CHAPTER THREE

HUMAN TEETH AS ANALYTICAL SAMPLES

Apatite = απατάω (I deceive) (McConnell 1973, 3)

3.1 Introduction

The application of isotope analysis to identify migration requires the recovery of information from an individual's past that is related to their place of origin and can be contrasted to their ultimate place of residence. In contemporary studies of animals or humans there are a wide variety of tissues that may be used depending on the time interval being investigated; body fluids, soft tissues, nails, hair, bone and teeth can all be analysed. In most archaeological populations however, the hard, skeletal tissues of bones and teeth are usually the only remains to survive burial. As discussed in the previous chapter, the bony skeleton is a living, metabolically active tissue that is continually regenerated throughout an individual's lifetime. In contrast to bone, teeth do not remodel or re-grow. Once formed, therefore, a tooth represents an enduring archive that offers the opportunity to reach back into the past and retrieve information about an individual's diet and environment during childhood. Together, the deciduous and permanent human dentition provide a time record of perhaps twenty years from before birth until the mid to late-teens, when the third molar root is completed.

There is little value in analysing human remains whilst understanding neither their history (both ante- and post-mortem) nor what is actually being measured. The formation processes of teeth are crucial to interpretation of the data obtained and the meaningful application of isotope analysis to studies of past populations. Moreover, it is not just their macroscopic formation but formation on the microscopic scale that must be understood, and how this relates to the elements and isotopes chosen to track mobility. When, exactly, during the life of a tooth crown are Pb and Sr incorporated, and do they have any relationship to the incremental structures visible in both enamel

and dentine, as many researchers have suggested? The creation of enamel is complex and the timing of the processes behind the incorporation of mineral elements such as lead and strontium, as well as the incorporation mechanisms themselves, have proved difficult to unravel.

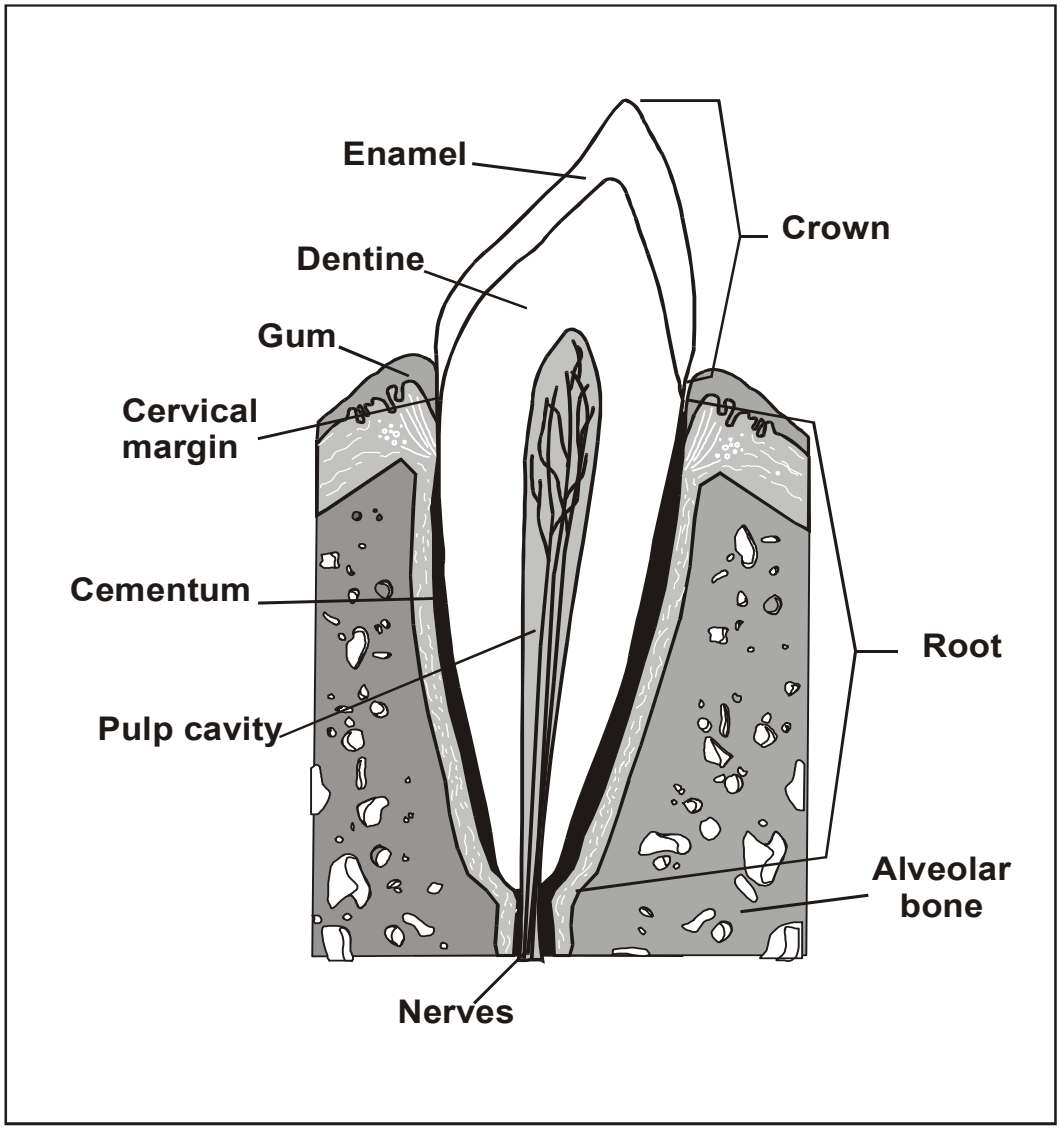

Figure 3.1 Longitudinal tooth section with anatomical terms used in this study. Source: Author.

Furthermore, most archaeological remains have been exposed to post-mortem contamination of one form or another and the possibility that their isotope composition may ultimately reflect little more than the burial soil must be considered. It will be shown that enamel appears to be remarkably resistant, although not entirely immune, to diagenetic alteration, primarily due to its unique structure and high degree of mineralisation but that the integrity of dentine is doubtful. This chapter will, therefore,

consider in detail the macroscopic and microscopic formation processes of tooth tissues in both the permanent and deciduous dentition, their behaviour *in vivo* and post-mortem and how this should inform sampling choice, methods used, analysis and interpretation of the data. Tooth anatomy is illustrated in Figure 3.1.

3.2 Dental notation system

Throughout the study individual teeth are referred to using a dental shorthand system. For all analytical samples, the type of tooth selected for analysis and its precise position in the mouth was identified and recorded, where possible, using the abbreviations below adapted from those recommended by van Beek (van Beek 1983, 6).

Table 3.1 Dental shorthand system used in the study

Tooth name	**Abbreviation**				
	Deciduous Dentition		*Permanent Dentition*		*Left or Right*
	Maxillary	*Mandibular*	*Maxillary*	*Mandibular*	
First or central incisor	di^1	di_1	I^1	I_1	L or R
Second incisor	di^2	di_2	I^2	I_2	"
Canine/cuspid	dc^1	dc_1	C^1	C_1	"
First premolar/bicuspid	-	-	P^1	P_1	"
Second premolar/bicuspid	-	-	P^2	P_2	"
First molar	dm^1	dm_1	M^1	M_1	"
Second molar	dm^2	dm_2	M^2	M_2	"
Third molar	-	-	M^3	M_3	"

Although alternative systems such as Zsigmondy system and that of the Fédéderation Dentaire Internationale (FDI) are available, the one outlined above offers a number of important advantages. This system uses a combination of letters and numbers and, in practice, requires very little dental or anthropological knowledge to classify or decipher

which tooth is being indicated. It is suitable for computer entry, relatively intuitive and did not demand previous familiarity with the system, thus reducing the chances for error (van Beek 1983, 6). This proved an important consideration as several people who either used different systems or were unfamiliar with any at all, dealt with sample information or the samples themselves.

Note that although the premolars of the permanent dentition are recorded as P1 and P2, it is more correct to describe them as P3 and P4: the first and second being absent in the human dentition. Similarly, the molars of the deciduous dentition are actually deciduous premolars (Hillson 1996, 7). To avoid confusion, modern human dental terminology is used consistently, rather than the less well known, though albeit technically correct, terms sometimes used in anthropology and palaeontology.

3.3 Formation and eruption of dentitions

Humans produce two complete sets of dentition: the deciduous (or milk) teeth shed during childhood and the permanent (or second) teeth. There is a considerable period of overlap, approximately between five and twelve years of age, where teeth from both dentitions are present in the mouth. The deciduous dentition normally consists of 20 teeth in total and the permanent dentition 32.

The vast majority of archaeological and forensic studies dealing with dental development have been carried out in terms of determining age at death from surviving remains. Whilst this parameter has been recorded for individuals investigated in this study, the primary consideration and reason for using dental development tables is to ascertain enamel formation and mineralisation times. It is during mineralisation of the organic-rich enamel matrix that incorporation of strontium and lead occurs. Dental development tables however, give development, eruption and root closure times but fail to make any distinction between formation and mineralisation of tooth crowns.

3.3.1 The deciduous dentition

The deciduous teeth start to form *in utero* 14 to 16 weeks after fertilisation although timing may vary between individuals and sexes. The first teeth to form are the first incisors (di1) closely followed by the first molars (dm1). The full sequence is normally: di1, dm1, di2, dc, dm2 (Hillson 1996, 121). The timing of individual crown inception, completion, apical root closure and development at the time of birth used in this study are reproduced in Table 3.2. The table is reproduced from Hillson (1996, 124) and is based on the earlier study of Lunt and Law (1974)

Table 3.2 Deciduous tooth formation timing

Tooth	Initial cusp formation[a]	Development at birth	Crown complete[b]	Root apex closed[c]
di^1	13.0-16.0	Crown 80% complete	1.5	1.5
di^2	14.7-16.5	Crown 60% complete	2.5	2.0
dc^1	15.0-18.0	Crown 30% complete	9.0	3.3
dm^1	14.5-17.0	Occlusal surface complete	6.0	2.5
dm^2	16.0-23.5	Cusps joined into 'U' by distal margin ridge	11.0	3.0
di_1	13.0-16.0	Crown 80% complete	2.5	1.5
di_2	14.7-	Crown 60% complete	3.0	1.5
dc_1	16.0-	Crown 30% complete	9.0	3.3
dm_1	14.5-17.0	Occlusal surface complete	5.5	2.3
dm_2	17.0-19.5	Cusps joined into ring	10.0	3.0

[a] Age in weeks after fertilisation; [b] Age in months after birth; [c] Age in years after birth
Source: Hillson (1996,124)

As with the permanent teeth, the deciduous teeth erupt some time between crown completion and root closure but precise timings vary considerably (Hillson 1996, 139). Identifying gingival eruption (emergence through the gum) is inherently difficult with archaeological material where no soft tissue remains and when eruption is observed it is likely to be at an earlier age than that recorded in the living. The onset of alveolar eruption (emergence through the alveolar bone) is easier to identify but still difficult to

accurately assess consistently, particularly as many samples for this study were selected precisely because the alveolar bone was fragmentary and sampling would not involve any further damage. The normal sequence of eruption is as follows: di_1, di^1, di^2, di_2, dm1, dc1, dm_2, dm^2 (Hillson 1996, 141). The timing of root resorption of deciduous teeth prior to exfoliation is considered to be extremely variable and an unreliable indicator of age (Hillson 1996, 142). However, because resorbing roots are an inherently dynamic tissue and may be actively incorporating Pb or Sr, if tooth roots had started to resorb (as opposed to being incompletely formed) this was recorded.

3.3.2 The permanent dentition

Formation of the permanent dentition is initiated *in utero* with the first permanent molar at 28-32 weeks after fertilisation. This is followed shortly after birth by the first (or central) incisor. The initiation period of tooth crowns shows much less variability than the period of crown completion. Nevertheless, crown formation times are considered to be a more accurate age indicator than eruption or root formation (Ubelaker 1989, 64). The normal sequence of crown initiation is: M1, I1, I_2, C1, I^2, P1, P2, M2, M3.

The permanent dentition may start to erupt from the age of five years and all teeth, with the exception of the third molars, are usually in occlusion by the age of thirteen. As previously discussed for the deciduous teeth, permanent teeth erupt after the crowns are fully formed and mineralised but before the roots are complete. The normal sequence of eruption is as follows: M1, I1, I2, (P^1C_1), (C^1P_1), P2, M2, M3 although considerable variation exists between both populations and sexes (Hillson 1996, 141). When selecting and ageing teeth, archaeological specimens that had become detached from the alveolar bone were considered to have erupted only if wear facets were present.

The schematic diagram produced by Gustafson and Koch (Gustafson & Koch 1974) was used as the primary reference for the main development stages of all permanent teeth with the exception of M3. It is easy to use and has been shown to produce only small errors in accuracy (-0.10 ±0.37 years) when tested on recent archaeological remains of known age and sex (Liversidge 1994). Furthermore, it does not require prior knowledge of the individual's sex as data for males and females (who may be 1-2 years

ahead developmentally) are combined. This is an advantage with archaeological skeletal material because, in the absence of aDNA analysis, the sexing of juveniles is problematic. Gustafson and Koch produced data for the maxillary left and the mandibular right sides of the dentition and their diagram is summarised in numerical form in Table 3.3.

Table 3.3 Permanent tooth formation timing

Tooth	Onset of mineralisation	Crown complete	Eruption	Root apex closed
I^1	3-4 months	4-5.25	5.75-8.5	9-11
I^2	10-12 months	4-5.75	6.5-10	10-12
C^1	4-5 months	5.5-7	8-12.25	12-15
P^1	1.5-2	5-7.5	8-12	12-14.25
P^2	2-2.5	6-8.5	9-14	12-15.5
M^1	peri-natal	2.5-4.25	5-8	9-11.5
M^2	2.5-3	7-8	10-14	14-16
I_1	3-4 months	3.5-5	5-7.5	8.5-10
I_2	3-4 months	4-5	6-9	9.5-11
C_1	4-5 months	4.5-7	7.5-12	12-15
P_1	1.5-2	4.5-7	8-13	12-14
P_2	2-2.5	6-8	9-12.25	12-15
M_1	peri-natal	2.5-4	5-7	9-11.5
M_2	2.5-3	6.25-8	10-13.5	13.5-16.5

All figures are years of age unless otherwise stated
Source: Gustafson & Koch (1974)

It should be stressed that these ranges cover the expected variation *within a population*. For example, formation of all the lower second molars of a group of children would normally start between 2.5 and 3 years of age and all crowns should be completed between 6.25 and 8 years of age. This table does *not* necessarily imply that a specific tooth in a single individual will necessarily take 5.5 years to form; a tooth that starts to develop early is very likely to finish early. Tooth formation times are genetically

constrained and factors such as sex will also have a bearing on individual timings. The formation of teeth is, however, recognised as being the most resistant skeletal process to alteration by external environmental variables such as malnutrition and disease (Smith 1991, 143).

Although age at death was considered and recorded in the current study, the primary purpose in considering dental formation in the course of this research was to identify the dental ages between which individual tooth crowns form. In this respect, the tables are being used for a slightly different purpose to that for which they were developed. Furthermore, it is specifically the period of enamel mineralisation, and thus the time during an individual's childhood when lead and strontium were being incorporated into the particular tooth, that is the focus of interest. Precisely when the developing enamel receives the massive influx of mineral ions prior to crown completion, is poorly understood (David Whittaker pers. comm.). To avoid falsely narrowing the range, the full age range from initial calcification to crown completion is given as the period of possible mineralisation. In truth, the main period of mineralisation, and thus metal ion entrapment is likely to be considerably more constrained than this. In order to define the possible period of metal ion entrapment more precisely it is necessary to consider the formation of dental tissues in more detail.

3.4 Formation and maturation of enamel and dentine

The mechanism of enamel formation remains a mystery. (Simmer & Fincham 1995, 99)

The mechanisms of crystal initiation and subsequent control of crystal growth and morphology are central problems in enamel formation. (Robinson *et al.* 1997, 156)

A theory of tooth formation that is widely accepted is that teeth form incrementally from the cusp tip down towards the root apex so that the enamel caps form before the crown dentine and the crown before the root. This would imply that the occlusal enamel would represent earlier forming enamel than that of the cervical region and the enamel would represent earlier forming tissue than the crown and root dentine. This theory of "incremental growth" has led many researchers to believe that it is possible to

sample tooth enamel and dentine chemically along these incremental lines and obtain temporally discrete information spanning the period of formation (e.g. Ericson 1985, 508; Lee *et al.* 1999, 180; Lochner *et al.* 1999, 300; Molleson 1990; Schneider & Blakeslee 1990, 73). Unfortunately, this interpretation of the way teeth grow is a gross simplification and also belies a fundamental misunderstanding of the developmental dental terminology and particularly the word "formation". Furthermore, it ignores the very different mechanisms that produce the enamel and dentine of a single, mature tooth, which will be discussed in the following sections.

3.4.1 Formation versus maturation

Incremental structures within enamel and dentine are undoubtedly visible microscopically. Cross striations - alternate dark and light bands across individual enamel prisms - are considered to represent 24 hours growth per dark/light pairing (Hillson 1996, 156). Brown striae of Retzius, of which the neo-natal line is usually the first and most prominent, cross the prisms every 7 to 10 cross striations, i.e. 7 to 10 days. The exact number varies between individuals but appears to be constant for one individual and between all the teeth of that individual (Hillson 1996, 157), suggesting the presence of some unknown, internal metabolic rhythm that starts immediately after birth. Surface phenomena such as perikymata and enamel hypoplasia that are visible with the naked eye, can also be related by incremental methods to age during their formation.

In dentine, incremental growth layers are visible at various magnifications from beneath the cusp tips to the root apex. They can sometimes be related to those in enamel such as the brown striae of Retzius and include a neo-natal line and, although it is considered they do reflect systemic fluctuations during formation, they are often irregular and difficult to decipher (Hillson 1996, 189).

The theory of incremental growth is perpetuated by the formation tables themselves (see Table 3.2) which give the percentage of the crown generally present at birth together with what is visible radiographically *in vivo* and what survives post-mortem in juvenile archaeological burials. However, dental tissue has to reach a specific density of

mineralisation to be visible on radiographs and what dental material survives archaeologically is merely that which had reached a *sufficient* level of mineralisation. It does *not* mean that that was all that was present at the time the radiograph was taken or the individual was buried. In her comparative study of dental ageing methods on the Spitalfields skeletal collection, Liversidge found seven cases where cusp tips were visible within the alveolar crypt but *could not be detected radiographically* (1994, 40). As the visibility of dental tissue in radiographs is highly dependent on the level of mineralisation, this would suggest that tooth enamel is *not* fully mineralised as it is forming nor when recovered as tooth caps from juvenile remains.

An investigation of juvenile dentitions and the samples used in this project supports this observation and has also indicated that unerupted tooth crowns may be *fully formed* morphologically with the crown dentine present but the enamel not fully mineralised. Teeth at this stage of formation did not have the same appearance as adjacent, unerupted but earlier forming teeth within the same dentition which displayed white, hard, translucent enamel, but were matt, opaque and appeared to have taken on the same discoloration as the surrounding alveolar bone. Furthermore, they did not have the mechanical hardness of fully mature enamel when dissected. There was no evidence to suggest that this phenomenon was due to differential preservation, as it occurred systematically with the expected sequence of dental development in all dentitions. Rather, it would suggest that although the tooth had completed its morphological formation, the organic and water content was such that it caused the tooth to be affected by post-mortem contamination in a similar way to the surrounding bone. Fully mineralised enamel was never observed in incompletely formed tooth crowns.

Tables that give crown inception and crown completion ages are useful as they narrow the specific period of childhood represented by the tissue under study. However, it became clear during the study that although crowns may be fully formed *morphologically* with all fissures and cusps intact, they are not, of necessity, *fully mineralised*. Clearly, in the case of enamel, formation and the presence of fully mineralised, mature enamel are not one and the same thing. Enamel does not form from the cusp tip down towards the cervical enamel as a fully mature tissue, i.e. incrementally in one single process. The timing of maturation, as distinct from morphological formation, is not given in tooth development tables and is still poorly

understood (D. Whittaker pers. comm.). For humans, such information is, of course, difficult to obtain from *in vivo* experiments without invasive surgery or repeated radiography. However, it is precisely this process of mineralisation and, consequently, incorporation of Pb and Sr ions into the mineral lattice, as distinguished from formation or morphogenesis, that is crucial to interpretations made in this study but it cannot be simply derived from ageing tables based on tooth formation.

3.4.2 Mature enamel and dentine – description and composition

Dentine constitutes the main body of the tooth: the root and the crown mould upon which the enamel cap – usually the only part of the tooth visible in the mouth – is formed. The two tissues are closely intermeshed at the enamel-dentine junction (EDJ) and as dentinal structures project into the enamel there is no clear dividing plane between the two (Boyde 1997, 24). Enamel is the hardest and most highly mineralised but it derives much of its strength and ability to withstand pressure from the presence of the underlying dentine.

Enamel, dentine (and bone) are composite tissues of inorganic, organic and water fractions in varying amounts. In all three, McConnell (1973, 80/88) considers the inorganic phase is the crystalline calcium phosphate mineral dahllite, or carbonate hydroxyapatite, with the repeating unit cell formula:

$$(Ca, X)_{10}(P, C)_6(O, OH)_{26}$$

where X is considered to represent a variety of possible substitutions for Ca such as Mg, Na, Sr, Ba and Pb (McConnell 1973, 21). More recently, Aoba (1996, 213) proposed a stoichiometric model formula for enamel mineral where $y = x - q - u$, as:

$$(Ca)_{5-x}(Mg)_q(Na)_u(HPO_4)_v(CO_3)_w(PO_4)_{3-y}(OH)_{1-z}$$

It is frequently referred to, inaccurately, in the literature as hydroxyapatite, with the unit cell $Ca_{10}(PO_4)_6(OH)_2$ (McConnell 1973, 80). As carbonate hydroxyapatite is almost exclusively found in vertebrate tissues, it is also termed biological or biogenic apatite. The crystals are hexagonal prisms formed from the repeating unit cell and are

renowned for their non-stoichiometry, the crystal lattice accommodating a wide variety of distortions, substitutions and vacancies, and rarely conforming to any simple formula. Carbonate is present at concentrations of approximately 4% in the inner enamel next to the EDJ, falling fairly evenly to ~1% near the surface (Robinson *et al.* 1995b, 17b7). Magnesium and sodium are also present at sufficient concentrations to significantly affect the structure and properties of the enamel crystals (Aoba 1996, 210).

The familiarity accorded to biogenic apatite in the literature belies the fact that it is still physically and chemically poorly understood. Dentine and bone do not yield single crystals large enough to ascertain crystal structure by powder diffraction methods whilst the much larger enamel crystals, tightly packed and interwoven as they are, have so far proved impossible to isolate individually. As a result, most of the information available has been obtained by analogy and analysis of various model compounds, of which hydroxyapatite is one of the few where single crystals can be obtained for investigation (Elliott 1997, 54). However the growth of the unique and remarkable crystalline structure of enamel is biologically mediated and cannot be fully explained by analogies of apatite crystal growth in mineralogical or crystallographic studies.

Although enamel and dentine contain the same mineral phase, there are major differences between the organic fraction. Enamel is the most highly mineralised of all mammalian skeletal tissues and is almost entirely composed of inorganic mineral. The mature tissue is avascular and acellular; the enamel forming cells (ameloblasts) die once the enamel is complete, and it does not have a blood supply. Once mineralised, therefore, the tissue can neither regenerate nor remodel, hence its inability to re-grow after succumbing to caries, and the composition of the enamel prism is effectively fixed throughout life. The organic phase contains no collagen but consists of what appears to be remnants of redundant protein that were incompletely removed during development. This protein is located mainly on the peripheries of enamel prisms and the majority is found near the EDJ being concentrated in the cervical area and particularly in the region underlying the fissures and cusps of the occlusal surface. The enamel of these complex regions is, therefore, less well mineralised than that of the histologically simpler tooth walls (Robinson *et al.* 1995b, 169).

The composition of dentine is more easily compared to bone than to enamel. The much shorter carbonate hydroxyapatite crystals of dentine and bone - 20-100nm long (Hillson 1986, 151) - are found within and around fibrils of collagen type I and both tissues contain many of the same non-collagenous proteins. Dentine, however, contains at least three proteins that are not found in bone (Butler *et al.* 1997, 108). Like bone, dentine is a living, cellular tissue that can respond to blood-borne substances. It does not, however, contain blood vessels and, most importantly, is not subject to the continual remodelling behaviour of the osteoclasts and osteoblasts of bone. Consequently, the composition of the primary tissue is, like enamel, determined largely at the time of formation (Rowles 1967, 202; Veis 1989, 189) and in the absence of caries or attrition it exhibits no visible changes (van Rensburg 1986). Nevertheless, the cells of dentine, the odontoblasts, remain active, and formation of new, secondary dentine continues throughout life.

New tissue, termed secondary dentine, may be laid down regardless of other changes that may occur in the tooth, in layers lining the pulp cavity (Hillson 1986, 198; van Rensburg 1987). The presence of secondary dentine is used as one of the ageing variables in Gustafson's system (Gustafson 1950) devised for use on modern individuals who had completed their dental development. He found secondary dentine was absent from teeth of individuals aged 25 years and under, present in some individuals aged 28-48 years and present in all individuals aged 49 years and over (Gustafson 1950, 52). Although warning against the use of M3, Gustafson used a variety of teeth. However, these findings would indicate that formation of secondary dentine does not start until the third decade of life. It should not be confused with the term circumpulpal dentine which is the dentine (primary or secondary) lining the pulp cavity.

Irregular secondary dentine, sometimes termed tertiary dentine, may be deposited as a response to specific pressure, attrition or caries to seal exposed dentinal tubules and protect the pulp cavity (Hillson 1986, 198). However, the precise aetiology of secondary dentine formation is obscure and there is no clear relationship between cause and effect (van Rensburg 1986; van Rensburg 1987). From about 30 years of age, further deposition of dentine mineral within the dentinal tubules occurs, starting from the root tip and expanding up towards the crown. This process, visible as a translucent

zone of dentine, appears to progress at a relatively constant rate enabling it to be used as the basis of several adult ageing methods (Hillson 1986, 199). Any change to the composition and morphology of dentine over time is, therefore, largely of an additive nature as opposed to the constant removal and replacement of tissue that occurs in bone.

Table 3.4 Composition of permanent enamel and dentine

Enamel	**by weight**	**by volume**
Inorganic	≥ 96%	88(80-100)%
Organic	< 0.2 - > 0.6%	0.3%
Water	variable	variable
Ca:P ratio (weight)	1.91 – 2.17	
Ca:P ratio (molar)	1.5 – 1.68	
Calcium %	34 – 40	
Phosphorus %	16 – 18	
Dentine		
Inorganic	72%	50%
Organic	20%	30%
Water	variable	variable
Ca:P ratio (weight)	2.1 – 2.2	
Ca:P ratio (molar)	1.6 – 1.7	
Calcium %	26 – 28	
Phosphorus %	12.2 – 13.2	

The compositions of permanent enamel and dentine given in Table 3.4 have recently been quoted by Hillson (1996, 218) from various sources although, as Veis (1989, 192) notes, the variability of dentine composition is so great that any quoted figure is, at best, an average. According to Veis (1989, 192) the collagen component represents 85-90% of the organic fraction but occupies over 50% of the total dentine volume, considerably more than quoted in the above table. Deciduous enamel contains

approximately 3wt.% less mineral/inorganic than permanent tooth enamel (Brudevold & Söremark 1967, 248). More recently, Elliott (1997, 64) has determined the inorganic (carbonate hydroxyapatite) content of permanent, mature enamel as 98 wt.% and 96 vol.% and notes that these concentrations are significantly higher than those normally quoted in the literature. The discrepancies may arise from natural variation between and within teeth but there are also analytical difficulties when trying to ascertain the percentage of water, which may be present as free water between the enamel prisms, associated with the residual protein and bound to the prisms themselves.

Water content determination in apatite in general is problematic, as water is tenaciously retained (McConnell 1973, 84) and the situation is further exacerbated because results can vary depending on the method of sample preparation and analysis making *in vivo* determinations particularly challenging. This difficulty was noted over 30 years ago by Brudevold and Soremark (1967, 248) with respect to enamel, and by Rowles (1967, 206) with respect to dentine. As mentioned above, this is an attribute of apatites in general but despite advances in instrumental methods the situation is little changed.

3.4.3 Formation of enamel and dentine

The mechanisms that govern the initiation, formation and design of enamel have proved notoriously elusive, prompting Simmer and Fincham to lament (1995, 99) *"the mechanism of enamel formation remains a mystery"*. Of all the mineralised tissues, enamel has proved the greatest puzzle and has so far defied all attempts to recreate it *in vitro*. This process results in the most highly mineralised of vertebrate tissues with a hardness somewhere between that of iron and carbon steel yet one that, because of its unique construction and underlying dentine support, displays a surprising elasticity (Simmer & Fincham 1995, 86).

Enamel and dentine are closely associated in the mature tooth but they have entirely different cellular origins. Enamel is produced in isolation from the blood supply by epithelial cells whereas dentine, along with the other types of connective tissue (bone, cartilage, muscle, tendons, blood vessels and cement), has its origins in the mesenchyme. The interface between the epithelial cells (which differentiate to produce enamel-forming ameloblasts) and the mesodermal cells (which differentiate into the

odontoblasts of the dentine) is located at the future EDJ. The two sets of cells effectively move in opposite directions away from the EDJ secreting their respective tissue matrices into the created space.

3.4.3.1 Dentine formation

The initial dentine matrix, or predentine, is secreted by the odontoblasts. Dentine is secreted and mineralised as a two phase process whereby the retreating odontoblasts secrete the initial dentine matrix, or predentine, which is then mineralised by the deposition of short (20-100nm) crystals of carbonate hydroxyapatite within the collagen fibre matrix. The mineralising "front" follows the retreating odontoblasts, separated by a 10-40μm thick layer of newly secreted predentine (Hillson 1996, 184). As human dentine grows about 4.5μm per day (Hillson 1986, 158), this would suggest that dentine tissue goes from secretion to mineralisation in approximately 3-8 days.

The first type of dentine formed under the EDJ is a thin layer of mantle, sometimes termed von Korff, dentine. A dense accumulation of collagen fibres and relatively disordered carbonate hydroxyapatite crystals produce a layer that is poorly mineralised in comparison with the dentine as a whole (Hillson 1986, 153; Veis 1989, 192). The odontoblasts and their long, fine processes are contained within dentinal tubules which, in the mature tissue, run from the pulp cavity through the dentine towards the EDJ, a few passing through into the enamel. Lining the tubules is a layer of highly mineralised peritubular dentine with randomly oriented crystals and few collagen fibres. During the life of the tooth this layer can increase in thickness and may eventually completely occlude the tubule. In the tooth crown, this process can occur in response to attrition but it is most noticeable in the roots of teeth where it leads to increasing tissue translucency which is well enough age-related to form the basis of a forensic ageing technique (Gustafson 1950; Hillson 1986, 198).

The bulk of the crown and root dentine is referred to as intertubular or von Ebner's dentine. It is composed of ~100nm diameter collagen fibres interspersed with a non-fibrous component termed ground substance. Within this organic matrix the vast majority of carbonate hydroxyapatite crystals are assembled into spherical or paraboloid structures 1-50μm diameter (Hillson 1996, 185). As previously mentioned,

incremental growth structures are visible within the dentine but frequently prove too patchy or faint, or fail to yield an obviously consistent rhythm which makes them difficult to use in practice (Hillson 1996, 189). It is generally accepted that mineralised dentine is present before enamel matrix secretion begins (Arsenault & Robinson 1989, 114; Boyde *et al.* 1988, 1481; Diekwisch *et al.* 1995, 152; Fincham & Simmer 1997, 119; Sasaki *et al.* 1997, 32). Veis (1989, 190) states that *"the collagen fibres and mineral crystals of the mantle dentin* (sic) *are abundant well before any enamel is formed"*. Moreover, Boyde (1989, 313) giving advice on specimen preparation for microscopy, warns *"Although soft enough to be cut with conventional microtome knives, immature enamel is inevitably attached to the surface of dentine, which is as hard and mature as it will ever be"*. There is less agreement, however, on whether the subsequent enamel crystallites are nucleated independently of the pre-existing dentine (Diekwisch *et al.* 1995, 159), are promoted by it, or are extensions of existing dentine crystals (Arsenault & Robinson 1989, 117).

3.4.3.2 Enamel biomineralisation

Enamel biomineralisation has been described as *"The transformation of what was basically an organic gel to almost pure inorganic mineral (90% mineral by volume) by cellular and biochemical flow processing"* (Mann 1997, 264). This process is achieved through the following distinct stages (Figure 3.2):

1. **Secretion -** of amelogenins and nucleation of crystallites
2. **Assembly -** of the amelogenins into nanospheres
3. **Formation -** elongation of crystallites
4. **Transition/Resorption -** of the organic matrix and replacement with fluid
5. **Maturation -** massive increase in mineral ions and lateral growth of crystals

In the first of these stages, the layer of ameloblasts begins to "pull away" from the EDJ, in the opposite direction from the odontoblasts, secreting an extracellular protein-rich matrix into the vacated space (Figure 3.3). Enamel matrix, unlike that of the dentine, contains no collagen. It does, however, contain several proteins that are unique to enamel. New and existing proteins are still being isolated and characterised and the current knowledge of enamel biochemistry has recently been reviewed in detail (Fincham *et al.* 1999). In simple terms, however, these proteins can be divided into two

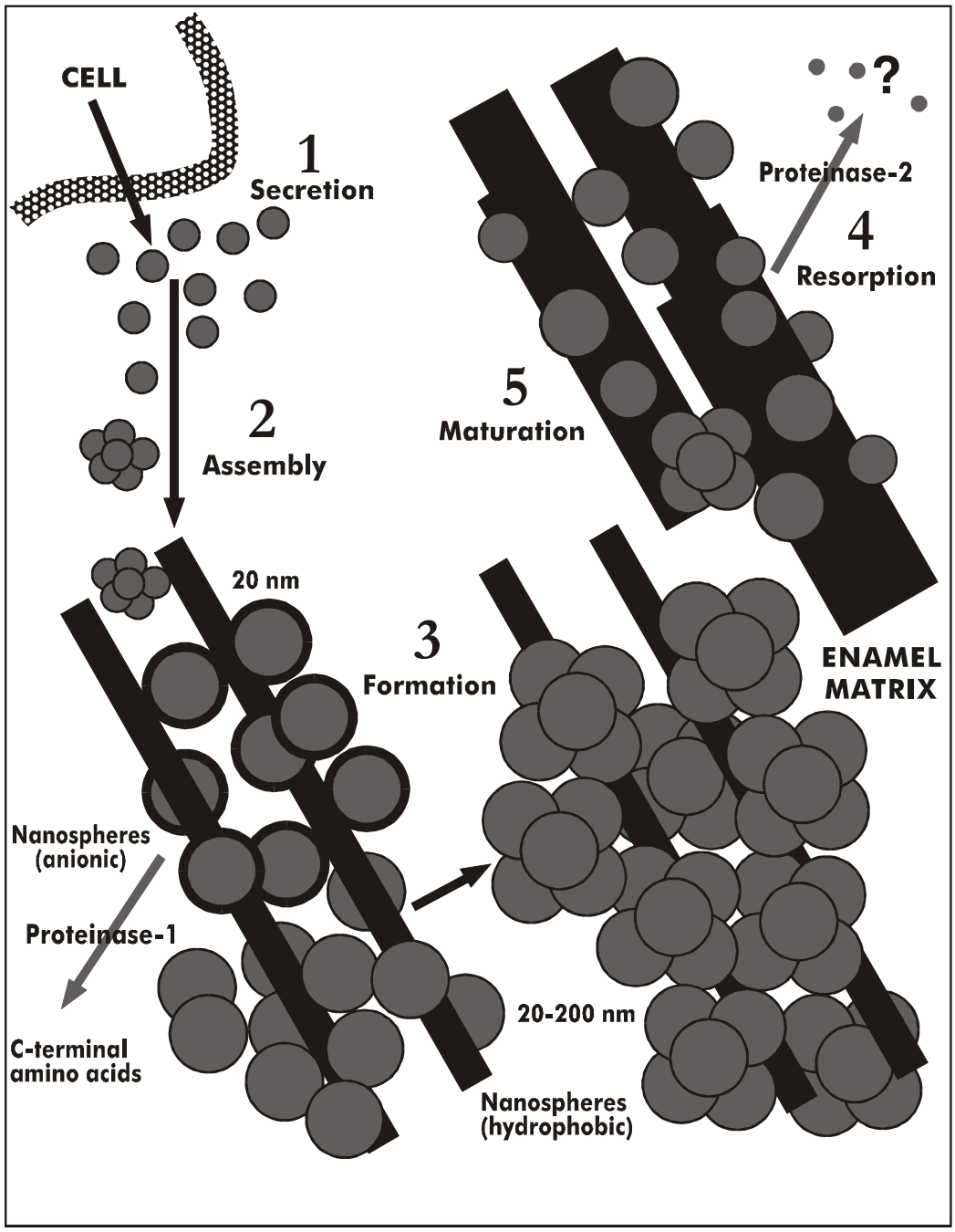

Figure 3.2 Schematic diagram of enamel biomineralisation. Note that at the formation stage (stage 3) enamel is only lightly mineralised. Full mineralisation (expansion of crystallites) only occurs during the maturation stage (stage 5).
Source: Redrawn from Fincham et al. 1999.

groups: the amelogenins and their processing products that comprise ~90% of the matrix bulk and the non-amelogenins, such as enamelin, ameloblastin and tuftelin which make up much of the remainder. Ameloblasts do not, however, simply secrete these two groups of proteins in a constant, homogeneous 10:1 mix and as a further complication, they also appear to secrete up to nine different mixtures of amelogenin

itself. Clearly, the proportions secreted vary during, and are related to, the different stages of development but the precise reasons and functions of most are still unknown (Robinson *et al.* 1995a, 147; Smith 1998, 134).

Tuftelin, ameloblastin and enamelin appear to be secreted first onto the mineralised dentine (Figure 3.3). Crystallites form almost immediately, 20nm apart, within the enamel matrix and crystal nucleation is closely followed by the subsequent secretion of amelogenin-dominated matrix. The crystallites then grow as extremely long, thin (1-2nm thick x 5-10nm wide) ribbons which can extend from the EDJ to the enamel surface and comprise 10-20% of the immature enamel volume (Fincham & Simmer 1997, 119; Mann 1997, 264). Whether these initial crystallites are carbonate hydroxyapatite or octacalcium phosphate is unproven but they remain optically visible in the mature tissue as an inner dark, carbonate-rich core that has a higher solubility than the surrounding mineral and is more susceptible to caries (Aoba 1996, 213; Robinson *et al.* 1995b, 177). Their routes through the matrix are neither random nor simply perpendicular to the dentine surface but are closely orchestrated by the ameloblasts. Mann (discussion recorded after Boyde's paper, Chadwick & Cardew 1997, 31) has described this process as *"the cell is reeling out the crystal in its wake, as though it were walking backwards and secreting an organized structure at the same time"*. As the ameloblasts move both spatially and in relation to one another, they weave a complex, sinusoidal pattern of crystallites, which produces the phenomenon of decussation seen in the mature tissue.

A putative role for amelogenin during this initial phase is prevention of lateral growth of crystallites, and crystal-crystal fusions, by the formation of strings of 20nm diameter "spacer beads" between individual crystallites (Figure 3.3) (Fincham *et al.* 1999, 290). The resulting tissue is, in effect, a plantation of tall thin saplings encased within a transitory protein framework. 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 mineralised (Figure 3.2, stage 3).

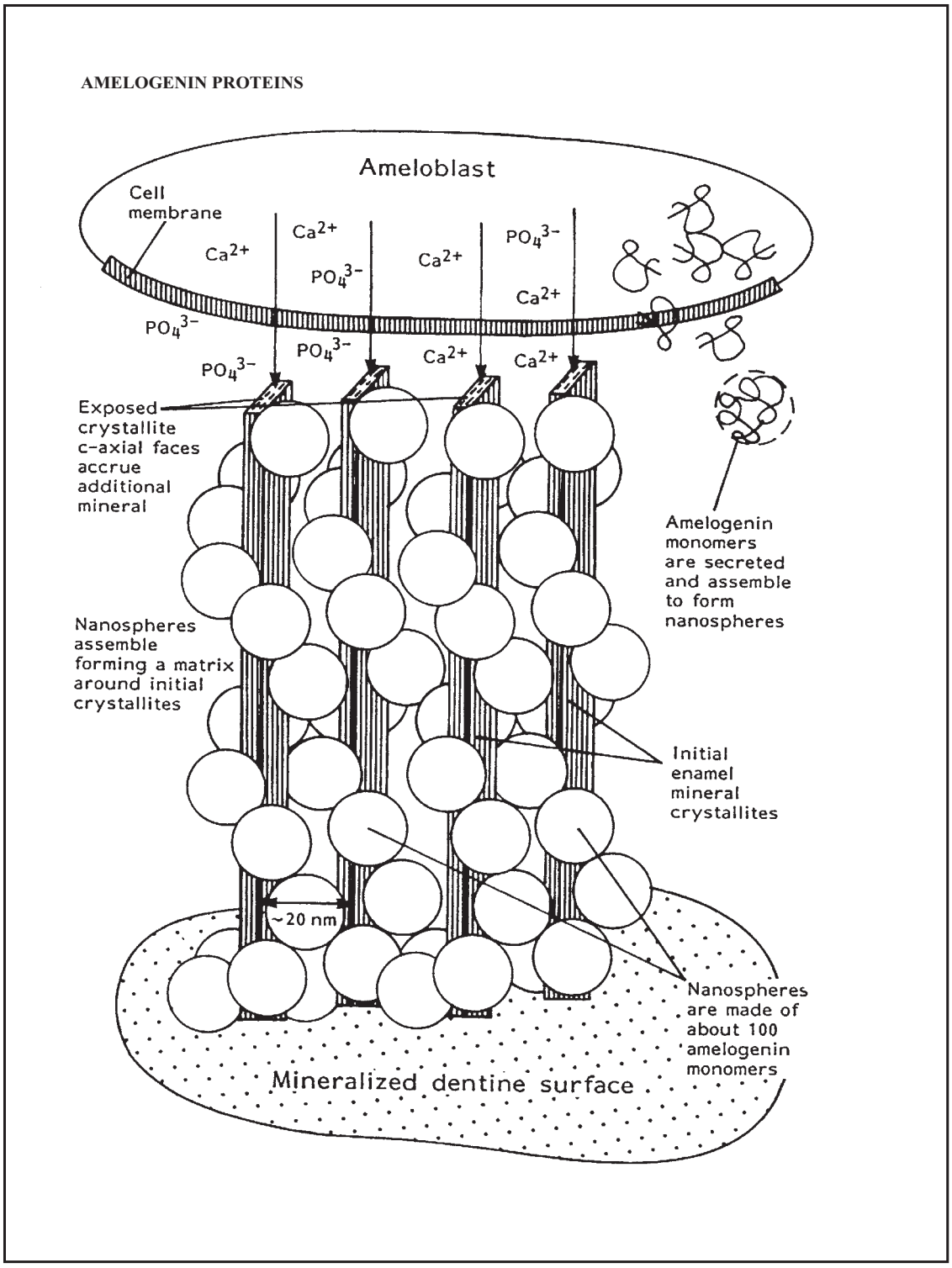

Figure 3.3 Schematic diagram of initial enamel mineral spacing and growth. Note the presence of mineralised dentine at the onset of enamel formation. Adapted from Fincham et al. 1999.

The ameloblasts cease matrix secretion as they approach the enamel surface and the enamel enters the second main phase. This transition/resorption phase involves the complete breakdown and removal of the ephemeral enamel matrix (Figure 3.2, stage 4),

in marked contrast to dentine matrix which is retained in the mature tissue. Amelogenin may be acting as either a physical space filler or a crystal growth inhibitor but its removal is considered vital in order to permit the immature crystallites to expand (Robinson *et al.* 1995a, 149). The protein matrix is processed and removed by the action of proteolytic metalloproteinases and serine proteinases secreted by the ameloblasts. As the matrix is withdrawn it is replaced by a large amount of tissue fluid which temporarily greatly increases the porosity of the immature tissue (Robinson *et al.* 1995a, 149). This fluid dries out quickly in extracted, developing bovine incisors, making the sharp boundary between transitional and mature enamel clearly visible (Robinson *et al.* 1995a, 149/150). The crystallites are now free to resume growth and during the maturation stage (Figure 3.2, stage 5) they may reach the 26nm thick x 68nm wide dimensions of mature crystals, and may extend throughout the full thickness of the enamel (≤ 2mm in humans) (Simmer & Fincham 1995, 91). The average mineral content is relatively constant until the end of the transition stage when it represents only 14% of the final mineral content. It then begins to rise steeply. During maturation there is, therefore, a massive increase in mineral ions as the bulk of the mature enamel mineral is deposited (Robinson *et al.* 1995a, 150; Smith 1998, 133). The final pore space available for water and ion diffusion between the enamel prisms is considered to approach atomic dimensions thus greatly restricting the subsequent passage of large and highly charged polyvalent ions (e.g. Pb^{2+} and Sr^{2+}) (Neuman & Neuman 1958, 103).

3.4.4 Structure of mature enamel

Approximately 1000 carbonate hydroxyapatite crystals are organised into each 5μm 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. Any residual protein is located principally between the enamel prisms, the greatest concentrations (<3%) being located near the EDJ and in areas of histological complexity rather than smooth, flat enamel (Robinson *et al.* 1995b, 184). Difficulty in efficiently removing the protein matrix from such areas and the less dense packing of prisms that arises with increasing complexity may both account for this phenomenon

(Robinson *et al.* 1995b, 168). Carbonate content is usually greatest at the EDJ (<4%) and decreases towards the enamel surface. The majority is believed to be located at the centre of the enamel prisms, resulting in the greater solubility and susceptibility to carious attack of the prism cores (Robinson *et al.* 1995b, 176).

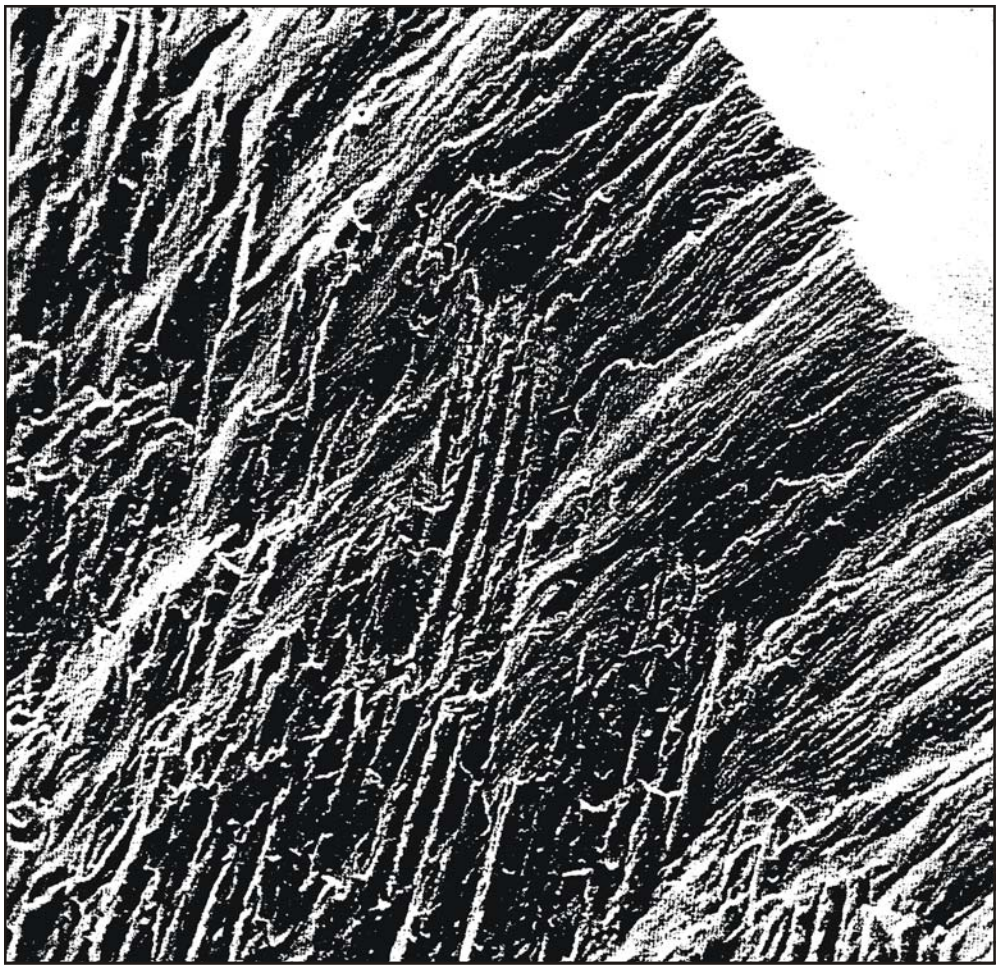

Figure 3.4 Micrograph of fractured human deciduous molar enamel. The enamel surface is in the top right corner. The contrast between the thin aprismatic surface layer and the prism direction of the bulk core enamel is illustrated. Field width 222μm. Adapted from Berkovitz et al. 1989.

At the EDJ and the tooth surface of both permanent and deciduous human enamel there is a thin (20-100μm at the surface) layer of enamel crystals in which the prismatic structure is absent (Figure 3.4). These layers are the first and final enamel secreted by the ameloblasts and are a product of the initial absence and terminal loss of the ameloblast cell process (Tomes' process) upon which the production and control of the prism structure is crucially dependent (Boyde 1989, 355; Fearnhead 1979, 910). Both layers are more highly mineralised than the core of the tissue and the crystals may run

parallel and straight but the structure can be extremely variable (Boyde 1989, 351/414/423; Brudevold & Söremark 1967, 251; Deutsch *et al.* 1997, 144; Fearnhead 1979, 910; Ripa *et al.* 1966, 46; Ripa *et al.* 1967, 39; Whittaker 1981, 247). The presence of the aprismatic surface layer varies both with tooth type (it is rarely found on permanent incisors for example) and location on individual tooth surfaces. There is some evidence that teeth where thick surface layers are more frequently found, such as molars, are more susceptible to carious attack and react differently to dental procedures (Whittaker 1981).

Other characteristic features in the structure of mature enamel, such as cross striations and brown striae of Retzius, are also related to the period of organic matrix secretion by the ameloblasts rather than the maturation stage when the majority of the mineral ions are incorporated into the developing tissue. As Hillson (1986, 161) cautions, *"In enamel, the incremental structures represent matrix formation only – they are seen exactly the same in immature enamel"*. In a similar vein, Boyde (1989, 386) warns *"Considering that the final degree of mineralisation at any one level in the enamel is achieved years after its initial secretion (in the case of man), it must be an event which occurs at the time of secretion or initial mineralisation (crystal-centre growth) that is of importance"*. Clearly, such features are produced by disruption to, or rhythms of, matrix secretion and cannot be related to periods of mineral ion incorporation during maturation. In effect, they are relics of the formation process that have been subsequently, and quite literally, fossilised into the structure of the finished tooth.

3.4.5 Timing of enamel maturation

It is difficult to ascertain from the literature whether the process of maturation in human enamel is rapid or takes place over several years. Most relevant papers skirt around the issue with much of the experimental work being carried out, for obvious ethical reasons, on animal subjects. As enamel formation is species specific, results are rarely analogous with humans (Boyde 1997, 19). Hillson states that each ameloblast spends ~300 days in the secretory stage and then switches over to maturation, but he gives no indication of the time maturation takes other than to say it may still be occurring whilst the tooth is in occlusion (Hillson 1986, 114).

Most researchers appear to agree that matrix secretion and initial crystallite elongation occur almost instantaneously (Figure 3.2). Boyde gives the time interval between the secreting cell and the crystal tip as 15 minutes in humans but there is considerable confusion when it comes to the question of the time required for these immature crystallites to mature into the finished product. He also asserts, somewhat vaguely, that *"Enamel crystals grow rapidly in thickness in human enamel. Thus they are soon not as thin as they were in the first instance"* (See recorded discussion following paper, Boyde 1997, 30&31). Fincham *et al.* (1999, 282) write *"the hardening of enamel, (results) from rapid crystal growth during the maturation stage",* whereas Sasaki *et al.* (1997, 32) state that *"Mature enamel…requires a long formation period from matrix production until the completion of maturation"*. In apparent contradiction of his comments above, Boyde (1989, 309) concurs, *"the process of increase in the degree of mineralisation known as maturation extends over a long period"* and more specifically states *"a human permanent tooth may take 5 years to harden its enamel before eruption"* (Boyde 1989, 441).

It is possible that the confusion arose because research is limited in this area but also because the situation is not simple either between or, more crucially, within teeth. Boyde (1989, 345) illustrates the internal complexity in the following passage:

> *"In human third permanent molar enamel, the tissue may reach 85% of the concentration of apatite within 100μm of the secretory enamel surface… however, the rate of increase of mineralisation from the secretory front is initially rapid and then slows down deeper in the tissue…Surveys of the increase in mineralisation in enamel during maturation using polarized-light microscopy, X-ray absorption or both show matching trends which do not relate simply to the sequence of formation and the relative age of tissue microvolumes…. To begin with, the tissue next to the enamel-dentine junction is generally substantially more mature than that formed later. This effect may be explained by a putative access of mineral ions via the pore spaces of the dentine side of the enamel-dentine junction. Secondly, in most regions of the tooth where matrix secretion has stopped, the more superficial layers of the enamel are substantially denser than those lying deep. This would be explained by the preferred access to the mineral ions necessary for growth of the crystal surfaces closer to the enamel organ cells. Thirdly, there is a trend for the more incisal or*

cuspal levels of the enamel to be more mature at any given relative thickness level within enamel than those formed later. All of these trends are superimposed upon the first trend mentioned, namely that opposite any given ameloblast the enamel which is formed first tends to be denser than that which is formed last whilst secretion is still underway."

Whilst this paragraph may not clarify the issue further - it is not, for example, clear precisely what "dense" signifies - it does serve to highlight that sectioning human tooth enamel in order to obtain time-resolved incremental data would be extremely problematic. The issue is further complicated by the realisation that the first enamel to form is to be found at the very centre of each mature enamel crystal (Boyde 1997, 18). Thus any attempt at incremental chemical analysis would need to be performed in much the same way tree ring analysis is carried out on ancient oak trees, albeit on a much smaller scale and without the firmly established annual growth pattern.

3.5 Lead and strontium incorporation in enamel and dentine *in vivo*

Pb and Sr are incorporated into enamel and primary dentine at the time of mineralisation. As neither tissue subsequently remodels or re-grows they are considered to retain Pb and Sr ingested during mineralisation (Brudevold *et al.* 1977, 1171; Koch *et al.* 1997, 425; Rowles 1967, 201; Shapiro *et al.* 1972, 470; Underwood 1977, 446; Wieser *et al.* 1996, 415). However, it has been variously claimed that erupted teeth both accumulate (Delves *et al.* 1982, 335; Ericson 1985, 506; Gulson 1996, 311; Purchase & Fergusson 1986, 248; Shapiro *et al.* 1975, 485), lose (Rabinowitz *et al.* 1993, 340) or exchange (Gulson & Gillings 1997, 823; Horn & Müller-Sohnius 1999, 263) Pb and Sr by various mechanisms during an individual's lifetime. Loss of Pb and Sr is not a problem but post-formation incorporation or exchange could change the original isotope signatures. Such contradictory conclusions, however, have often been reached because researchers used whole teeth or different tooth constituents in their analysis making direct comparisons problematic. Moreover, it is important to realise that exposure to Pb pollution has varied considerably even within the lifetime of modern subjects, whereas environmental Sr has not, and most modern children are exposed to greatly reduced Pb levels compared with preceding generations (Jaworowski 1990). As

a result, studies that compare the Pb concentration in teeth of elderly people with those of young adults or children and conclude that tooth Pb increases with age, may actually be finding evidence for declining Pb pollution not age-related accumulation. This artefact of perspective may explain why many contemporary studies conclude enamel-Pb increases with age.

Enamel and dentine are structurally and functionally very different and whole-tooth analysis will mask the individual behaviour of each tissue. Furthermore, each tissue exhibits within it characteristic Pb and Sr distributions and concentration gradients (different for each element) which make it crucial to understand precisely what tissue was included in the sample and which part of the tooth it was taken from. Neither can deciduous and permanent teeth necessarily be compared. For example, deciduous teeth appear to actively accumulate Pb in the shrinking root during the process of root dentine resorption and deciduous enamel is slightly more permeable than permanent enamel (Whittaker & Stack 1984, 40/1). Delves *et al.* (1982) also found inexplicable but substantial differences in Pb concentration between di1 and di2 from the same child, leading them to recommend standardisation of tooth type in future studies.

3.5.1 Distribution of Sr in modern human teeth

Sr concentrations are remarkably similar between the bone, dentine and enamel of an individual (Aufderheide 1989, 243; Brudevold & Söremark 1967, 259; Chaudhri 1995, 539; Parker & Toots 1980, 202; Underwood 1977, 446). Reported ranges for enamel vary considerably and are typically 50-300ppm (Brudevold & Söremark 1967, 260; Chaudhri 1995, 539; Curzon & Cutress 1983, 38/285; Losee *et al.* 1974, 129) but can exceed this in areas of high-Sr geology and drinking water. For this reason, concentrations within a localised modern population show surprising consistency but can vary considerably between geographical populations (Brudevold & Söremark 1967, 260; Underwood 1977, 445) thus making inter-population comparisons difficult. High tooth-Sr concentrations have been associated with a low incidence of caries (Curzon & Crocker 1978, 652).

It has been suggested that the enamel surface of erupted teeth may continue to take up Sr from the saliva by the cycle of de- and re-mineralisation associated with early caries

(Curzon & Cutress 1983, 292; Featherstone *et al.* 1981, 233) or by chemical exchange via the pellicle (Ericson 1985, 506; Horn & Müller-Sohnius 1999, 263). However, in both erupted and unerupted teeth in both sets of dentition, primary dentine and enamel concentrations from a single healthy tooth are very similar, although dentine may contain slightly more Sr than enamel (Brudevold & Söremark 1967, 260; Hillson 1996, 223; Rowles 1967, 221). Results from this study (Chapter Six) suggest that, if anything, enamel may contain slightly more Sr than co-genetic dentine. Recent *in situ* studies using LA-ICP-MS have confirmed that Sr/Ca profiles through longitudinal sections of tooth crowns produce Sr distribution profiles that mimic those of calcium. They conclude that Sr is relatively evenly distributed with depth in both enamel and dentine with no obvious peaks that are systematically related to surface accumulation, tissue structures or tissue type (Lee *et al.* 1999 182; Montgomery *et al.* 1999, 292).

3.5.2 Distribution of Pb in modern human teeth

In modern teeth, dentine usually contains more Pb than the enamel of the same tooth (Brudevold & Steadman 1956, 430; Gulson & Gillings 1997, 822; Purchase & Fergusson 1986, 239; Shapiro *et al.* 1972, 468). Reported enamel-Pb concentrations are typically <0.1 - 80ppm (Chaudhri 1995, 539; Fergusson & Purchase 1987, 22; Lane & Duffy 1996, 393; Manea-Krichten *et al.* 1991, 186; Purchase & Fergusson 1986, 242; Shapiro *et al.* 1972, 468/9; Solis *et al.* 1996, 360). However, most quote mean or median values below 30ppm and several studies found no examples above 10ppm (Al-Naimi *et al.* 1980, 723; Farmer *et al.* 1994, 594; Losee *et al.* 1974, 129). In a recent extensive study by TIMS of deciduous and permanent teeth from immigrants to Australia, Gulson *et al.* (1997, 789) found only two teeth (both from Bulgarians) with enamel-Pb concentrations exceeding 7ppm. In further studies of inhabitants of the Broken Hill Pb mining community they concluded that low exposure resulted in enamel-Pb of <2ppm whereas high exposure produced enamel-Pb of ~2-10ppm (Gulson 1996, 310; Gulson & Wilson 1994, 281).

Gulson & Gillings (1997) found a significant correlation between enamel-Pb and dentine-Pb in both permanent and deciduous teeth; the analysis was done on root tip dentine in permanent teeth and crown dentine in exfoliated deciduous teeth. The ratio of enamel to dentine varied between 1:1.2 and 1:8 in permanent teeth and between

1:1.5 and 1:4.1 in deciduous teeth. Brudevold & Steadman (1956, 430) quoted 1:3. Purchase & Fergusson (1986, 242) also analysed circumpulpal dentine to obtain a three-way ratio of 1:2:6 (enamel:dentine:circumpulpal dentine) whereas Al-Naimi *et al.* (1980, 723) found a ratio of 1:3:~10 in children under 17 years of age and 1:6:~30 in adults over 40. These variations may stem from the inclusion of different dentine components in the analysis of teeth from subjects of different ages.

Although neither dentine nor enamel remodel, there is evidence that Pb concentration can increase with advancing age in both dentine (Al-Naimi *et al.* 1980, 723) and enamel (Brudevold & Steadman 1956, 434; Lane & Duffy 1996, 395; Manea-Krichten *et al.* 1991, 199). Such increases may be more artefactual than real however, as Pb exposure has varied considerably during the 20th century. Crown dentine usually has less Pb than root dentine (Delves *et al.* 1982, p334; Gulson & Wilson 1994, 281; Stack *et al.* 1974, 63). If present, the thin layers of permanent tooth secondary dentine contain progressively higher concentrations, with the final layer adjacent to the dental pulp showing the highest Pb levels of any tooth tissue (Al-Naimi *et al.* 1980, 723; Shapiro *et al.* 1972, 468/9). Both root and secondary dentine are subject to continuing age-related dentine formation. Dentine-Pb also rises along the EDJ (Fergusson & Purchase 1987, 29). In contrast to Sr, high tooth-Pb concentration has been associated with an increase in caries in humans (Brudevold *et al.* 1977, 1170; Gil *et al.* 1996, 189). In the offspring of rats fed a high-Pb diet, Watson et al. (1997, 1025) reported a 40% increase in caries. Appleton (1991) demonstrated the formation of a "lead-line" in the mineralising dentine of rats that had been injected with Pb-acetate but curiously, could find no Pb in the region of the line. "Lead-lines", which have the appearance of thick Harris lines on long bone radiographs, are normally considered to be the result of heavy Pb deposition at areas of active bone growth (see section 2.4). However, the "lead-line" consisted of pathological, hypomineralised dentine rather than a concentration of Pb in the tissue and he observed a markedly irregular structure in the dentine that formed after the injection. The result of a high-Pb intake appeared, therefore, to be disruption of the mineralising process rather than the passive incorporation of Pb^{2+} in place of Ca^{2+} into the carbonate hydroxyapatite crystals (Appleton 1991, 381).

Pb concentration exhibits a steep gradient within the surface enamel of erupted and unerupted teeth in both permanent and deciduous teeth (Brudevold *et al.* 1977, 1167;

Brudevold & Steadman 1956, 432; Purchase & Fergusson 1986, 243; Shapiro *et al.* 1972, 469). The high surface concentration falls sharply within <100 μm to a constant level throughout the core enamel; this distribution is very similar to both Zn and fluorine (F) (Brudevold *et al.* 1977, 1169; Hillson 1996, 224; Lee *et al.* 1999, 183; Purchase & Fergusson 1986, 239). The surface enamel peak appears to be spatially correlated with the change in orientation and crystal structure that occurs in the surface enamel (Boyde 1989, 351, 414, 423; Brudevold & Söremark 1967, 251; Fearnhead 1979, 910; Whittaker 1981, 247). Because of the very small amount of material available when analysing the thin surface layer and the possibility of surface contamination, quoted concentrations vary considerably but are frequently >1000ppm (Fergusson & Purchase 1987, 29). Results were obtained by various methods such as pooling numbered layers from many teeth to obtain an average, or *in situ* acid-etch biopsies where it is very difficult to ascertain the exact amount of material dissolved; concentrations may, therefore, not be reliable. Nevertheless, recent semi-quantitative LA-ICP-MS transverse profiles through modern tooth crowns confirm the presence of the peak at the EDJ and the much larger and extremely variable high-Pb peak in the outer enamel layer (Budd *et al.* 1998, 126; Lee *et al.* 1999 184; Montgomery *et al.* 1999, 292). They also verify that Pb concentrations are spatially variable in dentine but remarkably constant throughout the core enamel.

3.5.3 Implications for Sr and Pb tooth analysis

It is clear from the preceding sections that enamel and dentine exhibit fundamental differences in how they incorporate Pb and Sr. Furthermore, the dissimilarity between Pb and Sr distributions themselves is puzzling given that the accepted incorporation route of both is substitution for Ca, suggesting perhaps that there are other mechanisms controlling Pb concentration. Sr, like Ca, is relatively homogeneously distributed throughout and between both tissues whereas Pb exhibits a very different profile, which appears to be related to within-tooth structures rather than Ca content. The vast majority of studies appear to accept unquestioningly that Sr and Pb are “bone-seekers” of similar habit that substitute for Ca, although there is very little hard evidence in the literature to prove that Pb does so. The few researchers who have suggested that this may not be the case have been ignored. Indeed, as Appleton (1991, 381) has pointed out Neuman and Neuman (1958, 94/95) clearly changed their minds when they studied

the question specifically: *"Preliminary observations of the deposition of lead in a model system in our laboratory indicate that its entry into the crystal does not involve a simple displacement of calcium as heretofore believed (Neuman & Neuman 1953). This "bone-seeker" needs reinvestigation."*

Once formed, enamel and primary dentine are not remodelled. Mineralised enamel is not in contact with the blood supply and is isolated from metabolic processes. Any changes must occur by diffusion or absorption from the surface or via the EDJ. Pb-peaks at both these locations together with a low and constant Pb concentration within the core enamel suggest that any inward movement into the dense, core enamel is severely restricted. However, as this profile is present in unerupted teeth also, it is by no means clear whether it is a product of mineralisation or life-long accumulation. It has been pointed out that surface concentrations of F and Pb decrease where tooth wear is greatest and any post-eruptive uptake is insufficient to keep pace with attrition rates even in fluoridated and high-risk lead communities (Brudevold *et al.* 1977, 1169). Attrition rates can vary considerably between individuals and between teeth but there is evidence that surface-Pb concentrations are higher in the cervical region and on the lingual side of the tooth (Fergusson & Purchase 1987, 30) which are both areas of low attrition. Likewise, with Sr, the constant concentrations present in core enamel and its correlation with Ca distribution suggest age-related accumulation is not happening from either the inner or outer enamel surface.

The processes of de- and re-mineralisation of the enamel surface has been proposed as a mechanism for accumulation. However these processes are associated with early caries (Featherstone *et al.* 1981; Robinson *et al.* 1995c, 234) and not with the surface of structurally normal, healthy teeth. Re-mineralisation would result in the deposition of mineral in a manner similar to calculus formation, discontinuous with, and very different from, the original, highly-ordered, carbonate hydroxyapatite crystalline structure produced by the ameloblasts. It has been suggested that dicalcium phosphate (monetite, $CaHPO_4$) or dicalcium phosphate dihydrate (brushite, $CaHPO_4 \cdot 2H_2O$) are the most likely minerals formed by this process (Robinson *et al.* 1995c, 236). In healthy teeth, there is no evidence that this happens as the thin layer of aprismatic surface enamel is a product of amelogenesis; it is not acquired after eruption (Ripa *et al.* 1966, 46; Ripa *et al.* 1967, 46). There is evidence, however, that both Sr and Pb

concentrations are higher in carious enamel and dentine (Chaudhri 1995, 539) which may result from accumulation during the development of carious lesions.

Like bone, calcification of secondary dentine is a lifelong process and the accumulation of Pb and Sr here is, perhaps, unsurprising. Circumpulpal dentine is in constant contact with the blood supply and Pb and Sr concentrations may be expected to be higher here, even if secondary dentine is not present. Furthermore, it has been established that although regarded as bone-seeking elements, Pb and Sr have a greater affinity for smaller, rapidly forming carbonate hydroxyapatite crystals and are therefore preferentially incorporated into mineralising rather than mature bone, trabecular rather than cortical bone, dentine rather than enamel (Aufderheide & Wittmers 1992, 813; Brudevold & Söremark 1967, 260; Likins *et al.* 1960, 2156). This discrimination against the incorporation of Pb and Sr into the existing carbonate hydroxyapatite lattice would be greatest during post-formation incorporation into enamel, so it is perhaps not surprising that mature, core enamel is resistant to subsequent change and thus retains pre-eruptive isotope ratios.

However, the resulting difference in Pb and Sr concentrations between enamel and dentine may also stem from differential incorporation during mineralisation or subsequent age-related increases. This would depend on what the analysed sample included: whether it was root/whole tooth dentine/primary crown dentine or if circumpulpal dentine was left intact and whether secondary dentine was present. Although primary dentine is not remodelled, additional Sr and Pb may be incorporated during secondary dentine formation or the infilling of tubules that produces root sclerosis; both are age related processes. What difference they will make when included in analyses will depend on the age of the individual when the tooth was extracted. Secondary dentine, is difficult to identify and isolate (van Rensburg 1986, 629) and although many researchers claim to have analysed or removed it, it was not found by Gustafson (Gustafson 1950, 52) in any individuals under the age of twenty-eight. Moreover, whilst the mineralisation of enamel and crown dentine in a single tooth is contemporaneous, root formation may not be completed for up to seven years later, during which time Pb or Sr exposure may change significantly. A clear distinction must be made, therefore, between analyses that utilise surface enamel and core enamel, and primary crown, primary root, circumpulpal and secondary dentine.

In order to analyse a consistent set of samples taken from a wide range of spatially and temporally unrelated individuals it is necessary to standardise the tissues under analysis as far as possible. Clearly, possible age-related phenomena such as surface increases, secondary dentine and root sclerosis should be removed if teeth from children and adults are to be compared. **Enamel samples should consist of the core tissue only and must be free from all surface enamel and of the EDJ. Dentine samples should be of primary, crown dentine, not root dentine, and cleaned of all circumpulpal, and consequently, any secondary dentine. These precautions should ensure that as far as possible samples are standardised to contain only tissue mineralised during childhood and thus enable teeth from individuals of varying age at death to be compared.**

3.6 Post-mortem behaviour of enamel and dentine

In addition to the biological processes that create and change dental tissues *in vivo*, teeth that are extracted from archaeological remains have also survived a post-mortem history that has not been metabolically constrained and that cannot now be exactly recreated. Clearly, there is an *a priori* assumption in trace element and isotope studies that *in vivo* signatures can be retrieved and any exchange, substitution or equilibration between the biogenic tissue and the burial medium has been negligible. For this reason, an understanding of ante-mortem skeletal and dental parameters is vital in order to identify the secondary effects of post-mortem diagenesis and how individual tissue characteristics may impact on the process of diagenetic change (Hancock *et al.* 1989; Robinson *et al.* 1986, 51; Tuross *et al.* 1989).

3.6.1 Diagenetic changes in skeletal tissue

For the vast majority of archaeological remains the burial medium is soil and preservation (or survival) is a result of the physical, chemical and biological interaction between soil and skeleton. As many researchers have pointed out this can vary on both large and small scales, from cemetery to cemetery and between two teeth from the same jaw (Henderson 1987, 43; Radosevich 1993, 285; Sponheimer & Lee-Thorp

1999, 148). This makes the assessment of current soil conditions problematic even before the extra dimension of change over time is considered. Preservation does not appear to be directly related to the length of time that skeletal remains have been buried; *"diagenesis is only incidentally a time-dependent process."* (Parker & Toots 1980, 200). However, several studies have concluded that chemical alteration can occur remarkably quickly and then remain relatively stable thereafter (Koch *et al.* 1997, 428; Sponheimer & Lee-Thorp 1999, 148). Pate and Hutton (1988, 730) give the three main processes for post-mortem chemical bone diagenesis as:

1. Precipitation of separate mineral phases e.g. calcite, in small voids and fractures
2. Ionic exchanges between the soil solution and carbonate hydroxyapatite lattice sites
3. Recrystallisation and crystal maturation involving the conversion of carbonate hydroxyapatite into a larger, well-crystallised geological apatite

Extrinsic factors such as site hydrology, soil composition, Eh, pH, temperature, element transport, uptake and diffusion, flora and fauna, burial depth, associated grave goods can all affect survival. Isolating and ranking the effects of these factors in order to create an over-arching model of bone diagenesis has engaged but eluded researchers mainly because under different sets of burial conditions the dominant factor will be different and preservation depends, ultimately, on what has actually been buried. Age, sex and pathology, factors intrinsic to an individual skeleton, can all impact upon the macroscopic and microscopic mineralisation, porosity and size of the skeletal tissue and thus affect survival. The majority of researchers conclude that physical and chemical bone diagenesis is ubiquitous even if macroscopic preservation is good, but predictable only in so far as it will undoubtedly occur (Bell 1990, 90; Kohn *et al.* 1999, 2744; Lambert *et al.* 1990, 467; Nelson *et al.* 1986, 1948; Radosevich 1993, 316; Sponheimer & Lee-Thorp 1999, 144; Vuorinen *et al.* 1996, 158). Site hydrology, for example, appears to exert a major influence on bone preservation (Hedges & Millard 1995, 162; Nielsen-Marsh & Hedges 2000a, 1139) and would be especially significant in the temperate climate of Great Britain. However, many of the intrinsic factors that make bone and dentine susceptible to diagenetic change do not necessarily apply to enamel.

In a variety of biochemical and isotope studies, enamel is considered to be stable and resistant to structural and chemical change over geological (Bocherens *et al.* 1994, 791; Glimcher *et al.* 1990, 219; Horn *et al.* 1994, 360; Kolodny *et al.* 1996, 168; Michel *et al.* 1995, 154; Michel *et al.* 1996, 117; Rink & Schwarcz 1995, 255; Wang & Cerling 1994, 288) as well as archaeological time scales (Budd *et al.* 2000a, 691; Elias *et al.* 1982, 2578; Ericson 1993, 169; Koch *et al.* 1997, 428; Lee-Thorp & van der Merwe 1991, 349; Montgomery *et al.* 2000, 376; Nielsen-Marsh & Hedges 2000b, 1158; Price *et al.* 2002, 127; Price *et al.* 1994a, 417; Robinson *et al.* 1986, 31; Vernois *et al.* 1987, 83). As previously discussed, enamel and dentine contain the same carbonate hydroxyapatite mineral phase but their structure, formation process, crystal size and organic content are very different. These differences reflect the specific functions for which each tissue is created and as a consequence dentine bears far more similarities to cortical bone than to enamel. It should, therefore, be expected that enamel would also react in dissimilar ways to dentine and bone when subjected to post-mortem taphonomic and diagenetic processes.

Certain parameters intrinsic to skeletal tissue, such as porosity, and hence surface area and density, appear to have a major effect on how readily the skeleton interacts with the soil (Hanson & Buikstra 1987, 561; Hedges *et al.* 1995, 205; Kyle 1986, 411; Nielsen-Marsh & Hedges 2000a, 1147; Von Endt & Ortner 1984, 252). As a result, diagenetic histological change is not random but appears to be specific to both the features of bone structure (Bell 1990, 101) and the burial site (Hedges *et al.* 1995, 203). Mature enamel has considerably higher density and much lower porosity than any other skeletal tissue and is kinetically more stable. At the point of burial it is virtually entirely mineral, in effect already fossilised. During burial, it retains the morphology created during matrix formation over millions of years and is normally indistinguishable microscopically from modern tissue (Boyde *et al.* 1988, 1488; Kolodny *et al.* 1996, 168).

The dense, felted structure of enamel leaves little room for cracks and voids that facilitate groundwater diffusion or deposition of secondary minerals. The enamel crystal surface area available for ion exchange, considered the major mechanism for Sr and Pb incorporation, is far less than for bone or dentine (Neuman & Neuman 1953, 12). In fully mineralised enamel the pore size approaches atomic dimensions and whilst accessible to univalent ions, the higher charge and larger size of polyvalent ions (i.e.

Pb^{2+} and Sr^{2+}) prevents them from entering the pore spaces (Neuman & Neuman 1958, 103). Furthermore, bone and dentine are composite tissues of mineral and collagen and are thus susceptible to organic decay. This increases porosity and de-stabilises and softens the tissue (Beeley & Lunt 1980, 371/3) leading to carbonate hydroxyapatite re-mineralisation and crystal growth (Hanson & Buikstra 1987, 561). Enamel, however, does not contain collagen and has a very low residual protein content that plays no part in the stability of the tissue. Moreover, carbonate, often considered to be the Achilles Heel of carbonate hydroxyapatite both *in vivo* and post-mortem due to its higher solubility, is concentrated at the EDJ (<4%) and decreases towards the surface enamel. However, as it appears to be encapsulated at the centre of the enamel crystals (Robinson *et al.* 1995b, 176), and it is the crystal surfaces only which can participate in ion exchange, most enamel mineral would be effectively isolated from diagenetic fluids. Porosity may also be increased by microbial tunnelling of micro-organisms which appear to attack both bone and dentine (but not enamel) shortly after burial (Hanson & Buikstra 1987, 559; Hedges *et al.* 1995, 203; Nielsen-Marsh & Hedges 2000b, 1158; White & Hannus 1983, 321). The high level of mineralisation and the very much larger crystal size, and hence lower surface area, renders enamel kinetically stable and greatly reduces opportunities for surface ion uptake which is the predominant mechanism for ion incorporation in mature carbonate hydroxyapatite (Neuman & Neuman 1953, 36).

3.6.2 Methods developed to identify diagenetic change

Several methods have been developed to identify diagenetic change in skeletal, predominantly bone, tissue such as increased crystallinity, changes in the Ca/P ratio, histology, discoloration and incorporation of rare earth elements. Many researchers recommend that such screening should be performed prior to any chemical or isotope analysis (Price *et al.* 1992, 525). For Sr and Pb isotope analysis, their utility and validity are however, doubtful because it is unclear how such proxy tests prove the presence or absence of chemical or isotope change (Reeser *et al.* 1999, 230). Clearly, as perceptively argued by Burton et al. (1999, 614), *"these tests of integrity may be independently passed or failed without directly confirming or negating the hypothesis that Ba/Ca and Sr/Ca reflect biological levels"*. Likewise, in geological studies, visible appearances are considered no guide to isotope alteration (J. Evans pers. comm.).

For example, it cannot be automatically assumed that either good macro-morphological preservation, lack of discoloration or pristine histology indicate that *in vivo* chemical and isotope signatures are preserved (Beeley & Lunt 1980, 376; Bell 1990, 90; Hanson & Buikstra 1987, 561; Kolodny *et al.* 1996, 168; Reeser *et al.* 1999, 230). Conversely, conditions leading to poor organic preservation will not necessarily indicate that the mineral phase, of enamel in particular, is isotopically altered. Radosevich (1993, 314) has argued that good histological preservation occurs precisely because the bone is rendered less soluble due to the incorporation of fossilising elements such as F and Sr: *"Although the relative intactness of histological structures may indicate less diagenetic alteration in some cases, it is equally likely……that the reason these structures remain intact is* because *of incipient fossilization"*.

The ability to retrieve a Ca/P ratio to within the range of modern bone does not necessarily mean that the tissue is unaltered – both elements could have increased or leached yet together produced a seemingly "normal" ratio (Radosevich 1993, 307). For example, Beeley and Lunt (1980, 377) retrieved normal Ca/P ratios (and radiographs) from all their dentine samples irrespective of whether or not there had been extensive organic decay. Furthermore, modern clinical studies use Ca/P ratios and the crystallinity index to follow age, biomechanical and disease-related mineralisation changes. Neither crystallinity nor Ca/P ratios are homogeneous within a single bone but depend upon the maturity of both the chosen site and the individual: *"any macroscopic piece of bone contains mineral particles that have just been recently deposited and some that have been there for various lengths of time. This spatial heterogeneity is compounded by a temporal one. It has been observed that bone mineral changes with the age of the animal which means that it changes with the age of the tissue itself. In growing animals…the first mineral deposited is different in appearance from the same mineral in older more mature tissue."* (Grynpas 1993, S57). In modern bone, Ca/P molar ratios can vary between 1.3 and 1.66 and crystal size is much smaller in osteoid or recently formed bone. Consequently, Ca/P ratio and crystallinity will vary depending upon the maturity of both the chosen site on the bone and the individual (Grynpas 1993, S57; Neuman & Neuman 1953, 25). Indeed, this variation in density on the microscopic scale has recently been harnessed in order to obtain bone fractions representative of the last few days to ~18 years of life (Bell *et al.* 2001). Moreover, it has been demonstrated

that, within structural limits, the Ca/P ratio of bone is intimately dependent on that of the blood, and blood Ca/P can vary with diet (Neuman & Neuman 1953, 29).

Clearly, this variation *in vivo* would make the application of these two techniques to bulk archaeological bone problematic as any observed deviation from "normal" values may be due to the localised rate of active *in vivo* bone modelling and turnover rather than post-mortem change. Crystallinity and as a result, Sr concentration, have been shown to increase during the 10 years since death in animal bones that have never been buried (Tuross *et al.* 1989, 665). Enamel does not undergo *in vivo* remodelling or modelling and there is little room within the tissue for enamel crystals to mature further. As a consequence, neither its Ca/P ratio nor crystallinity index should alter appreciably. Little evidence has been found of increased crystallinity in even fossil enamel (Sponheimer & Lee-Thorp 1999, 148).

3.6.3 Methods developed to address diagenetic change

As already mentioned, most archaeologists and palaeontologists acknowledge that diagenesis is a problem and many believe that archaeological bone cannot be analysed without resorting to chemical or physical pre-treatment procedures (Horn *et al.* 1994, 360; Lambert *et al.* 1990, 468; Price *et al.* 1992, 525). Reliance on such pre-treatment procedures has enabled claims to be made that *"diagenesis is not a substantial problem in Sr isotope analysis"* (Grupe *et al.* 1997, 520) and *"Post-depositional contamination of bone and tooth (diagenesis) is not a significant problem in strontium isotope studies"* (Price *et al.* 1998, 408). In comparison to the large body of work dealing with Sr diagenesis, there has been little chemical investigation into the fidelity of biogenic Pb in archaeological skeletal tissue (Millard 1998, 98). As the mechanism of uptake is not as apparent as, and is possibly very different from, that of Sr, chemical pre-treatments have not been developed specifically to remove diagenetic Pb. Few studies employ Pb isotope analysis; the vast majority measure elemental concentrations in order to investigate Pb-exposure in historical and prehistorical periods.

Pb uptake post-mortem has been demonstrated in teeth and bone (Waldron 1981, 396; Waldron 1982, 195; Waldron 1983, 39; Whittaker & Stack 1984, 41). Using fast particle activation analysis, Waldron (1982; 1983) found that Pb in modern teeth was

concentrated in the circumpulpal dentine whereas in an archaeological example it was concentrated on the tooth surface. He interpreted this to mean that the tooth had absorbed Pb onto the surface during burial. Whilst this may be the case, the surface enamel is clearly susceptible to Pb accumulation even before the tooth has erupted into the oral cavity. This makes it difficult to establish with certainty whether the high-Pb peaks observed in archaeological surface enamel result from *in vivo* or post-mortem processes and suggests the peaks occur as a result of the different structure of the surface enamel itself. Many Pb concentration studies have either avoided the question of diagenesis (Aufderheide *et al.* 1981) or concluded it was not a problem (Corruccini *et al.* 1987, 238; Grandjean & Jørgensen 1990, 7; Vagn Nielsen *et al.* 1982, 147)

3.6.3.1 Pre-treatment procedures

Physical and chemical methods have been developed to combat the effects of post-mortem mineral deposition and recrystallisation of biogenic carbonate hydroxyapatite but doubts are still expressed as to whether these procedures are effective in removing the products of diagenesis entirely. Physical methods mechanically separate and remove tissue most likely to be intrinsically altered or to incorporate diagenetic phases, for example trabecular bone and surface enamel. However, there is doubt that such methods will successfully remove smaller soil-derived particles within the porous bone structure (Kyle 1986, 415; Lambert *et al.* 1990, 467). Whittaker & Stack (1984, 41) recommended physical removal of the more permeable surface enamel of deciduous teeth before Pb analysis. Whilst surface enamel appears to accumulate elements such as Pb both *in vivo* and post-mortem, the core tissue is considerably denser than bone or dentine and the space available for such extrinsic incorporation of diagenetic phases represents only a small proportion of the overall tissue volume. Furthermore, LA-ICP-MS profiling of Pb distributions through cross sections of archaeological teeth show that, as in modern teeth, the levels of Pb in the core enamel tissue are surprisingly and consistently low, with no apparent diffusion from either the surface enamel or EDJ (Budd *et al.* 1998, 134; Montgomery *et al.* 1999, 295). Careful sampling to ensure that both the surface peaks and all trace of dentine and the EDJ were physically removed, would isolate a sample of core enamel that has been relatively impervious to *in vivo* post-eruption or diagenetic change.

Chemical methods, such as the solubility profiling technique developed by Sillen principally for bone-Sr studies (Sillen 1986; Sillen 1989), involve washing or leaching samples in weak acids prior to analysis. The underlying principle rests on the assumption that post-mortem additive mineral phases and recrystallised carbonate hydroxyapatite contain more carbonate than the original biogenic apatite and are therefore, more soluble, or conversely, contain more fluoride which renders them less soluble (Sillen *et al.* 1998, 2468). The number and sequence of the washes that contain the biogenic Sr will depend upon the specific burial conditions of the samples under investigation (Sillen & Sealy 1995, 314). However, to be able to leach diagenetic Sr from bone whilst leaving the biogenic Sr intact in either a leach or residual tissue, the diagenetic Sr must be largely additive and, therefore, removable, and this will not always be the case (Sillen & Sealy 1995, 318). Such leaching methods will not distinguish nor remove diagenetic Sr that has exchanged with the biogenic Sr ingested *in vivo* and is located within the biogenic apatite.

Many researchers have used chemical leaches experimentally and found that whilst they may lead to some improvement, neither the remaining insoluble portion nor any of the leaches constitute wholly biogenic apatite (Elliott *et al.* 1998, 430; Hanson & Buikstra 1987, 561; Horn *et al.* 1994, 361; Koch *et al.* 1992, 284; Koch *et al.* 1997, 426; Nelson *et al.* 1986, 1947; Nielsen-Marsh & Hedges 2000b, 1158; Sealy *et al.* 1991, 412; Sillen & LeGeros 1991, 385; Trickett 1999, 61; Tuross *et al.* 1989, 666). Furthermore, acid leaches may actually bring about alteration of the existing tissue. The pre-treatment method employed by Price et al. (1992, 525) which involves leaching in 1M acetic acid in order to return the Ca/P ratio to that of modern bone, has been criticised for altering the carbonate hydroxyapatite crystal structure in archaeological specimens (Lee-Thorp & van der Merwe 1991, 349). Sillen and Sealy (1995) also claimed the ashing at 500ºC and 50:50 (v/v) acetic acid/H_2O leaching procedure of Nelson et al. (1986) produced a similar alteration. Lambert et al. (1990) tested four pre-treatment methods on archaeological bone and concluded that 1N acetic leaches were extremely harsh, removed large quantities of biogenic bone and were unsuitable for trace element studies. They recommended a combination of physical abrasion and Sillen's solubility profiling method.

Several studies have applied chemical leaching methods specifically to enamel samples (e.g. Ezzo *et al.* 1997; Grupe *et al.* 1997; Price *et al.* 1994a; Price *et al.* 1998; Price *et al.* 1994b; Price *et al.* 2000; Sealy *et al.* 1995). However, they did not analyse enamel-Sr isotope ratios before and after chemical pre-treatments so it is difficult to ascertain whether the process significantly improves bulk enamel Sr isotope ratios. In the few studies that have obtained before and after Sr isotope ratios, the removal of a more soluble, diagenetic component does not appear to have a significant effect on the bulk enamel Sr isotope ratio. Horn *et al*. (1994) used a weak acid leaching process to remove diagenetic Sr from ~700ka fossil stag enamel. They concluded only ~6% of the total enamel-Sr was soil derived and of different isotope composition to the remaining enamel. Koch et al. (1992, 284) applied a modified solubility profiling method to tooth enamel from a late Miocene fossil salmon buried in a terrestrial context to attempt to recover the seawater Sr isotope ratio. Although the enamel was obviously heavily contaminated with terrestrial Sr isotope ratios, progressive acetic acid leaches were virtually invariant.

On much younger archaeological remains, Trickett (1999) applied the solubility profiling method of Sillen separately to bone, dentine and enamel from each of two individuals. Bone and dentine Sr isotope ratios moved progressively further away from the soil Sr isotope ratio toward those of the enamel with each leaching stage. However, he found only a small change (< 0.0006) in the Sr isotope ratio between the enamel leaches, final residue or the original, untreated (but physically abraded), tissue. Whether there is any advantage in using chemical leaches on enamel will depend on the precision to which the Sr isotope ratios need to be determined. Archaeological interpretations may require changes in the second or third decimal place of the Sr isotope ratio to clearly differentiate individuals from the variation found within the local signature. Chemical leaching of a small amount of diagenetic Sr may, therefore, make negligible difference to the final Sr isotope ratio of the bulk tissue. Only if provenancing to source is required may such a treatment be necessary or justified.

Many researchers have concluded that diagenetic Sr incorporation occurs not only by addition but also by exchange with the Sr ingested *in vivo* which chemical pre-treatments are deliberately designed not to remove (Budd *et al.* 2000a, 692; Nelson *et al.* 1986, 1947; Radosevich 1993, 311; Tuross *et al.* 1989, 668). This conclusion was

also reached by Kohn et al. (1999) on ~3Ma old, discoloured, fossil enamels. Their results indicated that enamel was altered, although not to the same extent as dentine and both contained concentrations of rare earth elements (REE's) and U above the very low levels normally found in modern enamel and dentine. They believe such increases specifically indicate diagenetic chemical and isotope change of Ca-site elements such as Sr and Pb, and therefore recommend REE and U analysis as a valid means of assessing trace element fidelity in fossil remains (Kohn *et al.* 1999, 2744). Parker & Toots (1980) however, found a number of elements in fossil bone occurred in secondary minerals deposited in pore spaces, e.g. Pb, U, Al, Si and Ba (but not Sr) whilst *"others appear to substitute in to the apatite structure"*, i.e. Pb did not (Parker & Toots 1980, 207). Their conclusions regarding Sr were that *"Sr is a particularly useful palaeobiological tool in that it appears to be unaffected by diagenesis over a wide range of conditions."* (Parker & Toots 1980, 203). However, Radosevich (1993) warns that *"Sr may be immune to diagenesis over a wide range of conditions but these conditions do not include those found in soils most favourable to bone preservation….in conditions most favourable to the preservation of archaeological bone (pH of approximately 7 or more) bone can easily become a strontium sponge"* (Radosevich 1993, 314/5). That is, if it has survived it is implicitly altered, thus rendering pre-treatment methods redundant. The results of the Monkton study (Chapter Six) where excellent bone preservation was coupled with Sr dentine diagenesis would support this hypothesis.

As a final consideration, it is noteworthy that apatite, either mineral or biological, is used for *in situ* remediation of contaminated land as it immobilises radioactive isotopes and polluting elements such as ^{90}Sr and Pb (Laperche *et al.* 1996, 3326; Ma *et al.* 1993, 1810), although as previously mentioned, in the case of Pb it appears that immobilisation occurs by dissolution and reprecipitation of the less soluble hydroxypyromorphite rather than Pb incorporation into the apatite lattice (Lower *et al.* 1998b, p147; Ma *et al.* 1993, 1810). Such a mechanism may also account for Pb accumulation in, or leaching from, buried archaeological bone.

3.6.3.2 Soil analysis

Burial soil analysis has only recently come to prominence in archaeological studies that concern bone geochemistry. A few isotope and trace element studies of archaeological skeletal material have recommended soil analysis in order to assess the availability of soluble and exchangeable ions in the burial soil (Horn *et al.* 1994, 360; Horn & Müller-Sohnius 1999, 263; Pate & Hutton 1988, 736; Vuorinen *et al.* 1996, 158; Waldron 1981, 397). However, many studies do not incorporate soil analyses (Åberg *et al.* 1998; Cox & Sealy 1997; Latkoczy *et al.* 1998; Price *et al.* 2000; Sealy *et al.* 1995; Yoshinaga *et al.* 1998) or use whole soil analyses (Ghazi 1994, 628; Grupe *et al.* 1997, 519; Price *et al.* 1994b, 322).

For trace element concentration studies the amount of an element present in the bulk soil is not necessarily a good indicator of its availability: *"The measurement of concentrations in two elements does little to acquaint us with the geochemistry of the site's soils"* (Radosevich 1993, 275). Elements may be present in large quantities precisely because they are immobile and are thus accumulating over time. Pate and Hutton (1988, 736) recommend extracting both the water soluble cations of the bulk soil solution and the exchangeable cations of the more tightly bound hydration layer in order to model the interaction between burial soils and archaeological bone. They point out that in arid, alkaline soils the soluble cations will dominate the soil solution, whereas the contribution from exchangeable cations will increase in more humid climates. Their study on arid, alkaline soils demonstrated Sr and Ca were highly soluble and, although it was not analysed in their study, expected Pb, along with transition metals like Cu, Zn and Fe to be immobile due to adsorption by silicate clays (Pate & Hutton 1988, 734). This would perhaps indicate that Sr is mobile in alkaline soils, whereas Pb is mobilised under acidic conditions.

It is still, however, difficult to ascertain how much Pb or Sr is derived from the burial environment without performing isotope analysis of both the burial soil and skeletal tissue. Isotope analysis has been suggested as a method of discriminating between diagenetic and non-diagenetic Pb and Sr (Millard 1998, 98; Price *et al.* 1992, 525; Waldron 1981, 397). Whilst there has been a considerable number of Sr isotope ratio studies there are relatively few that have analysed Pb isotope ratios in archaeological

human skeletal material. In isotope studies, characterisation of the soil isotope ratios can be used to ascertain the isotope ratios of the mobile soil cations available for incorporation into buried skeletal tissue in the same way that the mobile isotope ratios are characterised for biosphere food web studies (see section 2.3). Nevertheless, it has to be considered that this ratio may not have remained constant throughout the entire burial history of the analysed remains. If isotope signatures are obtained from skeletal material that are significantly different from the burial soils, it indicates that such a signature must have been obtained from some other source, biogenic or diagenetic. However, if soil and skeletal isotope ratios are very similar it is difficult to establish if the skeletal ratio is biogenic or diagenetic since the mobile burial soil ratio may be identical to that of the local plants and animals. In such cases, it will not be possible to distinguish between local individuals or diagenetically obscured remains, whereas skeletal tissue that produces an isotope ratio very different from either the local signature or the burial soil is clearly different.

Enamel is intrinsically more resistant to diagenetic change and whilst the isotope ratios it contains may not be completely unaltered, they will represent a *minimum* difference from soil ratios (Grupe *et al.* 1997, 524; Koch *et al.* 1992, 284; Price *et al.* 2002, 127; Price *et al.* 1994a, 417; Sealy *et al.* 1995, 296; Sillen *et al.* 1998, 2470). This may well be sufficient for the purposes of the study. Koch et al. (1992, 284) concluded that whilst their fossil migratory salmon enamel samples were altered *"migrational analysis was still possible because the attenuation of the freshwater Sr isotopic signal was not complete."* Whether or not a biogenic isotope ratio can be retrieved will depend on how high the *in vivo* concentration of Pb and Sr were and how much of the two elements has been taken up from the burial environment. For example, the *in vivo* Pb isotope ratio of an individual buried in a Pb coffin is highly likely to have been swamped with large amounts of coffin-derived Pb, whereas high-exposure individuals buried in a low-Pb burial context may still retain a predominantly biogenic Pb isotope ratio. As Koch et al. (1992) demonstrated, it is still possible to distinguish between freshwater and marine habitats even in very old, heavily fossilised and altered material. Clearly, in the case of (comparatively recent) archaeological enamel, survival of *in vivo* isotope signatures that will allow individuals to be identified as different may be expected. However, as enamel is resistant rather than entirely immune to diagenesis, provenancing to a specific source may thus prove problematic.

3.7 Conclusions

It can be seen from the preceding discussion that the enamel cap is morphologically formed long before mineralisation is complete and crown dentine is mineralised before the enamel. Not until the massive influx of ions during the, possibly late, maturation stage is the bulk of the enamel mineral deposited. Although the phases of secretion, transition and maturation move in waves from the cusp tip to the cervical region, this is by no means a well understood or simple process and other, smaller scale, processes may be superimposed on this broad trend. Consequently, mineral may be deposited at the cusp tip at the same time as it is deposited in the cervical enamel, and enamel near the EDJ may be more mature than that at the cusps. Moreover, because maturation may take several years after the tooth is fully formed, any incremental information deposited during matrix secretion may be smoothed or removed entirely during subsequent phases.

Enamel is not mineralised according to visible incremental structures, rather these structures are subsequently fossilised in the completed tissue. Any meaningful investigation of incremental structures in enamel would need to be at the individual crystal level in a method analogous to investigations of tree rings, as enamel also grows incrementally from the centre of each crystal to the outside. Unfortunately, there is currently no instrumental method that can provide *in situ* trace element or isotope information at this level of resolution. Although archaeological textbooks such as Hillson's *Teeth* (1986) and *Dental Anthropology* (1996) clearly point out the distinction between formation and mineralisation/maturation, it does not seem to have filtered into mainstream knowledge, perhaps because the word "formation" has been misconstrued.

Empirical observations of juvenile archaeological dentitions undertaken during this study appear to confirm that complete enamel mineralisation occurs after the enamel is morphologically formed. In contemporary studies, the division between transitional enamel and the hard, white tissue of mature enamel can be clearly seen if developing teeth are removed from the jaw. The large amount of water that has replaced the organic matrix in transitional enamel dries out quickly producing a sharp boundary and although this has been clearly shown on developing foetal bovine incisors (Robinson *et al.* 1995a, 149/150), there is little comparable data on human infants. Such a study

could, however, be carried out on archaeological remains as there is clearly a disparity in resistance of transitional and mature enamel to diagenetic alteration during burial although it is not clear whether any colour difference is present before burial or is solely an artefact of burial. The implications of this observation on the integrity of the *in vivo* isotope signature in enamel that is not fully mineralised are also uncertain.

Formation tables must be understood for what they actually are. Ages are dental development ages which may not relate directly to the actual age of the individual in question. Furthermore, those developed from radiographs of jaws are following crown mineralisation and not matrix formation. As noted by Liversidge (1994, 40) and Hillson (1986, 179), teeth will only be visible on radiographs once they reach a level of *sufficient,* although perhaps not complete, mineralisation. As a consequence, if Pb and Sr are incorporated into enamel along with the vast majority of the mineralising ions this will occur during the maturation phase, towards the end of the crown formation period. The timing post-dates and is therefore, not directly related to, that of visible incremental structures. In dentine however, where formation and maturation occur within a matter of days, such incremental information may be obtainable.

Diagenesis provides a further complication with the analysis of archaeological teeth. Current methods to identify and remove diagenetic changes, with the possible exception of REE and U analysis, do not necessarily isolate diagenetic as opposed to biogenic differences and, more importantly, are not valid indicators of isotopic change. They may also bring about changes in the original tissue. Furthermore, for Sr and Pb isotope analysis of archaeological enamel, chemical pre-treatments developed for archaeological bone may be redundant if, as it appears, they do not alter the enamel isotope ratios to any significant degree. Neither is it clear that Pb and Sr behave in the same manner under the same burial conditions; most avenues of investigation appear to indicate that they do not, although few studies specifically address this problem.

A variety of soil leach approaches appear to be more representative of the present mobile Sr and Pb isotope ratio than whole soil analyses. It is not clear, however, which type of leach, if any, represents the diagenetic fluids that have surrounded the skeletal material throughout the entire period of burial. It would be interesting to compare soil

leach isotope ratios with those of diagenetically altered archaeological tissue. For this purpose, dentine would be better than bone as the ontogenetic period of primary, crown dentine and enamel overlap considerably and both do not subsequently remodel thus greatly reducing the opportunities for *in vivo* change. Bone is continually turning over, so there can be no certainty even in very young children that its isotope ratio and that of enamel were still the same at the time of burial.

Dentine would appear to behave in a very similar manner to archaeological bone whilst enamel is a very different tissue and inherently not susceptible to the same alteration post-mortem. Even in extremely old fossils, enamel appears to retain some biogenic information long after dentine and bone have equilibrated with the burial environment. A sampling procedure for human teeth must take account of the various structures within, and the processes that form, the different tissues such as the EDJ, the surface enamel layer, mantle dentine, secondary dentine, crown versus root dentine and the decreased mineralisation that can be present in histologically complex areas of enamel. This is further complicated by the implications of the 3-D structure of teeth. It is critical that the varying thickness of the enamel cap and the undulating shape of the underlying dentine of each tooth type be known prior to sectioning. To be able to compare Pb and Sr from a tooth of an adult with that from a child it is vital to remove any tissue that may have been altered or formed after eruption. Isolating the core enamel by removal of the surface and EDJ and also preparing a sample of crown dentine that is heavily abraded to remove circumpulpal, secondary and root dentine will give the best chance of selecting comparable tissues. It will also produce enamel and dentine samples that have had the tissue most susceptible to diagenetic alteration removed.