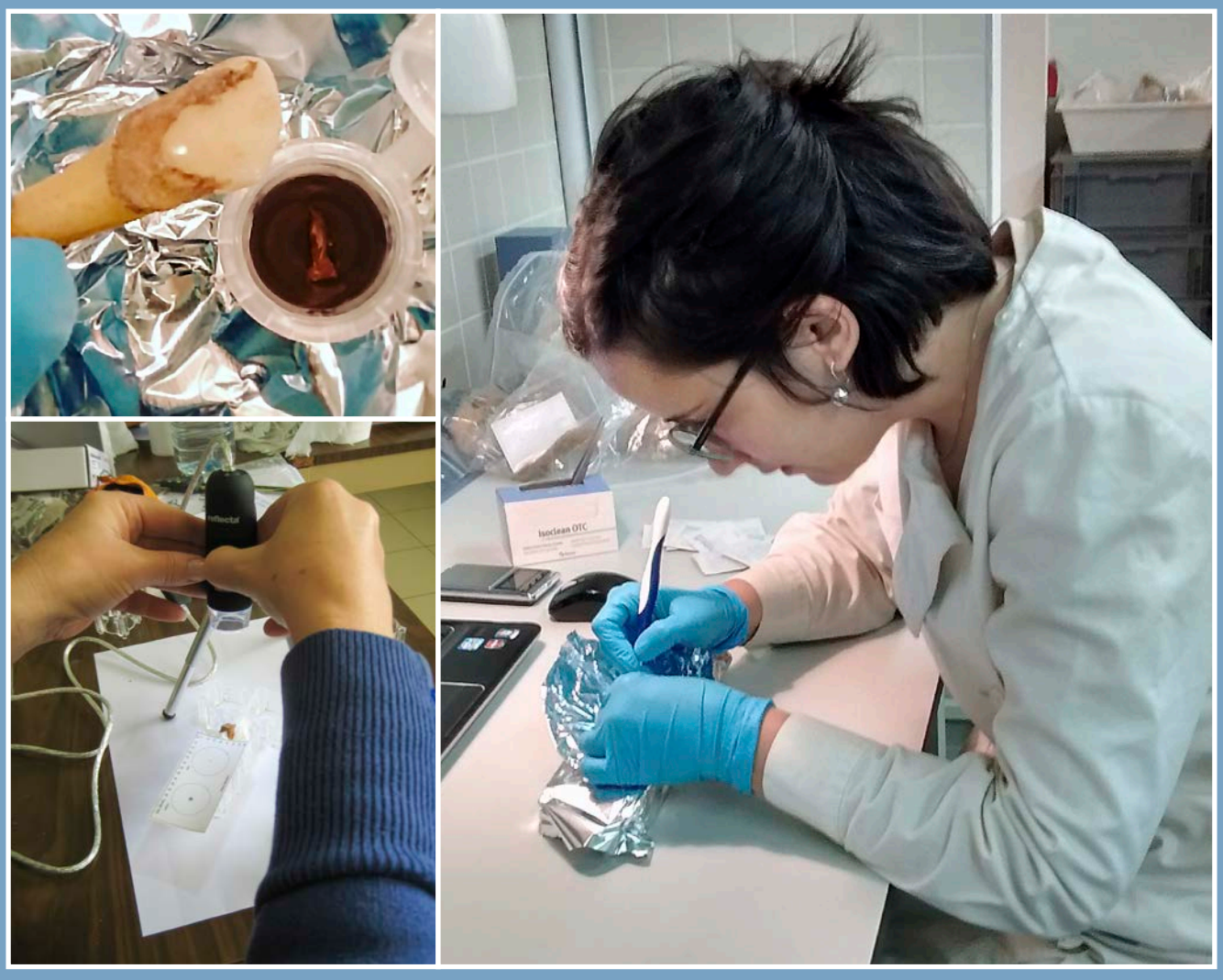
2023

Science and the Dead

Destructive sampling of archaeological human remains for scientific analysis

Second edition



Advisory Panel on the
Archaeology of Burials in England

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Executive summary

Human remains are the most direct archaeological evidence we have for understanding people's lives in the past. However, because they are the remnants of once-living people, dealing with human remains involves legal frameworks and ethical sensitivities not encountered in other areas of the discipline. Recent scientific advances have greatly increased what we can learn from human remains. Some of these involve destruction of samples of bones or teeth. Museums, and other institutions holding archaeological human remains, are receiving increasing numbers of applications from researchers to access remains in their care, and often these include requests for destructive analyses. This raises ethical dilemmas for curators. For example, whilst the application of techniques that require destructive analyses potentially result in the generation of new knowledge, loss of material, however small it might seem, will inevitably constrain future research.

Clergy, and others responsible for historic churchyards and other burial grounds, are also receiving more requests from those wishing to exhume ancient burials for research purposes, and these usually envisage destructive analyses. In addition to considerations associated with the destruction of remains, such applications need to be considered in the light of the presumption of the Church of England against the disturbance of human remains.

In addition to the ethical dilemmas, the scientific considerations are often complex and new developments are rapid. It may be difficult for those caring for human remains to balance the arguments for and against an application. The purpose of this document is to provide a framework to help organisations in responding to requests for destructive analyses of human remains. The document replaces the first edition of Science and the Dead, published in 2013. The remit remains as before, skeletal remains more than 100 years old (herein termed archaeological) from England.

An overview of some of the legal, ethical and scientific considerations pertaining to destructive sampling for the purposes of scientific research is set out. There then follow sections devoted to some of the more commonly applied laboratory techniques: radiocarbon dating, stable isotopic analyses to study ancient diets and to study geographical origins of people, ancient DNA studies, proteomics (the study of proteins), and microscopy. In each of these sections, the science behind the technique is summarised, the sorts of information that it can yield are outlined, and the bone or tooth samples that may be needed are described. Some case studies are then given for illustrative purposes.

The main advice is as follows:

- In general, the benefit of generating new knowledge by the application of techniques that require destructive sampling needs to be weighed against the benefit of preserving skeletal collections intact and, in the case of Church burial grounds, the presumption of the Church of England against disturbance of remains.
- Researchers should have considered ethics in their research design and should demonstrate an awareness of current ethical standards for the treatment of human remains
- Any application should be directed at addressing clear research questions
- When considering a proposal for destructive sampling the following need to be assessed:
  - The likelihood of obtaining useful knowledge and the value of that knowledge
  - Whether that knowledge could be obtained by non-destructive analyses
  - The background, experience and knowledge of those who intend to undertake the work
- The effects of the destructive analyses on the future research potential of the remains
- Expert casework advice should be sought, if needed, from the Advisory Panel on the Archaeology of Burials in England (APABE) or other sources such as the British Association for Biological Anthropology and Osteoarchaeology (BABAO) or a suitably qualified human osteologist
- If sampling is approved, it should be minimally destructive, commensurate with the purposes of the research, and removal of any material should be adequately documented
- Decisions concerning destructive sampling should be made in the public interest and in an accountable manner

1. Introduction

Scientific research on ancient human remains may use a mixture of non-destructive and destructive methods. The former include visual observation and measurements of bones and teeth, augmented by imaging techniques such as radiography. Destructive analyses entail the removal of small samples of bones or teeth, either for biomolecular analyses, of which radiocarbon, DNA and stable isotope analyses are perhaps the most familiar, or for the purposes of microscopic examination.

Archaeological science is a rapidly changing field. Over the last decade, some major innovations in laboratory techniques for the study of bone or tooth samples from human remains have taken place. Palaeoproteomics, the identification and study of ancient protein residues, has emerged as an important research field. The advent of next generation sequencing has revolutionised the way in which we study ancient DNA. Sampling requirements for many techniques have altered. For example, many stable isotope studies now use samples from teeth rather than bones. The study of dental calculus (calcified dental plaque) is a rapidly growing field. Calculus preserves a wealth of information, and a variety of biomolecular and microscopic techniques have been used to study it.

These sorts of technical advances have increased the information that can potentially be obtained from destructive sampling, so the number and diversity of requests from researchers has increased. At the same time, there are also more options for mitigating the impact of destructive sampling on skeletal collections. For example, there are more sophisticated ways of making images of skeletal parts prior to sampling. Advances in information technology for data storage and sharing mean that there are increased options for making the primary data from destructive analyses more widely available. This updated edition of Science and the Dead takes account of these developments.

The purpose of this guideline remains as for the first (2013) edition: to assist institutions with responsibilities for ancient human remains in decision-making regarding destructive sampling, both at the level of casework (i.e. in dealing with specific requests from researchers) and in helping institutions to formulate more general policy. The target audience is museum staff and any others responsible for curating or otherwise caring for ancient human remains. These latter include a broad range of organisations, ranging from the Church to commercial archaeological contractors.

A few, larger museums may have specialist staff who also conduct research on human remains, but most applications received by museums to study remains will come from external researchers, principally from the university sector. The majority of museums will not have specialist osteology curators on their staff to give casework advice on such applications.

Clergy and others responsible for churchyards and other historic burial grounds may face requests for exhumation of specific burials (for example, those thought to be of known historical figures) for research purposes. Usually, such requests involve research that entails destructive analysis.

An archaeological contractor conducting a fieldwork project on an archaeological site will hold the excavated remains until their transfer to a museum or other institution (or reburial) upon completion of the post-excavation (assessment, analysis) phases of the project. Sometimes destructive sampling is carried out as part of archaeological fieldwork projects. For example, radiocarbon determinations from burials may be commissioned at the assessment or analysis phases of a project in order to help date the site. The project osteologist may commission destructive sampling for other types of analyses in order to address research questions regarding the buried population, either as part of the developer-funded analysis phase of a fieldwork project, or as standalone additional, collaborative work (e.g. with a university).

There are also other stakeholders who may be relevant to decision-making regarding destructive sampling as part of archaeological fieldwork projects. These include the museum who will receive the archive, and Local Authority Archaeological Officers. The latter, especially, are often closely involved in discussions about the extent and nature of analyses that assemblages should undergo in developer-funded archaeological fieldwork projects. A careful balance needs to be drawn between ensuring that a developer undertakes sufficient analyses to reveal the significance of a site whilst not unduly compromising the future research potential of remains destined for archive.

Fig 1 A sample being cut from a femur for radiocarbon dating. The small rotary electric saw is being used to remove the sample.

This guidance aims to provide non-specialists with responsibility for human remains with scientific and other information in order to aid them in their deliberations when they are faced with requests for access to human remains in their care for research that involves destructive sampling. However, it is recognised that additional advice may sometimes be needed. The Advisory Panel on the Archaeology of Human Burials in England (APABE) is available to provide support and specific casework advice on all aspects of archaeological human remains. This encompasses destructive sampling, including that for the purposes of applying techniques not covered in this guideline (https://www.archaeologyuk.org/apabe/).

This document begins with a brief outline of some of the legal, ethical and scientific considerations of destructive sampling. There then follow sections devoted to the different techniques. These give a brief outline of the science, examples of what can be learnt, and the nature of the samples that may be required. In a guideline such as this it would be impossible to cover every scientific technique that involves destructive sampling of human remains. Instead, we concentrate on those that are currently among the more frequently used.

In keeping with APABE's remit, the scope of this document is restricted to remains over 100 years old (herein termed archaeological) from burial sites in England (different constraints apply to remains less than 100 years old, which are subject to the Human Tissue Act https://www.hta.gov.uk/guidance-professionals/hta-legislation/human-tissue-act-2004). The focus is on skeletal remains, as these are normally the only parts preserved in archaeological burials in England. However, much of what is written here is relevant, in general terms, to soft tissue in the rare instances where this survives.

This document should be read in conjunction with the UK Governmental 'Guidance for the Care of Human Remains in Museums' https://webarchive.nationalarchives.gov.uk/ukgwa/+/http:/www.culture.gov.uk/images/publications/GuidanceHumanRemains11Oct.pdf and for Christian burials, with 'Guidance for Best Practice for Treatment of Human Remains Excavated From Christian Burial Grounds in England' (2nd edition) (https://www.archaeologyuk.org/apabe/)

Fig 2 Four intersecting cuts from an electric rotary saw, like that illustrated in Fig. 1, have been used to remove a quadrangle of bone from a femur for radiocarbon dating. An incomplete bone was selected in order to help minimise the impact of sampling upon the future research potential of the remains.

2. Considerations for destructive sampling

2.1 Legal considerations

In England, it is unlawful to disturb buried human remains without lawful authority. Permissions are administered either under secular or ecclesiastical law. The latter applies to burial grounds under Church of England jurisdiction, mostly churches and cathedrals and their associated burial grounds. Elsewhere secular law applies.

Secular burial law is generally aimed at regulating the way in which human remains or grave markers are cleared from burial grounds. Permission to excavate archaeological burials is administered via the Ministry of Justice. The secular legal system recognises the public benefit of scientific work on human remains. Destructive sampling of collections of human remains excavated from archaeological sites and curated in museums or other institutions is not normally subject to legal constraint. It is generally the curating institution which grants (or withholds) permission for destructive sampling of remains in its care. However, in cases where permission for exhumation is sought from the Ministry of Justice for the specific purpose of scientific research involving destructive sampling, the Ministry will evaluate carefully the proposals for destructive sampling when the application for the exhumation licence is considered.

In burial grounds under Church of England jurisdiction, human remains cannot be disturbed without ecclesiastical permission, usually issued in the form of a Faculty, or for cathedrals by the Cathedrals Fabric Commission. Ecclesiastical law is protective. It draws upon the principle that remains entrusted to the Church should normally lie undisturbed. This does not, however, mean that human remains should never be disturbed. Ecclesiastical law recognises that the living, including church congregations, have rights which may come into conflict with this principle. The Church also recognises that human remains, and the archaeological evidence for the rites that accompanied their burial, are important sources of scientific information and that this information is of legitimate academic and public interest. Analysis of human remains, including destructive analyses, is therefore potentially acceptable provided that the research aims are adequately justified. Under the Church system, as well as authorising exhumation of burials, the Consistory Court or Cathedrals Fabric Commission also regulates their treatment once exhumed, and therefore has the authority to grant or withhold permission for destructive sampling, and determine details of what samples may be taken. Proposals to remove and / or destroy parts of skeletons are subject to rigorous scrutiny. This is particularly so when

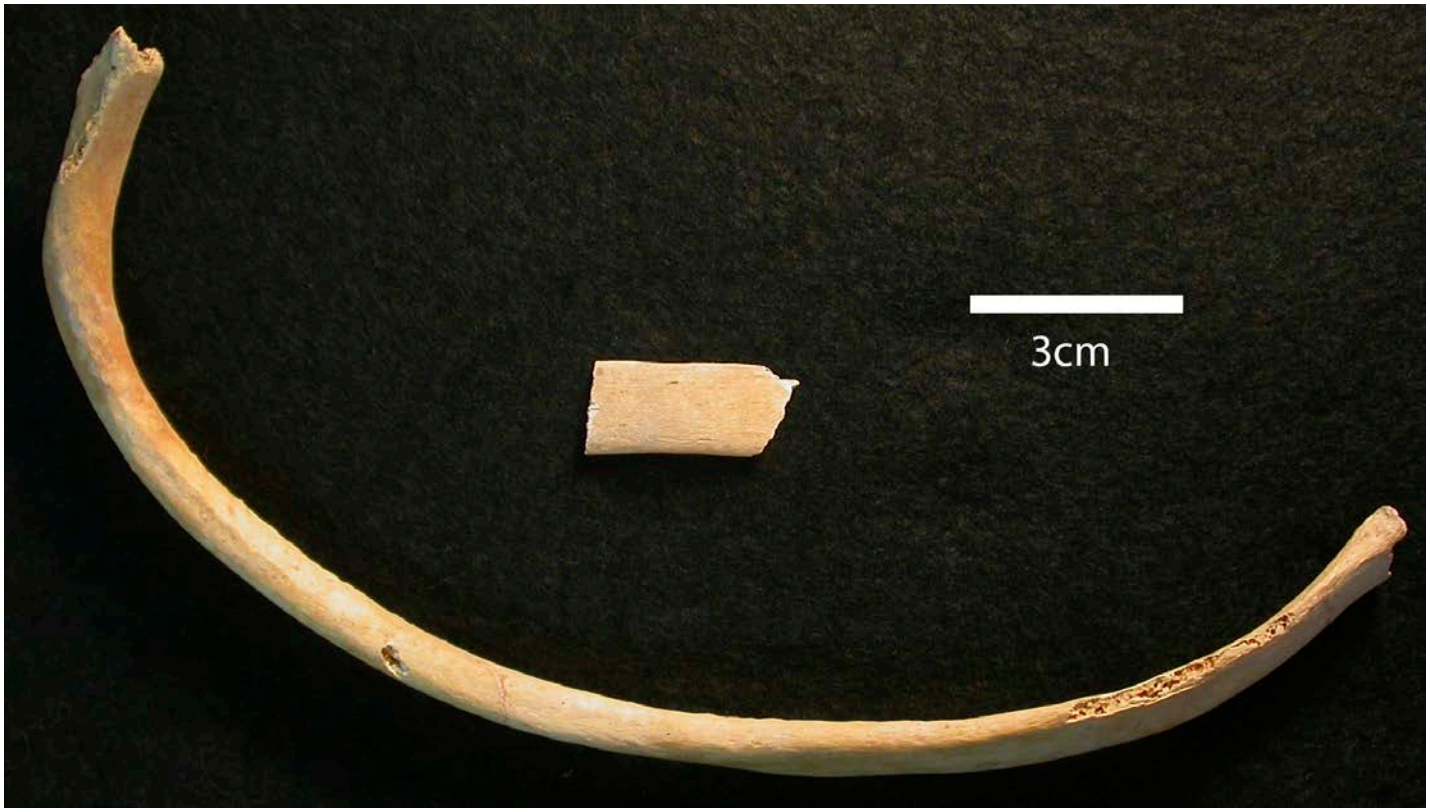


Fig 3 A rib fragment, weighing 0.9g, from an adult burial, used for carbon and nitrogen stable isotope analyses. It is shown alongside a fairly complete adult rib to indicate the approximate proportion of the whole bone that is represented by the fragment.

the personal identity of the individual is known and sensitivities are consequently heightened. In such instances, Consistory Courts or the Cathedrals Fabric Commission would expect petitioners to provide evidence that they have consulted living family members when they can be traced, and that the petitioner's project is not opposed. Courts or the Commission normally attach considerable weight to the views of family members when considering applications. There may be a requirement to destroy or return any material taken but not used during analysis.

2.2 Ethical considerations

Ethics is a framework of values concerned with moral aspects of the way in which we conduct ourselves. Archaeologists have become increasingly aware of a need to reflect upon the ethical implications of their work. Important aspects of ethics that are useful for guidance of conduct within archaeology include knowledge-based ethics (ethics associated with the generation or preservation of knowledge) and the recognition of the place of archaeology in the wider world, including how we engage the public in our work. In addition, because human remains are the remnants of once-living people, burial archaeology raises further ethical issues that do not arise in other areas of the discipline. These are associated with the need for respectful treatment of remains, and arise from the religious and other cultural norms that influence what broader society considers acceptable treatment of the remains of the long-dead. Anyone dealing with archaeological human remains needs to be aware of current guidelines concerning the ethical treatment of archaeological human remains, and links to various professional guidelines relevant to remains from England are provided in **Section 10** of this document. The paragraphs that follow concentrate on ethical matters specifically relating to destructive sampling.

Some ethical considerations specifically relevant to destructive sampling relate to knowledge-based ethics. Analysis of human remains offers important insights into the human past, and provides benefits of other kinds, for example contributing to the development of forensic science. Most people would consider that the accrual of knowledge is a significant benefit for humanity. A museum or other institution holding archaeological remains for research purposes may be considered to have stewardship of that material. That is, they hold it in trust for the benefit of the wider community, and for the benefit of future generations. There is, therefore, an ethical imperative toward the preservation of collections in ways which safeguard the information they contain. When it comes to destructive sampling there is a tension between the imperative to generate new knowledge and the imperative toward preserving collections intact. This dilemma lies at the heart of decisions concerning destructive sampling.

It is worth stressing that there is currently no consensus as to where the balance should lie between generating new knowledge using destructive analyses and preserving collections intact and thereby maximising future research potential. There will be no right or wrong answers about whether a given programme of destructive sampling should go ahead; that is a value judgement to be made by the institution that holds the remains. Decisions should be made, taking expert advice as appropriate, in an open and accountable way.

This guidance is primarily concerned with remains that are curated in long-term collections, rather than with those that are slated for reburial. It is APABE's position that collections of excavated human remains with long-term research potential be retained rather than reburied. Retention can be in a museum or similar institution or, for remains from Christian burial grounds, in church buildings (a Church Archive of Human

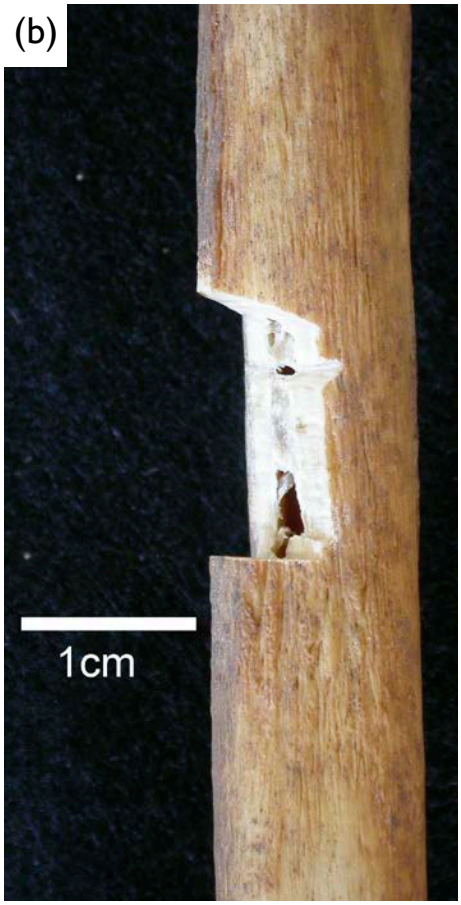


Fig 4a, 4b A fibula (one of the lower leg bones). A sample for carbon and nitrogen stable isotope analysis has been removed from the shaft of the bone. It is preferable to sample from an element that is already damaged or incomplete but that was not possible in this instance. Despite the bone's slender shaft, removal of the sample has been achieved without cutting completely through the bone, preserving it intact for measurement. The sample was removed using a small hand saw with a round-section 360° cutting blade. The lower part of this bone is thickened due to disease. The sample was taken from the upper part to avoid damaging this area.

Remains, or CAHR). Reburial of remains effectively means permanent loss of the information they contain, so that the ethical tension referred to in the previous paragraph will not apply. Nevertheless, competing demands of different researchers who may wish to sample the remains prior to reburial may still need to be managed.

In the case of requests to clergy for exhumations of skeletal remains from churchyards for research purposes, consideration will also need to be given to the Church's presumption against disturbance of remains. Particular care needs to be taken when there is a focus on remains of a specific known historic personage or other identified individuals. Ethical considerations in such cases are complex, but key questions include whether the project is in the wider public interest and whether it has the support of living descendants.

2.3 Scientific considerations

2.3.1 The nature of research

Research is not aimless data-gathering but should always be directed at answering clear and specific questions. Research in archaeology may be applied or methodological. Applied research involves using remains to find out about the past. In applied research, questions to be addressed may concern the ancient population represented by the burials in question, or they may be about the general time period or region that the population comes from, especially if the skeletal collection is one of several that are being used for a research project. In methodological research, remains are studied with the aim of testing or improving existing techniques of gaining information from skeletons (e.g. ways of assessing sex or age-at-death from skeletal remains, or methods for studying ancient diets) or developing new ones.

Sometimes research focuses on a single or some few skeletons, for example to study some unusual disease, to build up an 'osteobiography' of a person's life from their bones, or to identify or establish links with remains of a particular historical figure. As was mentioned in **Section 2.2**, in this last case it is important that the research questions are of wider interest – for example establishing family connections with some named person using analysis of an exhumed burial may be of great personal interest for the individual researcher(s) involved but be of little wider significance.

Although scientific studies can focus on single skeletons, most scientific research is quantitative. It involves identifying statistical patterning in data, so usually large numbers of skeletons (>100) are needed. This means that researchers often look for large collections. Alternatively, they may combine skeletons from a number of different sites, or else combine their results with data generated previously

Results of destructive analyses rarely make sense in isolation. Usually they require morphological data, recorded using non-destructive osteological techniques, to enable their interpretation. Therefore, research projects normally require access to collections to record some osteological aspects of the skeletons in addition to taking samples. Increasingly, projects are also combining different destructive techniques. This not only maximises the ability to address research questions

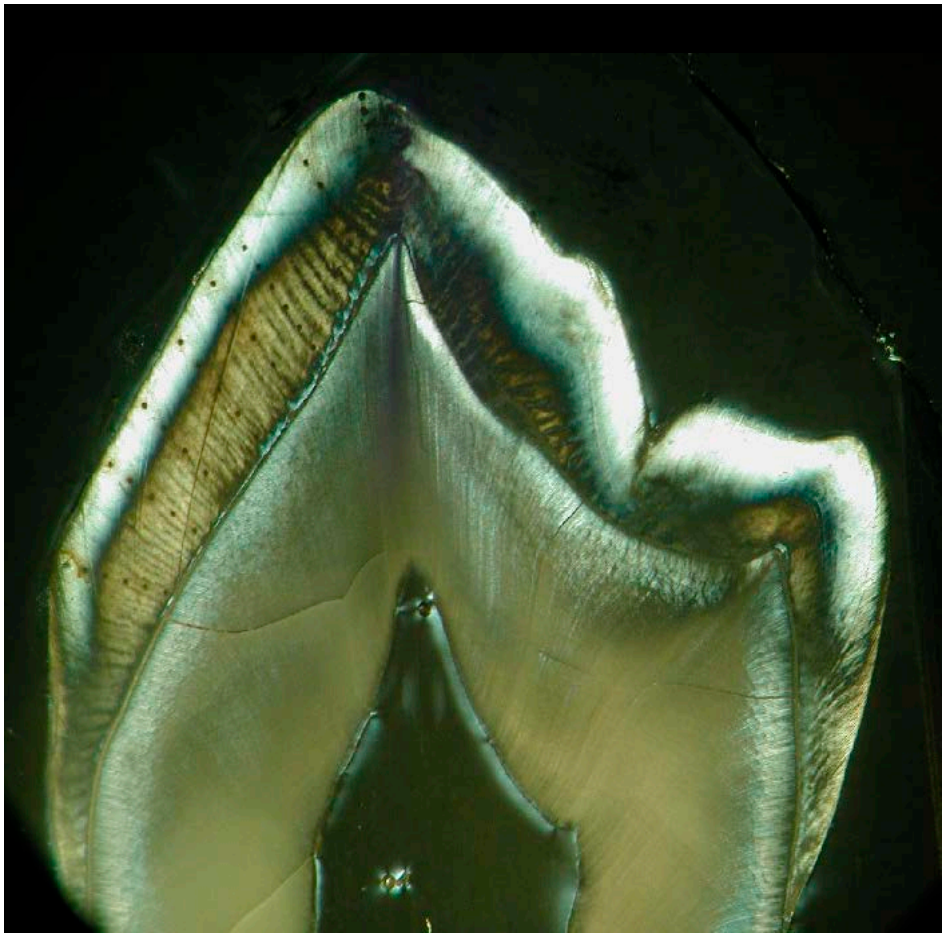


Fig 5 A thin ground section of the crown of a premolar tooth showing the central pulp chamber, the dentine core surrounding it and the enamel cap. Small dark dots in the enamel are laser ablation pits where microsamples were removed by laser for the purpose of analysis of trace element ratios.

but, because the same samples can sometimes be used for more than one type of analysis, it often helps to optimise information gain and minimise destruction.

Traditionally, the audience for scientific research in archaeology has been an academic one. However, researchers are increasingly aware of a need to disseminate results to a wider audience. This is particularly pertinent for work involving human remains, as this aspect of archaeology holds special interest for the public. For applied research, it is legitimate to ask researchers what their plans are for disseminating the results of their work to the wider public (particularly local communities) and to take this into account when considering applications. In addition, providing information to use as a basis for community engagement projects may itself be a legitimate reason to conduct destructive analyses, although this work should still be purposive and question-led. In view of the permanent loss that destructive sampling entails, any public engagement projects used to justify it should have demonstrable long-term value.

2.3.2 The nature of destructive sampling

As a generality, two trends have been apparent over recent years.

- Samples required for biomolecular analyses have become smaller. This means that, in general, application of these techniques is less damaging to collections than was previously the case. However, the situation is a little more complex than this. For example, a small sample removed from a tooth may result in greater information loss to future researchers than a larger bone sample taken as a rib fragment or removed from a long-bone: teeth are smaller and are complex structures that can potentially yield a lot of information when analysed using different destructive and non-destructive techniques.

- Microscopy of a cut section allows visualisation down to a level that is currently beyond that adequately captured by even the most sophisticated non-destructive methods. However, rapid advances in micro-CT and other imaging techniques have meant that studies of some microstructural features that once required sections to be physically removed from bones or teeth can now be accomplished non-destructively (although some advanced imaging techniques are currently limited in their availability to researchers).

There is every reason to believe that these two trends will continue in future, emphasising the need to assess requests for sampling rigorously.

The extent and nature of skeletal collections mean that extant archives of skeletal material, and our knowledge of earlier skeletal biology, vary temporally and geographically. Remains from earlier prehistoric times (Palaeolithic, Mesolithic) are rare in England, but curated collections become progressively more plentiful for more recent periods. Patterns of skeletal survival, intensity of archaeological investigation and other factors mean that regional disparities exist in availability of remains from different periods and from different types of burial grounds. Losses via sampling would be more significant for rarer material. In addition, certain important collections receive a disproportionate number of requests for access, and there is a risk of accrual of damage from repeated sampling. Such factors need to be taken account of when evaluating requests.

The nature of the research, the types of samples requested, and the measures to be taken to mitigate the impact of the destructive sampling need to be considered.

Research programme

- Any destructive analysis should be carried out within a coherent research programme and should stand a realistic chance of advancing knowledge.

- The questions to be addressed by the work should be of archaeological, historical or methodological significance, and clear hypotheses should be tested.

- Destructive analyses should only be considered if the research questions cannot be addressed adequately using non-destructive techniques.

- The researchers must be sufficiently competent and experienced to conduct the work proposed.

- Ideally, adequate funding for the work should be in place, but researchers often need permission in principle in order to apply for funding and so may contact curators on this basis. If permission is granted subject to funding being obtained, it should be time-limited rather than open-ended.

Sampling programme

- If the feasibility of a technique is questionable, then thought should be given to conducting a pilot study on a small number of samples, with permission to proceed further being contingent upon the results

- Sampling should be kept to a minimum, compatible with fulfilling the aims of the project. Oversampling, leading to stockpiling of material by researchers, is unacceptable

- The number, location on the skeleton, the size of samples, and the methods by which the researchers intend to remove them, should be made explicit

- The condition of the element to be sampled should be carefully checked to assess whether existing cracking or other damage means that sampling risks additional damage or fragmentation to an already fragile specimen

- The likely effect of sampling on future research potential of the remains is a key issue. To this end, the location in the skeleton from which a sample is to be taken should be carefully considered:-

    - Sampling from anatomical landmarks (points from which measurements are taken) or from areas important for inferring sex or age should be avoided

    - Unless the study specifically requires it, sampling from diseased bone should be avoided

    - If a tooth is to be sampled, then its antimere (the corresponding tooth from the opposite side of the jaw) should preferably be present

    - Samples should preferably be taken from bones or teeth that are already incomplete, damaged or fragmentary, unless the technique requires intact specimens

    - Coordinating sampling for different purposes may help to minimise destruction

    - In the past, chemical consolidants may have been applied to archaeological bone to try and strengthen it. This may interfere with some scientific analyses, so sampling such areas should be avoided

    - If appropriate, thought should be given to the visual impact of sampling – for example on the suitability of the specimen for future museum display

- Good record keeping of any sampling programme is essential so that it is clear what samples have been removed from which contexts. The exact nature of the records to be kept needs to be agreed by the holding institution with the researcher as a condition of access. Record keeping is normally undertaken by the researcher.

- Any un-used bone or tooth fragments or powder, as well as slides and embedded microscopy samples, should be returned to the collection

- Any un-used liquid extracts from samples usually remain with the laboratory that conducted the analyses, but should be returned to the organisation holding the collection if requested, and should be offered to them if the laboratory wishes to dispose of them. Use of these residues by researchers for purposes other than those that were part of the agreed project would require express permission

Mitigation programme

The level of mitigation needs to be agreed by the holding institution with the researcher as a condition of access. Mitigation is normally undertaken by the researcher.

Mitigation in this context means firstly making a record of the remains to be sampled so that observations are made before the part in question is destroyed, and secondly making available the data from the destructive analysis itself. The first element may take the form of traditional osteological recording (e.g. measurements, observation of osteological traits), together with photography and various casting techniques. The second comprises plans for management and sharing of the data from the researcher's own project.

What comprises an adequate level of osteological recording prior to sampling will depend, *inter alia*, upon the part being sampled, the state of the remains, the osteological studies that have been carried out in the past, and those intended by the researchers carrying out the project involving the destructive sampling. Sometimes no further osteological recording may be needed prior to sampling. A curator might ask the researcher for their plans in this respect and ask the advice of an osteologist regarding their adequacy.

- It is usual to take photographs before and after sampling. In addition, it may be appropriate to produce a surface cast of the parts to be destroyed or to conduct a surface scan or micro-CT scan or both. The aim in these cases is to produce a physical or virtual model of the part that future researchers may use for morphological studies. It is becoming increasingly common to ask researchers to mitigate the impact of their work in this way. In practice, this is more usually done for teeth, especially when crowns are sampled, but it is also appropriate in some instances for bones (for example, if they show alterations due to disease). Care needs to be taken that resolution of scans and quality of casts are sufficient to represent the part to be destroyed and to be useful for research purposes.

- Unless exhumation is for the specific purpose of research involving destructive analysis, sampling should not normally be permitted on-site during excavation.

- Publications arising from the scientific analyses should be lodged with the organisation which granted access to the remains.

- Data management plans, including provisions for the long-term storage and availability of data, are quickly becoming routine in large-scale scientific projects. Data resulting from studies should be published fully if appropriate. A copy of the raw data and recording protocols, or else access to the repositories where

these are held, should be deposited with the organisation holding the remains. This will aid collections management, and help prioritisation of future research applications that the holding institution receives. In instances where the raw data do not form part of the academic publication of the work, or have not otherwise been disseminated, the holding institution should normally be able to manage and control release of the data to other researchers as appropriate, perhaps following an embargo period agreed with the laboratory that generated it. Ability to interrogate data sets may, to some extent, reduce the need for future destructive analyses.

- DNA and proteomic analyses produce very large amounts of data. This may be disseminated using online data-sharing platforms. These are also becoming available as ways of disseminating other sorts of data.

- The implications of sharing DNA or other data from identified individuals require careful consideration.

3. Radiocarbon dating

3.1 The science

Isotopes are atoms of a chemical element with different masses. Some are radioactive and steadily decay, transmuting into other elements. Others are stable - they are non-radioactive and do not change in abundance over time. Carbon has three naturally occurring isotopes: ^{12}C, ^{13}C, and ^{14}C. These three isotopes do not occur equally, with carbon in the atmosphere and biosphere consisting of 99% ^{12}C, 1% ^{13}C and about one part in a million million of ^{14}C. ^{14}C is different from the other two isotopes in that it is radioactive with a half-life of 5730±40 years. From this it derives its name radiocarbon.

Radiocarbon is formed in the upper atmosphere by the interaction of neutrons, produced by cosmic rays, with nitrogen atoms. Once radiocarbon has been produced it rapidly forms carbon dioxide and mixes through the atmosphere, dissolves in the ocean, and enters the terrestrial food chain through photosynthesis. Consequently, the ^{14}C content of a living terrestrial organism is in equilibrium with that of the contemporary atmosphere.

When a plant, human, or animal dies it no longer takes in ^{14}C and thus over time the proportion of radiocarbon falls at a rate that is determined by the law of radioactive decay. By measuring the proportion of ^{14}C that remains, it is possible to estimate the time since the organism died.

Unfortunately, as the production of radiocarbon in the atmosphere is not constant, a year in the radiocarbon age timescale does not have an equivalent interval in the calendar timescale and for this reason calibration is required. Progress in the extent and resolution of the data available for calibration means the current internationally agreed calibration curve extends to 55,000 years before present. This provides a common standard and means that all calibrated dates are comparable.

Radiocarbon is present in such low abundance it puts a statistical limit on the precision of a radiocarbon determination. A fundamental aim during measurement is therefore to measure the isotope ratio as accurately and precisely as possible. The two main methods of measuring ^{14}C are decay counting methods (using liquid scintillation and gas proportional counters) and accelerator mass spectrometry (AMS) where the radiocarbon atoms are directly detected. Since the mid-1980s the introduction of accelerators for the direct detection of radiocarbon has allowed a whole range of much smaller samples to be measured. Nowadays, AMS is almost always used for measuring the amount of radiocarbon in samples.

Un-burnt human bone is one of the most complex materials commonly used in radiocarbon dating. Following the death of an individual, degradation of a bone's molecular structure and the incorporation of exogenous molecules as a result of chemical and environmental processes can influence subsequent radiocarbon measurements. Research into effective pretreatment methods to minimise the problems of contamination continues with the aim of reducing still further the contaminants present in the sample from the environment and to minimise the addition of further contaminants. With human bone and tooth dentine, attempts to improve on the widely used simple extraction of protein ('collagen') have included selection of large molecules using ultrafiltration, or the use of individual amino acids.

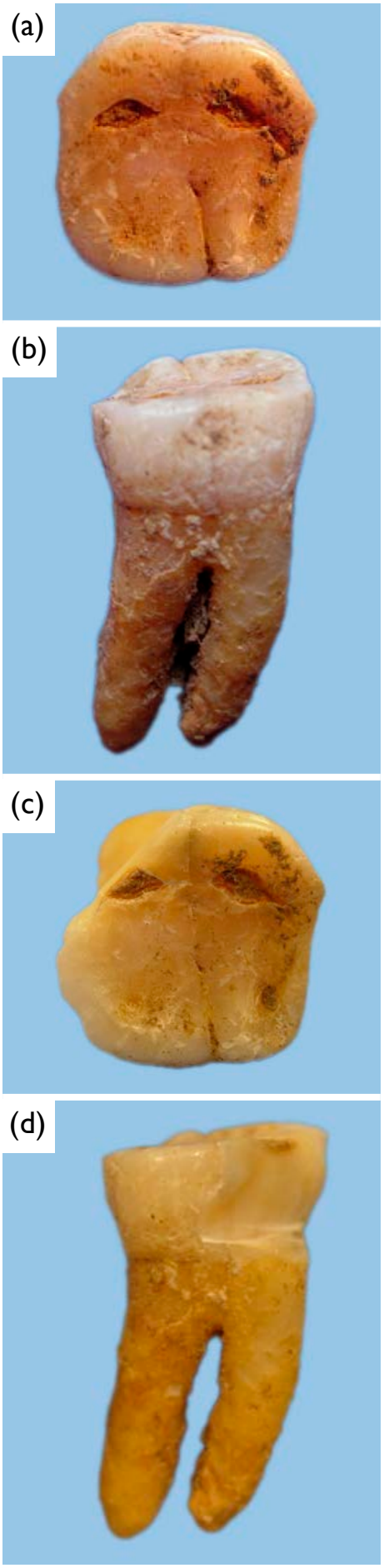


Fig 6 A molar tooth before (a, b) and after (c, d) removal of part of the crown for isotopic analysis. Although some measurement and other studies of crown morphology will now not be possible on this specimen, much of the crown has been preserved intact.

Bone that has undergone burning at high temperatures (i.e. cremation) no longer contains organic carbon and so until relatively recently has not been suitable for radiocarbon dating. In the last twenty years, the successful dating of the inorganic bone matrix (recrystallised bioapatite) content of cremated bone has meant it is now possible to date burned bone, allowing more widespread scientific dating for periods when cremation was the dominant funerary rite. Dating is only possible for thoroughly burned (calcined) bone; partially burnt (charred) bone cannot be dated.

Humans have a markedly variable and mixed diet and as such frequently derive carbon from more than one source (known as a reservoir). The measurement of carbon and nitrogen stable isotope ratios (see **Section 4**) can be used to determine the potential for diet-induced radiocarbon offsets if an individual has taken up carbon from a reservoir not in equilibrium with the terrestrial biosphere, for example – marine or carbonate-rich freshwater resources. This issue affects radiocarbon measurements on unburnt bone, but not those on cremated bone. In practice, dietary effects have not been found to be significant for interpreting radiocarbon dates on human bone from England from the Neolithic to the Viking period.

Calcined bone may exhibit an age offset derived from the incorporation of carbon from the pyre fuel during the cremation process (if for example the wood was from long-lived species, e.g. oak). The scale of such offsets is currently uncertain, as is their prevalence in the past.

Age offsets may also exist in un-burnt human bone. The offset arises from the time it takes carbon from the diet to be incorporated into bone collagen. As individuals become older than this, the average difference between the time when bone collagen was laid down and the date of death goes up, particularly in men. Bone turnover offsets are unlikely to be of practical relevance except for the most high-precision applications.

3.2 What can we learn from radiocarbon dating?

Chronology provides a fundamental structure for understanding the past, with timing unravelling the sequence of past events and the tempo of change. Increasingly refined chronological frameworks from burial grounds, e.g. Barton-upon-Humber, are enhancing understanding and appreciation of the value of such assemblages, particularly when combined with other investigations such as stable isotope analysis.

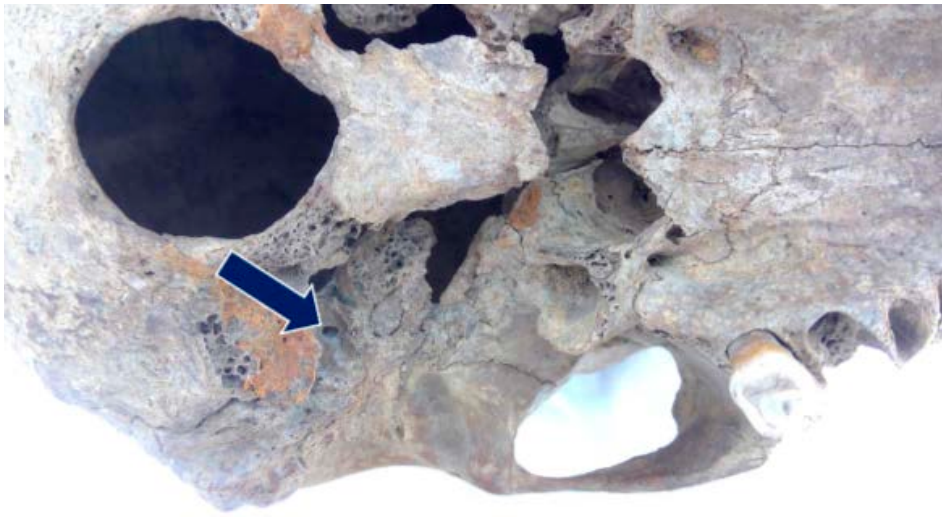


Fig 7 A cranium which has been sampled for DNA using the cranial base drilliing technique. The arrow indicates the site from which the sample was drilled.

The ability to chronologically divide the human population for cemeteries such as St Mary Spital, London (10,516 bodies) using radiocarbon dating and archaeological phasing means that it is possible to track developments in demographic change and variations in the health of the population that lived and died in this part of Mediaeval London. The radiocarbon dating programme for this site also identified cases of pre-Columbian syphilis, and mass burial pits predating the Black Death of AD 1348.

Radiocarbon dating of a selection of the 50 or more bodies once present in Aveline's Hole, Burrington Combe, Somerset confirmed the site as one of the largest early Mesolithic burial sites in Europe. The results suggest use of the cave for burial over, at the most, a century or two, in the mid to late ninth millennium BC.

Exploiting information about the relative age of formation and eruption of human teeth, radiocarbon dates on slices of dentine from the first and third molars of individual burials from Palace Green Library, Durham were 'wiggle-matched'. Wiggle-matching involves matching the shape of a series of radiocarbon dates separated by a known number of calendar years against the calibration curve. The chronological evidence suggests the burials are those of imprisoned Scottish soldiers from the Battle of Dunbar in AD 1650.

When considering individual radiocarbon dates, it must be remembered that the uncertainty of calibrated radiocarbon dates is not only a function of the errors quoted on radiocarbon determinations and on the calibration data, but also on the shape of the calibration curve. Thus, for some time periods the uncertainty is relatively large, for example c 750–400 BC for a person who actually died in 500 BC, i.e. where the actual ages falls on a 'plateau'. It can also be relatively precise: AD 1400–1470 for a person who died in AD 1425, where it falls on a 'steep' section of the calibration curve.

In the last twenty years the use of a Bayesian approach has proved to be the most effective method available for producing statistically reliable estimates of chronology. In archaeological terms this means that we analyse new data we have collected about a problem (the 'standardised likelihoods' — radiocarbon dates) in the context of our existing experience and knowledge about that problem (our 'prior beliefs' —for example the stratigraphic relationship between graves). This allows us to arrive at a new understanding of chronology which incorporates both our existing understanding of the problem and our new data ('posterior belief').

3.3 Sampling for radiocarbon dating

The most common human remains submitted for dating are unburnt bones from which typically 1g of bone is needed for AMS dating. Samples from the larger dense bones of the body (femur, tibia, humerus, or mandible) are preferred as these typically have better collagen preservation and less sedimentary

contamination than more porous bone.

Sampling of complete unburnt bones and teeth for AMS dating is usually undertaken with a mechanical drilling kit and special care should be taken to avoid any areas that may have been consolidated or treated with chemical preservatives.

The preservation of unburnt bone can be greatly influenced by the burial environment, resulting in chemical and physical degradation. Over 90% of the collagen content can be lost in some environments, which restricts the potential for radiocarbon and stable isotope analysis. A rapid technique, determining the %N content of whole bone, that requires very little material (<5mg bone), has been shown to be very successful in predicting whether a bone is suitable for dating. This pre-screening method reduces the amount of destructive sampling, in addition to saving time and money spent on unsuccessful dating.

For teeth, the preferred samples are incisors, canines, and molars, and attempts should be made where possible to leave enamel in good condition for other researchers (e.g. strontium and oxygen isotopes) when sampling the dentine.

For cremated bone, a 2g sample of fully calcined bone is required.

In exceptional circumstances, other material suitable for dating can also be preserved, e.g. hair, skin, and soft tissue.

For large human bone assemblages, the use of Bayesian simulation models to identify the minimum number of samples needed to provide meaningful answers has proved especially valuable.

4. Stable isotopes and ancient diets

4.1 The science

Most chemical elements exist as mixtures of two or more stable isotopes. For some elements, the stable isotope ratios differ in different classes of foods, and these differences are passed on to the tissues of the consumer. Hence, measurement of stable isotope ratios in skeletal remains can be used to study ancient diets. The most widely used elements in this respect are carbon and nitrogen.

Carbon stable isotope ratios differ in plants using different photosynthetic pathways to manufacture carbohydrates from atmospheric carbon dioxide. Most temperate zone vegetation uses the so-called C3 pathway. Some plants that are native to warmer regions, such as maize, use the C4 pathway. In addition, both carbon and nitrogen stable isotope ratios differ in marine and terrestrial foods. In England there are no indigenous C4 foods, so most stable isotope work has concentrated on studying marine contributions to diets. For nitrogen isotopes, there is a small trophic level effect, meaning that ratios increase as one ascends a food chain. In principle, this means that it is possible to say something about the relative importance of animal products (meat, dairy) versus plant foods, but limitations in our knowledge of other sources of variability in nitrogen isotope ratios in bone mean that this is often difficult in practice. Because exclusively breastfed infants are only consuming a product of the mother's body, they are one trophic level higher. Nitrogen isotope ratios have been used to study the duration of breastfeeding in past societies.

Dietary stable isotope studies normally focus on collagen from bone or tooth dentine. In living people, collagen in bone is continually renewed. During infancy, this process is rapid, but by adulthood it slows down so that analysing adult bone collagen gives a measure of diet averaged over years or decades. Collagen in dentine is not renewed, so this gives indications of diet whilst the dentine was forming as the tooth developed during childhood. All the nitrogen in collagen, and most of the carbon, comes from dietary protein, so results tell us mainly about the protein part of the diet. Carbon stable isotopes can also be analysed in tooth enamel (the mineral contains carbon in the form of carbonate). Like dentine, enamel is not renewed once formed, so this too gives a child-diet signal, but unlike collagen results appear to reflect whole diet rather than being biased toward protein.

Although the great majority of dietary isotopic analyses involve carbon and nitrogen, other elements are also sometimes used. Of these, sulphur is the most important. Sulphur stable isotope ratios differ in marine and terrestrial environments and, depending upon local geology, may be different in terrestrial versus freshwater foods. Sulphur stable isotope ratios in collagen may help identify consumption of foods from marine and coastal, and in some instances, freshwater ecosystems.

Researchers usually find it useful to have local isotopic values from archaeological faunal remains (and plant remains if possible), to provide a baseline to help interpret the human data. These can either be obtained from the literature or from conducting archaeofaunal and archaeobotanical isotopic analyses as part of the project.

Usually, bone and tooth samples from burials on English archaeological sites contain sufficient intact collagen for successful stable isotope determinations, so stable isotope work normally produces usable results. However, in cases where collagen survival is uncertain, measurement of nitrogen content, as described in Section 3.3, can be used as a pre-screening technique. Dietary information cannot be obtained from isotopic analysis of cremated bone.

4.2 What can we learn about diet from stable isotope analysis?

To study diet, carbon and nitrogen stable isotopes are usually used together. Most work attempts to address questions of broad archaeological or historical interest, so sampling involves multiple skeletons rather than single burials. Currently, most studies use anything from about 30 to more than

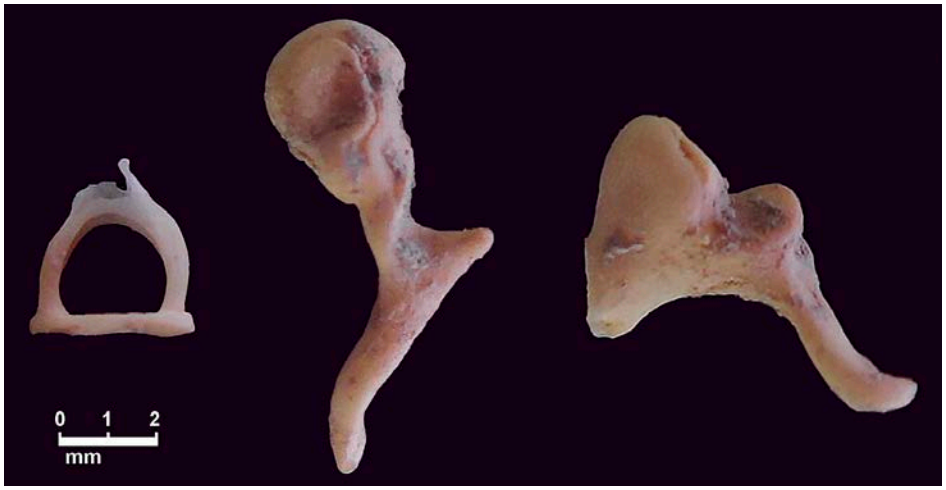


Fig 8 The three auditory (ear) ossicles. From left to right: stapes, malleus and incus.

100 skeletons, often from several archaeological sites, depending upon the questions to be investigated.

Many studies have looked at the way in which diet changed with the advent of farming in the Neolithic period. In Britain, results show that prior to the Neolithic, coastal groups relied heavily on seafood, but these resources were largely abandoned with the introduction of farming. In Britain, this change in diet generally occurred abruptly, but in some other parts of Europe it was a much more gradual process with marine foods continuing to be exploited in significant quantities well into the Neolithic.

In Romano-British times, isotope data suggest that consumption of marine foods was greater than in immediately preceding or succeeding periods. Trading networks associated with the Roman Empire meant that these foods were available inland as well as on the coast, although in some locations at least, they were more available to the wealthy than to the poor. Work on burials from the succeeding Early Anglo-Saxon period found that, although consumption of marine foods was generally lower, compared with inland locations, slightly elevated carbon stable isotope ratios at coastal sites and slightly raised nitrogen stable isotope ratios at estuarine locations suggested that communities at these locations did make some use of local aquatic resources within a predominantly agrarian economy.

In the Mediaeval period, at the village of Wharram Percy, Yorkshire, nitrogen isotope data suggested that breastfeeding was continued until children were about 18 months old. This prolonged period of breastfeeding seems to have had beneficial results: infant mortality in that community appeared low by premodern standards.

4.3 Sampling for carbon and nitrogen stable isotope work

Typically, a bone sample of less than 1g is taken for carbon and nitrogen stable isotope determinations. Typically a rib is sampled. Rib collagen is renewed somewhat more quickly than in other bones such as longbones. Adult rib collagen provides a good indication of adult diet. In infants and children, rib collagen turns over rapidly, so may be used to assess age of weaning because the delay with which the weaning signal is manifest is minimised.

Archaeological bones are often fragmentary. If an appropriately sized bone fragment is present then this is normally taken for analysis. Otherwise, a small saw is used to cut a piece of bone of suitable size. Care is taken not to cut completely through an intact or minimally damaged bone and to minimise visual impact beyond the area sampled. An alternative is to clean the bone surface and then use a cutting burr to generate a sample of bone powder.

As mentioned above (**Section 3.1**), carbon and nitrogen stable isotope measurements are routinely conducted on bone samples submitted for radiocarbon dating. This is because for accurate radiocarbon calibration, we need to know something about the individual's diet. In particular, it is important to detect individuals who consumed significant amounts of marine foods, as incorporation of marine foods into skeletal collagen tends to make radiocarbon dates too old, and a correction is needed for this. When both radiocarbon dating and dietary studies are envisaged, with careful planning it may be possible to minimise destruction of material.

Sampling of teeth from adult skeletons can be used to study diet when that person was a child. Recent technical developments mean that many samples, of tissue which developed at different ages, may be obtained from a single tooth. For example, a first molar may yield about 10-15 dentine subsamples, each of which corresponds to about 9 months of development, and together they span the period from about birth to nine years of age. Unlike sampling bone of infants and children who died at different ages, it enables details such as duration of breastfeeding and other dietary transitions in children to be reconstructed for individuals who survived to become adults rather than those who died in childhood. Microsampling dentine is increasingly used as a way of studying breastfeeding and diet in infancy and childhood. Conducting this sort of analysis normally involves cutting the tooth in half vertically, and sampling one half.

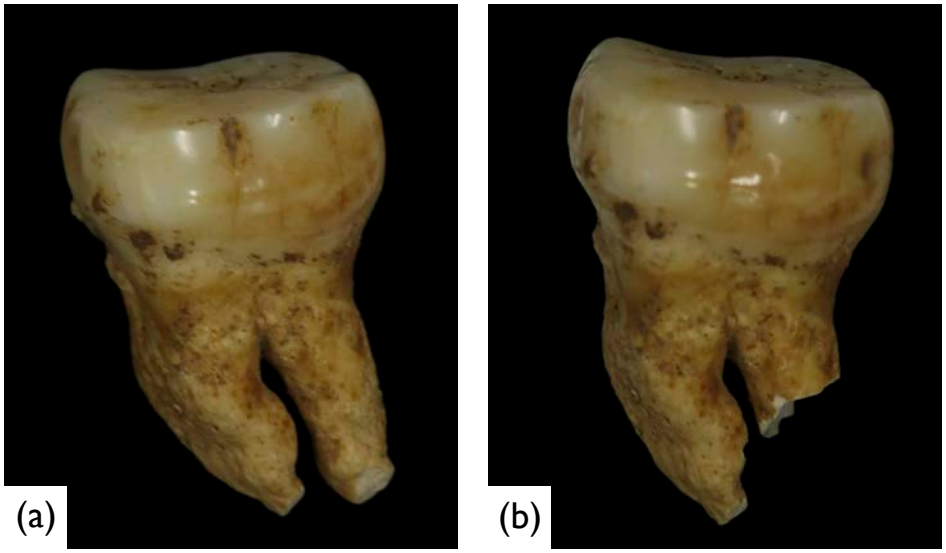


Fig 9a, 9b A molar tooth from an Early Anglo-Saxon burial before and after sampling. Part of the end of one tooth root has been removed (b), and in addition 10-30mg of powder has been drilled from within the root canal. This particular burial was part of a wider sampling programme at this and other similar cemeteries to investigate aspects of the spread of infectious diseases among communities at that time. The sampling served several purposes. Analysis for DNA of pathogens that may have been present at the time of death of this person was carried out (the material from within the root canal was targeted to facilitate recovery of pathogen DNA). Human DNA was analysed to assess the presence of genetic sequences that might indicate vulnerability to disease and / or adaptation of the immune system to infectious disease, as well as aspects of ancestry. Proteomic work was conducted to detect human proteins generated in response to disease as well as proteins that might be from pathogens themselves. This illustrates the range of information that can potentially be obtained from material taken from a single sampling site.

5. Stable isotopes and geographical origins of people in the past

5.1 The science

The two most important elements that have been used in isotopic studies to trace geographic origins are strontium and oxygen. Strontium isotope ratios vary in different types of rock. There are therefore systematic differences in plants and animals in areas with different geology, and these are passed on to the tissues of consumers. Oxygen isotopes vary in rainwater in different regions according to factors which include climate, altitude and distance from the coast. Oxygen isotope ratios vary in different living organisms, and hence in

different foods, but this does not usually matter very much for human studies as the isotopic composition of drinking water is the prime determinant of the oxygen isotopic composition of human tissues (an exception is suckling infants - during breastfeeding their oxygen isotope ratios are altered).

Unlike most carbon and nitrogen stable isotope work, strontium and oxygen isotope analyses use the mineral part of skeletal tissues and not collagen. The mineral part of bone and dentine is vulnerable to changes in composition during burial, but dental enamel appears highly resistant (as is cremated bone or dentine, due to structural changes undergone on burning). Therefore, most strontium and oxygen work on unburnt human remains uses dental enamel. Because dental tissues are not continually renewed, the isotopic composition of dental enamel reflects that in the locale in which the person lived as a child when the enamel was forming. An approximate local baseline for oxygen or strontium values in the location in which the individual was buried (and by implication lived immediately prior to death) can be established from geological or rainfall maps. More precise values can be obtained for strontium from local plant or water values or from archaeofaunal remains; for oxygen isotopes, modern local surface or well waters can be sampled. If the isotopic composition of dental enamel differs from baseline values, then the person likely spent at least part of their childhood elsewhere.

Oxygen isotope ratios in waters in Britain overlap with those in other locations, for example in continental Europe, particularly north-western areas and parts of the Mediterranean basin. Strontium isotopes will be similar in regions of similar geology, regardless of geographic separation. Oxygen isotopes are generally most useful for distinguishing among individuals on a fairly large spatial scale; strontium isotopes may, depending on geology, enable smaller spatial distinctions to be made. In practice, most workers use both strontium and oxygen isotopes in combination to narrow down the number of possible locations where a person may have spent their childhood. An exception is for cremated bone where only strontium isotopes are studied; alterations in oxygen isotope ratios during burning mean that they cannot be used for this purpose.

Some other elements in dental enamel are also sometimes studied. By far the most important is lead. In pre-industrial populations, lead isotope ratios tend to reflect local geology. Lead levels in the natural environment are generally low, so in societies with lead metallurgical technologies, most intake is from contamination from lead artifacts or industrial pollution, so isotope ratios reflect the lead ores used. In either case, lead isotopes in dental enamel can be used to help identify migrants in periods when the metal was used, and lead concentrations can be used to quantify lead burden, which can be high, especially in post-Mediaeval industrialised populations.

5.2 What can we learn about mobility from stable isotope studies?

A study of bone from some of the cremation burials from Stonehenge, dating from about the time of the monument's initial construction, showed that about 60% had strontium isotope ratios consistent with local geology. The remainder had signals indicating origins further afield. One of the places that would have been consistent with the data was west Wales, the likely place of origin of the famous bluestones used in an early phase of construction of the monument. These non-locals also had lower carbon isotope ratios in their bones. In cremations, the carbon isotope ratio does not reflect diet but appears to derive mainly from the wood used for the pyre. The lower ratios mean that the wood used in these cases came from a more heavily forested environment than that around Stonehenge. At least some of these non-locals may have died and been cremated elsewhere and their burnt remains brought to Stonehenge for burial.

Strontium and oxygen studies at the Roman fort at Catterick, North Yorkshire, indicated that burials dating from the 2nd-3rd centuries AD showed greater isotopic diversity than burials from the 4th century. This seemed consistent with the idea that, in the Roman army, an early policy of more diverse recruitment was later supplanted by more extensive recruitment from the local population.

Strontium, oxygen and lead isotopic analyses show that Roman towns contained migrants from continental Europe and further afield. That is perhaps of little surprise considering the far-flung nature of the Roman Empire. However, isotope data show that the smaller scale polities of post-Roman times still had far-reaching connections. Dental enamel strontium and oxygen isotopes from a cemetery at the 7th-9th century AD Royal centre at Bamburgh, Northumberland, revealed that non-locals were in the majority. Many were from western Scotland and Ireland, regions with links to Northumberland since Early Christian times. Others came from Scandinavia and nearby regions, attesting to the importance of trading, cultural and other links across the North Sea. Southern migrants came from as far as the Mediterranean. In Early Mediaeval times, the seat of the Kings of Northumbria was a cosmopolitan, multinational trading and political hub.

At a mass grave in Dorset, dating to about AD1000, the occupants, who had been executed by decapitation, showed origins outside Britain. The isotopic evidence was consistent with Scandinavia and nearby areas of Europe associated with Viking expansion. It is likely that this was a site of mass execution of Viking raiders captured by the English.

5.3 Sampling dental enamel for isotopic analysis

A vertical section of enamel, normally about 1mm thick, is removed using a rotary cutting disc. This causes noticeable damage to the tooth crown but, with care, leaves most of it intact. Because different teeth form at different times during childhood, sampling more than one tooth per individual allows patterns of mobility over longer periods of childhood to be constructed. For example, the enamel crowns of the three permanent molars mineralise at between approximately the first few months of life and three years, 3-8 years, and 9-13 years. Provided they have not been worn down by use, analysing enamel from all three allows coverage of most of infancy and childhood.

The enamel may be analysed as a bulk sample, but more detailed migration timelines can be built up by subsampling parts of the vertical section that formed at different times during the tooth's development. This can be accomplished by micromilling, a technique that allows very small samples to be removed. For strontium, an alternative is laser ablation in which a laser is used to remove multiple microsamples from the enamel section.

In cremated remains, the petrous part of the temporal bone at the base of the cranium can be used for for strontium isotopic work. The petrous part of the temporal bone preserves isotopic signals

from the earlier part of life and like other cremated bones, it resists changes during burial. Dental enamel is unsuitable because it shatters into tiny fragments when exposed to high temperatures.

6. DNA

6.1 The science

DNA contains an organism's genetic information. It is encoded in the sequence of chemical bases which form part of the repeating subunits (nucleotides) which make up the molecule. In human cells, DNA is located in the chromosomes of the nucleus and also outside the nucleus in the mitochondria. The chromosomes consist of the X and Y sex chromosomes, plus the autosomes. Nuclear DNA comes from both parents; mitochondrial DNA comes solely from the mother. In the skeleton, DNA is present in bone, and in the dentine and cementum of the teeth. It is not present in dental enamel. Studies of ancient DNA (aDNA) in archaeological human remains have concentrated on human DNA and on DNA from pathogens from infections that were present at time of death.

DNA molecules decay rapidly in the soil, undergoing progressive fragmentation and other damage. However, since the 1980s, techniques have been available to analyse aDNA. Traditionally, this involved single-locus PCR, in which a specific part of a DNA molecule was targeted, amplified and the sequence of bases determined. This process has now been almost entirely superseded by next generation sequencing (NGS), also known as high throughput sequencing. Rather than targeting a specific sequence, NGS permits all the DNA in a sample to be sequenced (although most workers chemically enrich the sample in the DNA of interest because most DNA in ancient samples is just contamination from the soil). This produces vast amounts of sequence data so bioinformatic techniques (computer algorithms) are used to analyse it. This allows much more of the genome to be studied. It permits sequencing of DNA fragments too small to be accessed by single locus PCR, important given the degraded nature of aDNA. It also enables new techniques for the authentication of ancient sequences to be applied, another key advantage given the issues of contamination with modern DNA that bedevilled the early days of aDNA work.

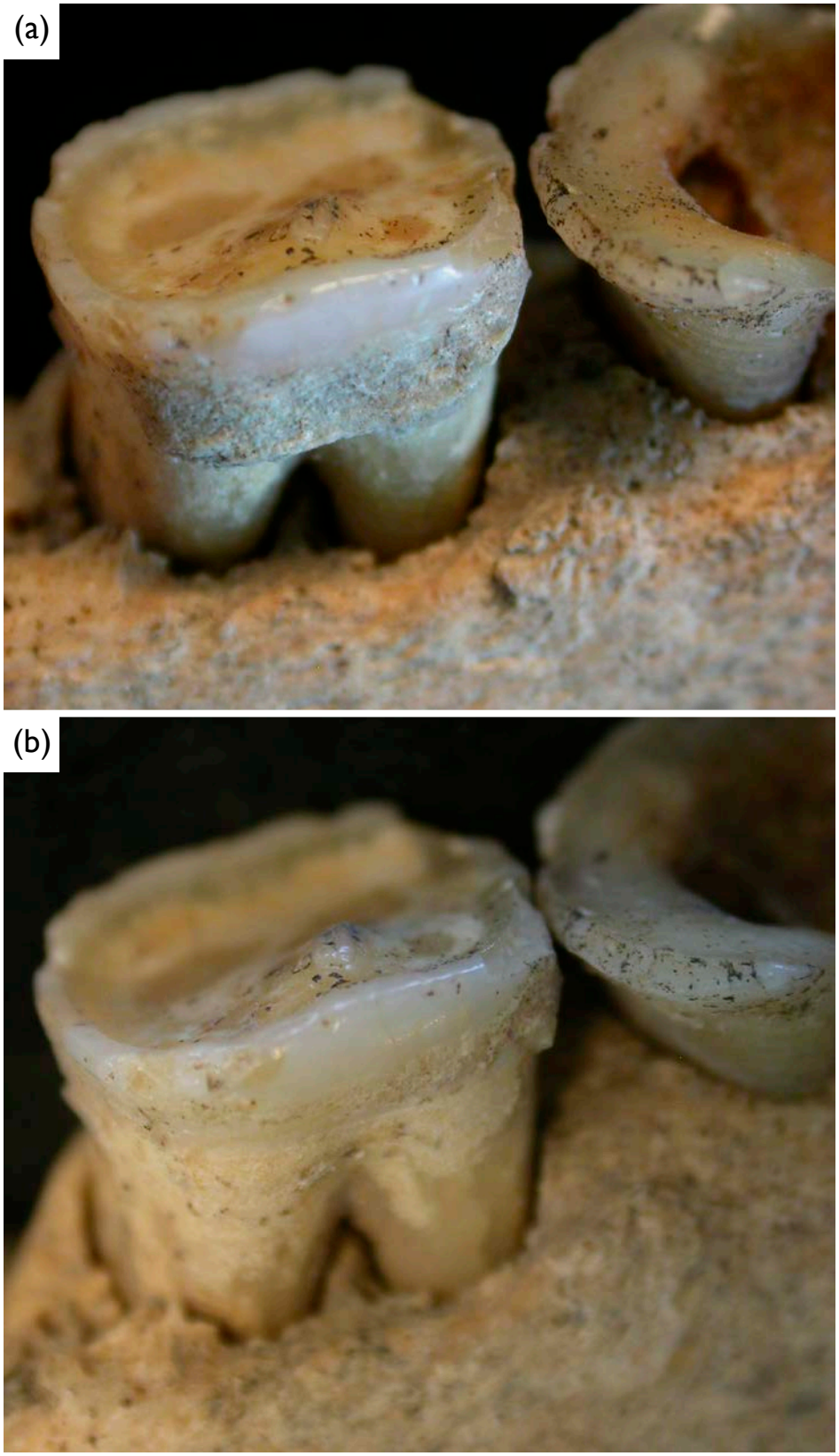


Fig 10a, 10b A molar tooth from a Mediaeval burial before (a) and after (b) removal of an approximately 15mg sample of dental calculus for the purpose of analysing for microscopic inclusions. Analysis revealed evidence for starch granules from the consumption of foods made from wheat and oats, together with particles from the living environment, including fragments of textile fibres (bast (most likely from nettles), flax/hemp, and wool), animal hairs, burnt wood from fires, and charcoal from fires and burnt material from cooking/on food.

Because DNA deteriorates rapidly in the soil, analyses of human remains sometimes fail to provide useful data. Nevertheless, the advent of NGS means that this happens less often than previously. Although single-locus PCR is still occasionally used (e.g. for some studies of pathogen DNA), the advantages of NGS make the balance between destruction of material and the likelihood of producing useful information much more favourable.

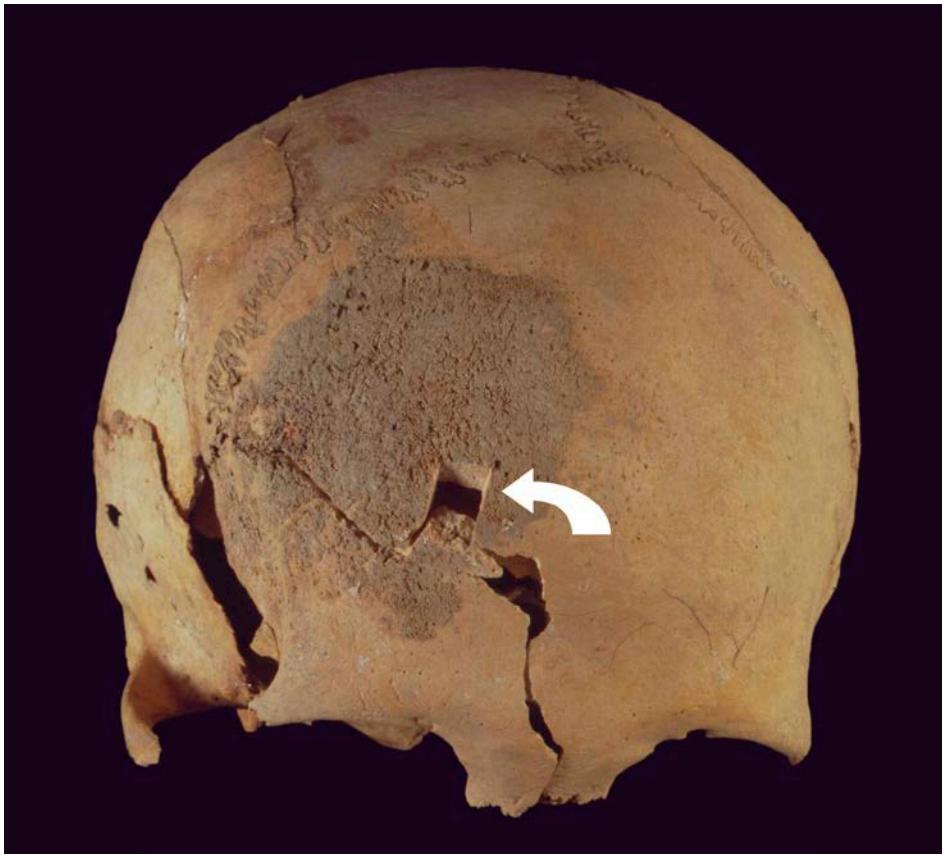


Fig 11 A cranium showing an area of pitted bone on its surface. Within this diseased area, a cube of bone (arrowed) has been removed to provide samples for light microscopy and scanning electron microscopy analysis. The sample was taken from an area that was already rather damaged. The nature of the bony changes suggested that this was a case of prostate cancer that had spread to the skeleton, and the histological examination of the bone sample supported this.

6.2 What can we learn from aDNA?

At a broad level, study of ancient human DNA can tell us about relationships between different human populations, and about migrations and population history. At a smaller scale it can be used to reconstruct patterns of biological kinship within burial grounds and communities. It can be used for probabilistic determination of sex of skeletons where this is not possible on osteological grounds. aDNA can also be used to make probabilistic statements about some phenotypic (bodily) features such as hair, skin or eye colour, as well as aspects such as lactase persistence (which determines whether a person can digest milk) or genetic predisposition to various diseases.

Because of the genome-wide nature of data from NGS, studies aimed at broad population-historical objectives normally also produce genotypic data relating to the sex of individuals and to phenotypic features. Typically, studies looking at population history / migrations involve sampling hundreds of burials from sites spread over wide temporal and geographic areas so that secular and spatial trends can be discerned. Often, the data from newly analysed burials are combined with archived data from previous aDNA studies, and DNA data from modern populations are also used to aid interpretation. Work aimed at some of the other objectives discussed above characteristically uses smaller numbers, often concentrating on one specific burial ground or even one particular burial that may be of special interest.

To study the question of whether the arrival of the Beaker cultural package at the Neolithic-Bronze Age transition in parts of Europe was primarily a process of cultural diffusion or whether it involved significant migration of people, a DNA study of 400 burials from over 130 prehistoric European sites was undertaken. These data were combined with previously published information to produce a dataset from nearly 700 burials stretching over Europe and western Asia. Of the analysed burials, 120 were from Britain, where results showed a genetic discontinuity between Neolithic burials, primarily from communal tombs, and Beaker period and later burials. This appears to support the idea of significant immigration at around the Neolithic-Bronze Age transition in Britain, but the nature and scale of the migration required to produce the observed DNA results is still debated.

A DNA study to investigate kinship was undertaken at a small 7th century AD burial ground in Germany. The interments were accompanied by rich grave goods. In all cases where sex could be determined, they were male. The burial ground was thought to belong to a household of a powerful family. The DNA study showed that some were indeed close biological kin, but this was not so in all cases, and one individual had ancestry far away in southern Europe. Biological kinship was a prime determinant of membership of this household group but it was clearly not the only factor, other considerations, presumably social or political allegiances, sometimes with individuals of very different ancestry, were also important.

Sex determination in adult skeletons is usually straightforward based on osteological features. However, the relevant features may be missing or damaged and in children they are insufficiently developed to be reliable. In instances where sex cannot be identified using osteological markers, DNA studies may be used if this would address a pressing research question. Among Romano-British populations, infanticide (killing of newborn children) seems to have been commonly practiced to limit family size. Some have suggested people in Roman times preferred male children, so more female infants may have been killed. However, aDNA analysis of newborn infant bones from Roman sites in Britain does not appear to support this, boys and girls being present in similar numbers.

Study of phenotypic traits may help provide information for facial reconstruction (for example, for presenting archaeology to the public) and sometimes for helping to put a name to a skeleton. Studies of the skeleton from Leicester thought to be Richard III showed that there was a 96% probability of blue eye colour and a 77% probability of blond hair. These results were consistent with other findings that pointed to this skeleton being that of King Richard. Trends through time have also been investigated. For example, data from studies primarily concerned with population history have also shown that, in Europe, Mesolithic people probably showed a variety of skin pigmentations; DNA associated with lighter tones only increased in later prehistory.

Study of DNA from pathogens in human remains can help us to understand infectious disease in ancient times. It can help confirm diagnosis

when the skeletal lesions are ambiguous. It also opens up the possibility of studying infections that leave no trace on the bones. The bubonic plague bacillus has been detected in mass graves linked to documented plague outbreaks, confirming what some had doubted, that this bacterium was indeed responsible for the Mediaeval and post-Mediaeval outbreaks in Europe. Perhaps more surprisingly, it has also been detected in Neolithic and Bronze Age burials from Europe and Asia showing that there has been a long relationship between this pathogen and human populations.

Some pathogens, for example those that cause tuberculosis and leprosy, show phylogeography – different strains exist in different parts of the world. Studying pathogen DNA from diseased skeletons from different places and times helps us to understand how these diseases spread among human populations in the past. DNA from oral bacteria (oral microbiome) is relevant for understanding general health and also susceptibility to dental disease.

6.3 Sampling for aDNA

For ancient human DNA, the best sampling site appears to be the otic capsule. This is a section of the petrous temporal bone at the base of the skull that contains the sensory apparatus of the inner ear. The method of accessing this site that minimises damage is the so-called cranial base drilling technique. A small (3-4mm) cutting burr is used to generate 200-300mg of powder by drilling from the cranial base. Each otic capsule can effectively be only be sampled once using this approach, but the powder generated is sufficient for several analyses as 50mg is enough for this. Ear ossicles (see below) should first be removed if present in the ear canal. For rare specimens, a CT scan can be undertaken prior to sampling to plan the trajectory of the drill through the petrous bone to minimise damage to anatomically informative areas.

An alternative source for ancient human DNA is the ear ossicles. There is less published research on DNA yields than for the petrous temporal bone, but using ossicles obviates the need to cut or drill and speeds up laboratory processing, and laboratories are beginning to use them routinely. Ossicles are often preserved in sediment within the ear canals, and can be dislodged without damaging the skull. One ossicle (we have three in each ear) may suffice. Although they are very small, ear ossicles can still be used to study ear disease amongst other things, so destroying one of them still entails loss of knowledge, as would be the case with any other bone. Tooth cementum may also be useful for aDNA studies but more work is needed to confirm this. When the above tissues are not available, tooth dentine is a better source of aDNA than the rest of the skeleton.

Petrous temporal bones and ear ossicles are not good sources of pathogen DNA. For this, skeletal lesions that were active at the time of death (if there are any) appear to be good sampling sites. For diseases spread via the bloodstream, dentine from tooth roots may also be a good option. Dental calculus contains DNA from the oral microbial community, which has an important role in human health.

Cremated bone is not generally suitable for DNA studies.

7. Proteomics

7.1 The science

Proteins are made up of amino acids. Different proteins have different sequences of amino acids. This can be used as a basis for identifying protein fragments preserved in an ancient sample. Modern laboratory techniques potentially enable hundreds of different proteins to be identified in a single sample.

A protein may differ slightly in its amino acid sequence depending upon the organism which manufactured it. For example, bone collagen sequences differ between human and faunal remains. One kind of proteomic analysis, known as 'zooarchaeology by mass spectrometry', or ZooMS for short, can be used to identify whether a bone fragment is human or not in instances where this is not possible on morphological grounds.

Proteomics is a major focus in the study of dental calculus. Most of this work is directed toward the study of diet, but there are difficulties. Meat proteins are difficult to assign to species, and hence to distinguish from the human host. Distinguishing plant proteins is more straightforward, and they can provide useful information, but they are often found in low abundance, despite the fact that plant foods must have been ubiquitous in most diets. Reasons for this are unclear, but may include poor survival of these proteins. β-lactoglobulin, a protein that specifically occurs in milk, appears to survive rather better and is quite often found in calculus. This helps in the study of consumption of dairy products in the past.

A variety of other types of biomolecular analyses have also been carried out on dental calculus, and researchers often combine different types of analyses. DNA work has chiefly focused on the study of the oral microbiome, the bacteria that live in the human mouth. Metabolomics looks at the array of small molecules derived from dietary and other sources. This potentially provides insights into the nature of the oral biofilm, and into dietary components and other ingested substances. Metabolomics can also be carried out other skeletal tissues, including bone.

Proteomics offers another option for sex identification in human remains. In humans, the amino acid sequence of amelogenin proteins, which play a part in tooth enamel formation, show minor differences between males and females. Peptides (protein fragments) from these remain in trace amounts within the enamel after it has formed. These peptides are present in both the deciduous and permanent teeth. The crowns of the former begin mineralising when the child is still in the womb, so the technique can potentially identify sex from foetal life onward. Depending on results, for males the method may provide a definite sex, but for females, sex determination is not certain but always probabilistic, although in the studies published to date, sex assignments generally carry a high degree of certainty.

7.2 What can we learn from proteomics?

A late Mesolithic shell midden in Scotland contained some disarticulated human as well as animal remains. There were also some bone fragments that were considered morphologically as possibly human. It was important to confirm this identification, as human remains from this period are scarce, and it would open the way to further analyses that would shed light on lifestyles at that time. The fragments were subject to analysis by ZooMS. Fourteen out of 20 proved to be human. Once identified, they were subject to radiocarbon dating and carbon and nitrogen stable isotope analysis. This enabled important additional dietary data to be collected for this key transitional period in prehistory. Identification by proteomics can release dormant research potential within unidentified bone fragments.

β-lactoglobulin is found in animal milk (humans do not produce it), specifically in the whey fraction. Protein fragments from β-lactoglobulin in ancient dental calculus may be identifiable to species. For example, calculus from British Neolithic remains showed evidence for consumption of cow, sheep and goat milk, as did calculus from late Bronze Age burials from Mongolia. These groups not only lived in different environments but also practiced very different types of subsistence, the former being settled agriculturalists, the latter steppe herders. The results show that, despite these differences, both exploited multiple species for dairy products.

Sex assessment from peptides in tooth enamel is a new technique and is still being perfected. A potential advantage over sex assessment using DNA stems from the fact that proteins are more resistant to degradation in the soil. Both proteomic and DNA sex assessment were carried out on 55 archaeological burials from California and the results compared to conventional osteological sex assessment, a method of proven reliability in adults. The proteomic method invariably produced a sex assessment, and there was good agreement with the osteology. The DNA sometimes failed to do so or else produced results that were in conflict with the osteological and / or proteomic sex when DNA survival was poor. There was no sample age effect for the peptides: the amelogenin signal remained stable over the 2000 years spanned by the interments. However, the DNA levels decreased in the older burials. Overall, the study supports the value of proteomic sex identification when osteological indicators are not available.

7.3 Sampling for proteomics

For ZooMS, a bone fragment is drilled to yield a sample. Normally less than 20mg is needed. Because the fragment will not have been previously identified as human, it will probably not be part of a human skeletal collection but more likely kept as part of an archaeozoological assemblage. Nevertheless, the general ethical and scientific principles for justification of sampling are similar. There should be some a priori morphological or other reason for suspecting that fragments might be human, and an archaeologically meaningful reason for identifying them as such. Collagen extracted for other purposes (e.g. radiocarbon dating) can also be used for ZooMS.

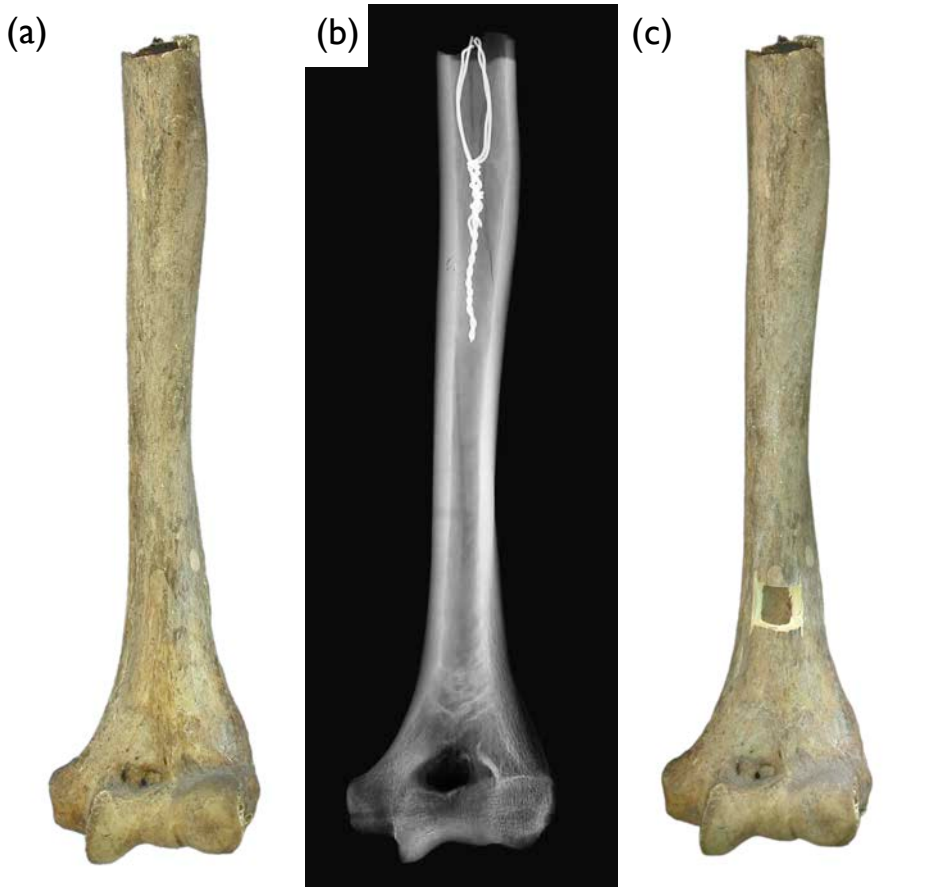


Fig 12 (a) Part of a humerus (upper arm bone) from a burial excavated in Victorian times. Attempts were made in the 19th century to repair a break by inserting a gutta percha dowel reinforced with wire (which can be seen just protruding from the top of the bone). The other limb bones in this skeleton were intact, so this one was selected for sampling for radiocarbon dating. An X-ray (b) was taken prior to sampling so that the dowel could be visualised. The wire shows clearly, and within the bone the end of the dowel, projecting just beyond the wire, is faintly visible. This enabled the 19th century repair, which forms part of the history of this specimen, to be avoided when the sample was removed (c).

For proteomics in dental calculus, only a very small sample (about 15mg) is generally needed. To put that into context, the total amount of dental calculus on the teeth of a skeleton varies from zero to 500mg or more. It tends to build up incrementally during the lifetime of an individual. This means that in general most adults show it, but deposits are less extensive and less common in children. For example, in a large Mediaeval rural population, calculus deposits were noted in 89% of adults but in only 22% of children. Often, dental calculus tends to separate from the tooth during storage of remains, so sometimes loose pieces can be taken from within the bag containing the dentition instead of breaking off a fresh sample.

In an initial application of sex assessment from peptides in tooth enamel, the procedure was to first abrade part of the enamel surface with a dental burr and then to etch it in acid to solubilise a sample of the peptides. This method was said to lead to minimal damage to the tooth crown, but the effects on biomolecular analyses and study of surface microfeatures are still unknown. The method is still being developed, and later workers have argued that a sampling approach that involves physical removal of a piece of enamel offers a more valid and sensitive basis for sex assignment, and hence is the method of choice. A sample of about 20mg of enamel is needed. This corresponds to a fragment of enamel measuring about 1 x 7mm.

8. Microscopy

8.1 The science

Under the microscope, skeletal tissues are not amorphous, but have a regular structure. Microscopic study of skeletal remains generally necessitates cutting sections of bones or teeth. Light and scanning electron microscopy are the chief modalities used for the study of archaeological human remains.

There are some microstructural differences between human and non-human skeletal tissue. Using these it may, in many cases, be possible to determine whether small bone fragments are

human or not by examining cut sections under the microscope. This may be especially useful where this distinction is not possible using proteomics, for example if the collagen content has been destroyed through burning.

Bone disease may cause alterations at the microstructural level, so microscopy may aid diagnosis. It is of more value in some diseases than others. It is of particular utility for conditions that upset bone metabolism, such as Paget's disease of bone or vitamin D deficiency. Paget's disease of bone is a disease of the elderly in which the bones become fragile, thickened and pitted. The gross appearance is not particularly distinctive, but the microstructural changes are firmly diagnostic. Vitamin D deficiency disease, primarily caused by lack of exposure of the skin to sunlight, was a significant health threat in the past, especially in the smoky cities of the Industrial Revolution. Rickets, caused by vitamin D deficiency in children, is quite easy to recognise visually in child skeletons. Osteomalacia, the equivalent condition in adults, is much harder to diagnose because the bone changes are often very subtle, but the effects of vitamin D deficiency on bone mineralisation can be observed directly under the microscope, allowing firm diagnosis.

Dental enamel is deposited in incremental layers. Irregularities in these layers often relate to episodes of disease or malnutrition during childhood, so the study of these features can tell us about childhood conditions. Some of these layers outcrop on the surface (as perikymata), so they can be studied without cutting a section, either through simple visual inspection of the enamel, or through making an impression of the surface using dental impression material and using that to make a cast for microscopic study, which permits more detailed analysis (Although conventionally viewed as non-destructive, application of dental impression material may cause damage to very fragile specimens, so care is needed.) However, some layers of enamel are hidden inside the enamel crown, so for the study of these, it is necessary to section the tooth.

Cementum coats the tooth roots and helps anchor them in their sockets, but it is normally a thin layer that is hard to see with the naked eye. Unlike enamel, layers of cementum continue to form throughout life. Cutting a section from the tooth and counting these layers under the microscope holds promise as a way of estimating age at death in adult skeletons. Although the technique, known as cementochronology, is still being perfected, it is potentially quite important, as other ageing methods work poorly, especially once a person is past middle age. Study of cementum also has some other potential applications: for example, recent work suggests that the number of pregnancies that a woman experienced may be detectable.

Microscopy is an important focus of studies of dental calculus. Entrapped particles of food such as starch granules, can tell us about diet. Other particles found in calculus are non-dietary in origin, having been introduced into the mouth when objects are grasped between the teeth, or else having been inhaled. Fungal spores or pollen grains can give clues to the natural environment, dust particles or larger fragments from manufacturing processes may give clues to environmental pollution, activity patterns or even occupation.

Soil-dwelling micro-organisms attack the collagen of bones and teeth during burial. This process, known as bioerosion, causes progressive degradation of microstructural features. When severe this may limit the information that can be gained, for example, in diagnosis of disease from microscopic study of bone or dentine, although some information can normally be gained even from severely degraded sections. The study of microstructural deterioration of bone during burial can be a research aim in itself, helping us to understand the mechanisms by which skeletal tissues degrade and the timescales over which this occurs in different burial environments. Sometimes, post-depositional changes to cementum can prevent accurate line counts, making it hard to apply cementochronology. Whether this is a problem or not varies from site to site. A pilot study to investigate this may be worthwhile prior to large-scale sampling of teeth.

8.2 What can we learn from the microscopic study of bones and teeth?

Microscopic study of bone samples from four adult skeletons from a burial ground associated with a 19th-early 20th century psychiatric hospital confirmed what had been suspected from looking at the bones, that these individuals were suffering from vitamin D deficiency. Psychiatric treatment at that time would have involved some patients spending long hours confined indoors. In the days before foods were fortified with vitamin D, this would have led to vitamin D deficiency, something that even today continues to be a risk for institutionalised populations.

A study of microscopic sections of deciduous teeth among those that died in childhood in a prehistoric population found that those showing more disruptions in enamel formation in parts of the teeth that formed before birth tended to die younger. Prenatal problems, presumably associated with maternal illnesses and malnutrition, appear to have predisposed to death in early childhood.

In dental calculus, the most common dietary particles are starch granules. Sometimes they can be identified to species, in which case they can tell us something about the plants consumed. However, quantitative inferences about diet are difficult. An individual will ingest billions of starch granules over a lifetime but only a miniscule proportion of these (normally less than 100) will be recovered from a dental calculus sample. Other particles relate to non-food items. A wooden fragment recovered from a piece of calculus from a Neanderthal seems to derive from a piece of wood inserted into the mouth as a tooth-pick. Calculus from a Middle Neolithic burial from near the site of Stonehenge provided evidence for bast fibres, with nettles being the most likely origin. Nettles provide useful fibres, so these remains may be a result of fibre processing. Birch pollen, micro-charcoal and soot were also present, giving clues as to his living environment. Traces of lapis lazuli, a pigment used for blue colouration, were found in calculus from a female burial from a Mediaeval nunnery. This suggested she may have been involved in pigment preparation and / or manuscript production.

8.3 Sampling for microscopic studies

Samples for microscopy taken from bone generally need only be a few millimetres thick. The other dimensions of the section depend upon the purpose of the study. A section can be separated by making two closely spaced parallel saw-cuts, or else a plug of bone can be removed using a suitable drill. If a full-thickness of cortical bone is required, a half section is normally taken so as not to cut completely through the bone. Bone or tooth samples need to be prepared, a process that normally involves embedding in resin and grinding and polishing the surface to be examined.

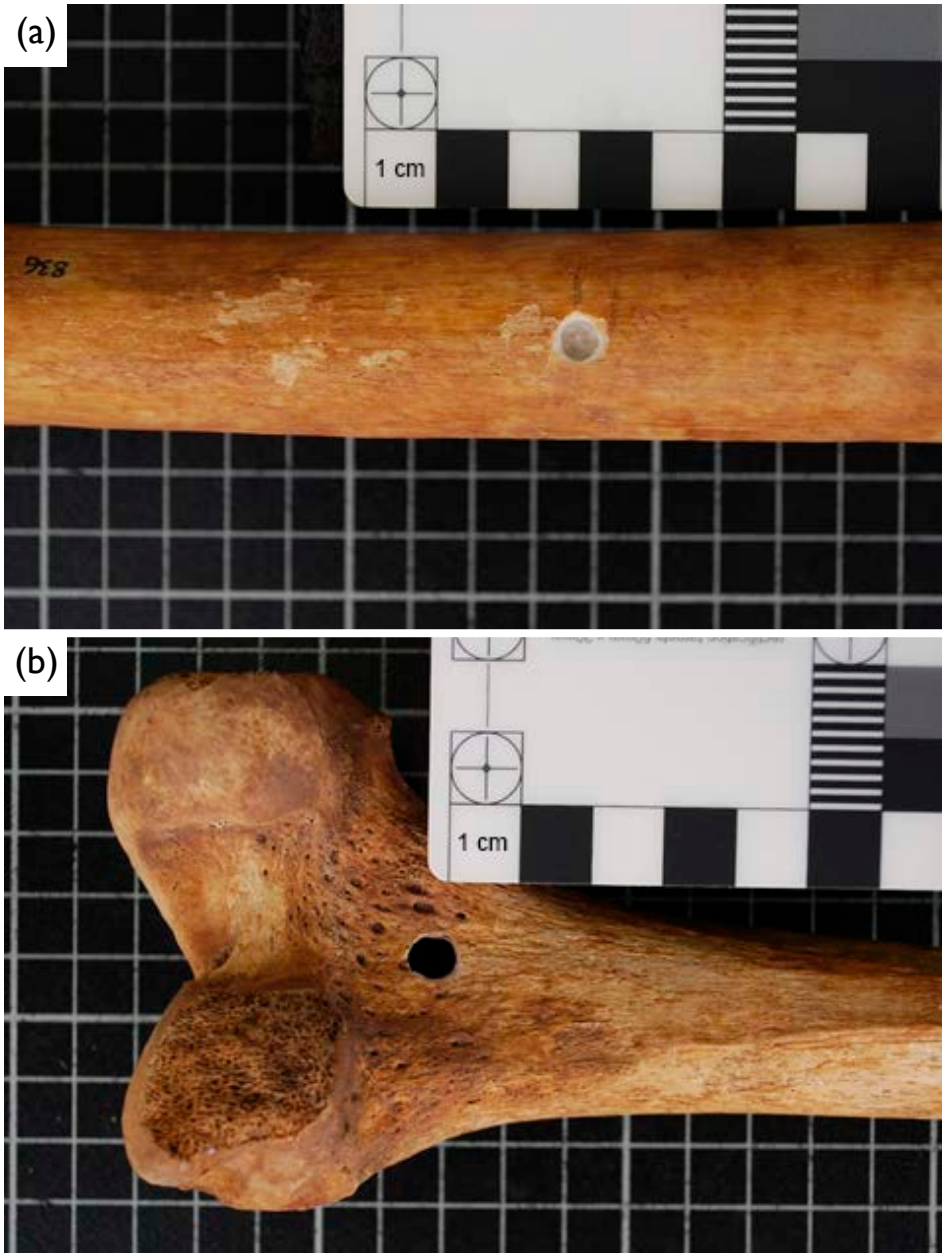


Fig 13a, 13b Final method approved for the sampling of the femurs from the Barton-upon-Humber collection for the analysis of metabolomics. The method requires sampling both the honeycomb-structured trabecular bone present inside the ends of the bone, and the dense cortical bone that makes up the bone shafts. Both areas are carefully sampled using a 6mm drill, which yields the small amounts of bone powder required.

To study disease, if distinct bone lesions are present (for example, a tumour), a section is generally cut from the lesion. Of course, this may compromise future studies of the lesion. For conditions where the metabolism of the skeleton as a whole is affected, such as rickets or osteomalacia, the sampling site is not constrained in this way. So, for example, most burials contain numerous rib fragments. These are of limited use for morphological studies, and might therefore be sampled.

A microscopic section of a tooth may be made to study growth markers and growth disruptions in enamel and dentine. The tooth is sectioned vertically. For cementochronology, on the other hand, a transverse (horizontal) section is used. The root is placed in embedding material. The area of interest is normally the middle of the root of a single rooted tooth. Enough is normally removed to make several (sometimes up to about eight) thin sections, as evidence is normally combined from different sections to produce an age estimate. In practice, most of the root may be removed and one, or sometimes two teeth per individual are sampled. The sections are normally mounted on glass slides for viewing using a light microscope.

For calculus, a sample of less than 50mg is normally taken, and then decalcified to release the inclusions. However, a larger sample, is likely to yield a larger and possibly more diverse number of microscopic particles.

9. Case studies

This section contains three case studies. The first illustrates a process by which curators of a collection considered an application by researchers to access human remains that involved destructive sampling. The second illustrates some of the issues involved in projects that seek to exhume the remains of specific, identified individuals. The third focuses on scientific studies on a single, internationally important museum collection.

9.1 The human remains held at St Peter's Church, Barton-upon-Humber

St Peter's Church Barton-upon-Humber is no longer used for worship and is under the care of the English Heritage Trust and Historic England. Part of the church is used as a Church Archive of Human Remains (see **Section 2.2**). Over 2800 burials excavated during archaeological investigations in the church and churchyard are stored here. The church is still consecrated. Placement of remains here satisfies a wish, expressed by the Church, that human remains should be returned to consecrated ground after excavation, and at the same time it allows continued access to the remains by researchers. It is an internationally important collection and much in demand for research.

A Barton Human Remains Research Committee (BHRRC) was set up to administer access to the remains at St Peter's Church. The BHRRC is comprised of representatives of Historic England (HE), the English Heritage Trust (EHT) and the local community, as well as external expertise in human remains. The aim was to assemble a committee with a mixture of backgrounds in curatorial, community engagement, archaeological and osteoarchaeological matters. It is Chaired by an osteoarchaeologist.

Of course, requesting research access to remains held by any institution always involves a formal application process, and in this respect the remains stored in St Peter's Church, Barton are no different from any other curated collection. In order to apply for access to the remains, applicants are required to complete *pro formas*. This helps to ensure that applications are treated equitably, and that suitable information is gathered to evaluate each case. For applications involving destructive sampling, the aim is to ensure that

information relevant to the main considerations regarding destructive analysis, as outlined in **Section 2.3** of this document, is gathered.

The BHRRC considers requests for access to human remains using the flowchart set out below. Where the proposed work involves destructive analyses, it considers the proposals against the criteria set out in **Section 2.3**. To illustrate this, a particular application that was considered by this committee is described below.

9.1.1 An application to access the St Peters Church human remains

An initial contact was received by the Chair of the BHRRC requesting access to the Barton-upon-Humber human remains. It came from the Tobacco, Health and History Project (THHP), which aimed to study tobacco use in the past. The project director had yet to receive funding for this project, but needed to show that she had approval to access the collections required for her work before her application for Research Council funding could proceed. In this light it was agreed that she should complete the Barton human remains access *pro formas*, and that the BHRRC would review them with a view to assessing whether it would grant her permission in principle to access the remains.

The THHP aimed to investigate tobacco use and its health impact in the past using remains from a number of different sites in Britain and continental Europe, of which Barton was one. Barton was important for the project as the burials at the site are divided into several well-dated phases. This allows burials to be confidently assigned to before and after the introduction of tobacco to Britain in the 16th century. The collection is also large enough to support adequate statistical studies of patterning in data.

The work would involve both non-destructive and destructive studies of the remains. The former included estimation of age and sex, and recording of signs of some diseases. There was already an osteological report on the skeletons published as part of the site report, and this included study of these aspects. However, the THHP could not use this existing data because, in order to comply with proper scientific practice, these aspects needed to be recorded in an up-to-date and consistent way across all the different burial sites used in the project.

The destructive analysis envisaged in the project comprised metabolomics, DNA and proteomics. These are aimed at shedding light on tobacco use and some diseases that might potentially be associated with it. These conditions include oral disease, respiratory disease (e.g. tuberculosis), vitamin D deficiency and cancer.

The main evidence for tobacco use would come from metabolomics. In this method, bone samples would be analysed to look for traces of products from the metabolic breakdown of nicotine and other molecules in tobacco that might be preserved in someone who smoked or otherwise used tobacco. Metabolomics in ancient skeletons was a very new field at the time of this application (2019), but the applicant indicated that she had already evaluated her approach in a pilot study and that this demonstrated that metabolomes in bone are potentially effective indicators of tobacco consumption.

Modern day smokers have a different oral microbiome (bacterial colony in the mouth) and this is part of the reason they are more prone to certain oral diseases. The project proposed using analysis of proteins in dental calculus to investigate whether differences in the oral microbiome between smokers and non-smokers could be identified.

Oral disease, rickets, cancer and tuberculosis would be identified through visual inspection of the skeletons. The project aimed to confirm tuberculosis in any cases with suggestive skeletal lesions using DNA analysis.

A programme of public engagement on smoking and health, and what the past has to teach us about this, was integral to the project. The project director undertook to place all data from the project in the public domain once the project had finished.

It was proposed that the following samples would be taken for destructive analyses:

- Metabolomics: 0.5g of bone, comprising a fragment of a rib, from *circa* 200 skeletons.
- Approximately 3mg of dental calculus from 60 skeletons.
- A sample for tuberculosis DNA, from maybe 10-30 skeletons, taken from a bone lesion.

Following receipt of details of the access request from the THHP, a summary of the project was prepared by the BHRRC Chair. The purpose of this was to facilitate discussion of salient points of the project among members of the BHRRC whose experience and expertise lies in different areas. The completed application *pro formas*, the Chair's summary of the project, plus the Chair's provisional overall assessment were circulated to the BHRRC for their comments and for general discussion.

The Chairs' initial assessment was positive. The BHRRC agreed in discussion that the THHP stood a realistic chance of shedding interesting new light on the past community at Barton, and that overall the THHP was a useful research project. It was agreed that the nature of the Barton remains, in comparison with other cemetery sites, rendered it particularly suited to the project aims. The THHP would also potentially present a valuable opportunity for community engagement work at Barton.

Even though conventionally described as 'non-destructive', studies involving visual examination only inevitably result in some wear and tear on a collection through handling, however careful the investigators. Curators of collections still need to be cautious, and to be convinced of the value of the work, before granting permissions, but the committee felt that the non-destructive recording requested as part of the THHP was justified and, given their overall positive view of the project, would be something that they could support.

It was noted that for the biomolecular works, the destructive aspect was significant, but that it was an integral and essential part of the THHP, and it was noted that the required information could not be obtained non-destructively. Weighing the potential benefits against the destruction of bone and dental calculus, the BHRRC approved the destructive elements of the work, up to the weights of samples and total numbers described in the application. However, they imposed the following conditions upon the destructive sampling programme:

- The BHRRC should be provided with full details of previous and any ongoing pilot studies on the metabolomics method in advance of sampling, so that they can satisfy themselves as to its viability, before any sampling takes place.

- Calculus samples should only be taken from a skeleton where that would not involve removing / using all the deposits that are present on the teeth.

- BHRRC to liaise with the THHP concerning sampling individual tuberculosis cases for DNA analysis in order that that the BHRRC can satisfy itself that the potential for future work on the lesions would not be unduly degraded.

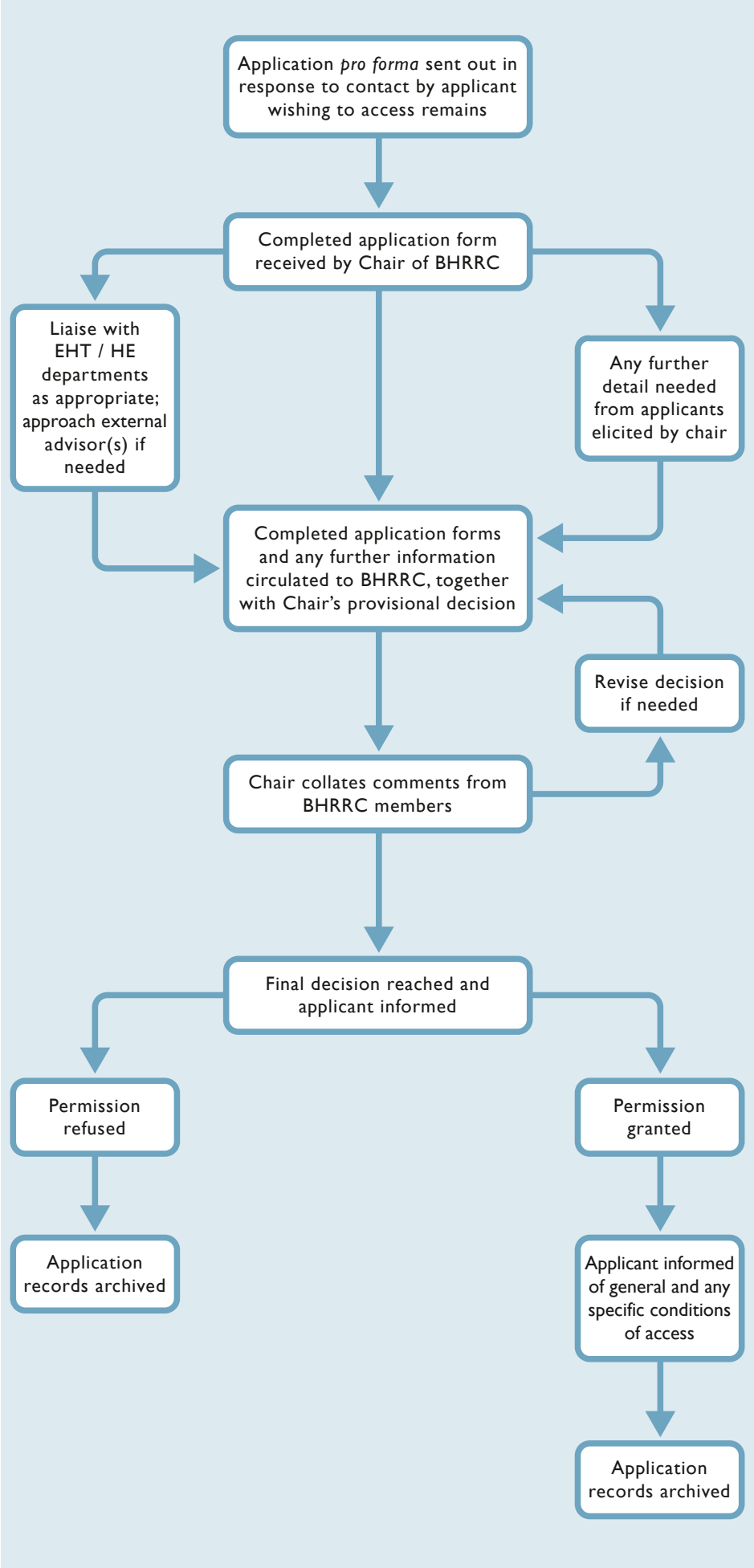
- The BHRRC would wish to liaise with the THHP on any public engagement activities connected with the project that might be organised at Barton in order to ensure that the benefits of these for the Barton community be maximised.

- That the BHRRC standard conditions of access are adhered to (these include matters to do with handling remains with respect, complying with reasonable requests from curatorial and other staff when on-site, acknowledging BHRRC in publications etc.)

- That suitable logistical arrangements for access were agreed with the BHRRC.

The THHP agreed to these conditions. In due course the THHP received research council funding and the project went ahead.

Soon after the project started, further pilot work on other remains revealed that rib fragments were problematic for the metabolomic studies involved in the THHP; limb bone pieces were needed instead. The THHP liaised with the Chair of the BHRRC to develop a new sampling strategy that would enable sampling of limb bones instead of ribs at Barton, whilst still minimising the impact on the remains. The sampling strategy developed involved using samples from the femur. Because this represented a variation on the sampling that had been approved, this was presented to the BHRRC for their consideration. After due deliberation, this was approved by the BHRRC. At time of writing the THHP is on-going (**https://www.tobaccohealthhistory.co.uk/**)



Flowchart summarising procedures for consideration of requests for access to the human remains stored in St Peter's Church, Barton-upon-Humber

9.2 Exhumation of remains thought to be of particular historical individuals

Cases involving the exhumation of single burials usually involve attempting to identify the remains of prominent individuals. The best-known project of this type is probably the excavation in 2012 in Leicester that recovered the skeleton subsequently identified as that of King Richard III. The resultant publicity led to a spike in requests to clergy and others responsible for historic burial grounds for projects aimed at exhuming and identifying and studying the remains of other historical figures. Over the years, projects have been proposed to exhume remains purported to be of Shakespeare, King Alfred the Great, King Harold II, the Mediaeval Duke of Clarence, and the early Saxon Christian St Eanswythe (this last was successfully carried out following advice from APABE, see below), among others.

In addition to the factors outlined earlier in this document, some other caveats need to be considered in projects that involve exhumation of burials from historic burial grounds. Prominent personages are often interred in elaborate tombs and these are often in a fragile state. Dismantling them to access the human remains may involve significant risk of damage. Reburial of human remains, and organic or other artifacts that accompany them, after examination, generally leads to their renewed deterioration.

The quality of information regarding archaeological context, and the nature of the supporting historical evidence concerning the targeted burial are crucial to the success of projects. These need to provide firm support that the remains of the specific, sought-after person lie within a particular tomb, or at a precise, marked location. If this is not the case, then it is unlikely that scientific analyses can establish identity. As a generality, burials beneath churches and in churchyards are very densely packed and intercut, with later burials disturbing and displacing earlier ones. Depositum plates bearing the identity of the deceased are rarely part of coffin furniture, even for the rich, prior to the 18th century. Historical evidence that a person was buried beneath a certain church, or a certain part of a churchyard, is normally insufficient to enable remains for analysis to be correctly targeted. For example, a project was conceived to test the hypothesis that a particular burial in a stone coffin at Bosham Church was that of King Harold II, killed at Hastings in 1066. There was some historical evidence that suggested Bosham as a burial site but it was weak – other locations are more likely. Even leaving this aside, there seemed no reason to think that this particular individual rather than any other buried under the church might be King Harold II, making the whole project rather speculative. This, and other concerns over the application, led to Faculty permission for the work being refused.

Even projects involving the exhumation and study of a single skeleton are a major undertaking and will necessitate the assembly of a multidisciplinary professional project team. Project costs may run into tens of thousands of pounds, and applicants should demonstrate that they have the funding in place or have proper plans to secure it once permission is granted.

As with any scientific work, the project needs to have clear aims. For example, human remains held in elaborately decorated mortuary chests at Winchester Cathedral are identified epigraphically as particular Anglo-Norman Bishops and Royalty, but despoilation during the Civil War now makes these identifications doubtful. A major scientific project was conceived to test whether the original remains are still inside. This was supported by the cathedral authorities as it had a clear hypothesis to test and, whatever the results, they would be important to the understanding and public appreciation of the history of the cathedral.

Although popular dissemination of work is often important (see below), there should be a commitment to publication of results in the scientific literature, and this is needed whatever the results. For example, the various analyses carried out on the skeleton identified as Richard III have been published in detail in scientific journals, enabling experts in the field to evaluate the validity of the identification and other interpretations.

Whilst, for ancient burials, personal identifications are unlikely to be unequivocal, they are most likely to be convincing when they are based on multiple lines of evidence. For example, contrary to popular belief, ancient DNA analyses are generally not conclusive on their own. In most cases, relevant scientific evidence is combined with the archaeological context and historical information relating to the place and manner of burial and (if available) to physical attributes of the person. In the case of the skeleton identified as Richard III, no one line of evidence was conclusive on its own in establishing identity. Nevertheless, combining the archaeological context in which the burial was found, historical information about Richard and his death and burial, osteological observations on the skeleton, and radiocarbon dating, DNA and other biomolecular analyses, enabled a compelling case to be assembled.

In most projects, a staged approach to post-excavation analysis is taken. If basic osteological study (e.g. age and sex determination) is compatible with the putative identification, then a first round of destructive sampling may be merited. Radiocarbon dating is often the first resort. Only if the date produced is compatible with the known date of death of the sought individual are further analyses (e.g. DNA, isotopes) generally worthwhile.

The public fascination for ancient human remains is magnified when they appear to be of known historical figures. There is thus likely to be considerable wider interest in projects, but care needs to be taken to ensure that this is handled sensitively and ethically. Some projects have been funded from the outset by television production companies. Whilst this is not necessarily problematic, care needs to be taken in such instances. It may be difficult to retain control over how the work is presented and, potentially, to avoid sensationalising or trivialising of results.

Properly handled, projects of this nature can provide important opportunities for community engagement. In Folkestone, the study of remains that had been concealed in a church wall at the Reformation, supported the idea that they were likely to be of 7th century St Eanswyth, one of the earliest female saints and an important figure in the adoption of Christianity in England. The study of the remains was used not only to support the identification but to explore aspects of her life. Engagement with the local community was integral to the project. The work was associated with the development of a much broader public appreciation of the history of Folkestone, and what has made it significant and distinctive as a community.

9.3 Multidisciplinary study of a museum collection

The 18th – 19th century AD human osteological assemblage from Christ Church Spitalfields (CCS), London has been held at the Natural History Museum in London under a Faculty and with permission from the Friends of Christ Church Spitalfields since 1986. The collection is managed according to the same policies and procedures pertaining to other human remains held by the Natural History Museum, including those relating to destructive analysis. The CCS assemblage includes unidentified skeletons and skeletons of adults and juveniles who were identified from the inscriptions on their coffin plates, known as the coffin plate series. The entire assemblage has been used to investigate human health, environment, diet and life history in 18th and 19th century London. The coffin plate series has been used to test and develop a range of analytical techniques in forensic and clinical research as well as osteoarchaeology and to undertake other research that relies on the documented biographical details.

This section reports on some of the research projects involving destructive sampling that have been conducted on bone and dental tissues from CCS. Proposals for destructive sampling were evaluated by collection managers, who considered the significance of the research question, the suitability of the collection, the likelihood of success of the proposed methodology and the impact of sampling. Several proposals that would have involved extensive sampling, sectioning of bones, or replication of previous work were refused. The following studies are examples of the research that was permitted.

Nitrogen stable isotope values in bone samples from infants and children from CCS were used to explore nursing behaviour in 18th and 19th century London. The study demonstrated for the first time that elevation in nitrogen stable isotope ratios associated with breastfeeding could be detected in the infant ribs by about 40 days after birth, reflecting rapid bone turnover in new-born infants. This result could not have been obtained if age at death had not been documented for the infant skeletons used in the study. Samples were taken from anatomically uninformative rib fragments, either by selecting small existing fragments or in a few cases by removing a small piece of bone from the broken end of a broken rib. No whole ribs were included in the study. Any study involving the removal and subsequent return of multiple similar looking bones or bone fragments from a collection carries a risk of mixing, so appropriate procedures were followed to minimise this risk. The rib samples were placed in individually numbered sealed bags. A note was placed in the skeleton box stating that a sample had been removed. The bone fragments were cleaned and sampled. Labels with the correct number accompanied the bone fragments and sample at each stage of analysis. A pilot study using a small number of ribs was undertaken before proceeding with the full analysis. Following completion of the study, remaining bone fragments were returned to the collection. The numbers on the sample bag and skeleton box were matched and the size and condition of rib fragments were considered before returning the fragment to the box. Complete analytical results were presented in peer reviewed journals and copies of the publications were added to the collection archive.

Cortical bone has a dynamic microstructure that is renewed throughout adult life by the gradual replacement of bone (bone turnover). This remodelling alters the microstructural organisation of the bone and produces secondary osteons. Individuals from CCS were included in a study that investigated biomechanical and other influences on bone microstructure. Polished thin-sections were prepared from bone blocks cut from the femoral midshafts and cross-sections from ribs. Secondary osteons were measured on digital images captured with a camera mounted on a microscope. Within the sample studied, the patterning of osteon dimensions was not clearly linked to age, sex, anatomical regions or inferred levels of physical activity. A subsequent study by a different team used the same bone blocks to investigate the presence of adult vitamin D deficiency osteomalacia. Analysis of the long bone sections using scanning electron microscopy confirmed the presence of microscopic features, consistent with osteomalacia, confirming the original diagnosis based on visual observations of the skeleton. The sampling of the femoral midsections generated valuable research results, but some other types of research involving the shape and bone microstructure of the femoral midsection that could not have been envisaged at the time of the original sampling have been prevented.

Growth markers occur in all dental tissues and capture a detailed record of the growth of those tissues. They are often studied using histological techniques. Teeth from five children from CCS who had a precisely documented age at death were studied to determine whether cross striations in enamel represent daily growth increments. The study demonstrated that the number of cross striations forming after birth corresponded to the number of days between birth and death in each child, confirming that these markers reflect a daily rhythm of enamel matrix secretion. For this study, histological sections of each tooth were prepared in a longitudinal plane from the cusp tip to the root apex. A small amount of enamel and dentine is lost during this process. The tooth blocks from each side of the section were retained. Teeth were only sampled if the antimere was present. Digital photographs of the sections were taken using a camera mounted on the microscope. The digital images, tooth sections and blocks could be used for further research.

Replicas (casts) have been made for both research purposes and to mitigate the loss of information caused by sampling prior to destructive analysis. High quality replicas can be used for research on tooth and bone surfaces that is not possible using CT scans. Some studies are preferentially conducted on high resolution replicas rather than directly on bones and teeth. Casting involves coating high precision dental impression onto the tooth or bone surface using a syringe, and gently prising this away from the surface once set. Since ths process involves a small risk of damage, requests to make casts were treated in the same way as applications for destructive testing. The condition of each tooth or bone was assessed and teeth or bones that were fragile, or those with a cracked or otherwise damaged surface were excluded. For teeth that were still in the jaw, protective measures were used to prevent the impression material spreading into the gap between the tooth and the jawbone.

In one such study, high resolution replicas of multiple tooth crowns from young adult dentitions from CSS were used to investigate the number and age distribution of enamel defects in the permanent teeth. The replicas could be studied at low pressure using scanning electron microscopy, which might have damaged the teeth themselves. Enamel defects were matched across the dentition by reference to tooth formation

schedules and by counting the number of perikymata (surface growth markers) between defects on simultaneously forming teeth. All of the dentitions exhibited at least one enamel defect that could be matched across simultaneouly forming teeth.

10. Further Reading

Professional Guidelines

BABAO, 2017-2019. *Guidelines and Standards*.
https://www.babao.org.uk/publications/ethics-and-standards/

Collections Trust (2017). Spectrum 5.0.
https://collectionstrust.org.uk/spectrum/

Mays S (ed) 2017. *Guidance for Best Practice for Treatment of Human Remains Excavated From Christian Burial Grounds in England*, 2nd edition. English Heritage / Church of England
http://www.archaeologyuk.org/apabe/pdf/APABE_ToHREfCBG_FINAL_WEB.pdf

Mays, S., Brickley, M., Dodwell, N. and Sidell, J. 2018. *The Role of the Human Osteologist in an Archaeological Fieldwork Project*. Historic England, Swindon:
https://historicengland.org.uk/images-books/publications/role-of-human-osteologist-in-archaeological-fieldwork-project/

Swain H (ed) 2005. *Guidance for the Care of Human Remains in Museums*. DCMS.
http://webarchive.nationalarchives.gov.uk/+/http://www.culture.gov.uk/images/publications/GuidanceHumanRemains11Oct.pdf

Scientific techniques

Mays S. 2021. *The Archaeology of Human Bones*, 3rd edition. Routledge, London.

Roberts, C. 2018. *Human Remains in Archaeology: A Handbook*, 2nd edition. Practical Handbooks for Archaeology, No. 19. Council for British Archaeology, York.

Case studies

Mays, S. 2012. Curation of Human Remains at St Peters Church Barton-upon-Humber, England. In: Giesen, M. (ed) *Curating Human Remains. Caring for the Dead in the United Kingdom*. Boydell, Woodbridge. pp. 109-121

Finding Eanswyth Project (2017). *Finding Eanswyth: The life and Afterlife of an Anglo-Saxon Saint*.
https://findingeanswythe.uk/about/

Molleson T, Cox M, Waldron AH, Whittaker DK. 1993. *The Spitalfields Project. Volume 2 – The Anthropology. The Middling Sort*. CBA Research Report 86. Council for British Archaeology, York.

11. Where to get advice

The Advisory Panel on the Archaeology of Burials in England (APABE) gives free casework advice to professionals involved in archaeological projects in England dealing with human remains. Its members cover a wide range of expertise, and its remit encompasses advice on ethical and legal matters as well as scientific advice. APABE can be contacted via its website: https://www.archaeologyuk.org/apabe/

12. Acknowledgements

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Advisory Panel on the Archaeology of Burials in England

APABE is a panel supported by Historic England, the Ministry of Justice and the Church of England. Its purpose is to provide a unified source of advice to professionals on the treatment of human burials from archaeological sites in England. APABE supports professionals and others in interpreting guidance documents on human remains that have been issued by the Department for Culture, Media and Sport (DCMS), by Historic England, and the Church of England. It also produces new guideline documents as necessary and provides casework advice on any aspect of archaeological burials. The objective is to foster a consistent approach to ethical, legal, scientific and other issues surrounding the treatment of burials from archaeological sites.
APABE will either give advice itself or refer the enquirer to the relevant expert organisations or individuals. The advice is free of charge. Enquirers are referred to https://apabe.archaeologyuk.org for further details.

Front cover:
Main picture and upper inset: Sampling of dental calculus for the analysis of the oral microbiome.
Lower inset: tooth being examined prior to sampling

Text compiled by Simon Mays, Joseph Elders, Louise Humphrey and Peter Marshall.

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