Unlocking evidence of early diet from tooth enamel

Louise T. Humphrey^{*†}, M. Christopher Dean[‡], Teresa E. Jeffries[§], and Malcolm Penn[¶]

Departments of ^{*}Paleontology, [§]Mineralogy, and [¶]Botany, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom; and [‡]Department of Cell and Developmental Biology, University College London, London WC1E 6BT, United Kingdom

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Recent developments in microspatial analysis of enamel chemistry provide the resolution needed to reconstruct detailed chronological records of an individual's early life history. Evidence of nutritional history, residential mobility, and exposure to heavy metals can potentially be retrieved from archaeological and even fossil teeth. Understanding the pattern and timing of incorporation of each trace element or stable isotope into enamel is crucial to the interpretation of the primary data. Here, we use laser ablation inductively coupled plasma mass spectrometry and ArcGIS software to map variation in calcium-normalized strontium intensities across thin sections of enamel from exfoliated deciduous teeth. Differences in calcium-normalized strontium intensities across each tooth reflect variation in tooth mineralization, implying that sampling location must be taken into account in interpreting results. Chronologically consistent shifts in calcium-normalized strontium intensities in teeth from children with known nursing histories reflect the onset and duration of breastfeeding and the introduction of nonmaternal sources of food. This tool is likely to be valuable for studying weaning and nursing behavior in the past. The distribution of normalized strontium intensities presented here is consistent with a model for the differential incorporation of strontium and calcium into enamel during the secretory and maturational phases of formation.

infant diet | laser ablation inductively coupled plasma mass spectrometry | strontium | weaning | calcium

A key question in paleobiology is whether it is possible to recover chronologically meaningful stable isotope or trace element distributions from tooth enamel that can be used to reconstruct age-related changes in an individual's behavior and environment during the period of enamel formation. Recently published studies show that crucially important information can potentially be retrieved in this way (1–3). Tooth enamel is a composite material with a crystalline ultrastructure and has a mineral component that closely resembles hydroxyapatite (4). The chemical constituents of hydroxyapatite are tolerant to substitution by a range of trace elements, which are readily incorporated into enamel forming at the time of exposure (5). The composition of subsurface enamel is fixed before tooth emergence and provides a durable record of changing environmental and physiological influences during the period of enamel formation. The information, which is literally embodied within this deep enamel, can provide retrospective evidence of dietary change, residential mobility, and exposure to toxic elements during the first few years of life.

Techniques now exist for analyzing the chemical composition of very small quantities of enamel, providing a basis for discrete multiple sampling investigations of variation in trace element and isotope composition through the tooth crown (6–8). Understanding the pattern and timing of incorporation of different trace elements or stable isotopes into developing enamel is fundamental to the interpretation of these signals (1, 8–12). Enamel formation involves two distinct stages, secretion and maturation, which can be defined on the basis of changes in the structure and function of ameloblasts and their effect on enamel composition (13, 14). During enamel secretion, enamel crystallites are seeded in a protein matrix located proximal to the ameloblast cell layer and elongate in the wake of retreating

ameloblasts to produce thin crystalline ribbons extending the full thickness of the enamel (13, 15). The daily appositional incremental growth of enamel is manifested structurally as prism cross-striations, and these preserve a permanent record of the timing of the onset of mineralization at each point in the enamel. At the end of the secretory stage the enamel crystallites account for $\approx 14\%$ by weight of the mineral content of mature enamel (14). Enamel maturation involves the degradation and removal of growth-inhibiting enamel matrix proteins and secondary mineralization at the sides of the existing apatite crystallites (16). During this stage, which transforms the partially mineralized immature enamel into a highly mineralized and durable tissue, $\approx 86\%$ of the mineral content of mature enamel is deposited (4, 14). This substantial increase in mineral content means that the chemical composition of mature enamel at any given sampling point is a time-averaged signal representing mineral deposition over a period of several months or even years. In view of this attenuated mineralization process, researchers have been cautious or even pessimistic about the likelihood of recovering chronologically relevant isotope and trace element sequences in enamel. Here, we suggest that differences in strontium and calcium transportation across the ameloblast layer contribute in a predictable way to the distribution of these elements in mature enamel and result in an enhancement of the physiological signal relating to the secretory stage of enamel formation.

Secretory epithelia provide a tightly regulated supply of calcium and are components of a variety of organs including the placenta, mammary gland, and enamel organ (17, 18). Active transport of molecules across a membrane involves transport up a concentration gradient by using energy derived from ATP. Calcium ions are transported across membranes by specific Ca^{2+} -ATPases. Enamel formation involves the selective transfer of ions across the ameloblast layer between the interstitial and enamel fluid to provide an optimal environment for the controlled synthesis of enamel during each stage of the mineralization process (17) (Fig. 1). During enamel matrix secretion, binding of enamel proteins at the sides of the enamel crystallites ensures that mineral deposition sites are only accessible at the tip of the growing crystallite, allowing expansion in length but not in width (17). This process requires low and stable calcium environment, which is achieved by mechanisms limiting the amount of calcium entering the enamel fluid and facilitating the removal of any excess amounts. The presence of Ca-ATPase at the basal (proximal) cell membrane during enamel secretion implies active calcium transport into the interstitial fluid, providing a mechanism for the removal of excess calcium out of the enamel fluid (17, 19).

Enamel maturation occurs after the secretory stage and is characterized by a substantial increase in the rate of mineral accretion (14). Accordingly, the rate of calcium transport across

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[†]To whom correspondence should be addressed. E-mail: lth@nhm.ac.uk.

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Fig. 1. Calcium transport across the ameloblast layer (enamel epithelium) during the secretory stage of enamel formation and the ruffle-ended and smooth-ended phases of the maturation stage. Active calcium transport is indicated by black arrows, and passive transport is indicated by white arrows. Strontium transport is passive throughout.

the ameloblast layer is up to 4-fold higher during enamel maturation than during enamel secretion, resulting in a high calcium environment in the enamel fluid that drives mineralization forward (17). The route taken by calcium into the enamel fluid varies according to the morphological state of the maturational ameloblasts, which undergo a periodic transition at the apical border from a ruffle-ended phase to smooth-ended phase. The apical (distal) location of plasma membrane Ca-ATPase in the ruffled ameloblasts indicates active transcellular transport of calcium from the interstitial fluid into the enamel fluid during the ruffle-ended phase. During the smooth-ended ameloblast phase there is transient loss of the distal tight junctions between adjacent ameloblasts, allowing components of the interstitial fluid to enter the enamel fluid through the intercellular spaces, but there is little accretion of mineral during this phase of the cycle. Instead, virtually all of the calcium that is incorporated into the expanding enamel crystallites enters via the ruffle-ended ameloblasts (14, 17). In total, an estimated 86% of the calcium present in mature enamel is actively transported through ruffle-ended ameloblasts during enamel maturation (17).

Although substantial research has been directed toward understanding the basis of calcium transport across the enamel epithelium (as reviewed in ref. 17), we have been unable to find a single reference to strontium transport across the ameloblast layer. In other calcium-transporting tissues, such as the placenta and mammary gland, where calcium follows an active transcellular pathway, the transfer of strontium follows concentration gradients, implying a passive mode of transfer (20, 21). Because the mechanisms responsible for the transport of calcium through the ameloblast layer are analogous in many respects to those in other secretory epithelia, it is reasonable to suppose that strontium transport through the ameloblast layer is not actively controlled.

We hypothesize that the amount of strontium present in the enamel fluid and incorporated into newly forming enamel reflects physiological (interstitial) levels during the secretory stage and does not exceed physiological levels during any phase of the maturation cycle. In contrast, calcium levels in the enamel fluid are lower than in the interstitial fluid throughout the period of matrix formation, but are up to four times higher during enamel maturation as a result of active calcium transport through the ruffle-ended maturational ameloblasts. Based on this information we can make the conservative assumption that the 14% of calcium incorporated into enamel during the secretory phase reflects physiological levels and that the 86% of calcium incorporated into enamel during the maturation phase is four times more concentrated than physiological levels. Assuming that strontium incorporation reflects physiological levels at each stage of enamel formation, the amount of strontium

Fig. 2. Outline of the longitudinal section of the buccal tooth cusps of a deciduous tooth, showing sampling points (black spots) and the position of the neonatal line. Areas shaded in gray indicate the position location in which normalized strontium intensities are predicted to be highest in teeth from children who are breastfed from birth (*Left*), formula fed from birth (*Center*), and initially breastfed but given complementary foods or formula at an early age (*Right*).

incorporated into enamel is 14 strontium units (per 14 calcium units) during enamel maturation and 21.5 strontium units (per 86 calcium units) during enamel maturation. On this basis we calculate that 39.4% of the strontium signal in enamel is laid down during the secretory phase of enamel formation compared with only 14% of the calcium signal during this phase.

Moreover, because the degree of secondary mineralization occurring during the maturational phase varies across the tooth crown, we suggest that the areas of enamel that are more heavily mineralized and therefore denser, contain a greater proportion of calcium and strontium deposited during the maturation phase. In contrast, areas of enamel that are less heavily mineralized contain a greater proportion of calcium and strontium deposited during the secretory phase. We therefore predict a higher calcium signal and a lower strontium/calcium (Sr/Ca) ratio in parts of the enamel that are more heavily mineralized. Finally, because age-related changes in dietary intake and physiological uptake are reflected in the Sr/Ca ratio of tissues forming at successive stages of development, our model implies that evidence of dietary transitions that occurred during the secretory stage of enamel formation should be preserved in the Sr/Ca ratios of mature enamel. The scenario presented here is essentially a working model, because it is not supported by experimental data, but it provides a testable mechanism and contributes to our understanding of the distribution of strontium and calcium in mature enamel.

This study uses laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to analyze calcium-normalized strontium intensities (Sr/Ca ratios) in enamel from deciduous teeth donated by children of known dietary history. ArcGIS 9.2 software was used to produce a continuous map of Sr/Ca ratios in each enamel section by interpolating between values measured at multiple discrete sampling points. The purpose is to explore whether the underlying distribution of Sr/Ca ratios within the enamel cusp reflects variation in the degree of mineralization and whether deviations from this pattern correspond with the timing of important dietary transitions (Fig. 2). Specifically, the following predictions can be made: (i) Sr/Ca ratios will be highest in the deepest enamel in the occlusal region of the crown and lower in enamel closest to the enamel surface, because mineral density is lowest in the deepest enamel beneath the tooth cusp and increases from the enamel dentine junction (EDJ) to the outer enamel in deciduous human teeth (22–24); (ii) Sr/Ca ratios will show an abrupt decrease between enamel formed before birth and enamel formed immediately after birth

Fig. 3. Interpolated surface maps of normalized strontium intensities in deciduous molar cusps from child B (exclusively breastfed after birth) (A), child G (predominantly formula fed) (B), and child D (initially breastfed with transition to formula at 5–6 weeks) (C).

in exclusively breastfed infants, because of differential transfer of strontium and calcium across the placenta and mammary gland (1, 20, 21); (iii) Sr/Ca ratios will increase between enamel formed before birth and enamel formed immediately after birth in infants fed on infant formula derived from cow's milk because formula-fed infants exhibit a higher intake and retention of strontium than breastfed infants (1, 25); and (iv) Sr/Ca ratios in enamel will increase when formula or nonmilk foodstuffs are added to the diet of a breastfed infant, because foods that are not maternally derived, and particularly those commonly given during weaning, have a higher Sr/Ca ratio than human milk (26–28).

Results

Interpolated surface maps reveal differences in the distribution of calcium-normalized strontium intensities between teeth from children with different nursing histories (Fig. 3). Teeth from children who experienced prolonged exclusive breastfeeding exhibit a marked reduction in normalized strontium intensities across the neonatal line (Fig. 3A). Teeth from children who were fed predominantly or exclusively on infant formula from within a few days of birth lack this “breastfeeding signal” and typically exhibit higher normalized strontium intensities in enamel that initiates formation immediately after birth than in enamel that initiates formation before birth (Fig. 3B). The early introduction of formula as a supplement or substitute for breast milk is associated with an increase in normalized strontium intensities ratios in a band of enamel that initiated formation at that stage (Fig. 3C).

Interpolated surface maps reveal an underlying trend for normalized strontium intensities to decrease from the EDJ to the enamel surface, such that the lowest values are recorded in enamel closest to the crown surface. Maps also reveal a tendency for normalized strontium intensities to increase from the occlusal to cervical enamel, but this varies between teeth. To account for these trends, a daily rate of change in Sr/Ca ratio was determined for intervals between adjacent sampling points along trajectories. This was calculated as the difference in Sr/Ca ratio between adjacent sampling points (value for outer point minus value for inner point) divided by the number of days represented by the interval between the midpoints of the two sampling points. Positive values indicate an increase in Sr/Ca ratio between the inner and outer sampling points, and negative values indicate a decrease in Sr/Ca ratio. The average daily rate of change in Sr/Ca ratio across the tooth crown was calculated as the average rate of change for each sampling interval that encompassed that day, using a minimum of three sampling intervals.

For each tooth, the average daily rate of change in Sr/Ca ratio for the period between 30 days before birth and 180 days after birth is presented in Fig. 4. The underlying negative trend of these trajectories reflects the tendency for Sr/Ca values to

decrease from the EDJ to the enamel surface. Interruptions in the underlying trend of the trajectories (peaks and troughs) correspond with major dietary shifts (Tables 1 and 2). Consistent records are recovered from enamel from deciduous canines and molars belonging to the same individual (Fig. 4 C and G). Changes in Sr/Ca ratio across the neonatal line reflect the diet given in the first few weeks after birth. Four teeth from three infants who were exclusively breastfed for the first few months after birth exhibit a strongly negative daily rate of change in Sr/Ca ratio at around the time of birth, reflecting a marked reduction in Sr/Ca ratio across the neonatal line. Three teeth from two infants who were fully or predominantly formula fed from birth exhibit a positive daily rate of change in Sr/Ca ratio at around the time of birth, reflecting an increase in Sr/Ca ratio across the neonatal line. Teeth from child D, who was given breast milk for up to 6 weeks, and child F, who was fully formula fed within 10 days of birth, have daily rate of change in Sr/Ca ratio that is close to zero at around the time of birth, implying that there is no marked shift in Sr/Ca ratios across the neonatal line.

After birth, Sr/Ca values are predicted to increase with the introduction of complementary foods or during the transition from breast milk to formula (Fig. 2). Consistent with this prediction, the introduction of significant amounts of formula or complementary foods is associated with an increase in Sr/Ca ratio in the enamel of three of four breastfed children. The tooth from child A shows positive average daily rates of change between 109 and 131 days after birth with a peak at 128–131 days, indicating an increase in Sr/Ca ratio. This peak corresponds with the introduction of vegetable-based complementary foods ≈18 weeks after birth (≈126 days). Both teeth from child C show positive average daily rates of change during the third month of life (at 46–99 days and 64–99 days) with peaks at 67–76 days in the deciduous canine and 72–77 days in the deciduous second molar. The increase in Sr/Ca ratio is consistent with the introduction of infant formula as a complementary food at ≈2 months after birth. The highest daily increase in Sr/Ca ratio in the tooth from child D occurs at 27–35 days after birth. This peak is consistent with the transition from breast milk to infant formula between 5 and 6 weeks after birth. In these three children (A, C, and D) the start of the weaning process is indicated by a peak of positive values in average daily rate of change. There was no pronounced change in Sr/Ca ratio associated with the introduction of complementary foods in child B or in any of the children who were fully or predominantly formula fed from within a few days of birth.

Discussion

Analysis of the distribution of calcium-normalized strontium intensities revealed two underlying trends that are independent of reported dietary intake during the period of enamel formation and cannot therefore be accounted for by changing physiological concentrations in the body associated with changing diet. The first trend is a decrease in normalized strontium intensities from the EDJ toward the enamel surface, and the second is an increase from the occlusal aspect of the enamel crown toward the enamel cervix. These underlying trends are broadly consistent with the variation in mineral density in human tooth enamel, which in turn reflects variation in the degree of mineralization in different parts of the tooth crown (22–24). The proposed model for the differential incorporation of strontium and calcium during the secretory and maturational stages of enamel formation explains and is consistent with both of these underlying trends, because we predict a higher calcium signal and a lower Sr/Ca ratio in parts of the enamel that are more heavily mineralized. An important implication of this finding is that sampling location must be taken in account in the interpretation of Sr/Ca ratios in enamel, and that variation within a tooth (and

Fig. 4. Average daily rates of change in normalized strontium intensities in deciduous teeth from children A–D (A–D) and E–G (E–G). In cases where two teeth are shown (children C and G) the canine trend is in black and the molar trend is in gray. Days from birth are counted from the neonatal line, such that teeth from the same individual are matched according to the timing of the enamel secretion.

by implication variation between teeth that are sampled differently) can occur in the absence of any change in environmental input.

Changes in Sr/Ca ratios in enamel that reflect physiological or dietary shifts taking place at birth were found to coincide with the position of the neonatal line in seven of nine teeth examined. These signals are evident in the interpolated surface maps and average daily rates of change in Sr/Ca ratio. Teeth from three children who experienced exclusive breastfeeding during the first few weeks of life show an unambiguous breastfeeding signal, with a marked and consistent decrease in Sr/Ca ratios across the neonatal line. The tooth from child D, who was exclusively breastfed for only 5 or 6 weeks, does not show this breastfeeding signal, which might reflect the shorter duration of exclusive breastfeeding. Further work will be needed to ascertain the duration of exclusive breastfeeding required to produce a clear

breastfeeding signal. Teeth from two of three children who were fully or partially fed on infant formula from within a few days of birth show an unambiguous “formula signal” with a consistent increase in Sr/Ca ratios across the neonatal line.

After birth an increase in Sr/Ca ratios corresponding with the introduction of complementary foods or infant formula is observed in teeth from three of four children who were previously exclusively breastfed. There is no equivalent signal in teeth from children who were already fed on infant formula. Results indicate that the initial change from a maternally derived source of nutrition to a nonmaternal source could be identified by an increase in Sr/Ca ratio in enamel being secreted at that age in seven of the nine teeth examined. The rate at which complementary foods are introduced and the types of food given may be crucial parameters in determining whether such a signal is detected.

Table 1. Criteria used for the recognition of dietary transitions

Dietary transition	Primary criteria	Secondary criteria
Breastfeeding at birth	There is a clear trough in average daily rate of change in Sr/Ca ratio across the neonatal line.	Average daily rate of change is negative across the neonatal line.
Formula feeding at birth	There is a clear peak in average daily rate of change across the neonatal line.	Average daily rate of change is positive across the neonatal line.
Subsequent introduction of nonmaternally derived foods	Average daily rate of change shows a local and absolute peak.	Average daily rate of change is positive at the age at which this peak occurs.

Table 2. Dietary histories, changes in Sr/Ca ratio across the neonatal line and subsequent postnatal increases

Child	Infant diet	Tooth	Birth	Postnatal
A	Breastfed from birth. Complementary foods from ≈ 18 weeks.	Canine	Decrease	Increase at 109–131 days (peak 128–131)
B	Breastfed from birth. Complementary foods from ≈ 12 weeks.	Molar	Decrease	None
C	Breastfed from birth with formula given from ≈ 2 months after birth.	Molar	Decrease	Increase at 46–99 days (peak 72–77)
D	Breastfed with transition to formula at 5–6 weeks.	Canine	Decrease	Increase at 64–99 days (peak 67–76)
E	Exclusively formula fed from birth.	Molar	None	Increase at –60 to 61 days (peak 27–35)
F	Exclusively formula fed within a few days of birth.	Canine	Increase	None
G	Predominantly formula fed within a few days of birth.	Canine	None	None
		Molar	Increase	None
		Canine	Increase	None

The model presented here implies that a higher proportion of the strontium content of enamel relates to the secretory stage of enamel formation than would have been predicted on the basis of the amount of enamel mineral deposited during enamel secretion and maturation. Furthermore, this proportion is higher in areas of enamel that are less heavily mineralized because those areas contain more “strontium-rich” enamel produced by the secretory-stage ameloblasts and less “strontium-poor” enamel produced by the maturational-stage ameloblasts. Importantly, the model is unique for strontium and cannot be used directly to support the chronological interpretation of any other trace element or isotope distributions within enamel, although analogous models may be developed in the future. Our model and the results of our analysis of teeth from children of known dietary history indicate that it is possible to recover chronologically meaningful variation in normalized strontium intensities in enamel that can be used to explore the type of milk given at birth and the onset of weaning in breastfed infants. A blind test of this technique would provide further confirmation of this model.

The process of weaning is a crucial parameter in demographic and life history research because different nursing practices can have a significant impact on the health and survival of infants in a population. During weaning, infants may be exposed to new sources of infection associated with contaminated foods and they become more susceptible to pathogens because they no longer receive passive immunity from their mothers (29, 30). Some infants may be weaned onto a diet that is nutritionally inadequate. These factors may cause delayed development, growth deficits, and increased mortality and morbidity, which, in turn, will be reflected in the health and demography of a population (31). Lactation is a significant component of maternal investment and is directly related to interbirth interval because of its effect on ovulatory suppression (32). The age at which complementary foods are introduced and rate at which breast milk is replaced are therefore important constraints on fertility and population growth.

The technique presented here will enable detailed investigations of changes in weaning behavior during human evolution and in association with major cultural and demographic transitions, including, for example, the shift from hunter–gatherer to agriculturalist ways of life, and changes in labor patterns and social organization that accompanied the industrial revolution. Importantly, this technique can be used to track dietary changes during infancy on an individual basis and will facilitate further research directions, including the relationship between infant diet and developmental indicators such as growth attainment, enamel growth disruptions, and childhood illness within populations.

Materials and Methods

Nine deciduous canines or second molars from seven healthy European children were used for this study (Table 2). All teeth were naturally shed and

caries-free. Information concerning diet in the first few months after birth was provided retrospectively by the mothers. The sample includes teeth from individuals who experienced a range of diets during the first 3 months of life. Three types of infant diet can be distinguished: (i) exclusive prolonged breastfeeding, (ii) exclusive or predominant formula feeding from birth, and (iii) a period of exclusive breastfeeding followed by partial or complete replacement of breast milk by formula.

Each tooth was sectioned longitudinally by using a slow-speed rotating Isomet (Buehler) diamond saw and one cut face polished with 3- μ m aluminum oxide power. The polished block face was fixed with epoxy resin adhesive to a glass microscope slide under pressure and a buccolingual section through the mesial cusps of the tooth, ≈ 100 μ m thick cut from the block together with the slide. The section was lapped planoparallel and polished. Using polarized light, a low-power ($\times 80$) digital photographic record of the prism paths in the buccal or lingual aspects of the crown and of the major long-period incremental chronology of the enamel markings was made. The neonatal line was used to identify the portion of enamel formed postnatally and to form a baseline for establishing a chronological framework for sampling within the enamel crown. Discrete samples of enamel were ablated at regular intervals along trajectories running from the EDJ to the enamel surface with an orientation approximately parallel to the direction of the enamel prisms. Rows of ablation points were spaced along the entire length of the tooth. After laser ablation, a higher-power ($\times 25$ objective) digital montage of the enamel containing the ablation pits was constructed. All ablation points in the crown were chronologically aged with respect to birth by tracking daily counts along prisms to each ablation point and extending down through the enamel along accentuated long-period incremental markings and continuing the counts out along new prism paths to each ablation point in turn in the chronological sequence.

LA-ICP-MS analysis was undertaken at the Natural History Museum by using a New Wave Research UP213 aperture-imaged, frequency-quintupled

Table 3. Analytical and instrumental operating conditions

ICP-MS	
Forward power	1,350 W
Gas flows	
Coolant	Ar: 13 liter·min ^{−1}
Auxiliary	Ar: 0.9 liter·min ^{−1}
Sample transport	He: 0.8 liter·min ^{−1} , Ar: 0.8 liter·min ^{−1}
Laser	
Wavelength	213 nm
Pulse width (FWHM)	3 ns
Pulse energy	0.05 mJ per pulse
Energy density	3 J cm ^{−2}
Energy distribution	Homogenized, flat beam, aperture imaged
Repetition rate	10 Hz
Spot size diameter	30 μ m
Analysis protocol	
Scanning mode	Peak jumping, 1 point per peak
Acquisition mode	Time-resolved analysis
Duration of analysis	60 s background, 60 s ablated signal
Dwell times	10 ms all masses

Nd:YAG laser ablation accessory operating at 213 nm coupled to a Thermo Elemental PlasmaQuad 3 quadrupole-based ICP-MS with enhanced sensitivity (s-option) interface. Helium was used as a sample carrier gas and mixed with argon before the torch. Discrete 30- μ m-diameter areas of the sectioned tooth were sampled during analysis. For each analysis data were collected for 120 s. During the first $\approx$ 60 s of collection, background data in the form of gas blank and electronic noise were acquired. The laser was then fired at the sample, and data were acquired for an additional $\approx$ 60 s. Background data were subsequently subtracted from the ablation signal in the tooth. Data were collected in discrete runs of 20 analyses, beginning and ending with two analyses of the National Institute of Standards Technology (NIST) standard reference material SRM NIST612. Initial data processing and reduction, including selection and integration of background and ablated signal intervals, were performed off-line by using LAMTRACE, a Lotus 123 macrobased spreadsheet created by Simon Jackson, Macquarie University, Sydney. Further data processing, including normalization of $^{88}\text{Sr}/^{43}\text{Ca}$ ratios based on the measured ratio in NIST612,

was performed with Microsoft Excel spreadsheet software. Analytical and instrumental operating conditions are presented in Table 3.

Data interpolation and elemental mapping were used to visualize trace element distributions. Enamel cusp boundaries, neonatal lines, and normalized elemental intensities were digitized and imported into ArcGIS 9.2. Inverse distance weighting (IDW) was used to produce interpolated surface maps of normalized trace element intensities within the enamel cusp boundary (33, 34). To optimize the IDW interpolation and account for directionality within the data we restricted the search area to an ellipse with a length of 2 and a width of 0.5 oriented along the main axis of the tooth. The ellipse was divided diagonally into four sectors with a maximum of nine and minimum of three data points required for each sector.

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