CHAPTER FIVE

SAMPLES AND METHODS

5.1 Tooth samples used in this study

The samples used in the study were from human deciduous or permanent dentitions plus a small number of animal teeth. Initially, a few archaeological teeth from unstratified contexts and donations of modern extracted or exfoliated teeth were used to investigate and test preparation and analytical procedures. Thereafter, most samples came from current or completed archaeological excavations where burial context information was well recorded and soil samples were available.

5.1.1 Recording and assessment methods

Samples from archaeological sites are normally assigned a unique classification number that identifies the individual skeleton at the point of excavation. These numbers were retained.

5.1.1.1 Sample recording

The type of tooth selected for analysis and its precise position in the mouth was recorded where possible using the abbreviations previously given in Table 3.1 and adapted from those recommended by van Beek (1983, 6). Unfortunately, archaeological dentitions frequently suffer from extensive attrition and wear, sometimes to the extent that all identifying crown features have disappeared. Similarly, deciduous teeth which are in the process of root resorption prior to loss, or permanent teeth with incomplete or absent root formation can be problematic to identify. As a result, isolated, single teeth that were not removed directly from the alveolar bone were sometimes difficult to identify precisely with absolute confidence. In such cases, where alternative samples were not available, teeth were identified as accurately as possible and any unidentifiable attributes (e.g. left/right, maxillary/mandibular) left uncategorised.

5.1.1.2 Preservation

Archaeological teeth can appear very different both during sample preparation and visually from modern examples although this varies enormously and is dependent on their individual post-mortem history. This is rarely a result of enamel degradation but stems from shrinkage, discoloration and softening of the underlying crown dentine, which normally adheres to and supports the overlying enamel. This softening is not related to dissolution of the mineral phase but appears to result from reduced cohesion as a result of collagen loss (Beeley & Lunt 1980, 376/7). There appeared to be little correlation between the length of time a tooth had been buried and its continuing similarity to a modern tooth; preservation and protein loss can and did, vary widely even between two adjacent burials

Archaeological teeth were assessed on their internal and external macroscopic similarity to their modern counterparts and their physical properties during preparation and scored using the criteria in Tables 5.1 and 5.2. The enamel and dentine from each tooth were scored separately; it was not assumed that post-mortem conditions would alter both tissues in the same manner. Any system of classifying teeth on such criteria is inherently subjective but in practice there are clear and obvious differences between the physical behaviour and appearance of modern and archaeological teeth. Whether, and in what way, these post-mortem changes affect the integrity of the Sr and Pb isotope signatures in enamel and dentine was not clear at the project outset; there is no straightforward relationship between macroscopic appearance and chemical alteration on the microscopic scale (Beeley & Lunt 1980, 376; Bell 1990, 90; Hanson & Buikstra 1987, 561; Kolodny *et al.* 1996, 168; Reeser *et al.* 1999, 230). Consequently, preservation information was recorded prior to the teeth being destroyed so it could be subsequently assessed against the isotope data.

It was found that enamel, though frequently cracked and sometimes loose to the point of falling away from the rest of the tooth when dental tools were applied, rarely remained discoloured after mechanical surface cleaning, whereas dentine usually stayed some shade of cream to dark brown. Physically, enamel behaved the same when bisected and cleaned with dental tools whether archaeological or modern, whereas dentine rarely did and was sometimes extremely soft.

Table 5.1 Enamel preservation classification

Score	Description	Notes
0	Modern	Enamel is hard, translucent, glossy and milky-white to creamy-yellow in colour.
1	Archaeological, but shows no evidence of burial.	Visually and mechanically indistinguishable from modern. As above with no visible cracks or post-mortem damage or change to surface enamel or EDJ.
2	Archaeological. Preservation excellent.	Surface shows signs of burial but, on cleaning, enamel is hard, uncracked, translucent and glossy with no obvious discoloration. EDJ is strong and intact.
3	Archaeological. Preservation good.	As above but fine cracks, post-mortem surface concretions or superficial plant root abrasion may be present. On cleaning, enamel is hard, translucent and glossy with no obvious discoloration. EDJ is intact but may be discoloured.
4	Archaeological. Preservation satisfactory.	Discoloration, plant root/stone abrasion, surface concretions, coatings and fine cracks may be present but enamel is hard, translucent and glossy when cleaned. Separation of enamel from underlying dentine at the cervical margin may be apparent. EDJ is weak with enamel separating easily from dentine.
5	Archaeological. Preservation poor.	Enamel is discoloured and cracked and may have other visible surface changes noted above. Separation of enamel from dentine at the cervical margin is apparent. On cleaning, enamel separates easily from dentine (some may already have become detached) and fragments easily. Discoloration may still be apparent after cleaning.
6	Archaeological but mineralisation incomplete.	Enamel is incompletely mineralised with incomplete crown dentine and/or root dentine formation. Although morphologically intact, enamel is brown, matt and opaque although EDJ is intact and strong. On cleaning, enamel is friable, fragments easily and remains discoloured.

Table 5.2 Dentine preservation classification

Score	Description	Notes
0	Modern	Dentine is hard, translucent near the EDJ and milky-white to creamy-yellow in colour.
1	Archaeological but shows no evidence of burial.	Hard on grinding and visually indistinguishable from modern. Properties as above with no visible cracks or post-mortem damage or change.
2	Archaeological. Preservation excellent.	Crown dentine is hard on grinding, uncracked and translucent near the EDJ. EDJ is strong and intact. Root dentine may be discoloured on the surface but with no obvious discoloration/de-mineralisation internally. Roots are intact with no post-mortem damage.
3	Archaeological. Preservation good.	Crown dentine is still hard on grinding and uncracked but may be opaque. Translucent zone near the EDJ is still apparent and EDJ is strong and intact. Post-mortem discoloration/de-mineralisation radiating from pulp cavity does not extend into crown dentine. Roots may display some minor surface changes such as plant root/stone abrasion.
4	Archaeological. Preservation satisfactory.	Crown dentine is opaque with no translucent zone at EDJ. EDJ is weak. Internal cracks may be present. Post-mortem discoloration/de-mineralisation radiating from pulp cavity may extend into crown dentine. Roots are discoloured internally with visible surface changes. On grinding, dentine is soft and powders easily.
5	Archaeological. Preservation poor.	Crown dentine is discoloured and opaque with no translucent zone at EDJ. Enamel has separated from dentine at EDJ. Roots showing signs of post-mortem breakage, attrition and decay. Friable, brittle and powdery or “cheesy” when ground.
6	Archaeological but mineralisation incomplete.	Incompletely mineralised and/or formed dentine.

Table 5.3 Attrition classification

Score	Stage of attrition	Description
0	Unerupted	Unerupted tooth with no attrition.
1	Negligible	Cusp tips intact. Polishing or small facets may be present. Mamelons may still be visible.
2	Slight	Blunting of cusps but no dentine visible.
3	Slight-moderate	Cusp tips removed. Pinpricks or thin lines of dentine visible on cusps.
4	Moderate	Discrete areas of dentine visible on individual cusps.
5	Moderate-severe	Exposed areas of dentine on cusps have coalesced.
6	Severe	All occlusal enamel has been worn away.
7	Very severe	Only cervical enamel remains – insufficient to sample.
8	Complete	Complete loss of crown with no enamel remaining.

Table 5.4 Stages of root formation and resorption

Score	Root status	Dentition
0	Crown dentine not completely formed.	Deciduous or permanent
1	Crown dentine complete but no root formation.	"
2	Roots ¼ formed.	"
3	Roots ½ formed.	"
4	Roots ¾ formed.	"
5	Roots complete, apices closed or closing.	"
6	Roots ¼ resorbed	Deciduous only
7	Roots ½ resorbed	"
8	Roots ¾ resorbed	"
9	Roots completely resorbed	"
X	Unable to score due to post-mortem damage	Deciduous or permanent

Source: Both tables adapted from Buikstra & Ubelaker (1994, 50 & 52)

Beeley & Lunt (1980) concluded from their study of the biochemical changes to archaeological dentine that "hardness" during grinding was a good measure of the extent of protein degradation. Although, poor macroscopic preservation did not always correlate with samples that subsequently appeared to have equilibrated isotopically with the burial environment, how the sample looked and behaved before and during preparation was recorded. Each sample was classified for the level of enamel wear (attrition) present using Table 5.3. If enamel attrition had occurred to the extent that the underlying crown dentine was exposed, it provided an opportunity for groundwater to penetrate inside the tooth crown as well as through the pulp cavity. This additional route increases the possibilities for diagenetic alteration of the crown, although it is also possible that penetration of the crown occurs through hairline cracks and fissures that are present in the enamel on most archaeological teeth. Samples were also scored for the degree of root formation observed, according to the categories given in Table 5.4. This was used as a means of classifying the formation stage of the tooth as a whole and to record incomplete closure of the root apex which potentially increases exposure to percolating groundwater.

5.1.1.3 Age and sex estimation

For the requirements of this study the key factor is the presence of particular fully mineralised teeth, known to form at a specific time of life that is of prime importance; whether the tooth had erupted and the individual's subsequent age-at-death are a secondary consideration. The choice of a specific tooth constrains the period of childhood being investigated to when the crown was mineralising and Pb and Sr were being incorporated. The years of life subsequent to this have a minimal effect on the composition of tooth enamel and, possibly to a lesser extent, primary dentine. However, an individual's sex or age at death may later prove to be a distinguishing feature or classification group. The age and sex information available for each individual was, therefore, recorded for completeness. The osteologists involved in post-excavation or production of the skeletal report, had already aged and sexed the population. For most samples, the whole skeleton was not available for inspection for this study and age at death and, in adults, sex, could not be directly confirmed. Existing data have, therefore, been accepted and reproduced in this thesis subject to the following considerations.

i. Juvenile age and sex

Where required, an estimated age at death of juveniles or by using individual teeth if that was all that was available, was carried out using the data in Table 3.2 and 3.3 and supplemented for the more variable M3 by Ubelaker's dental development diagram (1989, 64). In this diagram, data for males and females has been combined and the errors encompass both sexes, thus making attribution of sex unnecessary prior to use. Osteological sex estimation of the skeleton cannot be carried out with any certainty until the development of the secondary sexual characteristics of the adult skeleton. Although a few juveniles in this study have been sexed by analysis of aDNA, most were not and this increases the error involved in dental ageing, as dental development in females is usually 1-2 years in advance of males. The errors involved also increase with age and, therefore, ageing methods become less accurate as an individual reaches adulthood. Nevertheless, crown formation times are considered to be a more accurate age indicator than eruption or root formation (Ubelaker 1989, 64). Classification of, and references in the text to, juveniles was carried out using the standard age ranges recommended by Buikstra and Ubelaker (1994, 9):

Fetal	*(F)*	*<birth*
Infant	*(I)*	*birth – 3 years*
Child	*(C)*	*3-12 years*
Adolescent	*(AO)*	*12-20 years*

ii. Adult age and sex

Once individuals reach adulthood and all the teeth have erupted and epiphyses fused, age estimation becomes considerably more inaccurate and subjective. Although the later-fusing epiphyses of the clavicle, sacrum, iliac crest of the pelvis and the basal synchondrosis on the cranium may be useful in the identification of younger adults, for older individuals the majority of age estimation methods rely almost entirely on morphological, degenerative changes. Both microscopic and macroscopic methods have been developed but in archaeological contexts only macroscopic methods are widely used. Macroscopic methods have the advantages of being quick and relatively cheap to apply by experienced osteologists but lack the precision of the more complex microscopic methods. The broad age ranges assigned by macroscopic osteological ageing, whilst not perhaps constrained enough for identification in forensic cases are,

nevertheless, sufficiently accurate for the vast majority of archaeological studies (Ubelaker 1989, 92).

Rates of change resulting from degeneration of parts of the skeleton are not as consistent as those associated with growth and development. They can vary widely between individuals and populations as they are inherently dependent on the frequency and type of repeated movements performed by the individual in question. Of the two types of skeletal joint, cartilaginous and synovial, the less mobile cartilaginous joints, e.g. intervertebral, pubic symphysis, auricular surface of the pelvis and sternal rib ends are considered to be the most reliable indicators of age. The limited mobility ensures degeneration of the joint is more closely correlated with age than that of the more mobile synovial joint type, e.g. knees, elbows, hips. The Todd (1921a; 1921b) and Suchey-Brooks (1990) systems for classifying morphological changes to the pubic symphysis are considered to be the most reliable indicator of age-at-death of adults (Buikstra & Ubelaker 1994, 21). However, estimates using this method should, wherever possible, be supported by others such as the method developed by Lovejoy *et al.* (1985) for the auricular surface of the ilium and recommended by Buikstra and Ubelaker (1994, 24). An additional method recommended by Ubelaker (1989, 87) and developed by Iscan *et al.* (1984; 1985) is based on the degenerative changes of the sternal rib ends.

Due to the number of skeletons that were aged by different people, at different times and using different methods, adults analysed in this study were grouped into the following standard age ranges recommended by Buikstra and Ubelaker (1994, 9):

Young adult	*(YAd)*	*20-34 years*
Middle adult	*(MAd)*	*35-49 years*
Old adult	*(OAd)*	*50+ years*

For some individuals where preservation was poor or diagnostic features were absent or ambiguous thus making precise ageing impossible, the more general classification of *Adult (Ad)* has been made by the osteologists concerned. A further caveat to the ageing data presented here is that ageing methods are often derived from a particular sample population, i.e. North American Native Indians and European White Males, and therefore, may not be directly applicable to the archaeological individual in question. Furthermore, the archaeological individuals analysed in this study cover a date range

from the early Neolithic to the late mediaeval period (c. 5000 years) and it is not known if ageing methods can be applied with validity to such a temporally diverse sample of people. Tooth formation times, are however, genetically constrained and recognised as being the most resistant skeletal tissue to external environmental variables such as malnutrition and disease (Smith 1991, 143).

Osteological adult ageing methods usually require the prior determination of sex. Sexing of adults is done using the presence or absence of specific secondary sexual characteristics that start to develop at puberty. As these involve morphological bone changes to the skull (predominantly in males) and the pelvis (predominantly in females) along with measurements of the long bones, they require time to develop. This can lead to difficulties in the correct identification of young adult males whose male skull characteristics may not have developed fully and also in aged females who may develop male skull characteristics in later life (Walker 1995, 43). Most sex determinations are carried out on the whole skeleton and a conclusion reached as to whether most attributes point to male or female. In some cases the outcome may be indeterminate.

If preservation of the skeleton is too poor to make an osteological assessment of sex, the gender of the skeleton may be recorded using the grave goods included with the burial. If for example, the grave contains jewellery, the traditionally assigned gender would be female. Whilst this type of sexing was widespread at one time it is now recognised as misleading and aDNA analysis has shown that females can be buried with weapons and males with jewellery (Christine Flaherty pers. comm.). Grave assemblages are likely to have been chosen by those arranging the burial rather than the individual being buried and may be include symbolic gifts in addition to the personal property of the deceased. Sexing by grave goods is, therefore, indicated by “Mgg” or “Fgg” and should be regarded as evidence of the gender indicated by the grave assemblage rather than a proof of osteological sex.

5.1.2 Sample selection criteria

The type of tooth used was standardised wherever possible. The following factors were taken into account:

1. The prospective enamel yield. This is dependent on size, wear and complexity of cusp morphology; it is difficult to satisfactorily remove the outer and inner enamel surfaces from teeth with a complex fissure pattern, e.g. molars.
2. Suitability of the sample for other analytical methods. Incisors and canines are often the tooth of choice when assessing dental abnormalities such as enamel hypoplasia. Aesthetically, destruction or loss of these visible, anterior teeth is also undesirable.
3. Ease of removal from the alveolar bone. *In situ* teeth with two or three roots, e.g. molars, are often difficult to extract from the alveolar bone without force and resultant damage.
4. Time of formation. To maximise the possibility that an individual had moved, the tooth needed to be formed as early in childhood as possible. However, to ensure this had been formed from the diet of the child rather than the diet of the mother (*in utero* or during breastfeeding) a tooth more likely to form post-weaning was preferred.
5. Variability of formation times. The greatest variability between males and females occurs in the formation of the permanent canine (Ubelaker 1989, 64) and as sex cannot be determined reliably from juvenile skeletal remains this tooth may introduce the largest error. Of all the permanent teeth, the third molar exhibits the greatest overall variability during its formation.

It became apparent that when a choice was available, premolars would best satisfy the above conditions. In normal dentitions there are eight premolars. They are usually easy to remove, have relatively simple cusp morphology but thick enamel, and the crown forms and mineralises between the ages of 18 months and 8.5 years, depending on which premolar and which dental arcade (maxillary or mandibular) it originates from. Nevertheless, in some cases the choice of premolars was not an option as excavators or curators themselves chose and sent a requested tooth. Moreover, some of the younger juveniles in the study did not have fully mineralised premolars, and in some individuals

they were absent either through ante- or post-mortem loss or were too worn or carious to analyse. Teeth chosen were intact apart from normal attrition and free from pre-mortem dental modifications and pathological changes due to caries or enamel hypoplasia. Wherever possible, teeth were chosen to leave the antimere intact in the alveolar bone. The main consideration when selecting samples was whether enough enamel was available to provide sufficient and suitable material for analysis and whether the state of preservation would enable the enamel to be mechanically cleaned. For this reason, teeth that were clearly poorly preserved, severely worn or physically damaged were never deliberately selected. However, preservation on sites such as West Heslerton, where some burials were reduced to teeth only, was generally, but not exclusively, poor and for some burials there was no alternative sample available.

The analytical procedure is destructive and this obviously had a bearing on what samples were available for analysis. Attitudes to destructive analysis of skeletal material vary widely in archaeology and there are of course ethical issues involved (Cox 1997; Historic Scotland 1997). The main practical objection to destruction of the resource is the possibility of needing the particular tooth in the future for some existing or currently unenvisaged new technique. With care, sufficient enamel can be obtained from half a single premolar crown and perhaps only one quarter of a permanent molar for Pb, Sr and O analysis, *providing* attrition is minimal and the sample is sufficiently robust to withstand the cleaning procedure. Deciduous teeth have thinner enamel and are generally smaller and may require the whole tooth crown to be sampled. The ideal enamel sample weight for combined Pb and Sr analysis is 50-100mg the limiting factor being the Pb concentration which may vary by 4 orders of magnitude, but is obviously difficult to ascertain prior to analysis. However, enamel weights ranging from 20-140mg were successfully analysed in this thesis in samples ranging from 0.02-1540ppm Pb, the latter being a Pb coffin burial. Where the skeletal material was regarded as particularly important by the excavators/curators and destructive analysis was seen as undesirable, a replacement in the form of a morphologically exact porcelain replica was obtained from *Cosmadent Dental Laboratories Ltd*., Halifax, West Yorkshire.

5.1.3 Sample pre-treatment

One of the main aims of the study was to assess and compare the post-mortem isotope integrity of the two archaeological dental tissues in question, enamel and dentine, and to determine whether one or both had equilibrated with the burial environment. Consequently, pre-treatment procedures such as acid leaching or solubility profiling designed to remove integral diagenetic phases or chemical contamination from the samples were deliberately not used nor were samples selected using any criterion designed to identify tissues altered on a microscopic scale. Physical removal of age-affected tissues such as surface enamel and circumpulpal, root and secondary dentine was carried out to ensure samples consisted of primary core enamel and dentine and thereby enable teeth from adults and children to be directly compared.

5.1.4 Samples analysed

All samples were sourced from sites in Great Britain. Lists of samples and sites are contained in Appendix II. A list of the archaeological sites investigated is given in Table A5. Figure 1.4 shows their location and the age of the surface geology in the region. Archaeological human teeth samples used in the study are listed in Table A6. Table A7 contains details of four archaeological herbivore teeth used in the Lewis case study and Table A8 lists the modern human teeth analysed. All teeth were donated by colleagues, family and friends and have never been buried. Age given in the table refers to age as at 31/12/2001 although some teeth were extracted or shed some years prior to this date. More detailed information on sites, samples and sources is discussed within the context of the site in question in Chapters Six, Seven and Eight.

5.2 Soil samples used in this study

A list of all soil and bedrock samples analysed can be found in Table A9, Appendix II. Sub-soil and/or bedrock samples were collected and analysed in order to characterise the burial environment by extracting the bioavailable soluble and exchangeable Sr and Pb isotope signatures of groundwaters that were currently present in the vicinity of the burials. Some samples were collected straight from the excavation if it was ongoing,

whilst others were obtained from excavation archives or by taking fresh samples at, or as close as possible to, the site of the completed excavation.

It is difficult to be certain with archaeological soil samples that the isotope signature, pH or indeed, the soil itself, obtained today were either contemporary with interment or remained constant throughout the period of burial. The burial area may have been subjected to various anthropogenic activities as well as soil changes on the microscopic scale. Consequently, soil pH was not measured for this study. With hindsight and in the light of the different behaviour of Pb and Sr observed in the soils, it would have been useful to do so. Despite these uncertainties, it was, nevertheless, considered important to measure soil isotope ratios to assess whether the results from the enamel and/or dentine were identical to that obtained from the burial soil. If the enamel or dentine ratios were indistinguishable from those of the burial environment, it would be unclear whether the tooth result was diagenetic or biogenic in origin.

5.3 Analytical method: ID-TIMS

Sr and Pb isotope and concentration analyses were carried out at the NERC Isotope Geosciences Laboratory (NIGL) at the British Geological Survey by, and under the instruction of, Drs. Jane Evans and Barbara Barreiro. The majority of data was obtained using the established method of thermal ionisation mass spectrometry (TIMS). However, Pb isotope data for one batch of samples (Monkton-up-Wimbourne) were obtained by Dr. Barreiro using the newly installed multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) and analytical details have already been published (Montgomery *et al.* 2000).

Thermal ionisation mass spectrometry has long been the established analytical method to provide the high precision Sr and Pb isotope ratios required in isotope geochemistry. Details of the principles and background are given at length in Faure (1986) and Gulson (1986) and for the Finnegan MAT 262 multi-collector thermal ionisation mass spectrometer used in this project specifically, in NIGL Report Series No.121 (Royse *et al.* 1998) and No.78 (Kempton 1995). The main disadvantages of TIMS analysis are the lengthy laboratory preparation, slow sample throughput and for Pb, which does not

possess a non-radiogenic isotope ratio, the inability to monitor and correct internally for mass fractionation. This limits the precision obtainable and the accuracy to that determined by the external reproducibility of the standards. Nevertheless, external reproducibilities of ± 0.02-0.1% (Halliday *et al.* 1998, 928) are normally achievable for Pb and ± 0.003% for Sr (J. Evans pers. comm.) which can be corrected internally by monitoring the invariant Sr isotope ratio. Elemental concentrations are obtained using the isotope dilution (ID) technique. This involves the addition of a small, measured amount of "spike" that is highly enriched in one isotope, i.e. ^{84}Sr or ^{208}Pb, to a known quantity of sample prior to chemical separation. Although TIMS is able to obtain high precision isotope ratios on very low concentration samples, the amount of Pb available to load on the TIMS filaments after separation of the enamel Pb from the enamel matrix on the columns is at the lower end of this concentration range, i.e. ~5ng. Chemical separation of the sample Pb and Sr from the carbonate hydroxyapatite matrix proved necessary for TIMS analysis, as the high-Ca matrix appeared to suppress the ionisation of Pb and Sr (J. Evans pers.comm.).

5.3.1 Tooth sample preparation

By far the biggest problem in trace Pb analysis is the prevention of anthropogenic contamination from tools, reagents and airborne particulate. All procedures were developed with the aim of minimising laboratory contamination for both Pb and Sr. The possibility of contamination was continually assessed and considered throughout the whole project. Acid-leached Teflon beakers were used to hold reagents. Between samples, all workspaces, beakers and dental tools were thoroughly cleaned, and consumable items and reagents changed.

5.3.1.1 Dental tools

Several tungsten carbide dental burs (DFS, Riedenberg, Germany) designed for various tasks were used to extract the required samples. Tungsten carbide is an extremely hard material which introduces negligible Pb and Sr contamination (unlike agate which can introduce Sr and up to 18ppm Pb) (Royse *et al.* 1998, 3). It is recommended for grinding low-concentration geological samples prior to Sr and Pb isotope analysis and is, therefore, particularly suitable for enamel. There was, however, no small, rotary tungsten carbide saw available so a flexible, stainless steel saw with a diamond cutting

edge was used for the initial dissection of whole teeth. All saw cut surfaces were cleaned with a tungsten carbide burr and both saw and burr were subjected to strong acid leaches and analysed for Sr and Pb isotope ratios to assess and monitor contamination. All leaches from the tungsten carbide burrs produced insufficient Pb or Sr for TIMS analysis as did the Sr leaches from the stainless steel saw. Pb isotope ratios obtained from the stainless steel saw leach were less radiogenic and very different from the majority of tooth ratios:

$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
18.06	15.57	37.91

Dental tools were cleaned prior to use and between every sample using the following procedure:

1. Swabbed with Aqua Regia using a new cotton bud
2. Rinsed in Millipore Alpha Q H_2O
3. Ultrasonicated for 5 minutes in Micro cleaning fluid diluted with Millipore Alpha Q H_2O
4. Rinsed in Millipore Alpha Q H_2O
5. Ultrasonicated for 5 minutes in Millipore Alpha Q H_2O
6. Rinsed in Millipore Alpha Q H_2O

5.3.1.2 Tooth dissection

The archaeological teeth used in the study ranged from newly excavated examples to teeth from skeletons that had been curated for many years. All excavated teeth were pre-cleaned with a glass-fibre brush to remove any adhering soil and plant matter. All the teeth (archaeological and modern) were then cleaned ultrasonically for 5 minutes in Millipore Alpha Q H_2O (< 1ppb total heavy metal content) followed by a high-purity acetone wash (< 5ppb Pb and Sr) and left to air dry. Cleaned teeth were divided longitudinally to produce half-tooth samples for LA-ICP-MS and TIMS where necessary.

Sample preparation methods varied during the project depending on which and how many tissue samples were required from each tooth. Unless stated otherwise, the outer surface of the enamel was removed to a depth of >100μm with a tungsten carbide burr to remove any discoloured, carious or visibly damaged areas of enamel and the surface

enamel itself. Removing this surface layer ensured that the high Pb levels observed by LA-ICP-MS in the surface enamel (Lee *et al.* 1999; Montgomery *et al.* 1999) did not contribute to the analysis. This process is quite difficult to carry out with certainty on an undulating tooth surface, particularly if deeply fissured. Initially, the procedure was monitored by several methods such as measuring the amount removed with sliding callipers, the visible appearance of the cleaned enamel surface, comparative LA-ICP-MS transects of cleaned and uncleaned tooth halves (Figure 5.1) and ashing at high temperature, when the outer layer will turn black and the inner white (unpublished results and observations by C. Chenery). By careful sample selection of both the tooth type and where the enamel is taken from, i.e. not the deep fissures of the occlusal surface that are difficult to abrade consistently, it is considered that surface enamel and EDJ removal was carried out satisfactorily.

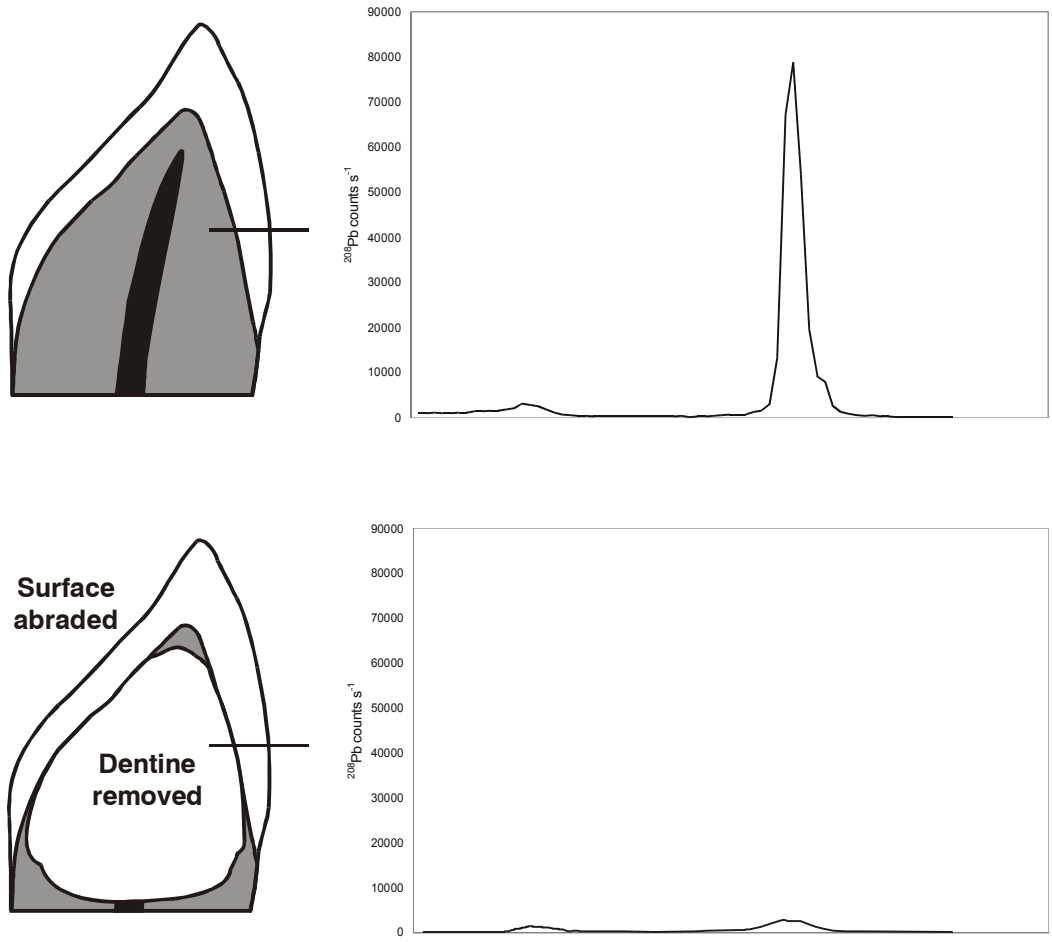

Figure 5.1 Time resolved analysis ^{208}Pb profiles across the enamel of two halves of a longitudinally sectioned tooth. The tooth was bisected and one half left intact. The other half had the enamel surface mechanically abraded to >100µm and all dentine removed internally. LA-ICP-MS was used to investigate the effect of the sample preparation on the Pb profile. The graphs show that the large surface Pb peak was almost entirely removed and the enamel-Pb adjacent to the EDJ reduced. The analysis was performed by Alan Cox, CAS, University of Sheffield.

Once surface enamel was removed, enamel samples were cut from the tooth with the flexible dental saw. With experience and the flexible blade, it is possible to cut just above and parallel to the EDJ thus effectively separating the two tissues with minimal sample loss. As leaching of the dental saw had produced sufficient Pb for analysis, all saw-cut surfaces were rigorously cleaned with a tungsten carbide bur to eliminate possible contamination from the saw blade. Enamel samples were also rigorously cleaned of any adhering dentine and the enamel bordering the EDJ. This procedure ensured that all enamel samples (unless otherwise stated) are of "core" enamel only and free of both surface enamel and the heterogeneous EDJ. Areas of histological complexity, i.e. occlusal fissures, were also avoided as these were not only more difficult to clean but are also known to be less well mineralised and therefore, more prone to post-mortem contamination than the smoother enamel of the tooth wall (Robinson *et al.* 1995b, 169).

For some teeth, enamel samples were obtained of both "surface-rich" and core enamel. The surface-rich samples were cut from the tooth in the same way as the core samples but no enamel was removed from the tooth surface. Instead, core enamel was removed from the inner surface with a tungsten carbide bur to produce a comparatively surface-rich sample. These samples were not composed entirely of surface enamel as it is impossible to abrade samples by this method to a thickness approaching 100μm without the sample breaking up into very small, unmanageable pieces. Samples of crown dentine were removed from the remaining tooth using the flexible dental saw. As with the enamel samples, all saw-cut surfaces were cleaned with a tungsten carbide burr to remove contamination and any adhering enamel. If the samples intruded into the pulp cavity, this was abraded to remove any remaining organic matter, circumpulpal and, if present, secondary dentine. This procedure ensured that all dentine samples were of primary crown dentine only and no root or circumpulpal dentine was used. All samples were sealed in clean, labelled containers and transferred to a class 100 HEPA-filtered cupboard for further preparation. Remaining unused material was retained for future use or returned if requested.

5.3.1.3 ID-TIMS laboratory preparation

Following initial sampling, all further preparation was carried out within a class 100, HEPA-filtered laboratory facilities at the NERC Isotope Geosciences Laboratory (NIGL), Keyworth, UK. Sample preparation and instrumental analysis was carried out under the supervision of Drs. Jane Evans and Barbara Barreiro and, with slight modifications, followed the Laboratory's procedures for geological samples (Kempton 1995; Royse *et al.* 1998) and the Laboratory's Common Pb "Recipe" sheet. The detailed procedure specific to tooth preparation developed for this project is given in Appendix III. The laboratory procedure used ion exchange chromatography and Teflon distilled reagents to individually isolate, prepare and spike the Pb and Sr content of the tooth matrix prior to instrumental analysis. Isotope dilution was used to ascertain Pb and Sr concentration using ^{208}Pb and ^{84}Sr spikes respectively. Pb was separated first from the tooth matrix using Dowex 1 x 8, 200-400 mesh anion exchange resin. Sr was separated from the resulting eluent using standard Dowex AG50W X12 cation exchange resin. In total, six separate batches of samples were processed and measured on the MAT262 thermal ionisation mass spectrometer along with procedural blanks and standards.

Initially, in batch No.1 the samples were subjected to a leaching step in HCl for ~5 minutes. Although this is routinely done with geological samples, it produced an unexpectedly vigorous reaction with enamel and particularly dentine samples. It raised concerns about sample loss but was done because the "surface enamel" samples could not be mechanically cleaned. In batch No.2 the leaching was repeated and the leachates from the "surface enamel" samples reserved for analysis in order to ascertain whether there was an isotopic difference between the leachate and the bulk analysis. As no further "surface enamel" samples were prepared, and all other samples consisted of mechanically cleaned core enamel, this chemical leaching step was removed from the procedure for future batches. The laboratory procedure given in Appendix III was that used in batch Nos. 3 to 6.

5.3.2 Soil sample preparation

Sampling procedures were devised to prevent contamination both at the sampling site and in the laboratory. Whole rocks were removed to the laboratory where samples for

analysis were taken from the core of the rock. Soil samples were removed from the centre of the auger core or direct from a cleaned surface at the burial site using clean Teflon containers and powder-free gloves and transferred to the clean laboratory. Rock samples were finely ground in a tungsten carbide ball mill because agate can introduce both Pb (up to 18ppm) and Sr contamination in samples containing small concentrations of these two elements (Royse *et al.* 1998, 3). Approximately 2g of soil or rock sample was leached overnight with water (Millipore Alpha Q, <1ppb total heavy metal content) or weak acid (10% vol. acetic) to extract soluble and exchangeable ions. Initially stronger acid leaches using 16N Teflon distilled HCl (see Table A4) as a reagent were carried out but were not continued as it was concluded they did not characterise the mobile Sr and Pb as well as water and acetic. Samples were centrifuged and the leachate pipetted out and dried down. Pb and Sr were isolated by ion exchange chromatography following the procedure given in Appendix III but omitting Day 1.

5.3.3 Instrumental analysis by TIMS

5.3.3.1 Sr mass spectrometry

Sr isotope ratios and Sr concentrations were measured in static mode on the Finnegan MAT 262 automated TIMS fitted with multi-collector Faraday cups. The sample was taken up in 8M Teflon distilled HNO_3 and loaded onto the filament with 1µl of 1M H_3PO_4 (Suprapure). Samples and standards (NBS 0987) were loaded onto outgassed single tantalum (Ta) filaments and dried down in a class 100 HEPA-filtered cupboard. Sr isotope ratio was normalised during run time to $^{86}Sr/^{88}Sr = 0.1194$. The majority of the samples achieved an internal run precision of better than ±0.000010 (1SE). A single run of the mass spectrometer comprises 13 samples of which one to three are usually standards. To enable data collected over a period of time to be compared and to facilitate inter-laboratory comparison, the sample data are normalised to values obtained for the international Sr standard NBS 0987 (accepted value: 0.710235) for that particular run. This will be the average result if more than one standard was run. The standard values vary through time and between static and dynamic mode. The standard value obtained on the MAT 262 mass spectrometer for NBS 0987 drifted during the 18 months spanned by this work. Values ranged between 0.710180 – 0.710300.

Consequently, the sample data was normalised to a fixed and acceptable value for the standard, of 0.710235. For any given period of analysis the precision on a set of standards was ± 0.000030 (2σ) or better. As an example, during December 1999 – January 2000 the mass spectrometer gave the following result: 0.710201 ± 0.000028 (2σ, n = 7).

Sr samples were spiked with ^{84}Sr and concentrations were determined by ID-TIMS. In homogeneous samples isotope dilution accuracy can exceed 1% (2σ) (Dickin 1995) in favourable conditions and this value is widely quoted for Sr concentration of rock powders (Evans 1996) and used to model the analytical errors in this study. However, teeth have not been well characterised with respect to compositional heterogeneity. LA-ICP-MS studies suggest core enamel displays the most consistent Sr concentration (Montgomery *et al.* 1999). Within-tooth concentration reproducibility for the current study was estimated by dividing the core enamel chips removed from one tooth into several replicate samples. Replicate enamel samples from one permanent tooth (BAB2, n = 4) and two co-developing deciduous teeth from the same individual (AM1, n = 3 & AM2, n = 3) were analysed. Results (Appendix I, Table A3) gave a range of 44.1-49.5ppm for BAB2 and 62.7-77.6ppm for AM1 and AM2 combined, which greatly exceeds the analytical errors involved in ID-TIMS but was a very small initial study. Reproducibility of the Sr isotope ratio was better than the Sr standard at ± 0.002% (2σ), i.e. ± 0.00001, even though the sample number is small. This is considerably better than the estimated minimum spread of 0.00032 between Sr isotope signatures obtained by Sealy *et al.* (1995, 297) from a single archaeological bone but is understandable given the constrained formation period of enamel when compared to bone which is continually remodelling *in vivo.*

All sample preparation procedures result in a mixing of the analyte in the sample with the same element in the reagents and the laboratory environment. The level of contamination can be determined by performing the entire chemical procedure in the absence of a sample, i.e. a blank run. During this study the mean Sr blank was 135pg (range 43-400pg, n = 10), which is well within the expected range. The best result of 43pg is close to the best value achieved in the laboratory to date of 31 pg. Enamel Sr concentrations were generally between 40-300ppm in samples weighing 20-140mg. A

small 20mg enamel sample containing only 40ppm Sr would produce 800000pg of Sr to load onto the filament. Accordingly, the average blank contribution of 135pg represents, at most, 0.017% of the Sr analysed and is consequently small in comparison to the sample Sr. This represents a blank contribution to the Sr isotope ratio of ±0.01% (i.e. 4th decimal place). Blank corrections were, therefore, considered unnecessary.

5.3.3.2 Pb mass spectrometry

Pb isotope ratios and concentrations were measured in static mode on the MAT 262 automated TIMS fitted with multi-collector Faraday cups. Samples were loaded onto individual outgassed rhenium (Re) filaments in a class 100 HEPA-filtered cupboard using the silica gel-phosphoric acid method which allows measurements of sufficient precision on nanogram-size samples (Gulson 1986, 197). Prepared filaments are loaded onto the turret along with the Pb standard NBS 981. As the lighter Pb isotopes are more easily volatilised off the filament than the heavier isotopes and unlike Sr, Pb does not have an isotope ratio that is invariant in nature, this fractionation has to be corrected for using multiple determinations of the standard. This correction assumes that standard and sample behaves identically in the mass spectrometer.

A single run of the mass spectrometer comprises 13 samples of which one to three are usually standards. To correct for fractionation and to enable inter-laboratory comparisons the sample data were normalised to values obtained for the international Pb standard NBS 981 for that particular run using the accepted values of (Kempton 1995, 114):

$$^{206}Pb/^{204}Pb = 16.9322$$
$$^{207}Pb/^{204}Pb = 15.4855$$
$$^{208}Pb/^{204}Pb = 36.6856$$

Average results of 5 NBS 981 runs were:

$$^{206}Pb/^{204}Pb = 16.92 \pm 0.1\%\ 2\sigma$$
$$^{207}Pb/^{204}Pb = 15.46 \pm 0.13\%\ 2\sigma$$
$$^{208}Pb/^{204}Pb = 36.60 \pm 0.16\%\ 2\sigma$$

During this study the mean Pb blank was 115pg (range 46 - 213pg, n = 10) with one blank of 860pg. Most blanks are, therefore, well within the expected range and small compared to the amount of Pb in the samples. The best result of 46pg is close to the

best value achieved in the laboratory to date of 33pg. As with the Sr results, blank corrections were not considered necessary.

The main source of error in isotope dilution is the precision achievable when removing exactly 200µl of Pb sample to form the spiked aliquots. Experimental aliquoting during this project suggested an error of ± 1.07% (2σ) and analytical errors on Pb concentration plots are modelled on this error. A further source of concentration error is inhomogeneity of the sample itself. Dental tissues have not been well characterised with respect to compositional heterogeneity. LA-ICP-MS studies suggest a degree of Pb variation within teeth with core enamel exhibiting the most consistent concentration (Budd *et al.* 1998; Montgomery *et al.* 1999). As with Sr concentration, estimates of reproducibility for the current study are based on replicate analyses of modern core enamel measurements undertaken for both permanent (BAB2, n = 4) and deciduous teeth (AM1 n =3 & AM2, n = 3). The Pb concentration range was 2.23-2.87ppm for BAB2 and 0.18-0.32ppm for AM, which greatly exceeds all other measurement errors involved in ID-TIMS but again, may result from the small size of this preliminary study. Reproducibility of the $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ ratios (disregarding a possible erroneous measurement of AM1) suggests variation in the second decimal place (i.e. ≤0.09) between samples from the same tooth and was in line with the errors achieved by the standard for BAB1 and exceeded analytical error for AM in the $^{206}Pb/^{204}Pb$ ratio only, although the data are preliminary and small in number. Individual sample results can be found in Appendix I, Table A3.

5.3.4 Data handling

Isotope and concentration data are presented in results Tables A1-A4 in Appendix I. Sr isotope ratios are given to the conventional 6 significant figures and Pb isotope ratios to 4 significant figures, Sr and Pb concentrations to 3 significant figures. Sr isotope ratio data in particular, is considerably more precise than is required to make archaeological interpretations. An individual's isotope ratio is the weighted mean of many different dietary inputs and in a community the influence of one source may vary seasonally and linearly through time, leading to a spread of ratios which define the field associated with that community. The natural variation observed both within a single tooth and between teeth from the same individual or co-habiting siblings (section 6.1) and also

the statistical distribution of individuals belonging to the same population is considerably greater than measurement error. It would, therefore, be unwise to identify two people as different in the context of this study based purely on their skeletal isotope ratios being out of analytical error of each other.

Error bars on isotope plots are 2σ and are those of the external reproducibility of the standards: $^{87}Sr/^{86}Sr$ ±0.004%, $^{206}Pb/^{204}Pb$ ±0.1%, $^{207}Pb/^{204}Pb$ ±0.13%, $^{208}Pb/^{204}Pb$ ±0.16%, $^{207}Pb/^{206}Pb$ ±0.05% and $^{208}Pb/^{206}Pb$ ±0.05%. Errors are displayed as 2σ error bars on a single data point on Pb plots. Attempts to put error bars on every data point inevitably resulted in busy, cluttered plots that were very difficult to decipher. Sr isotope ratio and concentration errors are mainly contained within the symbols.

As the Sr isotope ratio is univariate, results are presented plotted against Sr concentration (ppm), as this can provide additional information about either diet or diagenetic incorporation of Sr. They are also presented plotted against a Pb ratio where this provides additional insights. Pb isotope ratios are most usefully displayed in a form that best illustrates the separation of data points. All data are presented initially as conventional bivariate plots using the ^{204}Pb measured ratios $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ as these retain information relating to the U and Th content of the geological source. Axis scales vary between sites reflecting the geological variation present. The ratios $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ are widely used in the archaeological and environmental literature, as they avoid the poorer precision inherent in the measurement of the low abundance ^{204}Pb. These ratios are, therefore, included in the data tables (Appendix I) and used for plotting against Pb concentrations and Sr isotope ratios. The Pb ore isotope growth curve of Stacey and Kramer (Ludwig 1994) for conformable two-stage ore deposits is included on the bivariate Pb isotope plots to enable the reader to establish where the data points plot relative to this curve. The Pb isotope ratio fields for known English, Welsh, Scottish and Irish Pb ores (Figure 2.1) are also included on the plots where applicable using data obtained from Rohl (1996).

There has been considerable discussion over the presentation of trivariate Pb isotope data in order to properly represent and visualise any separation of data points and source groups (Baxter 1999; Baxter *et al.* 2000; Budd *et al.* 1993 with multi-authored

comments; Sayre *et al.* 1992b with multi-authored comments; Scaife *et al.* 1996). However, for this project it is the presence of individuals who clearly fall *outside* known British ore sources or the apparent field of variation exhibited by the local population that is of interest. As Sayre *et al.* (1992a, 333) point out *"one can find no two-dimensional projection showing significant separation between points or groups of points unless such separation truly exists in three-dimensional space. Thus any two-dimensional projection that shows significant separation between such points provides sufficient evidence that the points are truly separated. We feel that it is only sensible for demonstrating such separation to use the projection that best shows the separation."* Moreover, it has been convincingly argued such separation will usually be obvious graphically without having to resort to statistical methods such as probability calculations (Baxter 1999, 123; Scaife *et al.* 1996, 306). Pernicka (1993, 259) believes that *"There is, in fact, hardly any need for multivariate statistical methods at all. Searching for subtle differences in various projections of the data is likely to lead to overinterpretation."*

Obviously, resorting to complex statistical methods to try and separate individuals makes no sense if these data points are clearly within the range of biological skeletal variation. Problems with resorting to statistical methods are even further compounded by the likelihood that the non-normality of Pb isotope fields is more often the rule rather than the exception, thus rendering many statistical methods inappropriate (Baxter 1999, 123; Scaife *et al.* 1996). Indeed, as Baxter *et al.* (2000, 979) conclude, *"The use of simple graphical inspection has been advocated…..Although decision-making in such cases may, on occasion, be subjective and uncertain, the uncertainty is unlikely to be any greater than that associated with more 'objective' inferential statistical procedures where the underlying assumptions are incorrect."*

Individuals from different burial sites cannot be combined to form a single statistical population for the purposes of identifying migration (Horn & Müller-Sohnius 1999, 266) as each site has to be assessed by a different set of geological parameters and migration possibilities. Due to sampling constraints such as cost and availability, samples from all sites, with the exception of West Heslerton, are not sufficient in number to form a statistically valid population on their own. No statistical analysis has thus been attempted. Furthermore, no studies represent a true random sample of the

population as samples were either selected for specific reasons from those available or were all that was available to analyse. Consequently spurious statistical analysis has, for these reasons as well as those outlined above, been avoided. Correlations defined as r_s denote the use of the Spearman rank correlation coefficient, which makes no assumption of normality in the data set. Although this may result in real differences being missed, this was thought preferable to using parametric methods that may find differences where there are none. In the event, both methods produced similar results and neither would have affected the resulting conclusions.