Human Deciduous Mandibular Molar Incremental Enamel Development

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KEY WORDS   age estimation; cusp formation time; daily enamel secretion rates; developmental biology

ABSTRACT   Quantitative studies of incremental markings retained within human enamel have reconstructed the duration and rate (crown and cusp formation times, initiation and completion, daily enamel secretion rates) of permanent tooth development. This approach has provided one way of estimating human age-at-death, and facilitated comparative dental studies of primate evolution. Similar applications from deciduous enamel are inhibited because developmental reconstructions from incremental markings for these teeth are less frequently reported in the literature. This study quantified the duration and rate of enamel development for mesial (protoconid, metaconid) and distal cusps (hypoconid, entoconid) for first (dm1) and second (dm2) deciduous mandibular molars from an archaeological sample of modern human juveniles. Crown formation time can be calculated from the dm1 protoconid because growth initiates and completes in this cusp, and from the dm2 protoconid combined with the final period of hypoconid growth. The dm1 postnatal crown formation time included the time taken for the tubercle of Zuckerkandl to develop, and differed slightly compared to radiographic methods. The majority of dm1 protoconid cuspal (occlusal region) enamel formed before birth. The dm2 entoconid enamel formed mainly after birth. Birth reduced daily enamel secretion rates, changed the visibility of incremental markings, and disrupted enamel growth for 3 to 8 days. Findings presented here can contribute to age-at-death estimates for human infants aged 13-postnatal months or less, and should facilitate comparisons of primate deciduous incremental enamel development in an evolutionary context. Regression equations are included so that cuspal formation time can be estimated from enamel thickness. Am J Phys Anthropol 144:204–214, 2011.   © 2010 Wiley-Liss, Inc.

Human deciduous molar enamel development (crown and cusp formation times, initiation and completion, growth rates) has been documented from studies of radiographs, measurements of tooth length, and direct observation of the developing fetal dentition (e.g., Logan and Kronfeld, 1933; Schour and Massler, 1941; Kraus, 1959; Fanning, 1961; Moorrees et al., 1963; Gilster et al., 1964; Kraus and Jordan, 1965; Fanning and Brown, 1971; Demirjian et al., 1973; Liversidge et al., 1993; Liversidge and Molleson, 2004). Standards of formation time have been developed from some of these studies, which are routinely used to estimate age-at-death for human skeletons recovered from bioarchaeological and forensic contexts (e.g., Demirjian et al., 1973). Others have used a different methodology and calculated formation times from histological studies of incremental markings retained within enamel (Boyde, 1963). Though these histologically-derived formation times are well reported for permanent teeth in the literature (e.g., Reid and Dean, 2006), they are scarcely reported for deciduous teeth (FitzGerald et al., 1999; Katzenberg et al., 2005; also see Shellis 1984, and comments by Smith et al., 2006). This lack of data is mainly due to the poor preservation of these markings in deciduous enamel, compared to permanent enamel (e.g., Smith, 2004). Yet studies of permanent teeth have shown that formation times derived from incremental markings can be accurate, when compared to a known age-at-death (Antoine, 2000; Smith et al., 2006; Antoine et al., 2009), and display less variation compared to some other methods (Reid and Dean, 2006).

Other quantitative aspects of deciduous molar enamel development, such as cusp initiation and completion, have not been documented from histological studies of incremental markings within enamel. Molar daily enamel secretion rates (DSRs) have been reported from small samples (Beynon et al., 1998a,b; Macchiarelli et al., 2006; Birch and Dean, 2009; FitzGerald and Hillson, 2009), though variation between cusps and molars was not a focus in those studies. No study has undertaken a detailed reconstruction of deciduous molar enamel development from incremental markings. The absence of these data inhibits the few studies of evolutionary developmental biology that compare deciduous enamel between extant and fossil species (Beynon et al., 1998a; Macchiarelli et al., 2006).

The aims in this study are: to calculate total enamel crown formation time and cusp formation times (subdivided into pre- and postnatal; cuspal, and lateral region[1]) for human dm1 and dm2 (also called dp3 and dp4) from histological analyses of incremental markings so that these data can be incorporated into age-at-death estimations for infant skeletons; and to quantify other aspects of deciduous enamel development so that they

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[1]Each cusp is subdivided into a cuspal (occlusal) and lateral region by the first enamel layer to appear at the outer surface as a perikymata; see enamel growth section. Lateral enamel is sometimes further subdivided into cervical enamel towards the crown-root interface.

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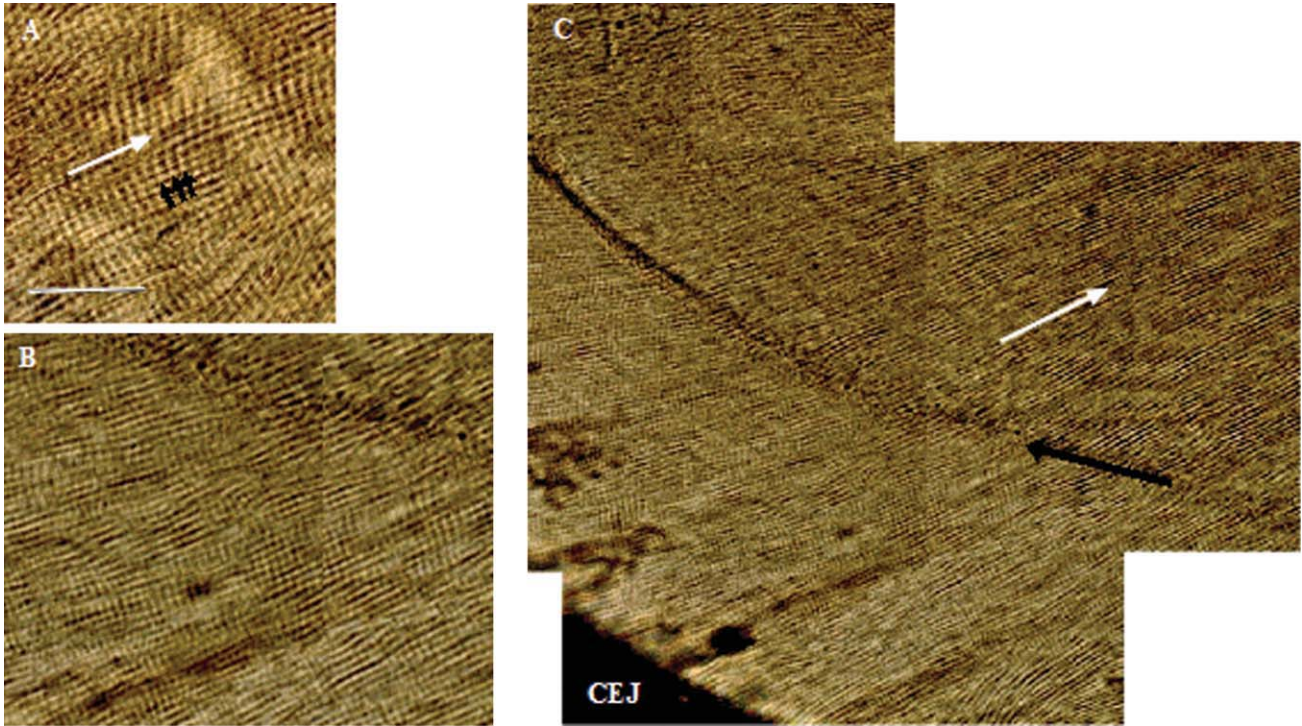
Fig. 1. Deciduous second molar hypoconid enamel (**A** = ×40, a close up of **B**. B = ×20, a close up of **C**. C = ×10, a montage of over-lapping images). In image A, the white arrow points in the direction of enamel prisms. Black arrows point to the cross striations used to calculate daily rates of enamel secretion (see text). Scale bar = 50 μm (dashed white line). In image C, the white arrow points in the direction of the enamel prisms and toward the outer enamel surface. Black arrow points in the direction of the neonatal line. CEJ is the cement enamel junction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

can be incorporated into comparative studies in an evolutionary context. These aspects of deciduous enamel development are: DSRs, Retzius line periodicity, the sequence of cusp initiation and completion, and the growth overlap between first and second molars.

ENAMEL GROWTH

Enamel-forming cells, or ameloblasts, secrete matrix through a distal cellular extension, the Tomes process (Boyde, 1989). Excluding the initial layer, the Tomes process produces prismatic enamel (Nanci, 2003) as it moves away from the enamel-dentin junction (EDJ) toward the future outer enamel surface (Ten Cate et al., 2003). Regular variations in ameloblast activity produce two types of incremental markings, which can be used to quantify the rate and duration of enamel growth (Boyde, 1963). The first are short period markings, or cross striations (see Fig. 1a,b). These lines reflect a daily 24-h rhythm in ameloblast activity (Schour and Poncher, 1937; Mimura, 1939; Risnes, 1986; Bromage, 1991). The second type are long period markings, or Retzius lines (Retzius, 1837; see Fig. 2), which indicate layers of enamel produced by ameloblasts every 6–12 days in modern humans (see Smith et al., 2007, their Table 2). These layers are visible under transmitted light within sectioned cuspal and lateral enamel, and emerge at the outer surface of lateral enamel as perikymata (Dean, 1987; Risnes, 1990, 1998; Shellis, 1998; Li and Risnes, 2004).

Periods of stress can produce accentuated markings (also called accentuated striae and Wilson bands) within forming enamel (e.g., Rushton, 1933). Prenatal enamel does not
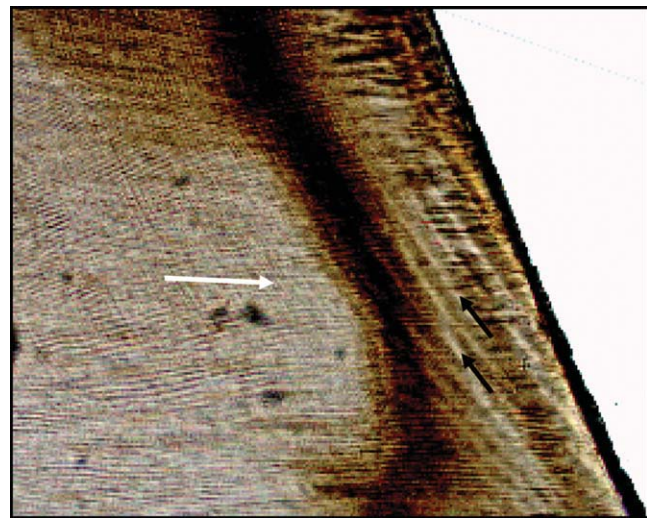

Fig. 2. Retzius lines in dm2 protoconid enamel. White arrow points in the direction of the enamel prisms. Black arrows point in the direction of the Retzius lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary. com.]

normally contain these markings in humans, so it is thought that the first one reflects the birth event, the neonatal line (Rushton, 1933; Schour, 1936; Weber and Eisenmann, 1971; Sabel et al., 2008). The neonatal line (Fig. 1c), and subsequent accentuated markings (e.g., Schwartz et al., 2006), can be used with cross striations and Retzius

lines to calculate pre- and postnatal enamel formation time, and also to determine the growth sequence between cusps and molars (e.g., Reid et al., 1998a,b).

MATERIALS

The dental sample is comprised of erupted unworn deciduous mandibular first (n = 12) and second molars (n = 13) from archaeological samples of modern human juveniles. Histological thin sections of the mesial cusps (protoconid, metaconid) and distal cusps (hypoconid, entoconid) were prepared. These sections formed part of a much larger sample (n = 108: dm1 and dm2 mesial and distal sections), some of which were used previously to report enamel thickness (Mahoney, 2010). The sections for this study were chosen because they retained incremental and accentuated markings. The remaining sections did not show these markings, which confirms that they are not well preserved in deciduous teeth (Fitz-Gerald and Saunders, 2005; FitzGerald et al., 2006). The juveniles dated to the British Bronze Age and Medieval period, and were curated by the Powell Cotton Museum, Hull and East Riding Museum, The National Museums of Scotland, and the University of Kent. The sex of the juveniles was not known.

METHODS

Sample preparation

Standard histological procedures were followed (e.g., Reid et al., 1998a). The molars were embedded in polyester resin to reduce the risk of splintering while sectioning. Using a diamond-wafering blade (Buehler® Isomet low speed), longitudinal sections between 180 and 200 mm were taken through the mesial cusp tips and dentin horns of each molar. A second section was then made through the distal cusp tips. Section obliquity was minimized following methods discussed by Mahoney (2010).

Each section was mounted on a microscope slide, lapped using a graded series of grinding pads (Buehler® Isomet lowspeed) to reveal the incremental markings, polished with a 0.3-mm aluminum oxide powder, placed in an ultrasonic bath to remove surface debris, dehydrated through a series of alcohol baths, cleared (using Histoclear®), and mounted with a cover slip using a xylene-based mounting medium (DPX®). Sections were examined under a high powered microscope (Olympus BX51) at 40× or 60× using transmitted and polarized light. Images were produced using a digital microscope camera (Olympus DP25), and captured using imaging software (Olympus Cell D).

Daily enamel secretion rates

Daily enamel secretion rates were calculated for cuspal enamel by dividing this enamel into three regions of equal thickness (inner, mid, and outer). Rates were measured along the long axis of an enamel prism around the center of each region (Mahoney, 2008: Fig. 3). A distance corresponding to 5 days of enamel secretion was measured, and then divided by five to yield a mean daily rate. The procedure was repeated a minimum of six times in each region, which allowed a grand mean value and standard deviation (sd) to be calculated.

Retzius line periodicity

Retzius lines were visible in the lateral enamel of one section from the entire sample. Periodicity is the number of
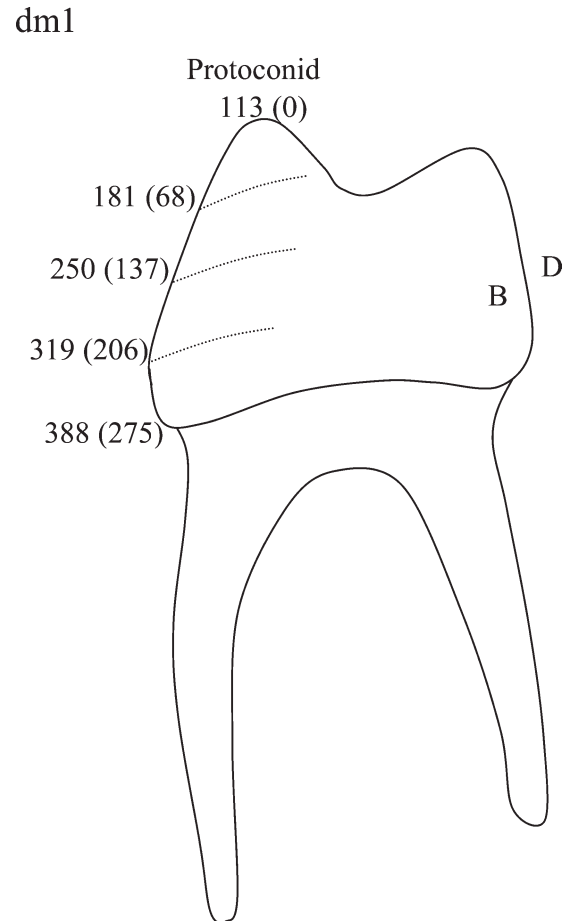

Fig. 3. Deciduous first mandibular molar enamel crown formation time. The figure shows mean enamel formation time for the protoconid subdivided into quartiles, which are indicated by lines and days of occurrence, with the corresponding chronological age in days from birth given in parenthesis. Values show that at birth (Day 0) prenatal enamel growth has occurred for 113 days and the protoconid cuspal—occlusal—region has formed. Lateral enamel growth continues, and crown formation is complete after 388 days (postnatal chronological age = 275 days). B = Buccal. D = Distal. Values taken from Tables 5–8 and recalculated.

days of enamel formation observed between two adjacent Retzius lines (i.e., an enamel layer). Periodicity for this section was calculated by measuring the distance between four lines along the long axis of the prism. The measurement was divided by a local DSR and then subdivided by 3 (i.e., three layers of enamel). This calculation gave the time taken to form the enamel between two adjacent lines.

Cuspal formation time

Cuspal enamel formation time was calculated in two ways. In four sections, cross striations were preserved throughout much of the cuspal enamel. For these sections, overlapping images of the entire sectioned cuspal molar enamel were produced at 20× using the imaging software. These were printed in color and a montage was recreated. Cross striations on the montage were marked with a pen, starting at the tip of the dentin horn and continuing to the outer enamel surface at the cusp

TABLE 1. First deciduous molar DSRs in μm

	Inner ($n = 8$)	Mid ($n = 9$)	Outer ($n = 8$)
Mean	3.47	3.87	4.76
Min	2.92	3.01	4.53
Max	4.36	4.65	4.95
±1SD	0.46	0.37	0.23

TABLE 2. Mean DSRs for two dm1 cusps in μm

	Prd[a]	Hyd
Inner	3.06	3.52
Mid	4.13	3.29
Outer	4.87	5.07
%Pn[b]	100	55

[a] Prd = protoconid. Hyd = hypoconid.
[b] Pn = prenatal enamel as % of cuspal enamel thickness.

TABLE 3. Second deciduous molar DSRs in μm

	Inner ($n = 8$)	Mid ($n = 11$)	Outer ($n = 4$)
Mean	3.68	3.91	4.92
Min	3.45	3.68	4.50
Max	4.20	4.20	5.18
±1SD	0.23	0.28	0.34

tip. The number of cross striations was then summed to give the number of days of cuspal enamel growth.

For the remaining and majority of the sections, cross striations were preserved intermittently. For these, cuspal formation times were calculated using a standard formula [(enamel thickness × correction factor)/DSR]. Enamel thickness was measured from the tip of the dentin horn to the position of the first Retzius line at the cusp tip tooth surface. A correction factor of 1.05 was used because decussation was not marked in this sample (Schwartz et al., 2003). Cuspal enamel was divided into three regions of equal thickness (inner, mid, and outer), and divided by the mean DSR from each of those regions (see above). The three formation times were then summed to give an overall cuspal formation time.

Lateral enamel formation time

Lateral enamel formation time was recorded using enamel prism lengths divided by local mean DSRs to navigate between accentuated markings (also see Mahoney et al., 2007: Fig. 1). A clearly visible accentuated marking in the lateral enamel was followed in an apical direction to the enamel-dentin junction (EDJ). Prisms originating at the boundary between the EDJ and this accentuated marking were traced toward another accentuated marking at or near the outer enamel surface. The procedure was repeated. The time taken to form these prisms was included in the estimate of lateral enamel formation time.

Overlapping images of the entire lateral enamel were captured at 20× for each section and printed in color. A montage was created. Accentuated markings upon the montage were traced with a pen, so that they were clearly emphasized. The tracing was used as a template to guide recording of another on-screen image of the lateral enamel taken at 40× or 60×, using the imaging software.

Cusp and crown formation times

The cusp enamel formation time was calculated by summing the time taken to form the cuspal and lateral enamel. The total crown formation time (total CFT) for dm1 was the time taken to form the protoconid (see Results). The total CFT for dm2 was the protoconid formation time summed with the period of additional and final growth that is recorded in the dm2 hypoconid only.

Prenatal enamel formation, sequence of cusp growth

Prenatal enamel formation time was calculated by locating the position of the neonatal line. The enamel thickness between this line and the dentin horn was measured and divided by a local DSR, taken around the center of the prenatal enamel.

The sequence of growth (initiation and completion) between cusps was determined by locating the position

of the neonatal line in the dm1 protoconid, and the position of subsequent accentuated markings. The time that elapsed between the neonatal line and the subsequent markings was calculated by dividing the enamel thickness by local DSRs, thus determining a chronology of growth disturbances. The chronology of disturbances was then sought in and matched between the remaining cusps. The same method was used to register growth between molars.

Statistical procedures

Pearson's correlation coefficient was used to measure the strength of the association between cuspal enamel thickness, measured from the tip of the dentin horn to the position of the first Retzius line at the tooth surface in μm, and formation time (data from all cusps combined for dm1, and then dm2). Once a positive significant association was established, a linear regression equation was calculated so that cuspal formation time could be predicted from cuspal enamel thickness in both dm1 and dm2 cusps. Pearson's correlation coefficient and the regression equation assume that the variables are normally distributed. Normality was checked with a one-sample Kolmogorov-Smirnov test. A Mann-Whitney U test was used to examine the distribution of the DSRs between regions in the dm1 (all regions) and dm2 (inner vs. mid enamel only due to the sample size: $n = < 5$ in the outer enamel). Tests were conducted in SPSS (15.0).

RESULTS
Daily enamel secretion rates

Mean DSRs increased from the inner to outer enamel region in both molar types. Mean DSRs from the dm1 hypoconid decreased slightly from the inner to the mid enamel, and then increased toward the outer enamel when compared to the dm1 protoconid. Descriptive statistics are shown in Tables 1 (dm1), Table 2 (hypoconid and protoconid), and Table 3 (dm2). Table 4 shows inferential statistics.

Retzius line periodicity

Retzius lines were present in the dm2 protoconid enamel of one section (see Fig. 2). The measurement across four adjacent lines was 104 μm. Dividing 104 μm by a local DSR of 4.01 μm and subdividing by 3 gave a

TABLE 4. Comparing DSRs between regions

	U	Z	P
dm1			
In vs. Mid	11.500	−2.008	0.045*
In vs. Out	0.000	−3.000	0.003*
Mid vs. Out	3.000	−3.123	0.002*
dm2			
In vs. Mid	9.500	−1.550	0.127

* Significant difference.

TABLE 5. First deciduous molar formation times in days (years)

	Prd[a]		Med		Hyd		Ent	
	Cu[b]	Lat	Cu	Lat	Cu	Lat	Cu	Lat
	155	243	115	129	–	–	73	163
	94	286	96	–	83	163	90	–
	138[c]	–	–	–	128	168	68	–
	118	277	93	129	–	–	–	–
	100	–	–	–	119	183	34	–
	66	299	–	–	117	–	72	–
	–	–	–	–	59	–	–	–
Mean	112	276	101	129	101	171	67	163
Cusp[d]	388 (1.06)		230 (0.63)		272 (0.75)		230 (0.63)	
Min	365		222		246		–	
Max	398		244		302		–	
±1SD[e]	15		16		31		–	
Total CFT[f]	388 (1.06)							

[a] Prd = Protoconid, Med = Metaconid, Hyd = Hypoconid, Ent = Entoconid.
[b] Cu = cuspal, the occlusal enamel region. Lat = lateral enamel.
[c] Direct count of prism cross striations in days.
[d] Cusp = Cusp formation time (cuspal + lateral formation time).
[e] sd = standard deviation calculated from the individuals with both cu + lat.
[f] Total CFT = total crown formation time: see text for calculation.

TABLE 6. Second deciduous molar formation time in days (years)

	Prd		Med		Hyd		Ent	
	Cu	Lat	Cu	Lat	Cu	Lat	Cu	Lat
	127[a]	324	110	236	107	335	120	198
	–	–	108	–	–	–	90	–
	117	–	147	–	150	365	157	265
	–	–	186	–	–	–	137	219
	–	–	–	–	150	268	207	146
	–	–	128	254	–	–	–	–
	–	–	–	–	115	–	176	–
	95	301	199	–	–	–	212	–
	71	312	–	–	143	249	85	218
	77	274	105	167	–	–	–	–
Mean	97	303	140	219	133	304	148	209
Cusp	400 (1.10)		359 (0.98)		437 (1.19)		357 (0.98)	
Min	351		272		392		303	
Max	451		382		515		422	
±1SD	42		56		53		46	
Total CFT	470 (1.29)							

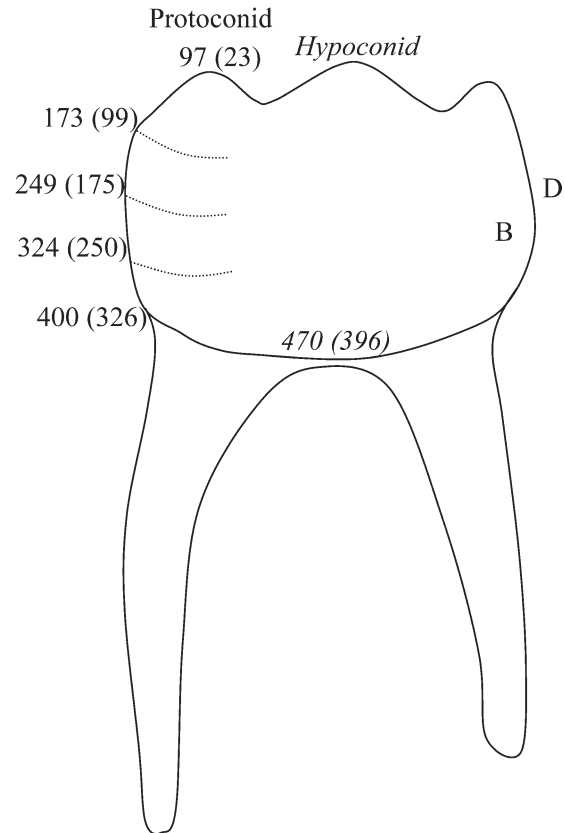[a] Direct count of prism cross striations in days.

dm2

Fig. 4. Deciduous second mandibular molar enamel crown formation time. The figure shows mean enamel formation time for the protoconid subdivided into quartiles, which are indicated by lines and days of occurrence, with the corresponding postnatal chronological age in days given in parenthesis. Enamel crown formation commences in the dm2 protoconid and completes in the hypoconid. Values show that the protoconid cuspal—occlusal—region has formed after 97 days of enamel growth (23 days after birth). Lateral enamel growth continues, and crown formation is complete in 470 days (postnatal chronological age = 396 days). B = Buccal. D = Distal. Values taken from Tables 5–8 and recalculated.

value of 8.6, or a Retzius periodicity of 9 days. Repeating this procedure in another region of the lateral enamel gave a value of 8.7, or a Retzius periodicity of 9 days.

Cusp formation times

The mean dm1 cusp formation time was 388 days for the protoconid, 230 days for the metaconid, 272 days for the hypoconid, and 230 days for the entoconid. Table 5 shows dm1 formation times (also see Fig. 3).

Cuspal formation time and enamel thickness were positively and significantly correlated in dm1 (Pearson's $r = 0.945$; $P = 0.000$). One linear regression equation was calculated from the combined data for the four dm1 cusps, where y is the cuspal formation time in days, and x is enamel thickness in mm: dm1: $y = 9.492 + (0.214x)$. For the dm1, 88% of the data variation was accounted for by the regression equation ($P = 0.000$).

The mean dm2 cusp formation time was 400 days for the protoconid, 359 days for the metaconid, 437 days for the hypoconid, and 357 days for the entoconid. Table 6 shows dm2 formation times (also see Fig. 4).

Cuspal formation time and enamel thickness were positively and significantly correlated in dm2 (Pearson's $r = 0.988$; $P = 0.000$). One linear regression equation was calculated from the data for the four cusps combined: dm2: $y =$

TABLE 7. *First deciduous molar prenatal enamel formation in days*

	Prd	Med	Hyd	Ent
	116	70	–	17
	94	77	70	54
	138[a,b]	–	–	–
	99	54	–	–
	120	–	60	36
	–	–	31	–
	–	–	58	21
	–	–	59	–
Mean	113	67	56	32

[a] Direct counts of prism cross striations in days.
[b] Assuming a gestation period of 274 days, enamel formation commenced in utero a minimum of 136 days after conception (274 days minus 138 days = 136 days) and a maximum of 180 days (274 days minus 94 days = 180 days) in this sample.
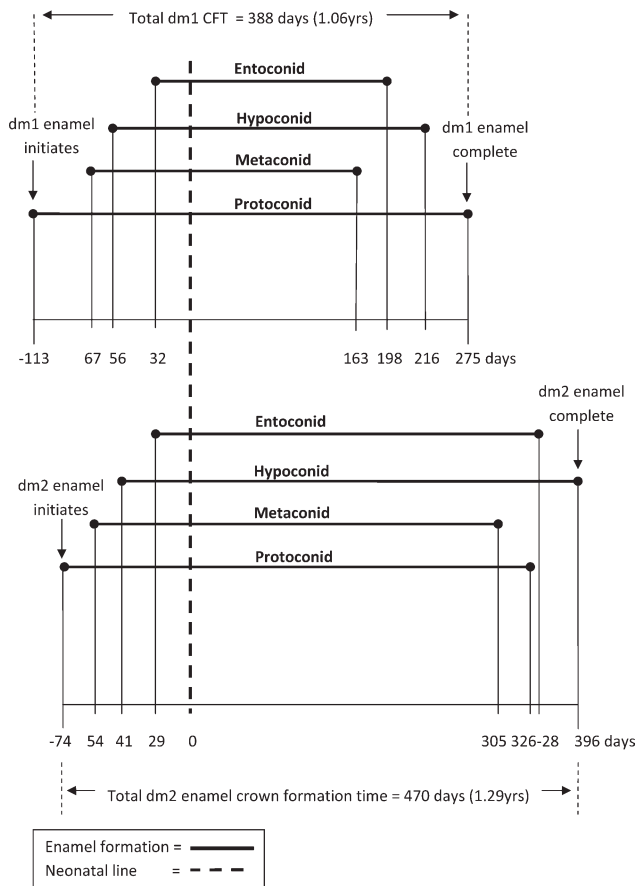

Fig. 5. Cusp initiation and completion in dm1 and dm2 in days (years). Data taken from mean values in Table 5–8.

$-0.942 + (0.247x)$. For the dm2, 92% of the data variation was accounted for by the regression equation ($P = 0.000$).

Prenatal enamel formation, sequence of cusp growth, and total CFT

On average, prenatal enamel growth was initiated in the dm1 protoconid 113 days before birth, in the metaconid 67 days before birth, and in the hypoconid and ento-

TABLE 8. *Second deciduous molar prenatal enamel formation in days*

	Prd	Med	Hyd	Ent
	101[a]	49	–	31[a]
	–	42	–	–
	80	68	51	43
	–	62	–	40
	–	–	43	30
	–	78[a]	37	10
	–	–	31	20
	60	39	–	27
	65	–	42	30
	63	40	–	–
Mean	74	54	41	29

[a] Direct counts of prism cross striations in days.

TABLE 9. *Comparing deciduous molar cuspal region DSRs in* μ*m*

	dm1		dm2	
Authors	Inner (min)	Outer (max)	Inner (min)	Outer (max)
Beynon et al. (1998)[a]			3.5	6.4
Macchiarelli et al. (2006)[a]			2.3	4.5
Birch and Dean (2009)[a]	3.0	4.4	2.3	3.9
This study[b]	2.9	4.9	3.5	5.1

Mean dm1 DSR of 3.4 ± 0.29 reported by FitzGerald and Hillson (2009) also lies within the range given in Table 9.
[a] Minimum and maximum values taken from the 10th and 90th centiles.
[b] Min and max values from Tables 1 and 3 in this study.

conid 56 and 32 days before birth, respectively. The first molar protoconid was the last cusp to complete formation. On average, 100% of the protoconid cuspal enamel formed in utero (metaconid = 66%, hypoconid = 55%, entoconid = 48%). Table 7 shows dm1 prenatal enamel formation times. Figure 5 shows first molar cusp initiation and completion times.

On average, prenatal enamel growth commenced in the dm2 protoconid 74 days before birth, in the metaconid 54 days before birth, and in the hypoconid and entoconid 41 and 29 days before birth, respectively. The second molar hypoconid was the last cusp to complete formation. On average 76% of the protoconid cuspal enamel formed in utero (metaconid = 39%, hypoconid = 31%, entoconid = 20%). Table 8 shows dm2 prenatal enamel formation times. Figure 5 shows second molar cusp initiation and completion times.

Because growth began and ended in the dm1 protoconid, this cusp recorded the total mean crown formation of 388 days, or 100% of the duration of enamel growth. No one cusp recorded the total period of enamel formation in the dm2. Growth began in the dm2 protoconid and ended in the hypoconid. When the mean period of additional postnatal enamel growth of 70 days recorded in the dm2 hypoconid was summed with the mean protoconid formation time of 400 days, it gave a total mean crown formation time of 470 days. The second molar cusps represented 93% (hypoconid), 85% (protoconid), and 76% (metaconid and entoconid) of the total crown formation time.

On average, enamel growth commenced in the second molar 39 days after growth had commenced in the first molar. Enamel growth was complete in the dm1 121 days before it was complete in the dm2.
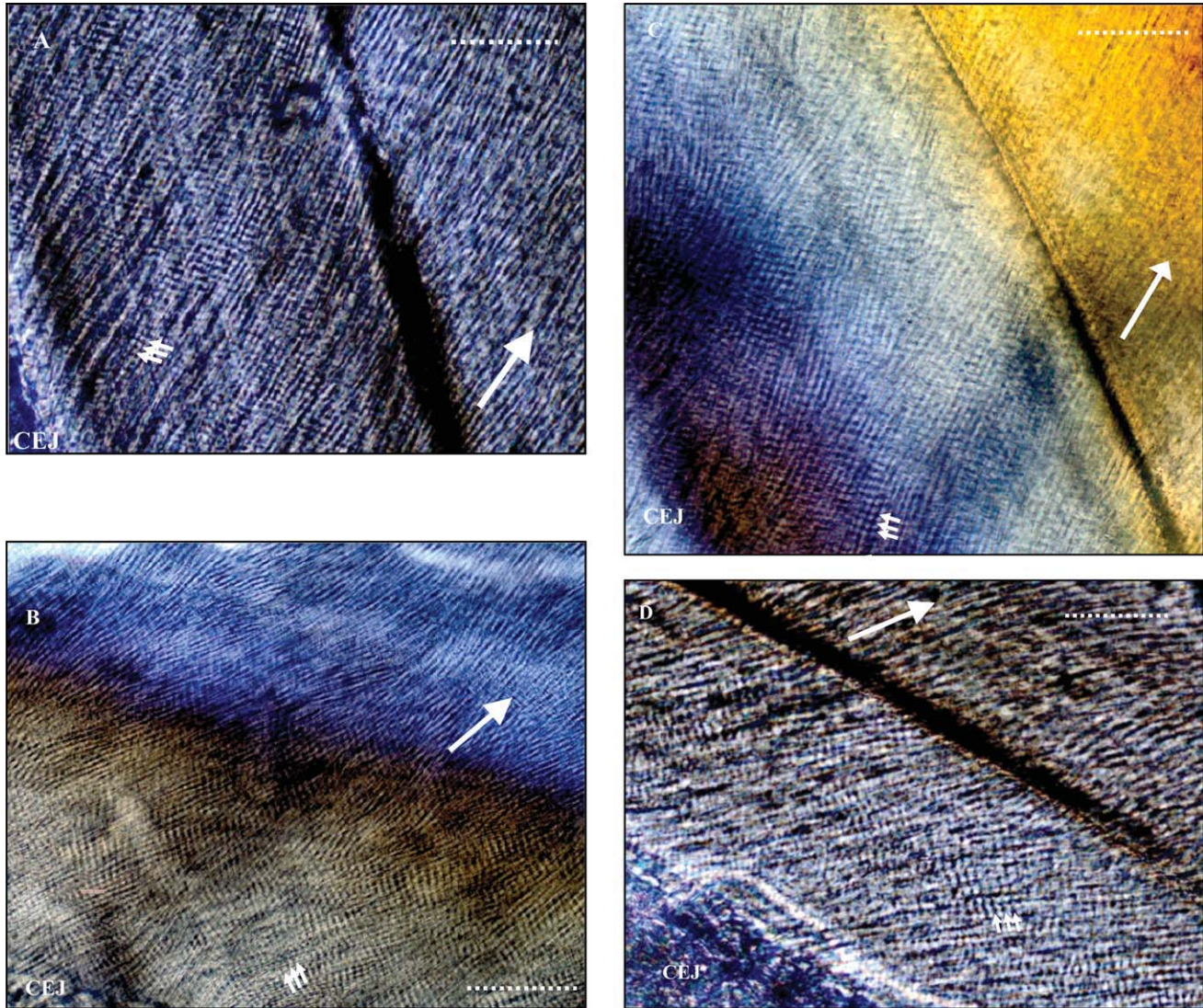
Fig. 6.   A–D. Cross striations and the neonatal line. Images A–D shows the change in cross striations before and after the neonatal line in four individuals. Cross striations are visible before the line but not after the line. Images A and D were produced using transmitted light at a magnification of ×40. Images B and C were produced using polarized light. The CEJ is to the lower left of each image. The large white arrow points in the direction of the prism, is adjacent to the neonatal line, and indicates postnatal enamel. Small white arrows point to cross striations. Scale = 50 μm.

DISCUSSION
Daily enamel secretion rates

The deciduous DSRs lie within the range of previously reported values (Table 9). Like other studies of deciduous and permanent molars (e.g., Beynon et al., 1991a, 1998a; Dean, 1998), daily rates of enamel secretion increased from inner to outer cuspal enamel, except in the dm1 hypoconid (below). Even though more data on enamel secretion rates are needed to determine if trends exist along the deciduous molar row, findings presented here support the proposal by Birch and Dean (2009) that differences in DSRs are apparent between deciduous and permanent teeth. Generally, mean DSRs from the deciduous teeth were not as slow adjacent to the EDJ or as fast in the outer cuspal enamel, compared to permanent molars (see Mahoney, 2008: Table 8).

The course of enamel secretion in the dm1 hypoconid differed when compared to the dm1 protoconid[2] (Table 2). The hypoconid showed decreased DSRs in the mid enamel, compared to the inner region, before accelerating towards the outer surface. The protoconid showed gradually accelerated DSRs through the three regions. An increasing number of studies have recently reported that DSRs may be influenced by periods of stress (Macchiarelli et al., 2006; Mahoney, 2008; Birch and Dean, 2009). Results reported for the dm1 hypoconid support this finding. On average, 55% of the hypoconid cuspal enamel formed in utero. By contrast, the majority of dm1 protoconid cuspal enamel formed before birth, and the neonatal line often emerged in the lateral enamel. One methodological implication from this finding is that comparative studies of daily enamel secre-

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[2]Cusps chosen because they retained the clearest cross striations.

TABLE 10. Comparing mandibular deciduous molar crown formation times in years after birth

Authors	dm1 Mean CFT (±1SD)	dm2 Mean CFT (±1SD)	Method
Logan and Kronfeld, 1933[a]	0.50	–	Sections, radiographs.
Fanning, 1961[b]	0.62 (0.49–0.76)	0.88 (0.77–1.00)	Radiographs. 50th percentile (25th and 75th percentile for males, not SD).
Moorrees et al., 1963[a,b]	0.45 (0.35–0.65)	0.75 (0.6–0.9)	Radiographs (SD estimated from diagram for males).
Gilster et al., 1964[a]	–	1.08 (0.79–1.30)	Radiographs (range).
Fanning and Brown, 1971	0.52 (0.47–0.77)	0.77 (0.51–1.01)	Radiographic. 50th percentile (3rd and 97th percentile for males, not SD).
Lunt and Law, 1974[a,b]	0.46	0.83	Review paper.
Liversidge et al., 1993	(0.4–0.8)	(0.7–1.4)	Tooth length measurements (range).
Liversidge and Molleson, 2004[b]	0.48 (0.3–0.66)	0.92 (0.66–1.18)	Radiographs, direct examination.
This study[c]	0.75 (0.71–0.79)	1.08 (0.97–1.20)	Histology.

[a] Recalculated from years to months.
[b] Mean age of attainment for enamel crown completion.
[c] Postnatal total crown formation time was calculated from mean values, and protoconid SD, in Tables 5 and 7 (dm1) and Table 6 and 8 (dm2).

tion rates should avoid comparing DSRs from a region adjacent to the neonatal line, with an equivalent region from another cusp that does not contain a record of birth.

Periods of stress such as the birth process can produce an accentuated marking, a neonatal line (Schour, 1936). This line was visible in the majority of the sections (Fig. 1c) and formed over a period of 3–8 days (width measurements of 10–25 μm divided by a mean DSR of 3 μm). Studies have previously shown changes to enamel microstructure across the neonatal line, involving prism direction, size, and crystallite concentration (Weber and Eisenmann, 1971; Whittaker and Richards, 1978), which continued into the postnatal enamel (Sabel et al., 2008). Findings for five individuals in this study indicate that the birth process may also affect the visibility of incremental markings. Cross striations were not visible in the postnatal enamel adjacent to the neonatal line, though they were visible in the prenatal enamel (see Figs. 1c and 6a–d). For each of these individuals, enamel prisms were visible before and after the neonatal line (i.e., the enamel did not become aprismatic). Therefore, while the birth process can disrupt enamel formation, producing a neonatal line and reducing rates of enamel secretion, it may also change the visibility of the short period markers. Further research is needed to confirm the latter proposal.

Formation time

Enamel on the buccal cusps of deciduous mandibular molars is thicker compared to the lingual cusps (Mahoney, 2010), which reflects similar differences seen in permanent mandibular molars (Schwartz, 2000; Grine, 2005; Mahoney, 2008). Thicker buccal enamel may increase resistance to greater functional demands, providing more resistance to wear as well as the potential for cusp fracture (Molnar and Ward, 1977; Khera et al., 1990; Grine, 2005). The thicker buccal enamel on the deciduous molars required a longer formation time, compared to the lingual cusps. This makes sense given the correlation between

enamel thickness and formation time in this sample. However, one unexpected finding was the similarity in the deciduous first and second molar protoconid formation time of 1.06 and 1.10 years, respectively, given that there are marked differences in the overall area of the sectioned mesial enamel cap between these molars (Mahoney, 2010). The similarity in cusp formation time is due to the much longer formation time required by the first molar protoconid lateral enamel, compared to the same region in the other cusps. The first molar protoconid lateral enamel covers the prominent dentin bulge named the molar tubercle of Zuckerkandl, which is not present in the other cusps, or the dm2 protoconid. Therefore, differences in the mesial area of the enamel cap between the two molar types relate mainly to the metaconid, and this is reflected by differences in metaconid formation times.

The postnatal deciduous total crown formation times can be used to calculate chronological age since birth (Fig. 3 and 4). The postnatal total CFTs are greater compared to the mean postnatal age of dm1 and dm2 enamel crown completion reported by the majority of radiographic studies (Table 10). The range of dm1 values reported here also overlaps only at the uppermost end of the range reported from two radiographic studies in Table 10, though there is much greater overlap in a comparison of the dm2 range. Others have previously noted differences in mean formation times derived from histology compared to radiographic methods (Beynon et al., 1991b; FitzGerald et al., 1999; Liversidge and Molleson, 2004; Reid and Dean, 2006), which may in part reflect the imaging techniques (Aiello and Dean, 1990; Beynon et al., 1991b; Beynon et al., 1998b). This might explain the findings in this study, particularly for the deciduous first molar. Lateral radiographic images record enamel crown completion from the mesial-distal aspect of the crown. Yet the buccal aspect of the dm1 protoconid continues to grow even after the mesial aspect of the crown is complete, due to the molar tubercle of Zuckerklandl. The tubercle can also obscure the mesial enamel completion stage on radiographs (Liversidge and Molleson, 2004). The additional growth required by the tubercle,

which is clearly seen in the lateral formation time for the dm1 protoconid, is most likely responsible for the slight difference in the finding between the methodologies.

Retzius lines were present in the outer lateral enamel of one individual. The periodicity of 9 days for this individual was based upon local DSRs, not direct counts of cross striations along one prism between adjacent lines, so this value may be inaccurate (see Smith et al., 2003). With this caveat in mind, the periodicity falls in the middle of the known range from modern human permanent dentition, which is 6–12 days (Smith et al., 2007). Like a previous study of Retzius lines in deciduous teeth from pigtailed macaques (Smith, 2004), the markings only appeared in the outer lateral enamel.

Sequence of cusp growth

Enamel growth commenced in the mesial cusps followed by the distal cusps. This sequence is the same as that reported for humans, and some nonhuman primates, using other methods (Turner, 1963, 1967; Nomato, 1964; Kraus and Jordan, 1965; Butler, 1968, 1992; Swindler et al., 1968). Enamel growth commenced in the deciduous first molar before the second molar. This sequence was mainly due to the early initiation of the first molar protoconid relative to the other cusps (see Fig. 5), which was also noted in Nomata's (1964) study of fetal dentition. Because growth initiates and completes in the dm1 protoconid, total crown formation time can be calculated from this cusp. For dm2, total crown formation time can be calculated from the protoconid cusp formation time combined with the final period of hypoconid growth.

The sequence of deciduous molar enamel initiation differs compared to others. Assuming a gestation period of 274 days, dm1 enamel growth initiated in utero between 20 and 26 weeks after conception, and between weeks 25 and 31 in dm2 (Tables 7 and 8). Nomata (1964) reports initiation in the two molar types in weeks 16 and 23, respectively (also see Kraus and Jordan 1965 and Onda 1959, for sequences). Part of the delayed initiation reported here, compared to Nomata's (1964) study, may be an artifact of the methodology. The staining of calcified tissues used by Nomata (1964) will reveal the initial prism-free mineralized layer of enamel, and the time taken to form this layer would have been included in their values. The methodology used in this study would not have accounted for initial prismless enamel because prism and cross striations are not formed until the ameloblast migrates away from the dentin. Alternatively, FitzGerald and Saunders (2005) identified potential problems when extrapolating standards from modern day samples to archaeological context, where differing responses to, for example, nutrition and the in utero environment could be reflected in dental development.

CONCLUSION

This study is the first detailed reconstruction of human deciduous mandibular molar enamel development from histological analyses of incremental markings. The postnatal crown formation times can contribute toward age-at-death estimates for infants aged 13-postnatal months or less. The quantitative variables should facilitate comparative studies of deciduous dental development in an evolutionary context, though some findings have methodological implications. The

birth process seems to alter the visibility of cross striations, and influence the rates of enamel secretion. Therefore, comparative studies of enamel growth rates should avoid comparing DSRs from a region adjacent to the neonatal line with an equivalent region from another cusp that does not contain a record of birth. Total crown formation time can be estimated for the deciduous first molar from the protoconid, and for the second molar from the protoconid cusp formation time combined with the final period of growth recorded in the hypoconid only.

ACKNOWLEDGMENTS

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