sept '69 to Nov '71	Jun '69
Lower Left 1	1.3475	0.0058	Dec '61 to Jul '62 or Jul '75 to Jul '77	0.5–3.5	Dec '59 to Jul '60 or Jul '73 to Jul '75	Jun '61
Upper Right 7	1.358	0.0058	Jan '62 to Sept '62 or Apr '75 to Oct '76	3.5–6.6	Jan '57 to Aug '57 or Mar '70 to Sept '71	Oct '71
Lower Left 7	1.2339	0.0052	Mar '60 to Feb '61 or Feb '82 to Jun '84	3.8–6.8	Nov '54 to Oct '55 or Oct '76 to Feb '79	Aug '79
Lower Left 6	1.2169	0.0036	Aug '58 to Feb '61 or Nov '83 to Jun '85	0.2–2.5	Mar '57 to Sept '59 or Jul 1982 to Feb '84	Aug '83
Upper Right 4	1.1328	0.0034	Jan '57 to Jun '57 or Nov '91 to Jul '94	2–5.5	Mar '53 to Aug '53 or Feb '88 to Sept '90	Sept '89
Lower Right 4	1.0883	0.0046	Feb '52 to Mar '54 or Jul '98 to Nov 2002	2–5.5	Apr '48 to Jun '50 or Oct '94 to Jan '99	Feb 2000

These trends can be accounted for by the fact that while primary dentine is laid down during tooth formation, a lesser amount of secondary (and tertiary) dentine continues to be laid down after this initial formation, and also, it is well recognized that the cementum also continues to be laid down after tooth formation. Thus, the activity is mainly a function of the primary dentine, but with contributions from the secondary (and tertiary) dentine and cementum. Therefore, in the case of the teeth

Table 1b Fraction modern ^{14}C activities in collagen from the roots of the same teeth, calibrated ranges for the average time of root formation, and dates of birth.

Tooth	Fraction modern (F)	Error (1 σ)	Calibration range for root formation	Root formation time (yr age)	Calculated date of birth range	Actual date of birth
Wisdom Tooth	1.1832	0.0050	Dec '85 to Dec '88	13.2–17.7	Jun '70 to Jun '73	Jun '69
Lower Left 1	1.5496	0.0065	Jun '63 to Sept '63 or Oct '68 to Aug '70	4–7	Nov '57 to Feb '58 or Mar '63 to Jan '65	Jun '61
Upper Right 7	1.2431	0.0046	Nov '58 to Nov '60 or May '81 to Nov '83	7.3–11.8	Apr '49 to Apr '51 or Oct '71 to Mar '74	Oct '71
Lower Left 7	1.1539	0.0044	Apr '55 to Feb '57 or Oct '89 to Jan '92	7.3–11.8	Sept '45 to Jun '47 or Mar '80 to Jun '82	Aug '79
Lower Left 6	1.1638	0.0046	May '57 to Mar '58 or Jul '88 to Jan '91	3.1–6.5	Jul '52 to May '53 or Sept '83 to Mar '86	Aug '83
Upper Right 4	1.0979	0.0047	Jul '53 to Apr '55 or Jan '96 to Jul '98	6.1–10.5	Mar '45 to Dec '46 or Sept '87 to Feb '90	Sept '89
Lower Right 4	1.0909	0.0043	Nov '97 to Apr 2002	6.1–10.5	Jul '89 to Jan '94	Feb 2000

formed post-1963 (on the down-slope of the bomb peak), the activity will be lower than the tooth formation time would predict, while for that sample formed pre-1963, the activity will be higher due to components laid down after tooth formation is complete. For the sample formed around 2000, the exponential curve is much shallower by this time, so any ^{14}C laid down after tooth formation will have little effect on the ^{14}C activity. This in itself is useful information as, in the cases where 2 possible calibration ranges are predicted (one on the up-slope of the curve and one on the down-slope), it enables the decision to be made as to which one is correct (Table 2). Thus, where Spalding et al. (2005) required 2 teeth corresponding to types that form at different times relative to birth, this method enables the estimate of birth to be achieved with a single tooth. However, we can also see a way in which the technique can be improved significantly. We now intend to analyze the collagen in the primary dentine that is situated immediately below the enamel in the crown of the tooth. This should give us an age range comparable to that of the enamel, but with the ability to analyze a subsample of this collagen for the stable isotopes ^{13}C and ^{15}N, which provides us with the opportunity to study the diet of the individuals and to identify any unusual diets that might influence the ^{14}C results, e.g. high consumption of marine resources. A further advantage of analyzing the dentine is that it contains much more carbon than the enamel. In addition to analyzing the collagen contained in the primary dentine from the crown, we will analyze the total collagen in the root of the tooth to allow us to predict, in those cases of ambiguity, whether the age range is pre- or post-1963.

CONCLUSIONS

^{14}C measurements made on the enamel of teeth typically provide 2 possible age ranges, one of which is a reasonably accurate reflection of the person's date of birth. The results generally confirm those of the study carried out by Spalding et al. (2005), and we found no evidence to suggest a significant lag between the ^{14}C activity in the enamel and the contemporaneous atmospheric activity. Additional analyses made on the collagen component of the root (combined dentine and cementum)

Table 2 Relative ^{14}C activity of the total collagen content of the root to the total ^{14}C activity of the enamel, as a predictor of the correct date of birth range of individuals, and comparison with the actual dates of birth.

Tooth type	Collagen ^{14}C activity relative to enamel	Implied time of tooth formation	Implied date of birth	Actual date of birth
Wisdom Tooth	Lower	post-1963	Sept 1969 to Nov 1971	June 1969
Lower Left 1	Higher	pre-1963	Dec 1959 to July 1960	June 1961
Upper Right 7	Lower	post-1963	Mar 1970 to Sept 1971	Oct 1971
Lower Left 7	Lower	post-1963	Oct 1976 to Feb 1979	Aug 1979
Lower Left 6	Lower	post-1963	July 1982 to Feb 1984	Aug 1983
Upper Right 4	Lower	post-1963	Feb 1988 to Sept 1990	Sept 1989
Lower Right 4	Same	post-1963	Oct 1994 to Jan 1999	Feb 2000

enabled us to make a decision as to which of the 2 possible age ranges was correct. We propose to develop this application to a study of: 1) the collagen component of the primary dentine located immediately under the enamel as a measure of the tooth formation time; and 2) the total collagen in the root as an indicator of whether the correct age range is on the up- or down-slope of the bomb peak. In addition, stable isotope (^{13}C and ^{15}N) analyses of the collagen will be used to identify individuals with diets that might influence the ^{14}C results.

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