A COMPARISON OF CALCIFICATION STAGING AND HISTOLOGICAL METHODS FOR AGEING IMMATURE MODERN HUMAN SPECIMENS

ABSTRACT: The past fifteen years have been a time of controversy regarding how to interpret dental development and chronological ages at death from skeletal specimens. A central focus of some of these debates concerns the comparison of ages derived from traditional radiological studies that identify calcification stages of developing dentition to ages derived from histological study of enamel microstructure. This study provides a detailed description of two unaged children from an archaeological context in the Middle East. These individuals are assessed according to previously published methods for the ageing of incremental growth in crowns and roots of different teeth. Scanning electron micrographs were taken to count the perikymata on enamel surfaces and histological sections of teeth were prepared and analysed for counts of internal striae of Retzius and cross striations. In line with previously published methods, total crown formation times were derived and root formation rates established in order to establish ages at death.

With the exception of first incisor crown formation, the results found here are in line with other studies for crown formation. The ages at death assessed by the histological methods are significantly shorter than those derived from radiological references standards and provoke questions: one child with first molar erupted is aged at 3.4 years of age and a second child, at second molar alveolar eruption, is aged at 5.8 years. These early ages are hypothesised to result from methodological flaws in the proposed method. By comparison with the other extant study of root extension rate, the human children studied here overlap ranges previously reported for non-human primates. The notions concerning distinctive differences are considered within this context. The present data extend the descriptive data base on population variability in perikymata counts and striae of Retzius observations for comparative studies of dental development in modern humans. The ages at death resulting from these methods are questioned, and some reconsiderations of the validity of these approaches are suggested.

KEY WORDS: Skeletal ageing – Dental calcification – Dental histology – Age-at-death – Immature human skeletons

INTRODUCTION

A number of paleoanthropological studies have focussed on the determination of the pattern and rate of tooth development in modern humans, aeps and in extinct hominines1, with the aim of investigating the significance that these may have in furthering our understanding of the development of maturational processes during the course of human evolution (e.g. Kaykendall, Conroy 1996, Macho, Wood 1995, Mann, Lampl, Monge 1991, Smith, Tompkins 1995). The goal of these studies is to provide insight into the evolution of the unique qualities that may characterise modern human growth and development and when these

1 In this paper, we follow Weiss (1987) in employing the subfamily Homininae, rather than the family Hominidae, to describe humans and our close immediate ancestors and relatives.
occurred in evolution, with particular interest directed to better understanding the prolongation in the developmental period leading to the attainment of physical adulthood and the acceleration of skeletal and dental development in modern human growth patterns.

One of the seminal features of these studies is a careful focus on the differences between the pattern vs the rate of dental development. While it is clear that the pattern of tooth development can be determined on both living and extinct species through the examination of the maturational state of the individual teeth in immature specimens, the rate of development in all skeletal samples has been more difficult to ascertain by traditional approaches. This is because rate assessments are time-dependent and require real time markers such as chronological age. This cannot be reliably identified in skeletal samples (Lamp, Johnston 1996) because of both genetic and environmental influences that manifest as individual and population differences in the chronological age of attainment of maturational events. The only well documented and generally acceptable way of assessing the rate of development in real time is with living individuals at specific moments in the course of their maturation, and sometimes, on samples of known age at death.

A number of research efforts have focused on observations of dental development patterns in human, ape and fossil juveniles, investigating calcification stages and eruption patterns in immature fossil specimens, or individuals where maturational states are not yet complete. The development of dental structures is a major consideration. For example, a study by Sacher (1975), who calculated the relationship between maturation rate and brain weight in extant mammals, in her exploration of the theoretical bases of rates of dental maturation in the Homininae, and constructed a model of hominine dental maturation rates. The possibility of direct assessment of dental development rates based on hard tissue remains has also been explored. Using external and internal features of enamel and dentin, Dean and Beynon (1987), Beynon and Dean (1991) and Broman and Dean (1985) suggested that a time ordered and feature-delineated sequence of enamel developmental events provides an accurate clock from which to infer dental development. This work is based primarily on limited experimental evidence that structures observed in the enamel of the developing fetus between 11 and 14 weeks since fertilization at Helsinki, and in the Solduz-Uzun Valley in north-western Iran (Muscarella 1989). These are two of the 246 skeletons excavated from Hassanne period IVB, which represents the final destruction of the settlement by conflagration in the 9th century BC. In general, the preservation of skeletal materials from this site is excellent. These two young individuals apparently were inhabitants of the city who were either executed by the invaders or killed by falling debris that followed the burning and destruction of the central part of the town (Muscarella 1989).

These two specimens were chosen for this analysis because they are relatively complete dental specimens who appeared to have died at gross maturation stages of significance for traditional skeletal studies: first and second molar eruption. Further, both specimens preserve right and left antinumers and, thus, the destructive sectioning required for this study involved only one side of the dentition and was considered conservative in terms of skeletal preservation.

Specimen 73.5-508 died at the time of first molar eruption. The dentition is partially isolated and only the maxillary left M1 and mandibular right P4 are embedded in bone. Excavation records document that these teeth belong to the same individual. On the basis of the extensive damage to the skull and the fragmentary condition of the postcranial bones, this child was apparently crushed by falling debris during destruction of the citadel. However, the isolated teeth are intact and in excellent condition, with even the very recently calcified roots well preserved and without cracks or chips. Specimen 60-20-226 (Figure 1) died at a time just after bone eruption of the M2 with the permanent I1, I2 and M1 at the occlusal plane. Alveolar bone of the maxilla has been broken and repaired, which permitted the removal of the erupted left I1, P1, the partially erupted M1 as well as the erupting C1. The general range of ages for these two specimens, based on dental eruption, estimates that the youngest child (No. 73.5-508) with first molar eruption was between 4.5 and 7.5 years of age, and the older child (No. 60-20-226), with erupting second molar, between 9 and 13 years of age at death (Eveleth, Tanner 1977, 1990).

For each of the specimens, the age at death, when assessed by calcification stage based dating, is compared to the age at death as assessed by histological methods. In order to exclude errors associated with the use of different methods of assessment (Liveridge 1994), all calcification stages reported here are based on examination of the teeth themselves and do not include radiographic assessment of teeth that remain in alveolar bone.

For the histological ageing, the method employed follows that outlined in Dean and Beynon (1991). The crown formation times are assessed from direct counts of striae in imbrication enamel added to the count of crossstriations in appositional enamel as outlined below. In this study, imbrication of striae on the enamel followed the principle that the most extensive damage to the skull and the fragmentary condition of the postcranial bones, this child was apparently crushed by falling debris during destruction of the citadel. However, the isolated teeth are intact and in excellent condition, even the very recently calcified roots well preserved and without cracks or chips. Specimen 60-20-226 (Figure 1) died at a time just after bone eruption of the M2 with the permanent I1, I2 and M1 at the occlusal plane. Alveolar bone of the maxilla has been broken and repaired, which permitted the removal of the erupted left I1, P1, the partially erupted M1 as well as the erupting C1. The general range of ages for these two specimens, based on dental eruption, estimates that the youngest child (No. 73.5-508) with first molar eruption was between 4.5 and 7.5 years of age, and the older child (No. 60-20-226), with erupting second molar, between 9 and 13 years of age at death (Eveleth, Tanner 1977, 1990).

METHODS

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FIGURE 1. Occlusal view of an immature maxilla from the site of Hasane (University of Pennsylvania Museum catalogue number 60-20-226).
TABLE I. Calification ages and histological counts for the present study.

<table>
<thead>
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<tr>
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</tbody>
</table>

*Calification stage ages from Moorsrees et al. (1963). It is noted that these data are mandibular references for all but incisor teeth and the second specimen here consists of maxillary teeth.

*All counts presented represent the average of three raters with standard deviations ranging from 1 to 5.

Perikymata counts * Striate of Retzius * Cross striations

<table>
<thead>
<tr>
<th>Specimen</th>
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<th>Male</th>
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<tr>
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<tr>
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</tbody>
</table>

*Perikymata counts are based on the average of three raters with standard deviations ranging from 1 to 5.

External enamel structures: Perikymata

All isolated or extracted teeth were moulded and epoxy casts were made using either both enamel or only the cemental teeth in the specimens, and served as replicates for microscopic analysis of enamel surface features. Moulding and casting techniques follow those described in Mann and Monger (1982). Casts were made using Reproil brand of polysiloxane and the replicates cast in epoxy resin (Hysol RIE6345NA/D1011). Casts were coated with gold-palladium and imaged using a Philips 500 scanning electron microscope (SEM). Perikymata were counted on montages of each tooth magnified to 80x. Only buccal or labial surfaces were counted.

Internal structures: Striae of Retzius and cross-striations

The original teeth were embedded in methacrylate and were sectioned either buccally or lingually using an Isomet low speed diamond saw. Only left side teeth were sectioned. The molars were sectioned through the apex of the protocone, the incisors through the mid-point of the crown and the canines through the tip of the cusp. Sections were etched for 30 seconds using a 0.5% solution of HCl and then carbon coated. Each tooth was imaged using either the Philips SEM at the University of Pennsylvania Museum, or the Joel SEM at the Material Science Engineering Facility at the University of Pennsylvania. Montages were made at 160x for analysis of the striae of Retzius and 320x for identification of the cross-striations. All counts are made on the buccal or labial surfaces. Cross striations are counted by following the course of several enamel prisms and counting the cross-striations from the tip of the dentin horn to the tip of the enamel, as previously outlined by Dean and Boyen (1991).

Crown formation times

For each individual, total crown formation time is calculated from the observed counts of these enamel structures. First, the internal line corresponding to the neonatal line is identified in the first molars of both specimens. Appositional enamel completion reflects the number of cross-striations counted, minus those found under the neonatal line, and imbricational enamel completion time is calculated from the striae of Retzius lines and an average cross striation periodicity.

Root formation times and age at death

For each tooth, the amount of root present is identified visually and measured. Root lengths and thus root formation times on the molars are derived from the mesial lingual root. The methods developed by Dean and Boyen (1991) and employed by them on an unaged, unsexed child from Christ Church Spielfields are repeated here: Commonly, unique hypoplastic striae are located on the adjacent incisors for these specimens. Perikymata subsequent to these hypoplastic bands become the clock for root formation as follows: The lateral incisor jags in development behind the central incisor. As the central incisor is completing its development and continuing to develop root, the lateral incisor is still in the process of crown formation. Theoretically, according to Dean and Boyen (1991), the time for root formation on the more rapidly developing central incisor can be indirectly identified. If it is assumed that incisor and root development are continuous, then the root formation time on the central incisor can be calibratd by the remaining crown formation time on the lateral incisor that exceeds that on the central incisor. The assumption underlying this approach is that root formation immediately follows calcification and occurs at a continuous rate. Thus, calculation of the continuing crown formation time on the lagging lateral incisor provides a clock for the root formation on the central incisor.

Historical ages at death are calculated as the sum of crown formation time assessed from appositional and imbricational enamel, together with root formation time. The ages are then compared to results from radiologically-based developmental ages according to Moorsrees et al. (1963).

RESULTS

Pattern-based method of assessment: Calculation staging

Both of these immature Hasalan individuals can be assigned an age at death with a broad range using the standards of Moorsrees et al. (1963) (Table I). For the younger specimen at first molar eruption, the age at death based on calculation stage encompasses a total range from 2.0 to 8.25 years of age when all teeth are considered, with an overall mean age of about 4.8 years (female, male reference respectively). It should be noted that the mean ages are heavily influenced by the relatively young ages assessed for canine teeth by comparison with the remainder of the dentition. As the canines are known to have been difficult to assess in the original study (Hunt; pers. comm.), and have often been noted to be very young when compared to other studies, it may be appropriate to exercise caution in interpreting these calculations.

The older specimen, at second molar eruption, is more difficult to interpret: The standards of Moorsrees et al. (1963) consist of maxillary ages only for the incisors; the remaining dental reference ages are determined based on mandibular dental No. 6-2 development. As the teeth under consideration are maxillary, the ages of the incisors from the maxillary reference range between 6.3 and 11.75, with a mean age of about 8.5 years. The mandibular reference applied to these maxillary specimens provides ages that range from 5 to 11.5, with a mean age of 7.3 to 7.8 (female, male respectively). By contrast with modern population data for second molar eruption (9 to 13 years) (Eisele, Tanner, 1976,1990), these averages would put this individual at quite a young age. Thus, it is likely that perhaps the specimen falls at the later end of the distribution of the Moorsrees et al. sample calcification ranges and the differences between mandibular and maxillary development are emphasised. Considering the population differences between these individuals and the reference standards, it is quite possible that they are being underestimated to this extent, in view of the significant differences found merely within regions of the United States (Maples et al. 1992).

Department of development methods

Enamel structures: Perikymata and striae of Retzius

All enamel structures counts represent the average of three raters with a standard deviation of between 1 and 5. Perikymata and striae of Retzius counts for these specimens are presented in Table I. The amount of abrasion on the first molar from 60-20-226 obscured clear visualisation of the perikymata and no counts were taken on this tooth. In general, there was good correspondence between the external perikymata counts and the internal striae of Retzius numbers and there is no statistically significant difference between the counts. There is no systematic error evident in the direction of estimation and the differences in the two estimates are within one standard deviation from the external observer error (only the molars are slightly greater).

Cross-striation counts were determined between the beginning of enamel formation at the tip of the dentine horn and the surface of the teeth. This was most clearly identified in the outer cervical enamel. In contrast to previous reports, finding consistent cross striations between striae of Retzius within individuals (Firzgerald 1998), a range of between 3 and 10 appear between adjacent striae of Retzius are found here, results in line with variability in cross striation counts reported by others (Huda, Bowman 1995, Rines 1998). In some cases, several cross-striations were present within the structure of the striae themselves. While the complexity of the counts is not illustrated by the means shown, the ages derived are based on the use of a mean count of 7 cross-striations between striae of Retzius, for comparison with previous reports.

Crown formation times

Specimen 60-20-226. A distinct neonatal line is evident in the first molar of this specimen, present just over the tip of the dentine horn in some of the very first layers of enamel. The age of 8 years is gauged from this point, as it marks the beginning development of the dentine since birth. Appositional enamel in the first molar (Figure 2), calculated from the cross-striations that extend from the tip of the dentin horn to the tip of the enamel (less those under the neonatal line), total 370 cross-striations. This is said to reflect 370 days of enamel formation time for the appositional enamel in the protocone, or 53 weeks.

Imbricational enamel in the first molar for this individual is estimated by the total number of striae of Retzius (Figure 3), and an average of seven cross-striations separating these markers. Thus, a total count of 51 striae of Retzius times 7 provides an estimated crown formation time of the imbricational enamel of the protocone of the first molar at 357 days, or 51 weeks. Taken together, these enamel microstructures identify an estimated 727 days (370+357 days) as the total crown formation time for the first molar, or about 2 years.

In a similar manner, calculations are made for the upper first incisor. Appositional enamel formation is estimated
at 217 days (217 cross-striations), or 31 weeks. Imbricalional enamel formation is estimated as taking 595 days (85 striae of Retzius x 7 days). The total crown formation time estimated for this individual’s incisor is 812 days, or 2.23 years.

Root formation time and age at death: Employing the method outlined by Dean and Beynon (1991) gives the following results: The lateral incisors lag behind the central incisors in both of these specimens, and thus, root formation on the central incisor precedes that on the lateral. The difference between the amount of enamel formed after the distinct shared striae to the end of crown calcification on the lateral incisor is said to provide a time estimate for the development of the root formation on the central incisor. A 28 perikymata difference between the central and lateral incisor (lateral exceeding central) is found beyond this unique structure, or 196 days, Following Dean and Beynon (1991), this calculation provides the basis for an estimate of root formation rate for this tooth in this child and, thus, the total time of tooth formation for this child can be estimated and an age at death can be derived. The central incisor contains 1.5 mm of root more than the lateral incisor. If this took 196 days to form, as per the enamel structure counts, then it can be estimated that the root formation rate in this incisor is 7.6 µm per day. At this rate, the total root formation time for the first incisor was 1,315 days (10 mm at 7.6 µm per day). Taken together, this provides an estimated age at death of 5.8 years.

According to Dean and Beynon (1991), the root formation time of the first molar can be calculated as follows: The largest estimate for crown formation time for the first permanent molar was 1.99 years. If the age at death according to the first incisor is 5.8 years, the 9.0 mm of first molar root may have taken as long as 3.81 years to form, at a calculated rate of 6.5 µm per day. Thus, the root formation rates for this individual are estimated at 7.6 µm per day for the incisors and 6.5 µm per day for the molar. The age at death as assessed from histological methods puts this individual at between 5.8 years of age based on a replication of the methods of Dean and Beynon (1991).

Specimen 73-5-308. The same method was applied to the forming incisors and first molar for this second specimen. After identification of the neonatal line, appositional enamel in the first molar was calculated from the cross-striations that extend from the tip of the dentin horn to the tip of the enamel. The total, less those under the neonatal line, is 330 cross-striations. This is said to reflect 330 days of enamel formation time for the appositional enamel in the protocone, or 47 weeks (0.91 year).

Imbricalional enamel in the first mandibular molar for this individual is estimated by the total number of striae of Retzius, and an average of seven cross-striations separating these markers. A total count of 90 striae of Retzius times 7 provides an estimated crown formation time of the imbricalional enamel of the protocone of the first molar at 630 days, or 90 weeks (1.73 years). Taken together, these enamel microstructures identify an estimated 960 days as the total time for crown formation of this mandibular first molar, or about 2.63 years.

For the lower first incisor, appositional enamel formation is estimated at 210 days (210 cross-striations), 30 weeks, or 0.58 year. Imbricalional enamel formation is estimated as taking 910 days (130 striae of Retzius x 7 days). The total crown formation time estimated for this individual’s incisor is 1120 days, or 3.07 years. Similar analysis was performed on the lower lateral incisor (Table 1, Figure 4).

Root formation time and age at death: As this individual is younger than the first one, and less root formation is present on the dentition, the time taken for root formation is shorter than in the older specimen. By a comparison of unique enamel characteristics on both of the incisors, 9 more perikymata occur on the lateral incisor than the central incisor after the common prominent enamel band. Calculating the root formation rate from the 0.5 mm difference in root length between the two teeth reflects a 7.9 µm per day root extension rate and the age at death of this specimen is estimated at 3.42 years.

According to the methods of Dean and Beynon (1991), it can be proposed that root formation time for the first molar required 0.79 year, or 288 days (derived from 3.42 years age at death based on incisor histology less the 2.63 years calculated years for enamel formation on the first molar). This provides a root extension rate of 14.6 µm per day (4.2 mm / 288 days) for the molar tooth.

**DISCUSSION**

A comparison of the age at death estimates derived by two alternative methods identifies significantly younger ages resulting from histological methods than those derived from calcification stage references. This is contrary to recent observations from a similar study employing non-human primate comparisons (Reid et al. 1998b).

The young specimen in this study parallels one that was previously published in discussions surrounding the usefulness of perikymata-based age at death estimates (Mann et al. 1990b). Dean and Beynon (1991) contested these observations and stated that they doubted such a specimen would stand up to "histological scrutiny" (Dean, Beynon 1991: 227). As in our earlier publication, the specimen in the present study has incisor perikymata counts (137) quite similar to those found on Srs 24 (135) as published by Bronmag and Dean (1985) and, thus, seemed a good candidate for histological scrutiny with the criteria and techniques set out in Dean and Beynon (1991), in terms of detailed assessment of crown formation and root growth. The histological investigation reported here provides an estimated age at death at about 3.4 years of age using the Dean, Beynon (1991) methods. These are estimates that overlap an age at death based only on perikymata counts. In fact, this child is the predicted age of Srs 24 (Bronmag, Dean 1985). These results provoke questions: Is it likely that this child, with first molar erupted, was indeed about 3.4 years of age at death?

The older individual studied here was aged, according to these histological methods, at 5.8 years of age. Again, these results raise the question of how likely it is that these ages are accurate and valid. For individuals at their respective dental maturity levels, first and second molar eruption, these are remarkably young ages by modern human standards. Indeed, it may be that our concepts and present descriptive data sets are not yet broad enough to accommodate such individuals. However, by contrast with
TABLE 2. A comparison of histologically-derived crown formation times.

<table>
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<th>Present study</th>
<th>Reid et al. 1998a</th>
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* Molar crown formation calculated for the maxillary molar (protoconid), the counts in the present study and those of Dean et al. 1991.

Crown formation times

A comparison of crown formation times found here with those reported previously on other samples points out that with the exception of the maxillary incisor, they are close to those reported by Dean and Beynon (1991) for an English child from Spitalfields, and somewhat shorter than Reid et al. (1998a) report for incisor and molar crown formation times assessed in four French skeletal studies (Table 2). A consideration of methodological differences between these studies includes both the methods of calculating both appositional and imbricalional enamel formation.

Appositional enamel methods: While the approach employed here parallels that of Dean and Beynon (1991), an alternative approach to the calculation of appositional enamel formation is used by Reid et al. (1998a). The latter study calculates crown formation times employing estimates of cumulative length (measurement of enamel thickness corrected for prism decussation) divided by the average cross section measurement. These alternative methods have been compared by Reid et al. (1998b) who report that crown formation times based on these two approaches, with overestimations of 2.5 to 21%, primarily in the anterior dentition, and underestimations ranging from about 4% to 8 percent, primarily in the molars, when the enamel thickness method is employed vs the striae count method. Thus, shorter crown formation times are predicted for the method employed by ourselves and Dean and Beynon (1991). Indeed, our results are less than those reported by Reid et al. (1998a) on the French specimens for the anterior dentition, although this is more pronounced in the maxillary incisor studied here. However, the molar crown formation times in our study are also lower than the range reported by Reid et al. (1998a), but in line with those reported by Dean et al. (1993a) for an African skeleton.

The results we report here contribute to the data base that is developing on crown formation times derived from histological methods. The known population affinity of our specimens also contributes to a perspective on what may be considered population and individual differences in these developmental processes.

Imbricalional enamel methods: In our calculation of imbricalional enamel we employ the number of striae of Retzius (as do Reid et al. 1998a), whereas Dean and Beynon (1991) used perikymata numbers. This should not influence our results as we document that there is high correspondence between our visualised striae of Retzius and the counted perikymata. In all of these histological studies, the time for crown formation is achieved by multiplying the visible line counts (perikymata or striae of Retzius) by corresponding number of days from the time of birth to achieve the number of days of imbricalional enamel formation.

In this study, we employ a seven day cross-striaion repeat interval for calculating imbricalional formation. The number seven was adopted in the early studies of histological age reconstruction, from the original hominid studies (Bromage, Dean 1985), where seven cross sections between molar perikymata on histological grounds, to the study of Beynon and Dean (1991), who identified seven cross striaions between striae of Retzius in their specimens. While Reid et al. (1998a) review a number of studies by their colleagues who find that the number of cross striaions between adjacent striae is always the same for an individual, we did not find this in our specimens. Whether this is due to population or individual differences unique to these specimens is unknown. Other studies have likewise observed variability in inter-striain cross section counts, ranging from 4 to 11 (Huda, Bowman 1995, Risnes 1998).

However, a requirement of this method is a constant cross striaion repeat interval between striae of Retzius/ perikymata for imbricalional enamel formation estimation and, thus, if deformation of molar crown formation is an interval, histological age estimation is impossible. We adopt a conservative approach to resolving this problem in employing a seven day periodicity between cross striaions for determining the number of days of crown formation. We use this number as it is in line with the assumptions underlying the theory of the method. In general, with the exception of the maxillary incisor, the crown formation times found here are in line with those reported by Reid et al. (1998a).

In summary, we posit the cross striaion variability between striae as a source of unknown error and agree with the recent statement of Risnes that "the Retzius line periodicity needs to be re-evaluated through further research in order to refine this tool for chronological studies" (Risnes 1998: 343).

Root formation estimation

We believe that a serious problem in this methodology is the method of inference concerning root formation time and that these formation times are having a strong influence on the ages at death of our specimens. Dean and Beynon developed the method used here and initially applied it to a Spitalfields’ European child (Dean, Beynon 1991) and one specimen of Gorilla gorilla (Beynon et al. 1991) reporting 2.85 mm per day growth rates for incisors and 2.9 to 3.8 mm per day growth rates for the molar in this human, and from 5.1 on an upper second incisor to 13.3 on a lower first molar for the gorilla. The methods posited to identify root formation time are based on an assumption that root formation progresses at a continuous daily rate. This is clearly erroneous: Both radiographic studies (Moorees et al. 1963) and histological and microstructural studies (Molnar et al. 1981), completed long before the propositions put forth by Dean and Beynon (1991), documented that there are times of variable velocity in root growth within a single tooth. It is thought surprising that the use of a single rate of growth for the entire root formation time of a tooth would considerably underestimate the overall time frame of tooth development. Employing the identical method, the calculation of root formation time in the first 0.5 mm of one of our subjects’ incisors, 7.9 µm per day, is more than twice that found in the earlier study by Dean and Beynon (1991), and the molar root formation rate is 9.1 µm per day on histological grounds.

Thus, the problem in the present study, is on the order of that identified for gorilla first molars (13 µm/day), by Beynon (1991). Either there is much higher variability in root formation time than previously imagined for humans and non-human primates, or there is a basic flaw in the method of derivation.

Thus, we raise questions about this methodology and the results that derive from it by all authors. When it was documented by Moorees et al. (1963) that "the rate of tooth development is not constant," these authors emphasised that in terms of root growth, the second quarter of development is two to three times longer than the final quarter in their exemplary case of canines and premolars. By contrast, Dean and Beynon (1991) stated that their method likely makes a relatively slow rate of root formation the de facto norm for root growth and set 9 µm as the growth rate as root formation proceeds. They accepted their calculations as demonstrating "that modern human root extension rates may be some three or four times slower for root formation than for root growth than in great apes" (Dean, Beynon 1991:226).

This concept of variable growth rates in root formation is incorporated in more recent work by Dean and colleagues (Dean 1995, Dean, Scannapieco et al. 1998). However, root deposition rates have been further investigated. They propose that the first 500 µm of root formation occurs at 1.4 µm per day and the remaining dentine occurs at an average rate of 5.5 µm per day in humans. While these figures do not reflect the radiographic rates, they provide another approach. The validity of this approach awaits further research. We believe that it is highly unlikely that single rates of root formation will be found to be representative of all human children.

Some questions regarding histological age at death estimates

While some researchers find that the histological method works well in predicting age at death of known individuals, others do not. For example, Stringer et al. (1990) used these methods on children of known age at death in their examination of 19 central incisors from the cemetery excavations at Christ Church, Spitalfields (Adams, Reeve 1987). In this study, the ages
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