Evidence for long-term averaging of strontium in bovine enamel using TIMS and LA-MC-ICP-MS strontium isotope intra-molar profiles

Janet Montgomery[a*], Jane A. Evans[b] and Matthew S.A. Horstwood[b]

[a] Division of Archaeological, Geographical and Environmental Sciences, School of Life Sciences, University of Bradford, BD7 1DP, UK.

[b] NERC Isotope Geosciences Laboratory, British Geological Survey, Nottingham, NG12 5GG, UK

*Corresponding author: Division of Archaeological, Geographical and Environmental Sciences, School of Life Sciences, University of Bradford, BD7 1DP, UK. Tel: +44(0)1274236551; Fax No: +44 (0) 1274 235190

E-mail address: J.Montgomery@bradford.ac.uk (J. Montgomery)

Abstract

High spatial resolution micro-sampling of tooth enamel offers the possibility of high temporal resolution isotope data to reconstruct climate, environment, diet and mobility. Questions remain about the duration and pattern of the maturation phase of enamel and the existence and direction of chronological "time-lines". LA-MC-ICP-MS measurements of c. 400 μm craters and TIMS analyses of transverse enamel sections of an archaeological bovine third molar were undertaken to investigate the long-term averaging of incorporated strontium. The same gradually increasing isotope profile was obtained from both approaches, indicating that the large increase in spatial resolution did not change the response profile obtained. The results suggest that even at the microscopic scale, strontium is incorporated over a period in excess of 12 months. Averaging of the input signal may result from both long-term retention of strontium in the skeleton and recirculation in the body pool, or long-term maturation of enamel on a microscopic scale. Whichever mechanism is responsible, it may not be possible to recover strontium isotope ratios with a high time resolution from cattle molar enamel unless there is a large imbalance in the amount of strontium supplied by different sources. Consequently, strontium isotope profiles may not be synchronous with those of lighter isotope systems.

Keywords: biomineralization; *Bos taurus*; enamel; LA-MC-ICP-MS, TIMS; strontium isotopes.

Introduction

High spatial resolution micro-sampling techniques for human and animal teeth are of interest to archaeologists and palaeontologists because they offer the promise of diachronic isotope data with a high temporal resolution that can be used to reconstruct changes in climate, environment, diet and mobility over periods of, perhaps, days in the life of the subject (Sponheimer *et al.* 2006). For this to be achieved, it requires a material in which a chronological "time-line" is present coupled with a sampling technique that can obtain high-precision isotope data from very small samples. In-situ laser ablation analysis clearly satisfies the second of these requirements, but whilst there is little argument that enamel matrix is *secreted* with a known high-resolution periodicity (Hillson 2005), there is equally little evidence that the progression of enamel *mineralization* follows matrix deposition either temporally or spatially (Suga 1982; Fisher & Fox 1998; Hoppe *et al.* 2004; Tafforeau *et al.* 2007).

The lightly-mineralized enamel matrix of hypsodont cattle molars is deposited in c. 100 um wide, overlapping, sleeve-like imbricational layers from cusp to the cervix, each individual layer extending from, and almost parallel to, the enamel dentine junction and terminating at the enamel surface (Hillson 2005). This implies that a line drawn from cusp to cervix and crossing each of these layers in turn, would represent a chronological time-line over the mineralization period of the tooth crown, which for the cattle tooth studied here is approximately one year. However, it is by no means clear that the subsequent maturation of the enamel, when the bulk of the mineral ions are incorporated, follows the same pattern or time scale. Over forty years ago, Brown *et al.* (1960, 9) noted that whilst the incremental lines of Retzius represented matrix

deposition, the *"final calcification of the enamel matrix begins at the incisal or cuspal tip when the full width of the enamel has been formed and proceeds in planes at right angles to the long axis of the tooth"*. Suga (1982; 1989) proposed that the maturation of the initial enamel matrix progressed in three further discrete waves of secondary (from the enamel surface inwards), tertiary (from the deeper layers outwards) and quaternary (the surface layer) mineralization making the chronological relationship between the incremental lines and the isotopic signal recorded in the enamel mineral even more tenuous. Moreover, he concluded that *"the progressive mineralization pattern is completely different between the matrix formation and maturation stages"* (Suga 1989, 194) and that this observation was true for all the animals studied to date. Deutsch *et al.* (1979) concluded that in deciduous bovine incisors, the length of time required for enamel maturation varied within a single tooth, with incisal enamel taking twice as long to mature as cervical enamel and therefore open to mineral incorporation for far longer. Fisher and Fox (1998) reported a 9-12 month lag in the seasonal oxygen isotope profile obtained from the surface enamel in mammoth teeth compared to that from co-genetic incremental tusk dentine. Hoppe *et al.* (2004) found that the duration of enamel maturation in horse molars and premolars was not linear along the tooth length and lagged behind matrix deposition by several weeks to months. Similarly, in their recent study of large herbivore enamel mineralization, Tafforeau *et al.* (2007, 217) concluded that *"lines of iso-density do not correspond to the developmental pattern of the matrix. Therefore, matrix formed at different times may exhibit similar maturation levels and matrix formed at the same time may exhibit variable maturation levels"*.

Thus, the time-line of mineral accumulation in mature enamel does not closely follow the time-line of organic deposition or a simple progressive cusp to cervix maturation, or at least, not in a straightforward chronological manner. Before diachronic strontium isotope information can be interpreted correctly and rigorously, we need to know the length of time that mineralization takes to complete, whether enamel simply records a running average throughout its depth and across its surface even at the microscopic scale, and if, where, and in what direction, the passage of time is recorded.

Nonetheless, oxygen isotope profiles have been obtained from herbivore intra-enamel serial sections and interpreted as reflecting temporal changes of the input signal, for example, seasonal variation in temperature (e.g. Fricke and O'Neil 1996; Fricke *et al.* 1998; Kohn *et al.* 1998; Zazzo *et al.* 2002; Balasse *et al.* 2003; Balasse *et al.* 2006; Wiedemann-Bidlack *et al.* 2008). Consequently, the chronological integrity of oxygen isotope information appears to be retained in large herbivore molars, although the primary signal is attenuated and averaged during the maturation phase and, given minimum enamel sample sizes necessary for isotope analysis by non-laser sampling methods, temporal resolution of a transverse enamel sample is, at best, six months (Balasse 2002; Passey and Cerling 2002; Kohn 2004; Zazzo *et al.* 2005; Zazzo *et al.* 2006). Kohn (2004) points out that as a consequence of this, cattle are unlikely to be good recorders of high resolution seasonality and short-term change.

To improve the time resolution of the measured signal it would make intuitive sense to reduce the volume and area of enamel sampled in each discrete analysis (Zazzo *et al.* 2005) but this can be constrained by the amount of tissue required for conventional isotopic analyses, particularly for strontium, and is a strong driver for the use of

higher spatial resolution methods such as laser-ablation (LA) sampling. The question remains, however, that even if the sample size can be reduced to the size of a small crater, i.e. *c.* 100 μm which would permit the analysis of individual perikymata, would that increase the temporal resolution of the isotope signal or, as suggested by Zazzo *et al.* (2005), is there little point sampling at a greater resolution than that at which the input signal is recorded? The aim of this study, therefore, is to investigate whether the gradient and shape of the enamel strontium isotope profile obtained from a cattle molar changes if the sample volume is reduced.

Materials and methods

To investigate whether reducing the sampling volume would increase and thus improve the time resolution of the measured strontium isotope profile, intra-tooth profiles were obtained from the same tooth using two methods: the well established method of thermal ionisation mass spectrometry (TIMS); and the relatively new high resolution method of laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS). A third molar was removed from a bovine mandible recovered from a pit of cattle bones surrounding an Iron Age chariot burial at Ferry Fryston, West Yorkshire (Brown *et al.* 2007). The crowns of mandibular third molars of modern cattle begin to form between 9 and 13 months of age and crown mineralization is completed approximately one year later (Brown *et al.* 1960). Clearly, differences between modern cattle and those living 2000 years ago may be considerable, but Brown *et al.* (1960, 33) found that modern cattle show *"No significant differences in the chronology of tooth development in the different breeds*

or sexes … in spite of relatively large developmental and genetic differences between dairy and beef cattle". The Ferry Fryston mandibular third molar chosen for analysis had intact cusps that showed evidence for only minor wear to the cusps of the mesial unit, minimal root formation and was in the final stages of crown mineralization with approximately 10 mm of incompletely mineralized cervical enamel visible on the distal unit (Figure 1). It is assumed, therefore, that strontium deposition was actively occurring in the cervical enamel of the distal unit at the time of death.

Adhering cementum was removed from the buccal surface of the fully mineralized mesial unit and the enamel surface abraded with a tungsten carbide dental burr (Figure 1). A longitudinal section from cusp to cervix was removed from this unit with a circular dental saw (Figure 2). All dentine adhering to the inner surface was removed with the dental burr: dentine is known to be highly susceptible to strontium uptake from the soil during burial which may alter the biogenic strontium isotope ratios (Hoppe *et al.* 2003; Trickett *et al.* 2003). The resulting enamel strip was bisected longitudinally. One half was sectioned transversely from cusp to cervix to produce 13 c.2mm sections which were dissolved and chemically separated for measurement by TIMS (Figure 2). The remaining half was measured by LA-MC-ICP-MS using c. 400 μm craters (samples 1-17). In addition, three discrete 2mm wide enamel sections (cusp, middle and cervix) were removed for TIMS from the lingual side of the third mandibular molar (M3) mesial unit (samples 18-20) to investigate whether differences were present between the opposing faces of the tooth unit, and a sample of dentine (sample 21) was taken to establish the diagenetic strontium vector (Montgomery *et al.* 2007). Finally, three enamel samples (cusp, middle and cervix) were measured by TIMS from the second mandibular molar (M2) (samples 22-24)

which commences mineralization around the age of birth and completes around the age of one (Brown *et al.* 1960) to investigate if the $^{87}Sr/^{86}Sr$ ratio changed during the first year of life.

Transverse sections of enamel (*c.* 30 mg) were dissolved under clean chemical conditions in a class-100 facility using high-purity reagents and Teflon labware. Strontium was chemically purified using Dowex© ion exchange resin and analysed by thermal ionisation mass spectrometry (TIMS) using a single (Re) filament and a TaF activator to enhance sensitivity and signal stability (after Birck (1986)). Long-term precision of this method at NIGL is demonstrated through the analysis of international reference material NBS987 which for static analysis gave a mean $^{87}Sr/^{86}Sr$ ratio = 0.710263 ± 0.000008 (n=50, 2σ). All session data were normalized to the reference material accepted $^{87}Sr/^{86}Sr$ ratio of 0.710250.

Laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICP-MS) analyses utilised a solid state 193nm laser ablation system, a 100μm ablation spot size and a dynamic (raster) ablation protocol to ablate a 425μm x 370μm crater. Analysis consumed 13-24μg of material (c.7-12ng Sr, assuming a concentration of 500ppm Sr), whilst ablating to a depth of 30-45μm. A total of seventeen analyses along the longitudinal enamel slice were spatially calibrated relative to the TIMS analyses using the co-ordination system of the laser ablation software and a handheld micrometer. Further details of the analytical approach, data correction procedures and uncertainty propagation protocol, are given in Horstwood *et al.* (2008).

Results

Comparison of the results from LA-MC-ICP-MS and TIMS

The LA strontium isotope ratios increase progressively from 0.7158 to 0.7187 from the cusp to the cervix of the bovine third molar (Figure 3). A comparable gradient and range of ratios from 0.7153 to 0.7183 were obtained from transverse enamel sections measured by TIMS. Although the TIMS ratios for most samples were lower than those obtained by LA, they are within analytical uncertainty and therefore indistinguishable using these techniques. Any remnant discrepancy likely represents small inaccuracies in the values used for the multiple compound corrections required in the laser ablation data.

None of the final ratios derive from the Magnesian Limestone from which the tooth was excavated, i.e. ≤0.7086 (McArthur *et al.* 2001). In the last cervical enamel sample, the ratio appears to be falling. This may indicate the consumption of food from an unradiogenic biosphere, such as limestone pasture, immediately prior to death. Unfortunately, because the cervical enamel was not fully mature, the incorporation of strontium from the limestone burial soil cannot be ruled out; this process is likely to explain the much lower strontium isotope ratio of 0.7137 obtained from the dentine (Figure 4). With the exception of Ferry Fryston, there are currently no strontium isotope ratios above 0.7150 published for animals or humans excavated from the sedimentary lithologies of England and they are only rarely found in Europe (e.g. Åberg *et al.* 1998; Schweissing and Grupe 2003; Price *et al.* 2004; Bentley *et al.* 2004; Bentley and Knipper 2005; Price and Gestsdottir 2006). One explanation for

this lack of comparable radiogenic samples, is that the granitic rocks required tend to produce acidic soils in which skeletal remains do not survive and this introduces an unavoidable bias into the archaeological sample. It is likely that the only time humans or animals from such regions will be found is if they are buried in alkaline soils such as those hosted by limestones or chalks or contained within voids such as stone cists.

The change in the strontium ratio from cusp to cervix is large and indicates a change, or changes, in the source of the food consumed by the bovine during the year the third molar crown was mineralizing. There is a presumption that the oxygen isotope profile within herbivore molars will reflect seasonal changes in temperature and that transhumance where animals are moved to different geological terrains could produce a similar seasonal strontium isotope profile (Balasse *et al.* 2006; Meiggs 2007). There is no evidence from the third molar, which takes approximately a year to mineralize, that the signal was sinusoidal and thereby suggestive of transhumance as has been found in other intra-enamel studies of archaeological cattle (Bentley and Knipper 2005). Moreover, as Figure 4 shows, the strontium isotope ratios obtained from the earlier forming second molar suggest that the third molar cuspal ratio of ~0.7153 is not the starting point of the rise in the measured signal but is a ratio produced through mixing of the two end-members (~0.7147 and $\geq$ 0.7183) and is, therefore, not directly indicative of place of origin. The strontium isotope profile in Figure 4 does not suggest seasonal movement but a process that occurred only once between birth and two years of age when the third molar crown was complete. Similar linear changes have been found in cattle teeth in other studies (Balasse *et al.* 2002; Towers *et al.* 2009.) It is difficult to identify a husbandry regime that can explain such a changing isotope profile if it signifies a true chronological record of real-time dietary change

over the period of two years. Possible, but unlikely, scenarios could be: carefully controlled, progressive supplementation of the diet with imported fodder, for example hay supplementation of grass-based grazing; or that the animal kept moving slowly for over a year grazing increasingly radiogenic pastures. Granitic rocks are the most likely source of the radiogenic strontium obtained but unlike sedimentary sequences, the geological boundaries of granites are far more likely to be abrupt and it is difficult to make the case for a geographic gradation in biosphere strontium when there is no realistic geological foundation for such a model.

The simplest model that would account for this is binary mixing of two end-members (Montgomery *et al.* 2007) contributing $^{87}Sr/^{86}Sr$ of ~0.7147 (TIMS end-member 1) and ≥ 0.7183 (TIMS end-member 2). That is, the bovine was eating food that supplied a strontium isotope ratio of ~0.7147 shortly after birth and subsequently changed to a food that supplied a strontium isotope ratio of ≥ 0.7183. For prehistoric cattle such as the one in this study, we propose the most likely mechanism is a single change of residence that occurred around the age of one before mineralization of the second molar crown was complete.

Binary end-member mixing

There are currently no published studies pertaining specifically to strontium that show how known abrupt or gradual changes of diet or residence are manifested in the enamel of herbivore teeth. Several light isotope studies have concluded that cattle are not good recorders of high resolution seasonal information due to the long (e.g. 6 months) maturation time of their enamel (Balasse 2002; Passey and Cerling 2002;

Zazzo *et al.* 2005; Zazzo *et al.* 2006). For example, in a landmark study of modern cattle transferred abruptly from a C_3 diet to a mixed C_3/C_4 diet at ~10 months of age, Balasse (2002) showed that the introduction of C_4 plants was first noticeable in the carbon isotope ratios of the cuspal third of the second molar enamel, and was the sole source of the signal in the cervical third. As the second molar is mineralized over most of the first year of life, it is apparent that the input signal is contributing to the measured signal in enamel that commences mineralization several months before the input signal changed. Clearly, as Balasse (2002, 162) concludes, *"when determining the age at which a change of diet occurred, this should not be directly inferred from the location in the tooth where the isotopic changes first occur, because the formation of that part of the tooth may have started before the diet changed".* The TIMS enamel strontium results obtained from the Ferry Fryston cattle (Figure 4), which are from transverse slices cutting across the incremental growth lines and are likely to contain enamel mineralized over several months (Suga 1989; Passey and Cerling 2002), suggest that strontium from both end-members is being deposited in the cervical half of the second molar and throughout the year of third molar enamel mineralization. This implies that the change in input signal occurred before maturation of the second molar was complete, i.e. before one year of age. One possible explanation for a change at this time could be separation from the mother and transportation to a different locality following weaning which could occur between six to ten months of age (Balasse and Tresset 2002).

For end-member mixing to persist in enamel that mineralized over a period of more than one year even allowing for non-linear rates and timing of enamel matrix formation and maturation, both sources of strontium must be available to be

incorporated into the enamel. Mechanisms which could produce a long residence time of an end-member are: very slow maturation of enamel, i.e. > one year; samples such as bulk transverse slices containing enamel mineralized over most of the crown mineralization period; or a long residence time of strontium in the body pool through recirculation of skeletal strontium which attenuates the input signal. Enamel maturation of one year is considerably longer than the six months estimated from stable light isotope studies, but in agreement with the recent work on rhinoceros enamel by Tafforeau *et al.* (2007) who concluded that maturation of enamel at a single point was 1.15 years and that for the teeth of large herbivores *"it seems impossible to obtain real high-resolution isotopic records from mammalian tooth enamel due to the time averaging linked to the maturation process"* (Tafforeau *et al.* 2007, 224).

Using the two-component mixing equation 13.5 in Faure (1986, 229) and assuming both end-members could produce the same enamel strontium concentration (i.e. 172 ppm – the mean concentration), the cuspal isotope ratio of 0.7153 in the third molar would result from 83% of end-member 1 (~0.7147) and 17% of end-member 2 (≥ 0.7183). An alternative scenario assuming a concentration of 151 ppm is contributed from end-member 1 and 218 ppm from end-member 2, the percentages are 88% of 1 and 12% of end-member 2. There is a positive correlation between enamel concentration and isotope ratio in the third molar (r^2 = 0.69), but given the multiplicity of dietary factors that can impact on the amount of strontium that is eventually deposited in enamel we do not consider the *c.* 88 ppm range in concentrations great from a biological reproducibility viewpoint (for example, compare the concentration difference between samples 1 and 18 or 15 and 16 in Table 1). However, the complex relationship between enamel strontium concentrations and

different types of food, calcium status, geology and environment warrants further study.

Comparison of sample sizes and implications for the time-resolution represented

If the enamel volumes containing end-member 1 and end-member 2 are spatially separate within the enamel, for example, within different imbricational layers, it should be possible to increase the resolution of the measured signal and reduce the attenuation through averaging by reducing the sample size (Passey and Cerling 2002). LA analysis has the potential to reduce the sampled volume to that of a shallow crater ~100 μm in diameter which is comparable to the width of individual cattle perikymata, which are ~100 μm through most of the crown height (Hillson 2005, 163). However, although the volume sampled here by laser ablation was larger (c.13-24 μg) this is still significantly smaller than the sample volume for the TIMS methodology used (c. 30 mg) and there is no such increase in resolution apparent between the two data sets (Figure 3). Both produce the same strontium isotope gradient within uncertainty and the laser ablation profile does not define a steeper shift towards end-member 2 which might be predicted if the smaller sampling volume increased the time resolution of the measured signal. Indeed, the same profile could be constructed by only three TIMS analyses from the cusp, middle and cervix (Figure 4). If the volume of enamel analysed from a surface crater provides the same strontium isotope ratio as the comparatively large, transverse enamel section beneath, it suggests that the decrease in sample volume is producing no increase in the resolution of the measured signal and whatever processes are creating the mixing of the two end-members, they are taking place on a scale far smaller than individual perikymata.

Discussion

Averaging of the strontium isotope signal on both the macro- and micro-scale as a result of mineralization processes

According to Suga's (1989) four-wave model of enamel mineralization, the final, quaternary, wave of mineralization is the rapid mineralization of the thin surface layer of enamel. In the cattle tooth analysed here, this thin layer was removed when the enamel sample was prepared for analysis with a tungsten carbide dental burr. However, these "waves" of mineralization do not explain how maturation proceeds on the microscopic scale. A tooth's final dimensional parameters of enamel thickness and volume appear to be established during secretion of the protein matrix when very thin enamel crystallites grow from the enamel-dentine junction to the tooth surface (Robinson *et al.* 1981; Fincham and Simmer 1997; Smith 1998). This immature, organic-rich enamel is thus "formed", i.e. its final dimensional parameters of thickness and volume are established (Smith 1998, 131), but it is only lightly mineralized. The resulting tissue is, in effect, a plantation of tall thin saplings encased within a transitory protein framework. The protein "scaffold" guides the nucleation and elongation of the enamel crystallites and is eventually resorbed during the transitional phase (*contra* dentine where it mineralizes *in situ*) to permit the enamel crystallites to expand widthways during the maturation phase and occlude the space previously occupied by the organic matrix (Mann 1997; Smith 1998; Fincham *et al.* 1999). As the organic matrix is resorbed, individual crystallites expand in a similar manner to the growth of tree rings with the first enamel to form at the very centre of

each mature enamel crystal (Boyde 1997, 18). During the maturation stage they may reach the 26nm thick x 68nm wide dimensions of mature crystals, and may extend throughout the full thickness of the enamel (Simmer and Fincham 1995). Approximately 1000 carbonate hydroxyapatite crystals are organised into each 5 um diameter enamel prism, their spiralling paths within the prism echoing the individual fibres of a twisted rope. Each prism is one of many that form the densely packed and highly organised array that constitutes the bulk of the mature tissue and gives it its strength.

No analytical technique can currently obtain strontium isotope data at the scale of individual enamel crystals and a laser ablation crater, even at 100 μm in diameter, is sampling, and thus averaging, strontium incorporated into each individual crystal over the whole maturation of that crystal. A diagram to visualise how binary mixing in enamel can occur at the microscopic scale and result in similar average values in samples of very different sizes is illustrated in Figure 5. Sleeve-like imbricational lines within the enamel represent the direction of the brown striae of Retzius which crop out at the enamel surface to produce perikymata. A transverse section for TIMS may cut through many of these layers. In cattle enamel, perikymata are approximately 100 μm apart (A) but to aid clarity are not shown to scale. Enamel prisms, shown in A in Pattern 2 form, are approximately 10 μm wide (B) and each prism is composed of many individual enamel crystallites 68 nm wide (C). On the right, the decreasing percentage of end-member 1 (black) and concomitant rise in end member 2 (white) is illustrated in individual crystallites along with the approximate position where each mixture was found on the Ferry Fryston M3 by both LA and TIMS. 25% corresponds to density estimates of the initial lightly mineralized enamel prior to the removal of

the organic matrix scaffold and the width-wise expansion of individual crystallites. Mixing resulting from slow crystal growth throughout the 12 months of crown development would explain the concordance between strontium isotope ratios from 400 μm diameter surface LA craters and underlying TIMS sections.

Averaging as a result of long term residence of strontium in the body pool

No level of sampling resolution will extract discrete short-term movement if the strontium isotope ratio is already an average of several months or even years of strontium ingestion *before* it is incorporated into the enamel. Such a reservoir effect was postulated to explain a similar intra-enamel gradient in a bovine M2 from South Africa (Balasse *et al.* 2002). There is little data on the residence time of skeletal strontium in cattle, but data exist for humans that demonstrate long-term residence of heavy "bone-seeking" elements, possibly as a result of storage and recycling by the skeleton through such processes as calcium homeostasis. Residence times of different elements in the body can vary considerably and will be dependent on bone turnover rates, calcium intake, age and health status (Papworth and Vennart 1984). For example, Gulson *et al.* (1999) showed that 50% of the lead circulating in the blood of pregnant women resident in Australia had been remobilised from old skeletal stores deposited prior to their migration to Australia. Strontium, like calcium, has a long residence time of 800 to 1600 days compared to 14 days for water, and studies using radioactive tracers suggest retention after 400 days can still exceed 10% of the original dose (Bowen 1979; Dahl *et al.* 2001). Elimination rates from bone can be age and sex dependent but rarely exceed 6% per year (Degteva and Kozheurov 1994; Tolstykh *et al.* 1997). Moreover, strontium incorporated by heteroionic substitution

into deep cortical bone during modelling will have a longer residency time than strontium in exchangeable pools of bone that participate in calcium homeostasis, e.g. bone surfaces, and will take longer to remobilise than that incorporated by a fully grown adult subject only to the processes of remodelling and surface exchange (Leggett *et al.* 1982; Dahl *et al.* 2001). Although the residence time of strontium in the skeleton of growing animals, such as the bovine investigated in this study, may be shorter due to a highly vascular and chemically active skeleton (Leggett *et al.* 1982), the results from humans imply that bone strontium incorporated into the skeleton during the first year of the calf's life will still be recirculating in the body and thus available for incorporation into tooth enamel over one year after initial ingestion. Further, it is possible that elements such as calcium, strontium and lead may be circulating in the body for a far greater time than lighter elements such as oxygen and carbon. Such a reservoir effect, may account for the discrepancy in estimates of the duration of enamel mineralization in this study and other light isotope studies. If residence times are different for different elements, it is possible that intra-enamel isotope profiles cannot be simplistically compared because they may not be synchronous.

Implications for long-term averaging of the measured signal

Whether the averaging is produced at a microscopic scale within the enamel, or within the body pool prior to deposition in the enamel, is difficult to determine with current analytical capabilities. Either scenario will attenuate the input signal obtained from bovine enamel and in migratory cattle will result in strontium isotope ratios that are a mixture of two or more end-members rather than discrete ratios characteristic of a

single lithology. How long before a change in residence becomes evident within the enamel will depend on various biological factors but because strontium is not an essential element and the amount deposited in skeletal tissue reflects how much is ingested, the averaging process will also be sensitive to the relative concentrations of strontium and calcium in the diet (Knight *et al.* 1967; Boivin *et al.* 1996; Meiggs 2007). If the strontium concentrations of the enamel are very similar, as found in the tooth in this study, it appears that the tissue may take a year to fully reflect a change from one locality to another. However, if there is a large imbalance between the *amount* of strontium metabolized from the two end-members, one may endure for longer or start to dominate the enamel ratio sooner. This suggests that how quickly a change in residence is apparent in the enamel may vary depending on the individual circumstances such as location, geology, and type of food, and the subsequent dietary and health status of the animal whilst the tooth enamel matures. Clearly, studies of modern migratory cattle of known origin, diet and residence are required if any of these variables are to be unravelled. Moreover, dentine could be sampled in modern teeth where it cannot be used in archaeological specimens due to diagenesis. The far quicker mineralization of dentine compared to enamel (Hillson 2005) could resolve the problem of distinguishing averaging due to the body pool from averaging as a product of lengthy maturation.

Conclusions

The equivalence of intra-enamel strontium isotope ratio profiles obtained from c.400 μm surface craters and transverse slices through the full width of the enamel, strongly suggests that both sample sizes contain strontium of a composition that represents many months of ingestion. This could result from a lengthy maturation process occurring in a spatially diffuse manner at the microscopic scale or long term residence of strontium, possibly buffered from skeletal stores, circulating in the body pool prior to deposition in the enamel. Both mechanisms may contribute, with their relative contributions varying depending on factors such as age, metabolic status and calcium intake. Neither scenario is likely to allow high-resolution temporal reconstruction of the input signal even with laser ablation sampling, unless there is a large imbalance between the amount of strontium supplied by the two end-members. The long residence time of the strontium end-member suggests that strontium is incorporated into third molar cattle enamel over a period of at least one year, even at the level of individual perikymata or incremental lines of Retzius. This is longer than the six months estimated for large herbivores such as cattle from stable light isotope data (Balasse 2002; Passey and Cerling 2002; Zazzo *et al.* 2005; Zazzo *et al.* 2006) but in agreement with the estimate of 1.15 years obtained from rhinoceros enamel by Tafforeau *et al.* (2007). This observation suggests that the intra-enamel isotope profiles obtained from a bovine molar for strontium and light elements such as carbon and oxygen may not be synchronous.

Acknowledgements

We are grateful to Angela Boyle at Oxford Archaeology for providing the cattle teeth from the Ferry Fryston chariot burial. Andrew Gledhill, Jacqueline Towers and Professor Jennifer Kirkham kindly commented on earlier drafts of this paper. The manuscript was considerably improved by the suggestions of an anonymous reviewer to whom we are indebted.

References

Åberg, G., Fosse, G. and Stray, H. 1998. Man, nutrition and mobility: a comparison of teeth and bone from the medieval era and the present from Pb and Sr isotopes. *The Science of the Total Environment* **224**, 109-119.

Balasse, M. 2002. Reconstructing dietary and environmental history from enamel isotopic analysis: Time resolution of intra-tooth sequential sampling. *International Journal of Osteoarchaeology* **12**, 155-165.

Balasse, M. and Tresset, A. 2002. Early weaning of Neolithic domestic cattle (Bercy, France) revealed by intra-tooth variation in nitrogen isotope ratios. *Journal of Archaeological Science* **29**, 853-859.

Balasse, M., Ambrose, S. H., Smith, A. B. and Price, T. D. 2002. The seasonal mobility model for prehistoric herders in the South-western Cape of South Africa assessed by isotopic analysis of sheep tooth enamel. *Journal of Archaeological Science* **29,** 917-932.

Balasse, M., Smith, A. B., Ambrose, S. H. and Leigh, S. R. 2003. Determining sheep birth seasonality by analysis of tooth enamel oxygen isotope ratios: The late

stone age site of Kasteelberg (South Africa). *Journal of Archaeological Science* **30**, 205-215.

Balasse, M., Tresset, A. and Ambrose, S. 2006. Stable isotope evidence (d13C, d18O) for winter feeding on seaweed by Neolithic sheep of Scotland. *Journal of Zoology* **270**, 170.

Bentley, R. A. and Knipper, C. 2005. Transhumance at the early Neolithic settlement at Vaihingen (Germany). *Antiquity* **79**(306), http://antiquity.ac.uk/ProjGall/306.html.

Bentley, R. A., Price, T. D. and Stephan, E. 2004. Determining the 'local' Sr-87/Sr-86 range for archaeological skeletons: a case study from Neolithic Europe. *Journal of Archaeological Science* **31**, 365-375.

Birck, J. L. 1986. Precision K-Rb-Sr isotopic analysis - application to Rb-Sr chronology. *Chemical Geology* **56**(1-2), 73-83.

Boivin, G., Deloffre, P., Perrat, B., Panczer, G., Boudeulle, M., Mauras, Y., Allain, P., Tsouderos, Y. and Meunier, P.J. 1996. Strontium distribution and interactions with bone mineral in monkey iliac bone after strontium salt (S 12911) administration. *Journal of Bone and Mineral Research* **11**, 1302-1311.

Bowen, H. J. M. 1979. *Environmental chemistry of the elements*. London: Academic Press.

Boyde, A. 1997. Microstructure of enamel, pp.18-31 in Chadwick, D.J. and Cardew, G. (eds.), *Dental Enamel. Proceedings of the Ciba Foundation Symposium 205.* Chichester: John Wiley.

Brown, F., Howard-Davis, C., Brennand, M., Boyle, A., Evans, T., O'Connor, S., Spence, A., Heawood, R. and Lupton, A. 2007. *The Archaeology of the A1*

(M) Darrington to Dishforth DBFO Road Scheme. Lancaster: Oxford Archaeology North.

Brown, W. A. B., Christoffersen, P. V., Massler, M. and Weiss, M. B. 1960. Postnatal tooth development in cattle. *American Journal of Veterinary Research* **XXI**, 7-34.

Dahl, S. G., Allain, P., Marie, P.J., Mauras, Y., Boivin, G., Ammann, P., Tsouderos, Y., Delmas, P. D. and Christiansen, C. 2001. Incorporation and distribution of strontium in bone. *Bone* **28**, 446-453.

Degteva, M. O. and Kozheurov, V. P. 1994. Age-dependent model for strontium retention in human bone. *Radiation Protection Dosimetry* **53**, 229-233.

Deutsch, D., El-Attar, I., Robinson, C. and Weatherell, J. A. 1979. Rate and timing of enamel development in the deciduous bovine incisor. *Archives of Oral Biology* **24**, 407-413.

Faure, G., 1986. *Principles of Isotope Geology*. New York: John Wiley & Sons Inc.

Fincham, A. G. and Simmer, J. P. 1997. Amelogenin proteins of developing dental enamel, pp. 118-134 in Chadwick, D. J. and Cardew, G. (eds.), *Dental Enamel. Proceedings of the Ciba Foundation Symposium 205*. Chichester: John Wiley & Sons Inc.

Fincham, A. G., Moradian-Oldak, J. and Simmer, J. P. 1999. The structural biology of the developing dental enamel matrix. *Journal of Structural Biology* **126**, 270-299.

Fisher, D. C. and Fox, D. L. 1998. Oxygen isotopes in mammoth teeth: Sample design, mineralization patterns and enamel-dentin comparisons. *Journal of Vertebrate Paleontology* **18**, 41-42A.

Fricke, H. C. and O'Neil, J. R. 1996. Inter- and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. *Palaeogeography, Palaeoclimatology, Palaeoecology* **126**, 91-99.

Fricke, H. C., Clyde, W. C. and O'Neil, J. R. 1998. Intra-tooth variations in $\delta^{18}O$ (PO_4) of mammalian tooth enamel as a record of seasonal variations in continental climate variables. *Geochimica et Cosmochimica Acta* **62**, 1839-1850.

Gulson, B. L., Pounds, J. G., Mushak, P., Thomas, B. J., Gray, B. and Korsch, M. J. 1999. Estimation of cumulative lead releases (lead flux) from the maternal skeleton during pregnancy and lactation. *Journal of Laboratory and Clinical Medicine* **134**, 631-640.

Hillson, S. 2005. *Teeth*. Cambridge: Cambridge University Press.

Hoppe, K. A., Koch, P. L. and Furutani, T. T. 2003. Assessing the preservation of biogenic strontium in fossil bones and tooth enamel. *International Journal of Osteoarchaeology* **13**, 20-28.

Hoppe, K. A., Stover, S. M., Pascoe, J. R. and Amundson, R. 2004. Tooth enamel biomineralization in extant horses: implications for isotopic microsampling, in MacFadden, J. (ed.) Incremental Growth in Vertebrate Skeletal Tissues: Paleobiological and Paleoenvironmental Implications, *Palaeogeography, Palaeoclimatology, Palaeoecology* **206**, 355-365.

Horstwood, M. S. A., Evans, J. A. and Montgomery, J. 2008. Determination of Sr isotopes in calcium phosphates using laser ablation inductively coupled plasma mass spectrometry and their application to archaeological tooth enamel. *Geochimica et Cosmochimica Acta* **72**, 5659-5674.

Knight, W. M., Bohman, V. R., Lesperance, A. L. and Blincoe, C. 1967. Strontium retention in the bovine. *Journal of Animal Science* **26**, 839-844.

Kohn, M. J. 2004. Comment: tooth enamel mineralization in ungulates: implications for recovering a primary isotopic time-series, by B.H. Passey and T.E. Cerling (2002). *Geochimica et Cosmochimica Acta* **68**, 403-405.

Kohn, M. J., Schoeninger, M. J. and Valley, J. W. 1998. Variability in oxygen isotope compositions of herbivore teeth: reflections of seasonality or developmental physiology? *Chemical Geology* **152**, 97-112.

Leggett, R. W., Eckerman, K. F. and Williams, L. R. 1982. Strontium-90 in bone: A case study in age-dependent dosimetric modeling. *Health Physics* **43**, 307-322.

Mann, S. 1997. The biomimetics of enamel: a paradigm for organized biomaterials synthesis, pp. 261-274 in Chadwick, D. J. and Cardew, G. (eds.), *Dental Enamel. Proceedings of the Ciba Foundation Symposium 205*. Chichester: John Wiley.

McArthur, J. M., Howarth, R. J. and Bailey, T. R. 2001. Strontium isotope stratigraphy: LOWESS version 3: best fit to the marine Sr-isotope curve for 0-509 Ma and accompanying look- up table for deriving numerical age. *Journal of Geology* **109**, 155-170.

Meiggs, D. C. 2007. Visualizing the seasonal round: a theoretical experiment with strontium isotope profiles in ovicaprine teeth. *Anthropolozoologia* **42**, 107-127.

Montgomery, J., Evans, J. A. and Cooper, R. E. 2007. Resolving archaeological populations with Sr-isotope mixing models. *Applied Geochemistry* **22**, 1502-1514.

Papworth, D. G. and Vennart, J. 1984. The uptake and turnover of ^{90}Sr in the human skeleton. *Physical and Medical Biology* **29**, 1045-1061.

Passey, B. H. and Cerling, T. E. 2002. Tooth enamel mineralization in ungulates: Implications for recovering a primary isotopic time-series. *Geochimica et Cosmochimica Acta* **66**, 3225-3234.

Price, T. D. and Gestsdottir, H. 2006. The first settlers of Iceland: an isotopic approach to colonisation. *Antiquity* **80**, 130-144.

Price, T. D., Knipper, C., Grupe, G. and Smrcka, V. 2004. Strontium isotopes and prehistoric human migration: the Bell Beaker Period in Central Europe. *European Journal of Archaeology* **7**, 9-40.

Robinson, C., Briggs, H. D., Atkinson, P. J. and Weatherell, J. A. 1981. Chemical changes during formation and maturation of human deciduous enamel. *Archives of Oral Biology* **26**, 1027-1033.

Schweissing, M.M. and Grupe, G. 2003. Stable strontium isotopes in human teeth and bone: a key to migration events of the late Roman period in Bavaria. *Journal of Archaeological Science* **30**, 1373-1383.

Simmer, J. P. and Fincham, A. G. 1995. Molecular mechanisms of dental enamel formation. *Critical Reviews in Oral Biology and Medicine* **6**, 84-108.

Smith, C. E. 1998. Cellular and chemical events during enamel maturation. *Critical Reviews in Oral Biology and Medicine* **9**, 128-161.

Sponheimer, M., Passey, B. H., de Ruiter, D. J., Guatelli-Steinberg, D., Cerling, T. E. and Lee-Thorp, J. A. 2006. Isotopic evidence for dietary variability in the Early Hominin *Paranthropus robustus*. *Science* **314**, 980-982.

Suga, S. 1982. Progressive mineralization pattern of developing enamel during the maturation stage. *Journal of Dental Research* **61**, 1532-1542.

Suga, S. 1989. Enamel hypomineralization viewed from the pattern of progressive mineralization of human and monkey developing enamel. *Advances in Dental Research* **3**, 188-198.

Tafforeau, P., Bentaleb, I., Jaeger, J.-J. and Martin, C. 2007. Nature of laminations and mineralization in rhinoceros enamel using histology and X-ray synchrotron microtomography: potential implications for palaeoenvironmental isotopic studies. *Palaeogeography Palaeoclimatology Palaeoecology* **246**, 206-227.

Tolstykh, E. I., Kozheurov, V. P., Vyushkova, O. V., Degteva, M. O. 1997. Analysis of strontium metabolism in humans on the basis of the Techa river data. Radiation and Environmental Biophysics 36, 25-29.

Towers, J., Montgomery, J., Evans, J. A., Jay, M. and Parker Pearson, M. in press. An investigation of the origins of cattle and aurochs deposited in the Early Bronze Age barrows at Gayhurst and Irthlingborough. *Journal of Archaeological Science.*

Trickett, M. A., Budd, P., Montgomery, J. and Evans, J. 2003. An assessment of solubility profiling as a decontamination procedure for the Sr-87/Sr-86 analysis of archaeological human skeletal tissue. *Applied Geochemistry* **18**, 653-658.

Wiedemann-Bidlack, F. B., Colman, A. S. and Fogel, M. L. 2008. Phosphate oxygen isotope analysis on microsamples of bioapatite: removal of organic contamination and minimization of sample size. *Rapid Communications in Mass Spectrometry* **22**(12), 1807-1816.

Zazzo, A., Mariotti, A., Lecuyer, C. and Heintz, E. 2002. Intra-tooth isotope variations in late Miocene bovid enamel from Afghanistan: paleobiological,

taphonomic, and climatic implications. *Palaeogeography Palaeoclimatology Palaeoecology* **186**, 145-161.

Zazzo, A., Balasse, M. and Patterson, W. P. 2005. High-resolution delta C-13 intratooth profiles in bovine enamel: Implications for mineralization pattern and isotopic attenuation. *Geochimica et Cosmochimica Acta* **69**, 3631-3642.

Zazzo, A., Balasse, M. and Patterson, W. P. 2006. The reconstruction of mammal individual history: refining high-resolution isotope record in bovine tooth dentine. *Journal of Archaeological Science* **33**, 1177-1187.

Figures

Figure 1

A photograph of the Ferry Fryston bovine mandibular third molar showing the buccal side of the mesial unit (left) cleaned of cementum prior to removal of the enamel and the striated, incompletely mineralized cervical enamel on the distal unit (right)

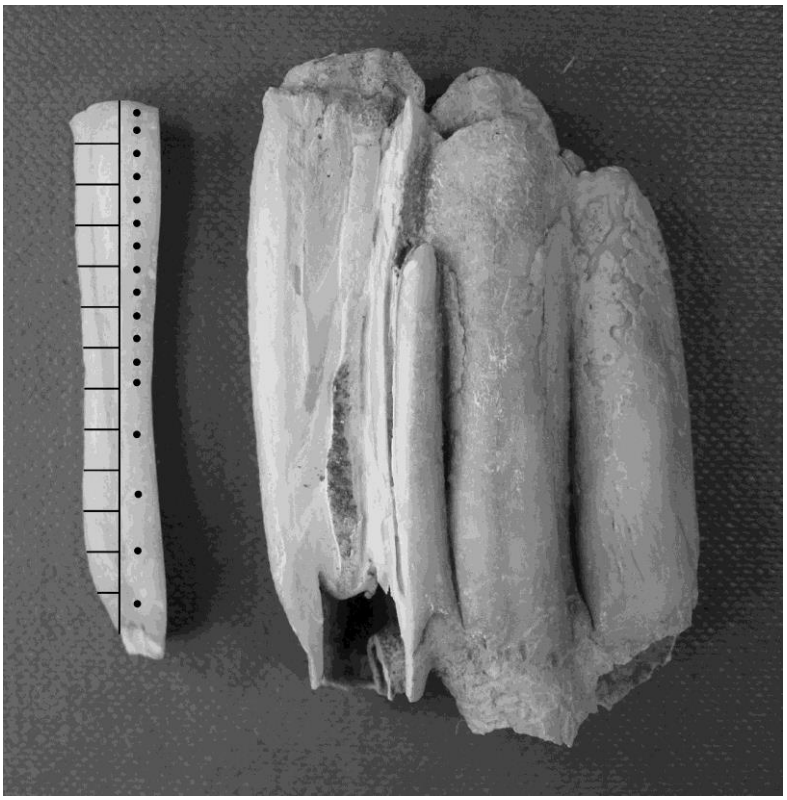

Figure 2

A photograph of the Ferry Fryston bovine mandibular third molar with the buccal side of the mesial unit removed and a schematic representation of how the section was bisected and sampled for TIMS (left) and LA (right)

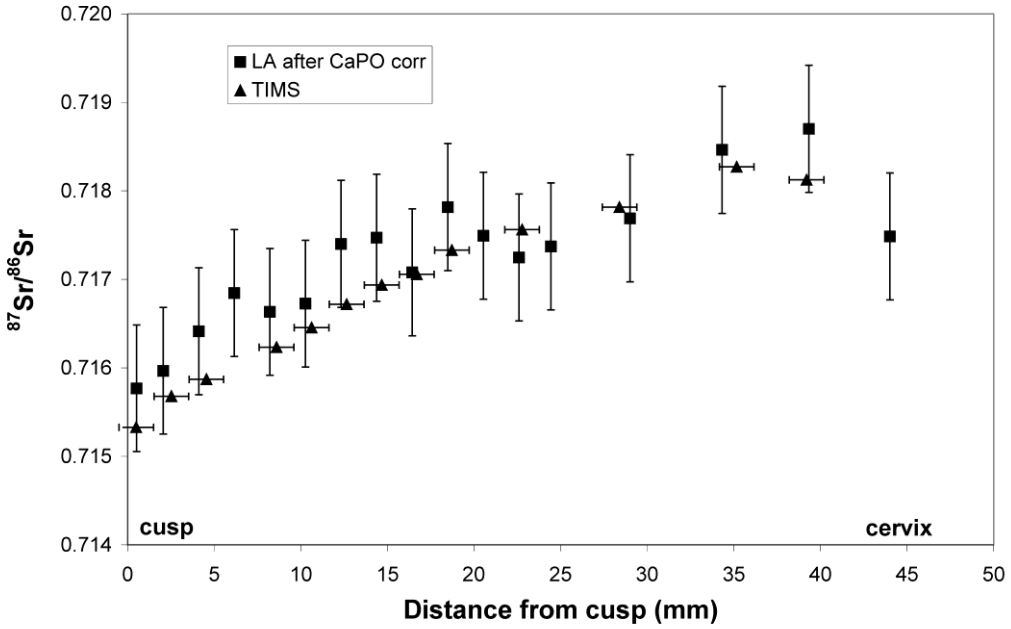

Figure 3

Plot showing $^{87}Sr/^{86}Sr$ TIMS data and corrected and propagated $^{87}Sr/^{86}Sr$ LA data for the Ferry Fryston bovine tooth. Figure adapted from Horstwood *et al.* (2008)

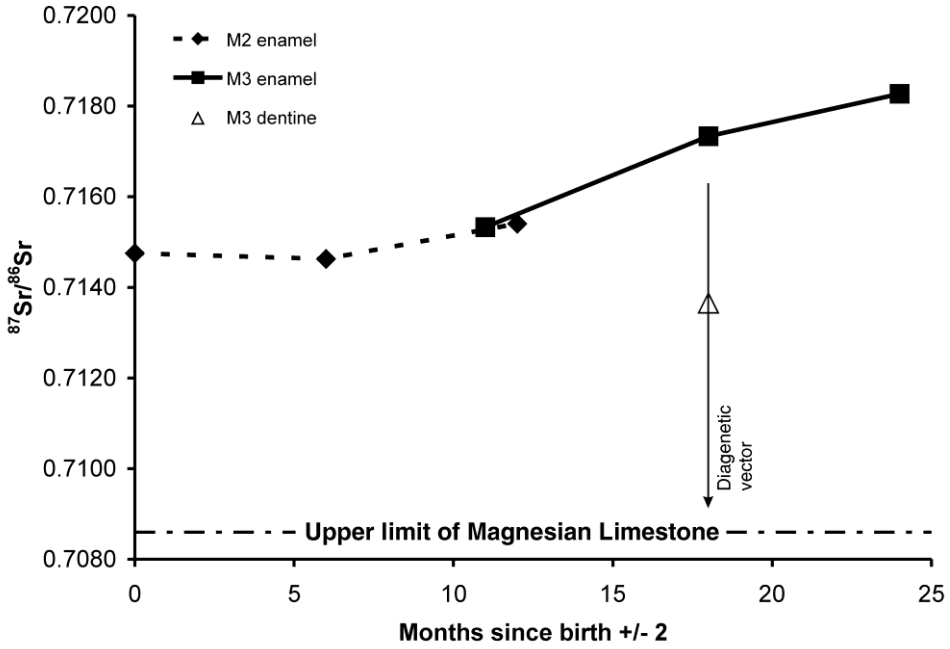

Figure 4

A graph showing $^{87}Sr/^{86}Sr$ data obtained by TIMS for three enamel samples (cusp, centre and cervix) from the second (M2) and third (M3) molars. Approximate age of crown formation is given along the x-axis following Brown *et al.* (1960). $^{87}Sr/^{86}Sr$ ratio for the Magnesian limestone is estimated from McArthur *et al.* (2001)

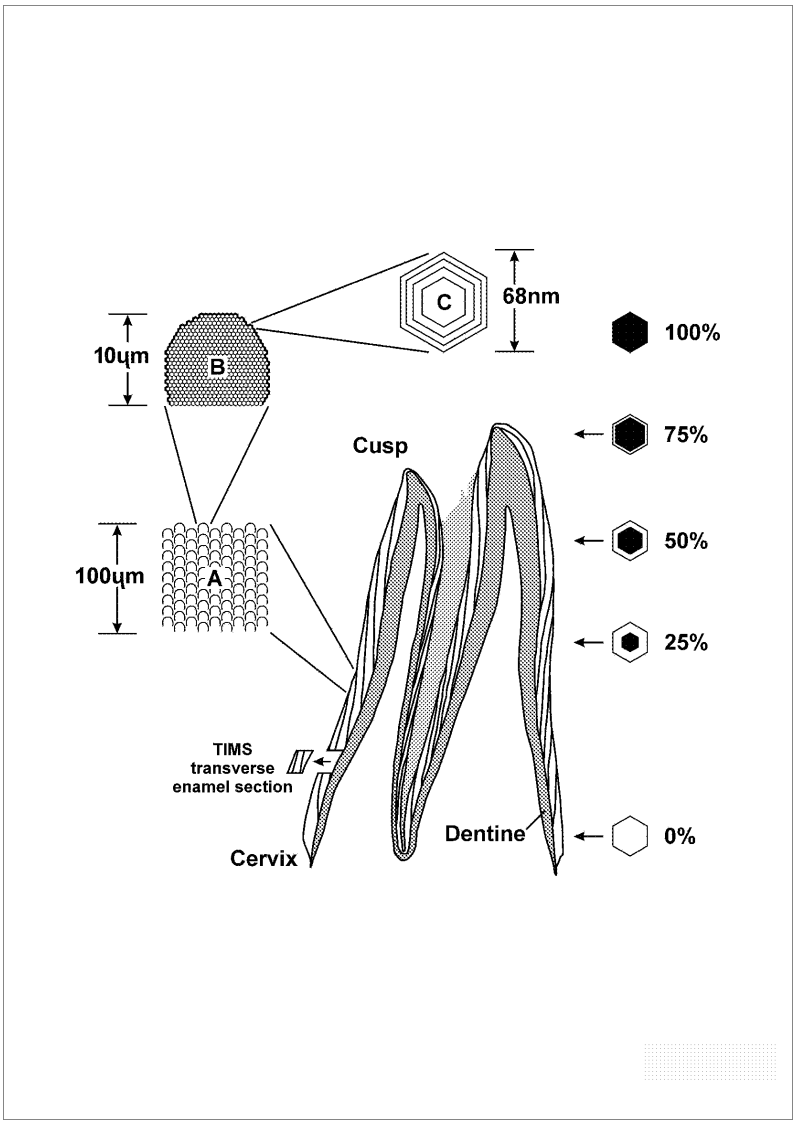

Figure 5

Schematic drawing of a cattle molar prior to root development and a model to illustrate the mixing of two sources of strontium within surface enamel sampled using laser ablation. Drawing of tooth adapted from Hillson (2005)

Tables

Table 1

$^{87}Sr/^{86}Sr$ ratio and concentration data for Ferry Fryston cattle tooth. Source of data for samples 1-17: Horstwood *et al.* 2008

	TIMS data			Laser data	
Sample No.	**Distance from cusp (mm)**	**Sr ppm**	**87Sr/86Sr**	**Distance from cusp (mm)**	**87Sr/86Sr**
Third mandibular molar - buccal side of the mesial unit					
1	0.50	158	0.71533	0.51	0.71577
2	2.53	151	0.71568	2.05	0.71597
3	4.55	163	0.71587	4.11	0.71641
4				6.16	0.71685
5	8.60	153	0.71623	8.22	0.71663
6	10.6	165	0.71645	10.3	0.71673
7	12.7	169	0.71672	12.3	0.71740
8	14.7	173	0.71694	14.4	0.71747
9	16.7	179	0.71706	16.4	0.71708
10	18.7	184	0.71733	18.5	0.71782
11				20.5	0.71749
12	22.8	197	0.71756	22.6	0.71725
13				24.4	0.71737
14	28.4	196	0.71782	29.0	0.71769
15	35.2	218	0.71827	34.3	0.71846
16	39.2	169	0.71812	39.3	0.71870
17				44.0	0.71749
Third mandibular molar - lingual side of the mesial unit					
18	cusp	130	0.71551		
19	centre	168	0.71733		
20	cervix	192	0.71807		
21	centre - dentine	173	0.71366		
Second mandibular molar - buccal side of the mesial unit					
22	cusp	189	0.71475		
23	centre	162	0.71463		
24	cervix	153	0.71541		