Incremental Banding in Dental Cementum: Methods of Preparation for Teeth from Archaeological Sites and for Modern Comparative Specimens

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ABSTRACT Analysis of incremental banding in dental cementum is a well-established means of determining the age and season of death of wild mammals. The dental cementum of domesticated mammals likewise can indicate age and season of death. Methods of preparation applicable to archaeological teeth differ from those used for modern specimens, however, and this paper describes two methods that have given excellent imaging on teeth of Bos taurus; one for modern teeth and the other for teeth from archaeological sites.

KEY WORDS: Cementum, increments, embedding, cattle, age, seasonally.

Preparation of teeth of Bos taurus for cementum analysis: I: modern specimens

Introduction

Cementum analysis is a well-established means of determining age and season of death of wild mammals. The dental cementum of domestic animals likewise can indicate age and season of death. A pioneering study that demonstrated this was conducted by Saxon and Higham on sheep, Ovis aries. ²³ Coy et al.⁴ observed increments in cementum of 605 taurus of both recent and archaeological origin. Age estimates for the oldest animals in their modern sample were approximately 7 years. Much of their sample was of unknown origin, however, and ages could only be estimated; the health of many specimens also was regarded as questionable. Before any confidence can be had of interpretations of teeth from archaeological contexts, it is essential to obtain baseline ages for the species under investigation by analysing incremental structures in teeth from animals of known age.

This paper is the result of research designed to provide the comparative baseline against which archaeological specimens of B. taurus can be aged more accurately. While the full results of this research will be presented in a future paper, it can be said here that our investigations of prehistoric cattle mandibles do not support the common belief that such animals would show retarded dental development when compared with modern cattle.

Teeth from the mandibles of cattle of various breeds, raised under a wide range of systems of husbandry were studied. Dental development and attrition was recorded by visual, radio-graphic and metrical examination. Younger animals can be aged from dental development.

Teeth for sectioning were, therefore, selected from older specimens in which all three mandibular molars were in full wear. The oldest animals were aged 15 - 16 years. Comparison of incremental banding in the dental cementum from many individuals, using several teeth from...
the same jaw of each, has demonstrated that, so long as due allowance is made for the timing of its development, any tooth can be used for estimation of age and season of death of *B. taurus*. For instance, approximately 2 years must be added to the count of annual layers of cementum of the first permanent incisor to obtain an accurate estimation of age. Since the first permanent mandibular molar (*M*<sub>1</sub>) is the first of the permanent teeth to develop, however, the ageing data recorded in its cementum need no adjustment.

Moreover, for archaeological reasons it is essential to base such studies upon the molar teeth, as the cheek tooth row is commonly recovered intact but with the incisor teeth seldom in place. *M*<sub>1</sub> is a tooth abundantly represented in our archaeological faunal assemblages, both in and out of mandibles. When found as isolated teeth, *M*<sub>1</sub> and *M*<sub>2</sub> may appear difficult to distinguish from one another, but a metrical parameter, cervical length (Beasley, Brown and Legge, in preparation) permits distinction between these two teeth in the majority of cases.

**Preparation of Tooth Sections**

The technique was perfected and refined using recently killed and modern dry and fresh material from a variety of sources. The tooth is padded with foam plastic for protection and fixed in the vice of a controlled feed and drive cutting machine. The feed moves the tooth towards a rapidly rotating, fine cutting wheel edged with diamond grit, which is water-cooled. Two cuts are oriented to remove the mesial root together with the molar pad while leaving the bulk of the crown and the distal root intact (Figure 1).

This provides a useful compromise between the need to observe as much as possible of the perimeter of the tooth and the necessity of having sections that are small enough to fit on to a standard microscope slide; it has the additional advantage of enabling the unused portion of the tooth to be replaced in the mandible of specimens from museum and teaching collections, so that their appearance is little altered.

Sections prepared using the standard histological techniques of total demineralization in a dilute acid or EDTA (Ethylenediaminetetra-acetic acid disodium salt), followed by wax embedding, cutting on a sledge microtome, staining, dehydrating and mounting in DPX xylene soluble mountant, were found to be only moderately successful. By contrast the method of section preparation described below is simple and quick and has proved wholly satisfactory for defining incremental banding in the cementum of mammalian teeth. Clear results have been obtained on teeth that have been dried and stored in museum and teaching collections as well as from fresh specimens.

The block of tooth (mesial root + molar pad) is demineralized in 5 per cent nitric acid and 2 per cent formalin. As stated by Klevezal the strength of commercial concentrated acid can vary, but slight variation in the strength of dilute acid made by adding a standard measure of concentrated HNO<sub>3</sub> to a standard quantity of water is insignificant in practice. During the demineralization process the solution should be changed daily. When the specimen has become sufficiently soft, usually by the third or fourth day for a molar and by the second or third day for an incisor, it is bisected longitudinally using a large scalpel or razor blade. This facilitates demineralization of the interior and gives two flat-cut surfaces from which to begin sectioning on the
freeze microtome. As a rule, specimens need to remain in the solution for 2 days more to complete the process. Arnim's ammonium oxalate test, can be used to monitor the demineralization process. In practice it has proved advantageous to arrest the process slightly before demineralization has been completed. This is because cementum on thin-sections of totally demineralized specimens tends to separate into ribbons, making slide preparation difficult. Removing teeth from the acid slightly before the point of complete demineralization is attained eliminates this problem. Daily checking ensures that the critical point is not missed. The inclusion of formalin in the demineralizing solution, however, has been found to make exact timing of the process less critical. Teeth processed in this solution are a little firmer than those treated in straight 5 per cent HN03 but this is no disadvantage and they do not become too hard.

The decalcified tooth is rinsed thoroughly in running water or by using many changes of water over a whole day. It is then put in distilled water and can be left overnight prior to sectioning on a freeze microtome. We have established that if it is not convenient to section a tooth immediately, it can be stored in a screw-top jar of distilled water in a refrigerator for several weeks after this rinsing. Owing to unforeseen circumstances some decalcified specimens had to be refrigerated for up to 6 weeks before they could be sectioned. The distilled water was changed every few days and no deterioration was observed. It is not necessary to add alcohol to the water. Indeed an important advantage of this method is the avoidance of distortion and shrinking of the specimen by dehydrating agents.

Sections ranging in thickness from 15 to 25 u.m are cut on a freeze microtome. An electric model in which circulating water serves as a coolant was found to be easier to use and more efficient than the type that has to be filled with frozen CO2. A very sharp microtome knife is essential. Tissuette supporting gel helps to support the specimen in place as it freezes. It is necessary to align the cut surface of the tooth with the blade so that sections are cut in the longitudinal plane as accurately as possible from the centre of the tooth. Longitudinal sections afford greater interpretive potential than transverse sections, for both incisors and molars. A wet, fine watercolour paintbrush is used to take each section from the blade to distilled water.

Sections are stained in Ehrlich's haematoxylin (Ehrlich 1885, as reported in Brain) until a good colour is obtained, usually 20-30 min, but a check after 10 min is advisable. The sections are passed through several changes of tap water, or running water, until blue, then transferred to a petri dish of distilled water. A curved needle, 40 cm long, mounted in a 100 cm wooden handle, is used to lift the sections from one vessel to the next. The translucent winter bands stain strongly and the opaque summer bands take up the stain more weekly. The petri dish containing the stained sections is viewed under low-power microscopy and the best sections are chosen for mounting.

A curved needle, as described above, is used to move the sections individually through a series of petri dishes containing increasing concentrations of glycerol (25 per cent, 50 per cent, 75
Sections remain for about 5 min in each dish and are then removed one at a time for mounting. After excess glycerol has been allowed to drain off, the section is placed on a glass slide and sealed with a cover slip. It is essential for the section to be completely flat. This is ensured if the section is positioned on the slide under low-power transmitted light microscopy. Some specimens are aligned easily; others require some careful manipulation with a fine brush and a curved needle. The Kyowa stereo microscope on its transmitted light setting at lowest magnification proved to be invaluable for this purpose.

For a reasonably durable finish the junction of the coverslip and slide is sealed by painting round the entire perimeter with Canada Balsam or DPX. For a really permanent seal the sandwich method of Romeis, as described by Klevezal, is used. The section is mounted in a drop of glycerol on a large coverslip (35 times 50 mm) and sealed with a small coverslip (22 times 40 mm). Several drops of DPX are put on to the surface of a glass slide and the pair of coverslips is turned down on to it in such a way that the smaller coverslip is sealed between the slide and the large coverslip.

**Summary**

1. Cut off mesial root and molar pad of tooth.
2. Decalcify in 5 per cent HNO₃ and 2 percent formalin.
3. Rinse
5. Stain with Ehrlich’s haematoxylin.
6. Pass from distilled H₂O through increasing concentrations of glycerol.
7. Mount in 100 per cent glycerol and seal.

**Cementum analysis**

Thin-sections prepared by the method described above are examined by transmitted light at low magnifications. The lowest magnification obtainable using the zoom lens on the Kyowa binocular microscope enables the whole specimen to be seen at once for initial orientation. Magnification is then increased until optimal visibility of the incremental bands is obtained; 25 - 30 times being generally good.

The molar pad, which contains cementum representing the earliest stage of root development, is examined first. In many specimens clear increments are visible in this region of the tooth. Nevertheless, increments in the dental cementum of *B. taurus* can vary considerably in thickness and clarity in different parts of the same root and on some specimens are irregular and undulating in all regions of the root, including the molar pad. In order to distinguish between supplementary lines and seasonal bands and to identify and interpret correctly seasonal bands that are partly divided, it is essential to trace bands along the full length of the root. For this purpose the lower magnifications have proved necessary. Higher magnifications are less suitable for counting annual layers in the cementum of *B. taurus* because the breadth of field is narrower than the full width of cementum in the molar pad and only a fraction of the total is visible at any one time.

Another drawback to high magnification is that accessory lines and minor laminations are revealed to such an extent that a general reduction in clarity of the major increments is experienced, the distinction between seasonal bands and supplementary lines is unclear and
Figure 2. Transmitted light photomicrograph of stained thin section of M. from a Chillingham cow aged 10 years. Month of death = January. Ten winter bands are visible in the molar pad of this tooth. This area between mid-root and apex apparently exhibits 7, whereas the developmental data for M. predicts 9 or 10 annual layers. However, bands 1 and 5 are probably coalescence of two bands each. Note how the layers undulate and are of variable width. (E) Edge of tooth root; (J) dentine-cementum junction: (1-7) winter bands; (1-9) interpretation from tracing bands around entire perimeter of root visible on the microscope slide.

Identification of the major seasonal bands becomes difficult. Because of the large area of the tooth sections from Bos, slides of 20 |.im thickness proved to be the easiest to handle. Although marginally too thick for very high magnification, sections of this thickness are entirely satisfactory when lower magnifications are used.

Results

The method described here has given good results on teeth from 47 cattle, including both cows and bulls of various breeds, raised under intensive and extensive systems and under near-wild conditions. Twenty-five animals, listed below, were of known age:

(i) twelve wild cattle from Chillingham, Northumberland, England (specimens by courtesy of Dr J. Clutton-Brock, the Hon. lan Bennett and the Royal Veterinary College, London);

(ii) two wild cattle from the Chillingham Reserve Herd in northern Scotland (by courtesy of the Hon. lan Bennett and A. Marshall Shepherd Esq.);

(iii) four bulls of the Spanish fighting breed (collection A.J. Legge);

(iv) one Devon Poll (Royal Veterinary College, London);

(v) one Highland Cross (Ancient Monuments Laboratory, London);

(vi) one Ayrshire, one Hereford, one Charolais, two Friesians (various slaughterhouses). In eight cases (four Chillingham cows and four Spanish bulls) the analysis took the form of a blind test. The age of these animals was documented, but was not revealed until after the cementum analysis had been completed. The arrangement of cementum bands in the teeth of B. taurus is complex (Figure 2), particularly in animals in excess of 10 years of age, but it nevertheless shows good correspondence with the age and season of death of the individual (Figure 3).
Research is in progress on incremental banding in the dental cementum of modern domestic cattle in Sweden. The first results obtained from this research likewise indicate that cementum banding exhibits good correspondence with age and season of death."

Conclusion

Cementum analysis is of value both in its own right and as a means of evaluating and calibrating systems of ageing based upon attritional criteria. The technique described here offers a quick, straightforward and satisfactory way of examining increments in cementum of any large teeth from modern sources. Results on the modern sample described above have been integrated with the results of radiographic investigations and crown height dimensions from both modern and archaeological cattle teeth in order to establish a sequence of dental changes that is related to age from 4 to 14 years and over (Beasley, Brown and Legge, in 'preparation) (Figure 4).
II: archaeological specimens

Introduction

Samples of cattle teeth were available from archaeological sites with a variety of soil conditions and varying conditions of preservation. These were as follows:

(i) A very large sample of archaeological cattle mandibles was available from two Bronze Age middens at Grimes Graves. Bone preservation is very good in the middens and, as was anticipated, the cementum, which is similar in composition to bone, also was well preserved.

(ii) The Neolithic causewayed camp group of Hambledon Hill/Stepleton has provided additional material for investigation. These sites have rather severe conditions for bone preservation; although the sediments are alkaline, because of the dry chalkland soils virtually all organic material has gone from the bones. Since it is the intermesh of collagen fibres that holds the mineralized component together it was anticipated that cementum analysis might prove difficult.

(iii) A fen-edge site at Haddenham near Ely. Here bones are preserved in waterlogged sediments, and preserved conditions are excellent. Cementum banding in this sample is, as expected, extremely well exhibited.

In conventional histological investigations it is the collagen fibres in the cementum that are stained. Although this approach has been attempted for archaeological material, the results are unsatisfactory. The loss of organic components, principally collagen, renders specimens too frail for normal histological preparation. Although varying amounts of collagen may survive in archaeological specimens, the structure of the organic component of the cementum has generally been destroyed by decay and this is therefore not available for staining. Mineral tissue that remains in an organized state is brittle and must be supported in a suitable embedding medium prior to sectioning. Epoxy resins have become an established embedding medium for archaeological teeth. The methodology described below uses relatively simple technology to obtain sections of high quality and is minimally destructive of archaeological specimens. In addition to having been used on archaeological cattle teeth, the method has been used successfully on teeth of horse from Solutre and on teeth of horse and red deer from Cough's Cave.

Resins

In this study various resins were tested. Initially Metset, a relatively inexpensive glass-clear resin with a low exothermic temperature, was used and satisfactory results were obtained. Metset is fairly viscous and when mixed using the proportions of resin and catalyst recommended by the manufacturers starts to thicken before evacuation of air has been completed. Reducing the amount of catalyst mixed with the resin delays setting and keeps exotherm temperature low. Storage in a refrigerator is necessary to avoid wastage.

Beuler's Epoxide is available in two-part sachets, easy to store and convenient to mix by removal of a pin. No control over the quantity of catalyst used is possible, however, making setting time shorter than the time needed for vacuum impregnation. More control can be exercised and results are satisfactory when Epoxide resin and catalyst are measured from separate containers, but Epoxide is less transparent than Metset.

Streuer's Epofix is an epoxy resin that was found to combine a number of advantages in use. It is glass clear and at 30°C is of very low viscosity. The resin has a good shelf-life and is packaged in a way that allows precisely measured quantities to be extracted in separate syringes of both resin and catalyst. Epofluor, a fluorescent green dye was added to the resin and penetration checked using a fluorescing microscope. The archaeological cattle teeth emit a little natural fluorescence but as far as could be ascertained it appeared that penetration was good using a double-embedding method with resin at 30°C. Epofix resin is less rigid when set than Metset and this slight flexibility was advantageous when thin-sections were being cut.
Thin-sections cut from blocks of any of these resins can be mounted with DPX or Canada Balsam and coverslip after air drying overnight.

LR White resin, a product with potential for good penetration of the specimen without a vacuum, but which requires curing at 50°C, was also tested. It is so fluid, however, that it escapes through the joints of two-part moulds and through the minutest perforation in a disposable mould. It reacts with DPX so cannot be mounted using this medium.

On balance Epofix was easiest to use and was found to be the resin of first choice for this work.

Technique of resin-embedding and tooth sectioning

In the following paragraphs all times are approximate and vary in practice depending on the exact details of the apparatus used and also according to the size and porosity of the teeth being embedded.

The roots of the tooth are embedded in Kerr red dental impression compound or wrapped carefully in polystyrene foam packing material. A water-cooled cutting wheel edged with diamond grit, as used for the preparation of sections of teeth from modern cattle (as discussed in an earlier section) is used to remove most of the crown and to bisect the roots longitudinally. One sectioned half of the roots is freed from the impression compound or unwrapped from the foam plastic prior to embedding in resin. The second half and the crown are saved for future reference. Impression compound has the advantage of continuing to support this second half of the tooth, but with very friable specimens there is a slight risk that traces of the final band of cementum may adhere permanently to the impression compound. This does not normally impede interpretation of age because we examine the whole perimeter of several cut surfaces of the specimen, including the cut surface of the block, which remains in the impression compound. The polystyrene foam, which offers sufficient protection for all but the most fragile specimens, is quicker to use and since there is no risk that cementum will adhere to it, is preferable when teeth are being prepared for seasonably determination.

The specimen must be thoroughly dry before the embedding procedure begins. Chemical dehydration should be avoided. Air drying at room temperature with good ventilation for at least 24 hours is simple and satisfactory.

The block of root (Figure 5) is embedded in resin under the degree of vacuum obtained with a commercial rotary vane vacuum pump. At first a single vacuum pump was used. Specimens, placed in individual moulds, were immersed in resin and evacuated in a dessicator. Mass-embedding of teeth in large moulds is not recommended because setting may occur before sufficient gas has been evacuated from the teeth and the resin. Also, when large quantities of resin are mixed the polymerization temperature may rise to such a level that specimens become overheated. Although reduction by up to 10 per cent in the quantity of hardener added to the resin reduces the generation of heat, deficiency of hardener beyond 10 per cent prevents complete hardening. Later a system with two stages of evacuation, described below, was devised (Figure 6).

The tooth to be embedded is placed in tube A. Sufficient volume of Epofix resin and hardener
to fill a mould is measured out into tube B. Tube B is placed in a beaker of water at 50–60°C. It is imperative that no water enter the tube. The resin is stirred with a thermometer. When its temperature reaches 30°C, tube B is connected to tube A, as shown, the joints having been coated thinly with silicone grease. The vacuum pump is switched on. Gases are simultaneously evacuated from the tooth root in tube A and the resin in tube B. After 5–10 min, when bubbles have ceased to rise to the surface of the resin, the apparatus is tilted so that the tooth falls into the resin. Usually, a few more bubbles appear at this stage and it is necessary to maintain the vacuum for a few minutes longer. The vacuum is released. Tube B is detached from the apparatus and its contents are quickly poured into a mould, with due attention being paid to aligning the tooth so that it will be well-positioned within the resin block. The mould and contents are placed in a dessicator and this is evacuated by a second vacuum pump for about 5 min to ensure that any air incorporated during pouring is removed. Small mouldings are left overnight in the dessicator to set. Where larger moulds are used, for example with some horse cheek teeth, it is preferable for setting to take place in a refrigerator to counteract any exothermic rise in temperature.

Our double-evacuation procedure results in good penetration of the resin through the specimen. We have found that processing large teeth individually, and smaller teeth in small batches, is convenient, and we maintain precise control over the vacuum treatment and embedding of the specimens.

Moulds for embedding can be two-part moulds of high-density polyethylene. The reusable moulds supplied by the manufacturers of the resins were useful for some of the smaller teeth, but do not accommodate our larger specimens. For this purpose two-part moulds were made from high-density polyethylene (Figure 7).

Disposable moulds are simplest to use, but further supplies of the size appropriate for cattle teeth are not being manufactured at the time of writing. Whichever type of mould is used, it must be greased well with silicone grease or petroleum jelly. This facilitates separation of the set resin block from the mould and minimizes risk of leakage of resin through joints in the two-part moulds or through any weak points in the disposable moulds.

Once the resin blocks have set, two or three thin longitudinal sections are cut from them using the controlled feed and drive, water-cooled, diamond grit edged cutting wheel. We have found that by taking great care at this stage even sections of 90–100 µm can be cut. After air drying at room temperature for at least 24 h these can be mounted directly on to glass slides using DPX and a coverslip. Although thicker
than normal sections, where transmitted light microscopy is used, these can be interpreted using transmitted light at low magnifications. Attempts to enhance the cut surface of sections by lapping proved deleterious. Lapping compounds penetrated any minute spaces between the specimen and the ground glass slide on which it was mounted, and although they could largely be extracted by ultrasonic cleaning, this added an extra stage to the process, and to no advantage, for sections ground to 30 μm retained little of their structural organization. Lapping was, therefore, abandoned. Our equipment produces such a smooth cut surface to the blocks that remain after thin-section cutting that these are suitable for examination by reflected light microscopy without further preparation or grinding.

As is shown in Figure 5 the sectioning technique described above makes available for analysis a considerable part of the perimeter of the tooth root yet much of the tooth remains intact. Occlusal wear patterns remain available for further study and teeth that have been removed from jaws can be replaced with little visible alteration to the specimen.

Summary
1. Embed the root of the tooth in dental impression compound or wrap the tooth carefully in foam plastic.
2. Using a water-cooled cutting wheel edged with diamond grit, cut off the crown.
3. Longitudinally bisect the root.
4. Remove the dental impression compound or plastic foam.
5. Leave at room temperature until thoroughly dry, normally overnight.
6. Embed one of the blocks of root under vacuum in transparent epoxy resin.
7. Cut thin-sections from this block.

Cut surfaces of blocks from stages 2 and 7 can be examined directly using reflected light. The thin-sections from stage 7 are examined by transmitted light. No staining is required.

Cementum analysis
After initial orientation (as in an earlier section), the cut surfaces of the blocks of root are examined using reflected light at an oblique angle at magnifications of 20–30 times. Light at an
Incremental Banding in Denial Cemenfum

Figure 9. Resin-embedded root of M, of *Bos taurus* from Grimes Graves Bronze Age viewed by reflected light (Nikon F2 + bellows). Winter bands are numbered. (E) Edge of tooth root; (J) dentine-cementum junction.

Oblique angle makes observation of incremental features easier because it minimizes the very fine surface lines produced on the cut surface by the cutting wheel. Siting the microscope in a dark room proved advantageous and easier on the eyes. Results from these blocks are in many cases so clear that examination of the thinner sections using transmitted light only serves to confirm interpretations of age made on the basis of the cut surfaces of the blocks. On blocks viewed using reflected light, summer bands normally appear lighter in and colour.
broader than winter bands which look darker (Figures 8–10). Interpretation of season of death is in some cases easier from the thin-sections. A final winter band can be hard to identify from a block — less so in the case of an experienced observer — and the translucency of the winter bands under transmitted light is therefore useful (Figure 11).

Conclusions
At the time that this investigation was begun, there was a widespread feeling of reservation about the application of cementum banding studies to archaeological material in general and to the teeth of domestic mammals in particular. This was due partly to the uncritical borrowing of unsuitable techniques from histology and to the use of unsatisfactory comparative samples. These investigations have shown that cementum banding is well attested in the molar teeth both of modern cattle and in suitable archaeological material. The described technique provides a tried and proven method for analysing cementum increments in teeth of cattle and other mammals derived from archaeological sites. It has proved possible to relate the age in years of a specimen as determined by cementum analysis with both the wear state of the mandibles and with crown height. Results of cementum analysis, from the samples from Grimes Graves, Hambledon Hill and Haddenham suggest that it may be reasonable to suppose that analysis of a small sample of teeth from a given site could be used as a means of calibrating the relationship between crown height and wear with age for the whole sample. This is intended to provide a more accurate scheme by which cattle mandibles from archaeological sites can be attributed a more accurate age at death. The results of this part of the investigation will be presented in a future paper. It is thought that with minor adaptations the technique could be used for any suitably

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preserved archaeologically derived teeth from whatever animal.

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