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Mary J. Benedix

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I am submitting herewith a thesis written by Mary J. Benedix entitled "Cementum increments and archaeology : an analysis of fallow deer (*Dama dama*) and white-tailed deer (*Odocoileus virginianus*) from Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.

Jan F. Simek, Major Professor

We have read this thesis and recommend its acceptance:

Walter Kippel, Murray Marks

Accepted for the Council:


Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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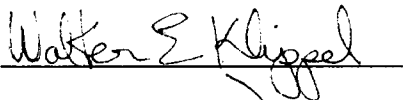
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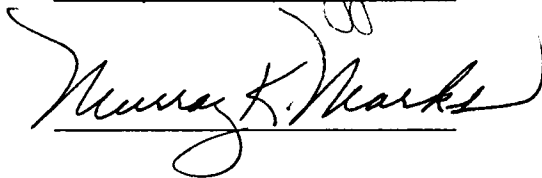
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Associate Vice Chancellor and
Dean of the Graduate School

CEMENTUM INCREMENTS AND ARCHAEOLOGY:
AN ANALYSIS OF
FALLOW DEER (*DAMA DAMA*) AND
WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)
FROM TENNESSEE

A Thesis
Presented for the
Master of Arts
Degree
The University of Tennessee, Knoxville

Mary J. Benedix

May 1998

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Abstract

This study concerns cementum increments in fallow deer (*Dama dama*) and white-tailed deer (*Odocoileus virginianus*) from Eastern Tennessee. Cementum increments have been used by wildlife biologists and archaeologists to determine age and season of death for many mammals. In theory, through both mineralized and demineralized tissue histology, one can examine the bi-annual growth and arrest lines found in the cementum.

Three goals were set forth at the beginning of this project. The first goal was to ascertain which method of thin sectioning, using six different protocols, produced the best results for a population of 8 modern fallow deer, 45 modern white-tailed deer, and 5 archaeological white-tailed deer. The second goal was to determine whether or not these methods were valid enough to be applied by archaeologists to prehistoric or historic faunal remains. The third goal was only applicable if the first and second goals were met with positive results. For this, the fallow deer and white-tailed deer molars were examined to determine if a within-family (*Cervinae*) comparison could be made, whereby a modern collection of white-tailed deer could be used in analyzing archaeological fallow deer.

The methodological aspect of the analysis was met with more new concerns than hypotheses answered. The mineralized sections resulted in a poor accuracy rate. There were good results for the decalcified modern and historic teeth, but a less than satisfactory outcome for the prehistoric specimens. As a preface and warning to archaeologists using cementum to infer age and season at death, there is tremendous room for error and interpretive problems. As for engaging in a within-family comparison, no such conclusion could be drawn.

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Chapter 1

Introduction

Cementum is an incremental growth tissue that forms the outer portion of the roots in mammalian teeth. It is constructed throughout the life of the tooth, although at varying rates, and provides a method for charting a mammal's summer growth and winter arrest periods. Theoretically, by microscopically counting and measuring the incremental lines, or annuli, one can determine the age and season at death for certain species of animals.

Histologically, cementum is comprised of cellular and acellular components, which both run in incremental lines parallel, or coat the surface of the root. The cellular area is located at the apical part of the root and may be difficult to discern in some teeth (Scott and Symons 1974). According to Stallibrass (1982), the acellular bands appear to alternate by season as either growth lines or arrest lines. It is unclear exactly why this mineralized tissue responds as it does; however, one can postulate that it is influenced by a variety of factors including nutrition, metabolic functions, climate, and the environment (Lieberman 1993).

This project examines the cementum increments of white-tailed deer (*Odocoileus virginianus*) and fallow deer (*Dama dama*) as a means of determining the season and age at death. Both species of deer were from eastern Tennessee. The white-tailed deer were wild, whereas the fallow deer, not being native to the United States, were raised on a farm. The two subspecies of fallow deer, *Dama dama dama* and *Dama dama mesopotamica*, were often cross-bred to yield a larger animal. This cross-breeding should not change the method's applicability to archaeology due to the uncertainty of

when and how the subspecies began to differentiate. There was most likely mating between the two subspecies while they were being introduced into new areas and forming specific niches (Chapman and Chapman 1975). This is discussed further in Chapter 3.

First Goal: Methodologies

The goal of this research project is threefold. Primarily, an analysis is performed to see if different methodological procedures would produce varying results. Two protocols for mineralized tissue analyses using plastic resin are employed, as well as three protocols for the demineralized paraffin technique. Despite the fact that many archaeologists claim that archaeological teeth should never be decalcified for sectioning (Ransom 1966; Kay 1974; Bourque et. al., 1978; Savelle and Beattie 1982; Koike and Ohtaishi 1985; Buie 1986; Spiess 1990; Pike-Tay 1991; Beasley et. al., 1992; and Lieberman and Meadow 1992), it may be possible to analyze certain teeth, depending upon time since death and depositional environment, if organic material is still present. Researchers in wildlife biology have had more success than anthropologists with aging specimens using cementum annuli because of their application of the decalcification technique (Low and Cowan 1963; Fancy 1980). However, according to Oetelaar (1981), it is possible to utilize the paraffin method when analyzing archaeological specimens. If this possibility becomes realized, it may provide the best scenario for when and how to thin section teeth.

Second Goal: Applicability to Archaeology

The second goal of this project is to explore the possibility that the actual use of the cementum annuli method may produce inconclusive and divergent results that would not benefit archaeology. Increments are often so variable within a tooth that accuracy plays a secondary role to subjectivity and uncontrollable biases.

Third Goal: Fallow Deer Versus White-tailed Deer

If the first and second goals of the project result in a positive determination of the best methodology for modern and archaeological samples, then the third goal can be thoroughly explored. The third goal is to determine whether or not species within the same family (Cervinae) have analogous annulation patterns. If this proves to be true, then the white-tailed deer cementum annuli, which have been extensively explored, could be used as an archaeological comparative tool for fallow deer, which have not been successfully examined. Although much has been written concerning the use of modern reference collections specific to the archaeological environment and species (Fancy 1980; Buie 1986; Lieberman and Meadow 1992), it may be possible to use animals within a family to expedite site analyses.

Concerns

As previously mentioned, histologists speculate that there are a number of factors that cause the formation of cementum to react in a seasonal manner. This fact can create problems when attempting to control variables and may foster many questions when doing such a study. There are many unknowns concerning the cementum annuli

method that should be kept in mind when doing such research. The modern populations of fallow and white-tailed deer may appear similar simply because they both live in the same basic environment. However, the fact that they experience nutritional differences could possibly create incongruencies (i.e., fallow deer are fed by humans and white-tailed deer are wild). According to Mitchell (1963), the cementum lines in a population of hand-fed red deer (*Cervus elaphus*) were not discernible. Conversely, Low and Cowan (1963) asserted that there were no significant differences between populations of wild and pen-raised mule deer (*Odocoileus hemionus*). For archaeological purposes, the environment and climate of modern Tennessee is not comparable to that of prehistoric, or even historic, southwest Asia or the Mediterranean region.

Concerns such as these are important to bring to the forefront of this research as a means of validating this project, as well as the myriad of other projects that have been done using various mammalian species. One cannot interpret the archaeological record using imperfect data. That would be unscientific and overly subjective. The purpose of this research is to control for and question the possible variables before attempting to use the knowledge for speculation concerning archaeological sites. One needs to carefully weigh the possibility that the lack of certainty associated with this method may not merit the destruction of a faunal artifact.

Summary

In summary of Chapter 1, it is the research goal of this project to systematically explore various methodologies of cementum increment analyses. Hopefully by doing this, poignant information will be drawn concerning the feasibility of using fallow and white-tailed deer for seasonality and age assessments in an archaeological context. It is

important to acknowledge the foreseen biases and concerns of this project that cannot be ignored or avoided. By exploring the problems, future researchers will be one step closer to validly controlling for the inconsistencies.

Chapter 2 explores some of the pertinent literature that has been published on the subject of cementum increment analysis. Researchers have extensively examined white-tailed deer and various other mammals for use in archaeological interpretations. Different protocols are previewed in this chapter which enables the reader to become familiar with the extent of methodological variation one is confronted with when developing a protocol.

Chapter 3 examines the manner that cementum increment analyses can theoretically be applied to archaeology. Both age and seasonality assessments are valuable tools for estimating past lifeways. This chapter also delves into the history of fallow deer and explores why it may be possible to compare the annuli of white-tailed and fallow deer.

Chapter 4 is concerned with the physiological structure of cementum. This must be understood prior to examining the teeth. This chapter also explains the hypotheses behind formation itself and the numerous problems that one may incur during analysis.

Chapter 5 contains the materials and methods used in this research project. Six protocols, using both mineralized and demineralized techniques, are thoroughly discussed in hopes that the methods used for this project can be easily replicated.

Chapter 6 reports the results that were gleaned from the analyses. This chapter provides an explanation for what was seen in the cementum of each slide with regard to age and season of death. That data is then compared to the actual age and season of death information for each deer.

Chapter 7 is the discussion section of the thesis. In this chapter, each goal is revisited with an explanation of how the data compared to the project's intent and purpose. Problems are explored, as well as methodological comparisons and archaeological implications.

Chapter 8 is the concluding section of the thesis. The findings of this project are reiterated and the unavoidable problems associated with this form of research are stressed. Some ideas are explored for possible future research projects that may end the confusion surrounding cementum, its formation, and scientists' interpretations.

The appendix of the paper includes a list of supplies for each protocol, a sample datasheet and a nomenclature section. The supply list and datasheet may aid someone wishing to replicate this research. The nomenclature section serves as a reference tool, providing access to relevant definitions.

Chapter 2

Literature Review

The first evidence that there was a correlation between cementum and age was discovered by Laws (1952) when he examined the teeth of the southern elephant seal (*Mirounga leonina*). During the 1960s, the cementum annuli method for age determination was embraced by people in the wildlife and game management industry for estimating population age structures (Stallibrass 1982). Since that time, archaeologists have also used this technique to develop age and seasonality profiles concerning archaeological sites. Numerous researchers have used this method to determine age and/or season of death for both herd management and archaeological collections. The cementum annuli of white-tailed deer have been examined with a range of little to great success by Gilbert (1966), Ransom (1966), Lockard (1972), Benn (1974), Kay (1974), Bourque et.al., (1978), Buie (1986), McCullough and Beier (1986), De Young (1989), and Spiess (1990). Many people that have used this method with other mammals include Low and Cowan (1963), Erickson and Seliger (1969), Spinage (1976), Grue and Jensen (1979), Gordon (1982), Savelle and Beattie (1982), Koike and Ohtaishi (1985), Naylor et. al., (1985), Charles et. al., (1986a and 1986b), Pike-Tay (1991), Beasley et. al., (1992), Lieberman and Meadow (1992), Landon (1993), and Burke and Castanet (1995).

White-tailed Deer

Gilbert (1966) examined the cementum annuli and dentine of incisors in white-tailed deer from Michigan, New Brunswick, Ontario, British Columbia, and Nova

Scotia. He found while the dentine was difficult to read, the cementum could be read at the "lateral portion of the terminal region of the tooth" (Gilbert 1966:202). He also found that the deposit of cementum begins before eruption as the root develops and that less cementum is produced as the animal ages. Both of these facts could skew one's results when determining age and season at death.

Both wild and pen-raised white-tailed deer molars were examined by Ransom (1966). He used a mineralized resin method and an eruption/wear method as a comparative point. He found it virtually impossible to age deer past eight years using the eruption and wear method (Servinghaus 1949). Ransom (1966:198) states that "the first light layer, nearest the dentine, was deposited during the first summer of a deer's life." If the tooth does not begin eruption until the deer is six months old and the animal is born in June, then this would point to the cementum being discernible before eruption.

Lockard (1972) examined the incisors and molars of 377 white-tailed deer, using histological sections. He observed bands in 88% of the molars. The teeth showing clear annulations came from areas with cold winters in comparison to deer from milder climates. He encountered a number of teeth with split annuli (see Figure 2.1), which are annulations that diverge within a season. He also found that the annual arrest bands did not begin formation before mid-winter.

Benn (1974) conducted research on the teeth of archaeological white-tailed deer from the Woodland period in Iowa as a seasonality determinant. He found that there were no statistically significant differences between annulation and eruption/wear studies (Servinghaus 1949). However, the cementum annuli method was more accurate for animals aged five years and older. He was able to see annulations in all of the

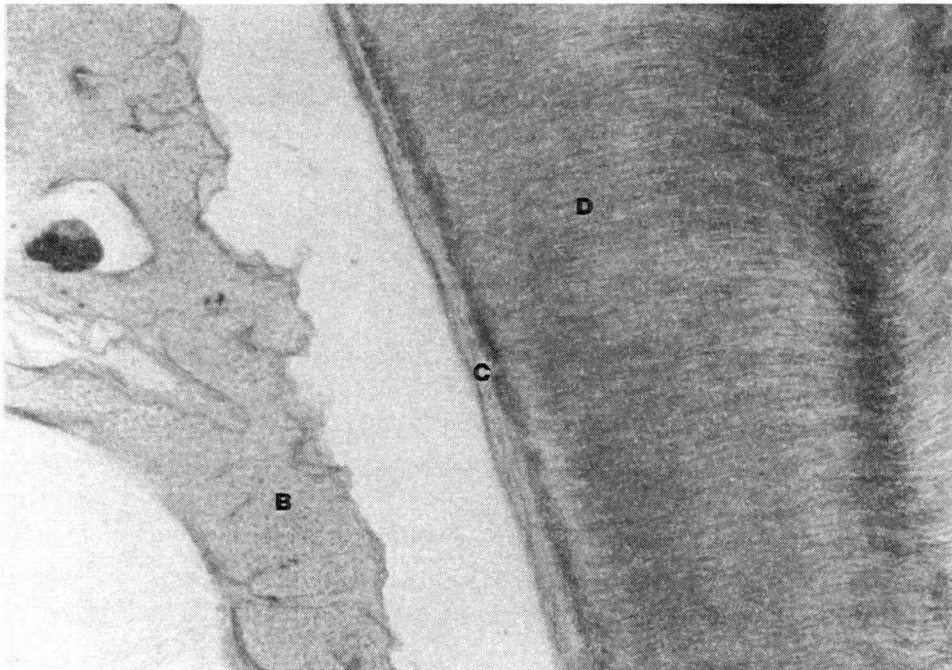


Figure 2.1: Split annuli (at x50)
B = Alveolar bone
C = Cementum
D = Dentine

specimens, although some were only visible in portions of the roots.

Kay (1974) studied the cementum annuli of mandibular and maxillary molars and premolars of white-tailed deer revisiting Ransom's (1966) method for use as an archaeological tool. However, he asserts that ideally first molars and incisors should be used for interpretation. Kay used 28 deer from the Mellor site in Missouri, dating to the Middle Woodland. He found accurate counts in only 71% of the thin sections due to difficulty in reading the incremental lines and problems with split annuli.

Bourque and co-workers (1978) conducted a study on white-tailed deer at the Turner Farm shell midden in Maine (5200-300 BP). The researchers' initial protocol was the solid sectioning method without resin. This produced adverse results, because the saw fractured the cementum. Next, Bourque et. al., embedded teeth in plastic, which allowed them to properly examine the annulations. They concluded that the site was occupied during all seasons, especially during the late Archaic.

Buie (1986) also looked at the annulations of white-tailed deer, but only had a 30% success rate. He unsuccessfully attempted to estimate a more exact date of death (month of death) by dividing the total cementum thickness by the cementum growth rate. He found this difficult due to great measurement error and an over-generalization of the date of birth. Overall, he concluded that dating an animal's death is clearer by using the tooth eruption and wear method.

McCullough and Beier (1986) studied both maxillary and mandibular first molars of 14 white-tailed deer and 23 black-tailed deer (*Odocoileus hermionus columbianus*). Their goal was to see if there was a difference between lower and upper teeth. They concluded that although the age estimates between molars were identical, the clarity of the annuli was more precise in the upper teeth.

The incisors of live white-tailed deer from Texas were aged using both the eruption/wear and cementum annuli methods (De Young 1989). De Young found that both were poor determinants of age and season at death but that the cementum method was somewhat more reliable with a 39% accuracy rate compared to the 35% accuracy rate of eruption and wear.

Spiess (1990) performed a cementum annuli study on white-tailed deer at the Turner Farm shell midden in North Haven, Maine (5200-300 BP), following the project of Bourque et. al., (1978). His goal was to estimate the seasonality of hunting patterns at the site. Before undertaking the archaeological project, he first developed a modern comparative sample from the same area. The season of occupation data that resulted was promising when combined with eruption and wear methodologies. Spiess had known death dates for the comparative sample and was able to coordinate the width of growth bands with the continuous, yet variable, deposit of cementum. He found that at the beginning of the growth season, cementum was laid down more rapidly than towards the end of the cycle. He also concluded that the growth rates of younger deer differed more from season to season than older deer. This manifested itself in the growth bands by being various widths each year.

Other Mammals

Low and Cowan (1963) had positive results when interpreting the cementum annuli of both wild and hand-raised mule deer (*Odocoileus hemionus*). Overall, they concluded that incremental line formation in the hand-raised deer was comparable to that of wild animals. However, they often found false annuli in wild deer that they did not see in raised deer. These false annuli can be compensated for if one can visually

determine a difference in appearance between false and true incremental lines. Low and Cowan claimed they were able to distinguish and account for the false annuli. As a result, they could estimate ages within one or two months of the actual ages of the deer.

Erickson and Seliger (1969) used the incisors of six wild and ten pen-raised mule deer (*Odocoileus hemionus*) for an age estimation study. With this group they had complete agreement between the actual ages and the ones derived from the cementum analyses. After solidifying their protocol, Erickson and Seliger looked at 116 freshly killed mule deer teeth and 110 from dry mandibles. They stained the sections with Harris' hematoxylin and examined the sections with fluorescent microscopy. They asserted that the method provided accurate results for 219 out of the 226 mule deer.

Spinage (1976) studied various mammals to discern whether or not increments could be seen in two different environments: unimodal and bimodal wet seasons. He looked at teeth from buffalo (*Bison bison*), black rhinoceros (*Diceros bicornis*), lion (*Panthera leo*), waterbuck (*Kobus ellipsiprymnus*), giraffe (*Giraffa camelopardalis*), and African elephant (*Loxodonta africana*). He found it difficult to interpret the annuli of the mammals dwelling in tropical climates with a constant wet season. Animals that lived in areas that fluctuated between dry and rainy seasons produced more distinct annulations. He noted experiencing much observer error and warned that different staining intensities of the sections may alter the appearance of the incremental growth structures.

Grue and Jensen (1979) worked with the decalcification technique on many different species of mammals. They also borrowed results from other researchers in a large comparative analysis of animals from numerous regions. They emphasized that there was intervariation and intravariation in species from one geographical area. This

suggests that there is more behind annulus formation than simply exogenous variation between seasons. The overall and specific condition of each animal most likely plays a large role in the cementum growth. In conclusion, Grue and Jensen (1979:41) warned that "the method has a sound theoretical base, but in practice it must be used critically."

Gordon (1982) examined the molars of caribou (*Rangifer tarandus*) from eastern Canada. He compared a modern sample from caribou collected during the summer and autumn months to archaeological specimens. Gordon used the plastic sectioning technique which proved to be beneficial especially when dealing with archaeological specimens. He determined that the sites in question had specific seasonal occupation periods, which was important for predicting prehistoric herd movements. These interpretations also provided information concerning the hunting patterns of prehistoric people. Over time, there was an increase in the hunting of young calves. This indicated either an increased human population or a decreased caribou population.

Savelle and Beattie (1982) thin sectioned 44 Paleoeskimo (3400 BP) and Copper Inuit (100 BP) muskoxen (*Ovibos moschatus*) teeth. They used three techniques: standard plastic, decalcification, and scanning electron microscopy (SEM). Of these methods only the traditional plastic method proved acceptable for all the specimens. The results were very good using the decalcification process for the historic Copper Inuit teeth, but it completely destroyed the prehistoric Paleoeskimo ones. SEM did not show ample contrast in the annuli to elicit any conclusions as to the age or season of death for the muskoxen.

Sika deer (*Cervus nippon*) were studied by Koike and Ohtaishi (1985). They extracted either the first or second molars from 14 sites in Japan and used the resin method. The authors found that annuli produced accurate estimates in animals older

than three to five years. This age information helped them calculate a life table explaining how hunting patterns of sika deer differed temporally between hunter-gatherers, rice agriculturalists, and sea-mammal hunters.

Naylor et. al., (1985) attempted to find an efficient, precise technique for examining the cementum annuli in humans. Their methodology included fixing, embedding, sectioning, etching, and staining. Human annuli are often difficult to discern, so photographs were taken of the three best sites along the roots. If there was a discrepancy in the number of lines, an average was taken. They concluded that transverse sections cut between 15% and 45% of the distance from the root tip to the neck of the tooth provided the clearest results.

Charles and co-workers (1986a) examined 42 premolars and canines from human cadavers. They selected these teeth because molars and incisors experience more ante-mortem loss than the other teeth. Their main concern was the accuracy of the technique. They used both demineralized and mineralized methods and found that mineralized sections appeared to have more variability in counts, even within the same tooth. This did not occur as often with the alternate method. The researchers also concluded that premolars produced more accurate results than canines. They stressed that multiple observers and sections be used to lessen subjectivity and error.

In a sister article by Charles et. al., (1986b), 80 clinically extracted human premolars were used for aging. They used the demineralized method because it proved more accurate in the previous study. They found that the standard error was between 4.7 and 9.7 years. This error would appear high if examining deer; however, humans live longer, providing time for greater deviations in line formation. They claimed that the health of the tooth and surrounding tissue, especially that of periodontal disease, can

skew analysis. The researchers also asserted that they experienced less error when counting the annulations in teeth from females.

Numerous red deer (*Cervus elaphus*) teeth from the European upper paleolithic sites Le Flageolet, La Ferrassie, Roc de Combe, Les Battuts, Gare de Couze, Le Morin, and Pont d'Ambon were studied by Pike-Tay (1991). She first analyzed a modern sample of elk using the plastic resin method and then employed that knowledge with archaeological specimens and found that the cementum annuli method produced analogous seasonality results when compared to the antler growth and presence-absence techniques.

Beasley et. al., (1992) sectioned the first molars of 47 modern cows (*Bos taurus*) for their study. Their results were fairly accurate using a decalcification method, providing them with a basis for examining archaeological teeth. Upon analysis of Neolithic and Bronze Age cow teeth, the researchers discovered that their method was unsuitable for these teeth, being too fragile for a standard histological sectioning because of collagen loss over time. To remedy this situation a plastic embedding method was employed and it produced readable growth and arrest lines. As a result, they stressed that the latter method always be used when analyzing archaeological specimens.

Lieberman and Meadow (1992) performed an in depth study of the physiological nature of cementum and sectioning techniques. They applied this knowledge by examining the teeth of modern and archaeological gazelles (*Gazella gazella*) from Israel for a seasonality study. They were able to reliably determine season of death for the modern gazelles in 17 out of 20 teeth. Archaeologically, they looked at 23 gazelles from both the Natufian (12,500 to 11,000 BP) and the Kebaran (17,000 to 14,000 BP) and all but two of the teeth were interpretable as to season of death.

Landon (1993) applied the cementum annuli method of determining seasonal cycles in a way that had not previously been studied with much depth. His sample population consisted of domestic cows (*Bos taurus*), pigs (*Sus scrofa*), goats (*Capra hircus*), and sheep (*Ovis aries*) from four urban and rural historical sites in Massachusetts (1630-1825). Landon used mineralized thin sectioning analyzed under polarized light and found that the seasonal slaughter patterns were similar for urban and rural areas during this time period. This suggests that people had not developed a long-term method of meat preservation; therefore, urban areas had to rely on the seasonal slaughter of animals from rural settlements.

Burke and Castanet (1995) explored the use of cementum increments in horse (*Equus caballus*) teeth as a seasonality determinant of prehistoric sites in southwest France (18,000-14,000 BP). They first examined 16 modern specimens of unknown age from Pennsylvania, Canada and France as their comparative collection. Their fossil sample consisted of 104 horse teeth from numerous sites. The protocols they used are as follows: undecalcified under polarized and transmitted light; scanning electron microscopy; decalcified and stained; and microradiography. They found the horse cementum difficult to decipher due to thickness differences in the cementum itself. Burke and Castanet asserted that 75% of the prehistoric teeth were legible enough to estimate season of death. Their data pointed to year-round occupations for all but one of the sites under scrutiny. Using this method for aging the horses was not reliable because their results were inconsistent with the eruption/wear data (Levine 1982).

Summary

The literature review provides an insight into the various ways that cementum increment analyses have been used in determining age and season of death. Through this examination of the literature, one can see that numerous protocols are employed. There are also vastly differing degrees of success rates seen by researchers. The lack of agreement concerning the best method to use and its applicability to archaeology immediately creates doubt for whether or not valid interpretations have been made.

Chapter 3

Archaeological Applications

Seasonality

Archaeologically, the *cementum annuli* method holds potential for creating subsistence profiles of how people lived many years ago. An important archaeological question concerns the season that sites were occupied. Succinctly, seasonality is “the coincidence of human activity with naturally occurring seasonal events” (Pike-Tay 1991:3). Before agriculture and animal domestication, people were nomadic and utilized sites at differing times of the year due to climatic changes and resource availability. The premise of seasonality creates the possibility that the environment is not only a natural landscape, but a social landscape as well. The environment can greatly affect the manner in which people subsist from one season to the next. It can define resource access, inhibit mobility, and create survival constraints (Cross 1988).

In turn, seasonal site dwelling may have affected population dispersal, demography, and belief systems (Monks 1981). While it is obvious that the population would be affected by seasonal occupations, it may be less clear as to why belief systems would be influenced by this nomadic lifeway. Certain places may have gained “spiritual” associations through site specific seasonal activities. As an example, if an area rich in chert could only be accessed during the summer, it is possible that the region became special to the people due to the unique resource that it offered. The chert would provide people with the raw material for making tools, which in turn would help them to survive.

There are numerous biological predictors, such as *cementum annuli*, that aid in

estimating seasonality. However, one must remember that there are often unpredictable, natural climatic and environmental occurrences that may alter archaeological interpretations. One can usually ascertain when a site was occupied, but one cannot positively determine when a site was *not* occupied. If there are a lack of seasonal indicators in the archaeological record, it does not mean that people were not there. Taphonomy, the process that occurs between death and recovery, may alter what is found during excavations (Lyman 1994). It is also possible that people occupied a site but did not leave anything behind that would be diagnostic of that specific season.

Although one can usually determine when an area was inhabited, false seasonal indicators may be present at a site. For example, a deer mandible may be excavated from a site that points to a winter occupation. However, the possibility exists that the deer may have died during the winter but did not enter the archaeological record until another season. It may have been used as a tool and carried between locales, or discovered post mortem and brought to the site for usage. Also, researchers cannot distinguish between a continuous residence during a year and a series of short occupations during different seasons but separated over time (Cross 1988).

These problems demand attention by using as many seasonal predictors as possible when analyzing sites. The most common seasonal determinants are presence and absence of animals due to migration and hibernation, seasonal skeletal changes (i.e., antler growth), tooth eruption and wear, epiphyseal fusion, and incremental growth structures (Monks 1981; Davis 1987).

Morey (1982) demonstrated an example of how seasonal determinants can give clues to prehistoric lifeways. He conducted research on how seasonal restrictions affected the Schmidt site, a Central Plains Tradition settlement in Nebraska, by

analyzing the incremental growth rings of catfish pectoral spines to denote season of death. These findings were correlated with the presence of migratory birds and tooth eruption/wear data in deer and bison mandibles at the site. His results demonstrated a pattern of fall and spring occupations. This modeled seasonal pattern is a result of a horticultural lifestyle coupled with bison hunting. Restrictive subsistence conditions demanded a seasonal pattern of occupation for survival.

Aging

Age estimation of animals is another benefit of annulus research which allows for aspects of site exploration that may have otherwise been ignored or impossible to deduce.

In assessing the nature of the exploitation of a wild species by man the most important parameters are the *age* and sex of the animals in the sample, the structure of which must be explained against the biology and behavior of the species (Chaplin 1969:239, emphasis added).

This knowledge can generate subsistence, hunting, and resource utilization profiles. In other words, an animal's age at death provides information about people's deliberate subsistence strategies through hunting. It is likely that the age profile of hunted animals would be different than the patterning of animals that were scavenged. Delving even further, the age profiles of animals culled after domestication would be unlike those hunted or scavenged. Understanding this shift in resource procurement provides knowledge about the development of complex thought processes as humans moved from scavenging to hunting to herding.

Fallow Deer and Archaeology

As previously discussed in the literature review, the cementum increments of white-tailed deer have been extensively studied and have proven fairly useful when determining age and season of death for deer on North American archaeological sites. Conversely, fallow deer have not been studied as comprehensively in this manner. This is likely due to the precarious history and evolution of the species.

In Europe there are two fossil species of deer, *Dama nestii* and *Dama clactoniana*, which date back 250,000 years ago and are similar in nature to the present day fallow. However, these predecessors eventually became extinct and are doubtfully direct ancestors to *Dama dama* (Chapman and Chapman 1975). Modern fallow deer are not truly seen in Europe until the Neolithic with the onset of agriculture and animal domestication (Hubbard 1995). In fact, there is no conclusive archaeological evidence of fallow deer in England until the medieval ages (Chapman and Chapman 1975:45).

There are a number of hypotheses as to the origin and migration of fallow deer. According to Chapman and Chapman (1975), European and Turkish fallow deer, *Dama dama dama*, probably evolved from the Mesopotamian subspecies, *Dama dama mesopotamica*. Archaeologically, the deer are first seen throughout the Levant region of Cyprus and Greece. Fallow deer have been described in Greek writings and found on coins from the Neolithic to the Bronze Age (4600 to 1900 BC). They even have their place in Greek mythology, whereby they were said to have arrived in Greece through the Oracle of Delphi, fighting serpents with their hooves. From their point of origin they were possibly traded in the Mediterranean region by the Phoenicians and later introduced throughout Europe by either the Gauls, Normans, or Romans (Chapman and Chapman 1975).

Hubbard (1995) proposes three scenarios for the introduction of fallow deer into Europe: (1) they immigrated to southeast Europe from southwest Asia, making one zoogeographical zone; (2) the deer were introduced by early agriculturalists and were never wild; or, (3) they were brought by early agriculturalists as feral animals and came under more direct human control during the Bronze Age. The first and third theories seem to be the most valid, as it seems highly improbable that fallow deer were initially under such strict human control. Hubbard states that

both botanical and zoological evidence indicate that farming was introduced into Europe and the eastern Mediterranean islands from several places in southwest Asia about the same time. Some colonists brought fallow deer that were released into the wild on arrival (1995:537).

Similarities Between Fallow Deer and White-tailed Deer

Although there are differences in the evolution, natural habitats, and biology of fallow and white-tailed deer, the animals may be similar enough to be compared on a histological level. Formerly, white-tailed deer were placed in the same genus as fallow deer when they were known as *Dama virginiana*. This caused much confusion for the international committees on nomenclature from 1916 until the matter was resolved in 1960, giving the separate genus affiliations that are used today (Chapman and Chapman 1975:22). Despite the fact that the most popular version of Latin names for the white-tailed deer is now *Odocoileus virginianus*, it is still proper to place the deer in the original genus (Hall 1981). The first description of the white-tailed deer was correct. Therefore, the comparison between the two species can be viewed as within-genus. However, to maintain a consistent approach, the white-tailed deer and fallow deer will be referred to as belonging to separate genera in this paper.

There are some phenotypic distinctions that place the white-tailed and fallow

deer into the separate subfamilies, *Odocoileinae* and *Cervinae*. Despite these differences, their developmental patterning is quite similar. Both deer undergo the rut at the same time of year and subsequently give birth during the late spring. According to Chapman and Chapman (1970:111), the order of eruption for fallow deer permanent teeth is M1, I1, M2, I2, I3, C1, M3, P4, (P3, P2), with the first molar beginning to erupt at about three to six months. Similarly, Servinghaus (1949) puts the order of eruption for white-tailed deer at M1, I1, M2, I2, (I3, C1), M3, (P2, P3, P4). The white-tailed deer experience eruption of the first molar at approximately six months of age.

In theory, much could be accomplished using cementum annuli studies of fallow deer in southwest Asia and the European Mediterranean to understand hunting profiles and culling practices once the deer fell under more direct human control. Concerning seasonality, cementum annuli analyses could aid in the determination of land and resource usage and could provide clues about the shift from nomadic to sedentary lifeways.

Summary

Theoretically, cementum increment analyses could aid archaeologists in interpreting the seasonality of subsistence patterns. These are both important factors to consider when estimating the lifeways of people in the past. The key is to determine the most accurate means of making such interpretations, which is where the validity of cementum annuli analyses comes into question. If this form of analysis is explored with the possibility of a within-family (genus) comparison, one must also examine the specific nature of fallow and white-tailed deer.

Chapter 4

Physiological Structure of Cementum

To fully understand the nature of cementum annuli and the manner in which this knowledge can help archaeologists, an explanation of the physiological aspect of teeth is required. Since the early nineteenth century the structure of teeth has been studied microscopically through histology, the science of tissues. According to Permar (1972:15), a tissue is “a group of more or less similar cells with intercellular substance and tissue fluid, combined in a characteristic manner and performing a particular function.” In concordance with this definition, all mineralized components of teeth (i.e., enamel, dentine and cementum) are tissues (see Figure 4.1). Of these three tissues, cementum not only continues to grow throughout the life of the animal, but also provides room around the base of the root for this growth to be microscopically visible. In fact, the thickness of cementum increases about three times between the ages of 11 and 76 (Scott and Symons 1974:252). Dentine also forms in an intermittent, yet predictable manner. However, dentinal increments get smaller with age because of limited growth space (Fancy 1980:242). This makes cementum a more beneficial area to look at when compared to dentine.

Cellular and Acellular Cementum

There are two types of cementum: cellular and acellular. The former is poorly mineralized and not suitable for annulation studies, especially for seasonality

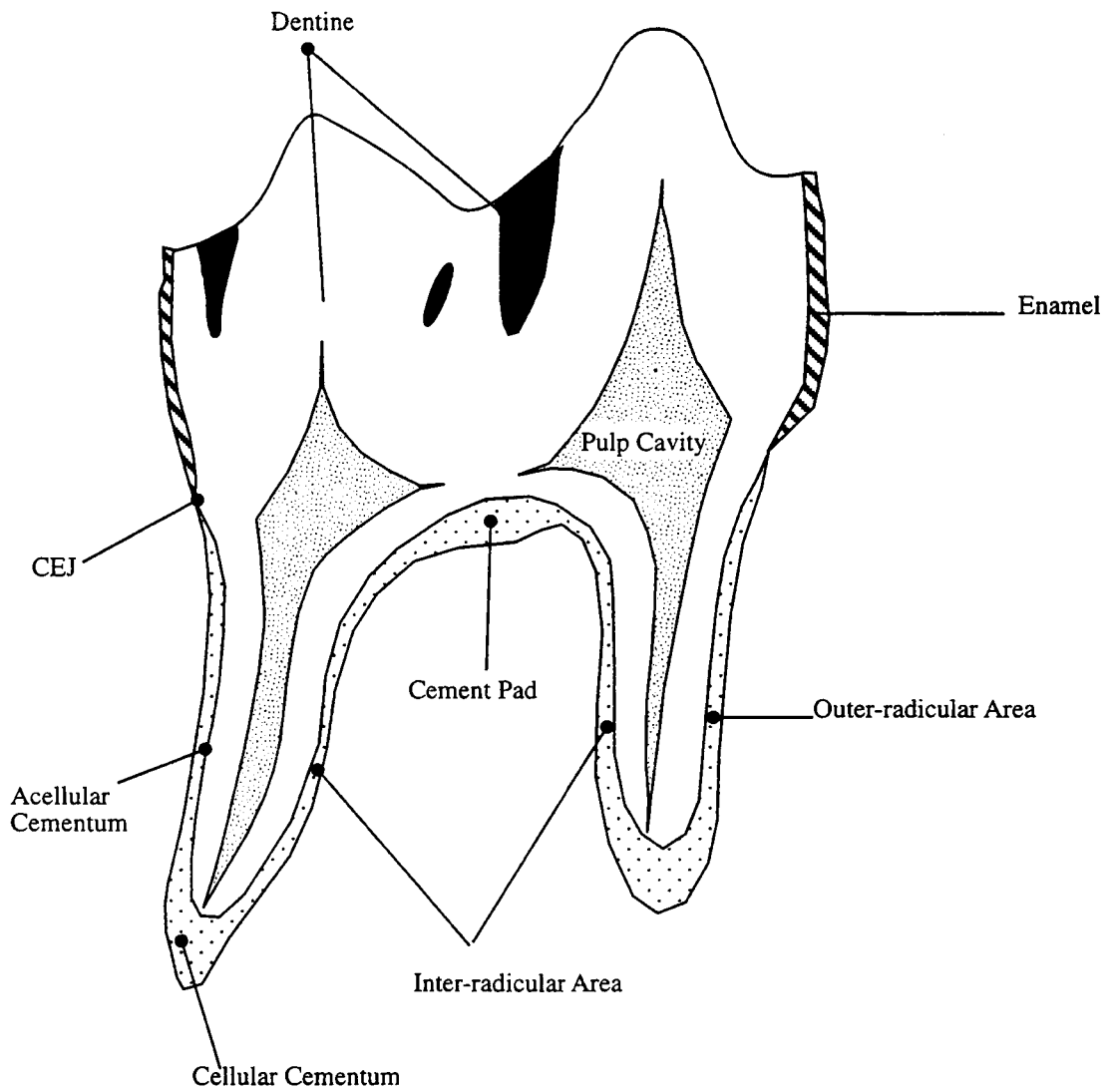


Figure 4.1: Mesio-distal thin section through a deer tooth.
 Modified from: Weinand, Daniel. 1997 *Increment Studies of White-tailed Deer (Odocoileus virginianus) from Coastal Georgia*. Unpublished University of Georgia Thesis: Athens, Georgia.

determinations (Lieberman 1994). It is very important to distinguish between cellular and acellular cementum when doing aging or seasonality research (see Figure 4.2). As an example, Grue and Jensen (1979) did not take the differences into consideration and reported problems reading the incremental lines around the apical portion of the teeth, which is the area containing cellular cementum.

Cementum consists of the organic matrix, collagen, hydroxyapatite, and an inorganic ground substance. The degree of mineralization in mature cementum is variable although both ground substance and collagen are mineralized. Theoretically, in fossil material where collagen has disappeared, the mineral shadows that are left behind should show the orientation of collagen fibers (Hillson 1996).

Both types of cementum are laid down by cementoblasts and are formed when the sheath of Hertwig disintegrates and the fibrocytes in the alveolar socket are converted to cementocytes (Permar 1972). These cementocytes are connected to one another by cytoplasm and occupy an area called the lacuna, or little space. The areas containing the cytoplasmic projections of the cementocytes are called canaliculi. The cementum is stabilized when Sharpey's fibers attach the periodontal ligaments firmly to the teeth. The Sharpey's fibers also attach the periodontal ligament to the bone of the alveolar socket (Permar 1972). This reorientation of the anchoring Sharpey's fibers is directly related to the continuous repositioning of the tooth and growth of the cementum (Charles et. al., 1989; Saxon and Higham 1969).

Before the cementum actually forms, a premineralized tissue called cementoid is formed. This contains many fibers and noncollagenous proteins. Cementoid is located between the outer layer of dentine, the Granular Layer of Tomes, and the first cementum that forms when the tooth erupts (Landon 1996; Avery 1992). The primary production

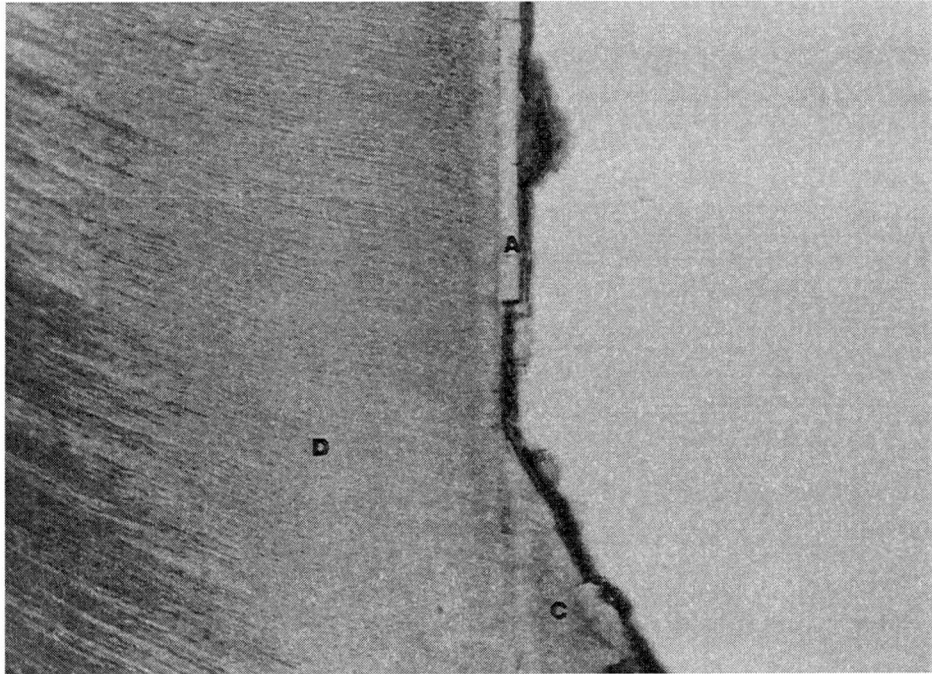


Figure 4.2: Differentiating between cellular and acellular cementum (at x100)
A = Acellular cementum
C = Cellular cementum
D = Dentine

of this may be the regulatory force behind the variation that is found in the cementum (Lieberman and Meadow 1992).

Cellular cementum is deposited quickly, whereby trapped cementocytes eventually decompose and produce lacunae. The cellular cementum can be seen at the apical root, increasing in thickness towards the apex. It is wider than acellular cementum and most likely fills a void between the root and the periodontal ligament when the tooth erupts (Lieberman and Meadow 1992). Scott and Symons (1974) assert that this type of cementum is formed when the tooth comes into occlusion and that it continues to be deposited as the tooth wears down to maintain the proper height.

This study concentrates on the examination of acellular cementum. This tissue gives the most accurate estimates of age at and season of death. In histological studies, it is the acellular cementum that appears to alternate between opaque and translucent bands, indicating periods of rapid and arrested growth. Acellular cementum begins at the cemento-enamel junction and continues halfway down the root, which is the outer-radicular area. It also exists at the cement pad and the inter-radicular area. Acellular cementum consists of layers of collagen fibers that help maintain function on the surface of the root (Avery 1992). This form of cementum is laid down slowly so that the cementoblasts are not embedded in the tissue, halting the incorporation of cementocytes or lacunae (Lieberman and Meadow 1992).

Formation

Despite the seemingly predictable nature of acellular cementum, Owens (1980) warns that its formation is later than believed, which may confuse aging. He conducted a study of acellular cementum in rat molars and suggests that the initial layer is actually a

thin unmineralized layer of predentine. If this holds true, the animal would appear older than it actually is.

The physiological analysis is quite conclusive that cementum bands do appear to alternate according to the seasons in temperate and arctic climates. However, the mere fact that this phenomenon occurs does not answer one of the most profound questions when addressing this topic: Why do the annuli form incrementally according to season? Many researchers have attempted to answer this question with a variety of hypotheses such as environmental, nutritional, hormonal, and metabolic changes, but a concrete reason has not been accepted by the scientific community to date. The reason most likely lies within a combination of factors.

When examining this issue, it quickly becomes obvious that this phenomenon is linked to the basic seasonality of actions that most animals exhibit, including deer. Antler casting is a seasonal occurrence starting at the end of March and lasting until the beginning of May (Chapman and Chapman 1975; Alvarez 1990). Male fallow deer and white-tailed deer enter the rut, the time between the first and last days of copulation, during October (in the Northern hemisphere) and the female deer respond in a timely manner (Appolonia et. al., 1990; Clutton-Brock 1991; Walther 1984). After a seven month gestation period, the fawns are born at the end of May or beginning of June (Alvarez 1990). This predictable date of birth creates a starting point when aging the animals. Seasonal activities such as these may not answer the question of why cementum increments occur, but they lead one to conclude that the answer probably does exist in inter-related, seasonally-induced occurrences.

Environment and Climate

The idea that the environment and climate influence annuli formation is pervasive in the literature. Not only is it believed that the environment affects growth and arrest periods, but the area where the animal lived notably influences the mere presence of such lines. Lockard asserts that there is a

tendency for bands to be more easily recognized in the pads of specimens from areas with cold, snowy winters than in the pads of specimens from areas with milder climates (1972:51).

Drawing a similar conclusion, Gilbert (1966:202) states that he could only see the annuli in animals from the far north.

The environment plays an obvious and large role in many physiological differences within nature, as explained by Bergmann's and Allen's Rules (Nelson and Jurmain 1991). This fluctuation between and within environments is pertinent. Even under the assumption that diet variability is the proximal factor in annuli variability, the available food resources are present due to the environmental or climatic changes. Therefore, the environment would be the ultimate cause of growth and arrest periods.

Diet

One hypothesis is that nutritional or dietary variation is the primary cause for the difference in annuli (Naylor et. al., 1985; Mech and McRoberts 1990; Lieberman 1994). Brown and Doucet (1991) note that as the winter months are accompanied by a lack of food, deer expand their diet. The browsing pressure forces the deer to become generalists and eat food that they normally would not prefer. This significant change in diet could influence the seasonal cycle that is ultimately controlled by internal factors, with the degree of contrast being exacerbated by external factors, such as food

availability and diet change (Stallibrass 1982).

Holter and co-workers (1977) conducted a study of the digestion, nitrogen balances and energy from caloric intake in white-tailed deer. They found that these physiological functions were significantly affected by seasonal change. If nutrition has an effect on the body at this biochemical level, it would not be out of the question to speculate that the diet would have a similar effect on the cementum physiology as well. The food that an animal ingests most likely affects it on many levels.

A more recent study by Lieberman (1994) applies this idea of nutritional variation and cementum annuli somewhat differently. He hypothesizes that the food consumption pattern is the direct reason for the banding, while the environment plays a lesser, secondary role. Lieberman (1994) believes that the alternating bands are caused by differing degrees of relative mineralization and collagen orientation. When animals chew tough food (i.e., winter diet), the collagen fibers orient vertically to keep the teeth from sinking. Consequently, when animals chew softer food (i.e., summer diet) the fibers readjust and orient horizontally. If an animal is nutritionally deprived, the bands may grow more slowly, but they should still be present in the cementum.

Weinand (1997) uses Lieberman's (1994) model to test the resolution of cementum bands from deer in southern areas where it was originally thought that the lack of climatic change could not produce beneficial results. His results differ from what Gilbert (1966) and Lockard (1972) profess to be problematic. He looked at deer on Skidaway Island, Georgia, and found that there was enough variation in the diet to produce significant results despite the relatively static climatic variability. This suggests that the environment plays a role only in that it results in a seasonal change of food type.

Hormones and Metabolism

A third theory is that metabolic or hormonal fluctuations are what truly create the seasonal annulations (Grue and Jensen 1979). This hypothesis suggests that an animal's internal cycle causes it to react to outside stimuli (i.e., the environment and food availability) in such a manner that would perpetuate the growth/arrest pattern. These metabolic or hormonal changes may occur primarily for the continuation of lactation, rutting and ovulation, while having a secondary effect on teeth. The annuli would essentially be a byproduct of the seasonal, metabolic forces that naturally accompany the life cycle of the animal. As an example of this, Sauer (1984) looked at the blood serum and phosphate levels in white-tailed deer. He found that a decrease in these levels during the winter slows down the cementum production at this time because both blood serum and phosphate levels must be high for prolific tooth and bone formation. This decrease in activity could produce microscopically visible differences in the teeth.

Saxon and Higham (1969) looked at the influence of the sexual and metabolic cycle of white-tailed deer. They noticed that hand-raised deer restricted their food intake during the winter despite the ample winter fodder, suggesting an internal metabolic factor being key in food intake. In a study on captive female white-tailed deer, Mautz, Kanter and Perkins (1992:660) assert that deer experience both behavioral and physiological changes that are a direct result of their seasonal metabolic fluctuations. On a macroscopic level the spring and summer months are accompanied by an increase in their physical activity and caloric intake. Through this they are also able to meet their nutritional needs for gestation and lactation. In this scenario, nutrition would be the proximate factor, while the metabolic rate would be the ultimate factor.

Problems with Analysis

Not only are there various possibilities and hypotheses for why the growth/arrest periods occur as they do, but to compound the difficulty in explanations, there are many intrinsic problems within the analysis of the deer teeth themselves. The bands can be difficult to read due to a number of reasons.

Split or False Annuli

There is the possibility of split or false annuli in some teeth, whereby one annulus appears as two (Lockard 1972; Spiess 1990), or an arrest period may be denticulated (Lockard 1972) (see Figure 2.1). This can only be controlled for if the researcher is able to discern an area of convergence. That is, one has to detect the point where the two lines come back together. If one cannot follow the annuli around the entire root, there is always the possibility that he/she is actually counting a single season as an entire year. That forces one to question whether or not an age interpretation should be made at all, unless the thin section is in pristine condition.

Split lines are often given the misnomer, rut lines. Reimers and Nordby (1968) report that during the rut, which is the deer mating season, males eat less, causing the mid-season annulus change. They postulate that under-nourished deer who are forced to eat for survival during the rut would not have these diverging lines. This assumption would lead to the idea that only males under certain conditions would produce these lines. However, it was later discovered that "divided lines do not occur every year, and they are not confined to males" (Lockard 1972:54). Therefore, the presence of so-called rut lines cannot easily be explained. They are probably caused by a brief interruption in metabolic patterns and cannot be predicted from one animal to the next (Grue and Jensen

1979; Stallibrass 1982). For reasons such as these, one should take extra precautions when assigning an age or death season to a deer.

Lack of Congruency

Even if split or false annuli do not occur in the tooth, an accurate estimation of age may still be difficult to estimate. According to Koike and Ohtaishi (1985), it was hard to identify the first winter increment of sika deer (*Cervus nippon*). The line was very thin and only at the cement pad (see Figure 4.1). Situations such as this make it essential to have all portions of the root present and examined (Gilbert 1966; Buie 1986). However, despite a thorough inspection of the root, the cementum annuli are often undiscernible in their entirety. They may only be present in portions of the root, or in many instances distinct annulations cannot be seen at all. It is unclear why this occurs. It is simply another manifestation of the problems that one may assume when studying cementum.

An additional problem concerns the number of months that are added to the results of the line counts. Age estimation can be problematic for this reason due to the uncertainty of when cementum can first be seen. It is questioned whether cementum is laid down during formation, eruption, or full occlusion (Spiess 1976; Grue and Jensen 1979). Hillson (1996) asserts that the unerupted teeth of young individuals have a layer of dental cement, albeit a thin layer. Another hinderance is that as an animal ages, the annuli become more irregular and difficult to read (Benn 1974). Despite the fact that cementum is laid down throughout a mammal's life, the incremental lines are visually less distinct and take up a smaller surface area as the animal becomes older. During the aging process, the surface of the cementum becomes irregular due to the calcification of

ligament fiber bundles (Avery 1992). This can make the annuli that were most recently laid down seem erratic in nature.

Summary

As one develops a deeper understanding of cementum physiology, it becomes apparent that there are numerous questions that remain unanswered. It is not enough to know that cementum is laid down by cementoblasts. One must now determine the triggering mechanisms behind cementogenesis, be it environmental, nutritional, hormonal, or a complex system of these inter-related phenomena.

Chapter 5

Materials and Methods

There are numerous and various methods for obtaining results from cementum increments. Some researchers claim that one protocol produces better results than others. Despite small differences in procedures, the main issue is whether or not to decalcify, or demineralize, the teeth prior to sectioning. A number of people propose that using a nondecalcifying method works best, especially when dealing with archaeological teeth. These researchers include Ransom (1966), Kay (1974), Bourque et. al., (1978), Savelle and Beattie (1982), Koike and Ohtaishi (1985), Buie (1986), Spiess (1990), Pike-Tay (1991), Beasley et. al., (1992), Lieberman and Meadow (1992), Landon (1996), and Hillson (1996). Although each researcher uses slight variations when employing this method, the basic protocol includes dehydration, embedding, sectioning, grinding, polishing, and examining the sections under a microscope using either transmitted, polarized transmitted, or reflective light depending upon if the sections were stained or not.

Low and Cowan (1963), Gilbert (1966), Permar (1972), Thomas (1977), Fancy (1980), and Bookhout (1994) found the finest results with the decalcification method. This protocol requires decalcifying, neutralizing, embedding with paraffin, sectioning, staining, and examining under transmitted light. This method is pervasive in the wildlife biology literature for working on modern specimens, and some researchers (Oetelaar 1981; Savelle and Beattie 1982) have claimed that it can work for archaeological teeth. However, most archaeologists stress that decalcifying can destroy the teeth because of their already fragile states. The archaeological teeth would most

likely have demineralized areas of low protein that could not support decalcification.

The Modern Sample

The mandibles of the deer used in this study were defleshed in three different ways. These various processing methods should not have an effect on the cementum annuli. All eight of the fallow deer, one male and seven females, were placed in an aquarium with dermestid beetles (Group 1). One group of white-tailed deer mandibles, 13 females and 10 males, was deposited in an open air site where natural insect activity and decomposition could occur (Group 2). The mandibles of the other group of white-tailed deer (17 females and 5 males) were macerated (Group 3).

Once the soft tissue had been removed, the white-tailed deer mandibles were aged by eruption and wear according to the guidelines of Servinghaus (1949). Some of the fallow deer had known ages and dates of death. Those that did not were also aged by eruption and wear using the Chapman and Chapman (1970) and Brown and Chapman (1990) methods. The first mandibular molars were used for the analysis. The first molars were chosen because of their early and relatively consistent eruption dates (Kay 1974; Buie 1986). These teeth were either extracted manually by moving them around until they loosened, or for more stubborn teeth, the mandibles were cut using a Bench band saw (Delta M 28-185) and embedded along with the molars. Zanon (1996) asserts that thin sectioning the teeth within the alveolus will lessen destruction to the cementum during manipulation

First Protocol: Mineralized

For this project, the initial protocol employed was the nondecalcifying, plastic method. The sample consisted of fallow deer that were defleshed via dermestids (Group 1), white-tailed deer that underwent natural decomposition (Group 2) and white-tailed deer that were macerated (Group 3). The specimens were dehydrated and embedded as follows: 50% alcohol for 24 hours; 70% alcohol for 48 hours; 90% alcohol for 48 hours; 95% alcohol for 48 hours; 100% alcohol for 48 hours; 100% acetone for 48 hours; 2/3 acetone, 1/3 resin for 24 hours; 1/3 acetone, 2/3 resin for 24 hours; 100% resin for 24 hours; and 100% resin in a 50 degree Centigrade oven (American Scientific drying oven DX-58) for 24 to 48 hours (after Gerard 1990). The embedding resin is actually a plastic (Spurr 1969). It is made when vinyl cyclohexene dioxide (a low-viscosity medium) is combined with a diglycidyl ether of polypropylene glycol (an epoxy resin to control plasticity), nonenyl succinic anhydride (a hardener), and dimethylaminoethanol (an accelerator to initiate polymerization) (Smith and Laragianes 1974).

After polymerization was complete, the samples were mounted onto resin blanks and cut longitudinally to follow the cementum around the entire root. At least three thin sections from each tooth were cut using a Leitz 1600 diamond blade saw. The saw was constantly cooled with running water to ensure a smooth cut. One side of each section was ground to create a smooth surface for mounting on the slides. Clear epoxy was used to adhere the sections to the slides. Both glass and plastic slides were used, although plastic were preferred because they would not shatter and were easier to grind and polish than the glass slides. The slides were then placed between two wooden blocks in a vice to secure the sections and allowed to dry for 24 hours.

After drying, the slides were ground and polished using a Mark V Lab grinder and polisher (3B 4B series). The grinding was done using both 1500 grit and 600 grit sandpaper sheets. The coarser grit was used first to take down the slide quickly to between 30 and 60 microns. To remove the deep etching lines for a more efficient polishing process, the finer grained sandpaper was used. Velvet and a liquid aluminum were used for polishing. Both the grinding and polishing utilized running water to obtain the desired outcome. If performed accurately, a translucent, smooth section would result, allowing enough light to pass through the section for an accurate reading under the microscope (see Table A.1).

At this point, the slides were ready for examination under a microscope. For this and for taking photographs of the slides, a Leitz Dialux 30 (BE14574) microscope was used. There were questions concerning what type of light to use: transmitted light or polarized light. Koike and Ohtaishi (1985) and Landon (1996) emphasized that a polarizing filter produced more distinct incremental lines, whereas Oetelaar (1981:115) used transmitted light by asserting that "polarizers...did not appear to enhance annuli visibility." To remedy this confusion the slides were examined using both transmitted and polarized light.

Light microscopy is based on an interaction of the tissue components with photons, which are units of retinal illumination. Polarized light employs the use of two filters. The first filter allows for normal light to pass through but only in one direction. The second filter is added with its axis perpendicular to the first filter, allowing no light to pass through.

If, however, tissue structures containing oriented molecules...are located between the two Polaroid filters, their repetitive, oriented molecular structure allows them to rotate the axis of the light emerging from the polarizer (Junqueira et. al., 1995:4).

This phenomenon is conceptualized by one's eye, appearing as bright structures against a black background.

Three of the slides were stained to determine if this aided in the analysis. For this, the magnification of 100x using minimal transmitted light produced the best visual results. The stain used on the plastic sections was Toluidine Blue (see Table A.2). This stain became effective when 5 ml of 1.0% Toluidine Blue was combined with 95 ml distilled water, filtered, mixed with 2.5 g sodium carbonate, and allowed to sit for 24 hours. At this point the stain was placed on the slide and warmed using a Corning PC 351 stirrer/hot plate. After three minutes the excess Toluidine Blue was removed from the slide, rinsed, and ready for microscopic examination.

Second Protocol: Mineralized

To see if slight variations in protocol could affect the reliability of the incremental lines, a second mineralized method was employed on five of the macerated white-tailed deer teeth (Group 3). The teeth were embedded in the same manner discussed for the first protocol; however, a new cutting saw was used in making the thin sections. The reasoning behind this was that the lower portion of the tooth could not be seen with the Leitz 1600 saw. Half of the resin block was situated under the blade so it was difficult to discern the precise location that the thin section was taken.

For the second protocol, the methodology of Marks and co-workers (1996) was followed (see Table A.3). For this procedure a Buehler Isomet Slow Speed Saw was used to cut the thin sections. The diamond wafering blade was constantly oil-cooled. The initial cut was made to allow the first section to be taken from the middle of the tooth. After the first cut, the surface was cleaned with alcohol and a glass slide was

fastened to the block remaining in the chuck with a cyanoacrylate adhesive (Duro Superglue). At this time the thickness of the section was adjusted for and cut. After the blade had completely freed the section from the block, the slide was cleaned with alcohol. The process was repeated for a second thin section. To ensure proper visualization, the slides had to be ground and polished using the same steps described in the first protocol.

Third Protocol: Decalcified with Hydrochloric Acid

As an additional measure in understanding the nature of cementum annuli, the paraffin method was used on two teeth that posed problems using the plastic methods. As an example, the molars from the left mandibles in Group 2 were examined using the plastic method. If one of these teeth produced poor results, then the molar from the opposite mandible was embedded in paraffin to see if demineralization created a better outcome.

The teeth were first placed in a decalcifying solution, comprised of 90% water, 10% hydrochloric acid, <0.1% Ethylenediaminetetraacetic Acid (EDTA), tetrasodium, <0.1% sodium tartrate, and < 0.1% potassium sodium tartrate. The decalcifying solution covered the teeth at least five to ten times the volume of the specimens. The teeth remained in this solution for one day under constant agitation from a Corning PC 351 stirrer. The agitation accelerated the decalcification process.

In order not to overly demineralize the teeth, the specimens had to be monitored very closely. It was important to withdraw the teeth from the hydrochloric acid as soon as the organic component had been removed. If the specimens remained too long in the acid, they would lose their shape and ability to be stained. This stage is where the

majority of problems with this method are encountered. When addressing this problem, Carson (1990:41) asserts that “quality should not be sacrificed in the interest of time, as frequently nothing is gained and all may be lost.”

There are three methods for determining if a specimen has been thoroughly demineralized. The mechanical method, which was used in this project, utilizes a needle. If the needle can easily puncture the tooth, it is ready. The second method is chemical and consists of sampling some of the used decalcification solution, mixing it with ammonium hydroxide and ammonium oxalate, and then determining if calcium oxalate is present. Radiography is the third method. By taking an x-ray of the specimen, one can tell if demineralization is complete (Carson 1990:40).

After finishing the decalcification step, the teeth were placed in varying alcohol percentages for dehydration and xylene for purification. The protocol for this was as follows: 70% alcohol for 45 minutes; 80% alcohol for 45 minutes; 90% alcohol for 45 minutes; 95% alcohol for 45 minutes; 95% alcohol for 45 minutes; 95% alcohol for 45 minutes; 100% alcohol for 45 minutes; xylene for 45 minutes; xylene for 45 minutes; paraffin for 55 minutes; and paraffin for 55 minutes. This was accomplished using a Shandon Citadel 2000 tissue processor. This machine contained solution compartments and a timer, which automatically placed the teeth in a different bath at a set rate.

In order to fit in the embedding cassettes, the teeth had to be cut longitudinally with a scalpel. Both halves of each tooth was then embedded in paraffin wax. They was embedded using a Shandon Embedding Center with a hot plate, cold plate, and paraffin center. Paraffin was used because it holds the cells and intercellular structure in place by completely infiltrating the specimen (Carson 1990).

After the teeth were embedded in paraffin, they were thin sectioned to four

microns using an American Optical 82D Spencer Microtome. Although neither was desired, it was better to under decalcify than over decalcify. If embedding had already taken place but the specimen still contained calcium and was too hard to cut, surface decalcification could be used. The tooth was exposed and placed in hydrochloric acid for a few minutes. Once the acid had taken effect, the paraffin block could be thin sectioned as normal (Carson 1990). The sections were then put in a Fisher TissuePrep Flotation bath (135) and placed upon a glass slide. The bath was kept at about 47 degrees Centigrade, which was warm enough to dewrinkle the sections, yet cool enough to prevent melting.

From this point, the slides were stained and again placed in the alcohol and xylene solutions, but in reverse order. This removed the paraffin from the slides and left behind the stained teeth. This process took approximately ten minutes and was done in the following order: 100% alcohol; 95% alcohol; water; hematoxylin; water; 0.25% ammonia water; water; eosin-y; water; 95% alcohol; 100% alcohol; and xylene. The critically timed steps were the hematoxylin and eosin baths, which took 3 minutes and 15 seconds, respectively.

For this project hematoxylin, a nuclear stain, and eosin - y, a counter stain, were employed. The stains work according to their nature as either acidic or basic compounds. Tissues that easily stain with basics, such as hematoxylin, are called basophilic. Conversely, acidophilic stains, such as eosin, stain with acids (Junqueira et. al., 1995). The active ingredients in hematoxylin are 8.6% ammonium sulfate and 0.4% hematoxylin. The active ingredient in the counter stain is 0.5% eosin-y (see Table A.4).

After the staining was complete, covers were placed on the slides and they were

analyzed under transmitted light using the Leitz Dialux 30 (BE14574) microscope. As with the plastic sections, a magnification of 100x with minimal light produced the best results.

The Archaeological Sample

The modern white-tailed deer teeth processed using the third protocol provided fair results. However, the question remained whether or not decalcification would destroy archaeological specimens.

The first molars from Archaic period (~5,000 BP) white-tailed deer were used for the prehistoric archaeological analysis. The teeth were from the Hayes site shell midden (40ML139) in Middle Tennessee along the Duck River. Beauchamp (1993) examined the first permanent molars and fourth deciduous premolars of white-tailed teeth from the Hayes site in a crown height aging study, which provided a basis for age and seasonality comparisons. If both cementum annuli and crown height analyses prove accurate, there should be no difference between the resulting age and seasonality data. Beauchamp found that sub-adults were mainly hunted during the early Middle Archaic, late Middle Archaic, and Late Archaic periods. As for seasonality, she asserted that these hunter-gatherers were killing white-tailed deer during the fall and winter for the late Middle and Late Archaic and during the fall for the early Middle Archaic.

A white-tailed deer tooth was also analyzed from the historic Gibbs house site (40KN124) in Knox County, Tennessee. This area was settled in the late eighteenth century by Nicholas Gibbs, where he built a log house in 1792. The Gibbs family grew numerous crops and raised livestock on their land. The house was family-owned until it was sold in 1971. Since that time it was subsequently bought by the Nicholas Gibbs

Historical Society in 1986.

From 1987 to 1991 Dr. Charles Faulkner, a professor of archaeology at the University of Tennessee, excavated the area surrounding the Gibbs house containing a cellar, outbuildings, and trash middens. The tooth that was used in this research project was located in an ash midden. This ash midden dates to the early occupation of the site, making the tooth roughly 200 years old (Lev-Tov 1994).

Fourth Protocol: Decalcified with Hydrochloric Acid

To initiate the archaeological examination, one tooth from the Hayes site was decalcified following the steps in protocol three. The same chemicals and embedding methods were used. Despite the fact that modern teeth took 24 hours to completely demineralize, the archaeological tooth had to be watched more carefully. Its frail state required a cautious approach to this stage of preparation. The tooth had to be monitored very closely as to not over-decalcify. The hydrochloric acid took 17 hours to complete the demineralization process of the roots. However, when it was time to section the tooth, the hydrochloric acid readily decalcified the roots, but failed to completely decalcify the enamel. If the tooth had been left in the decalcifying solution much longer, the roots would have been destroyed. Surface decalcification could not be used, because the enamel was still very mineralized.

Fifth Protocol: Decalcified with Formalin

Another chemical solution was used to determine the best substance for demineralizing archaeological specimens. To help prevent problems with this method, one historic and two prehistoric white-tailed deer teeth were both fixed and decalcified.

According to a study performed by Oetelaar (1981), fixing the first molars of ungulates prevents damage to the cementum during manipulation and decalcification. Fixation preserves the morphology and molecular composition of the tissue under analysis (Junqueira et. al., 1995).

The teeth was placed in a formic acid and formaldehyde decalcifying solution called Decalcifying Solution F (Baxter D1211). This solution simultaneously fixes and decalcifies the specimen while minimizing problems with nuclear staining.

One prehistoric archaeological specimen took 23 hours for fixation and decalcification to complete and the other took 22 hours. The historic tooth from the Gibbs site took 48 hours to decalcify. Afterwards, the specimens were embedded, sectioned and stained using the methodology previously described as the third decalcification protocol.

Sixth Protocol: Decalcified with EDTA

A third decalcification technique was employed using a white-tailed deer tooth from the Hayes site. The rapid decalcification from hydrochloric acid may be too strenuous on the roots of a prehistoric tooth, while not penetrating the enamel quick enough. Therefore, this protocol was initiated by using EDTA (Ethylenediaminetetraacetic acid) to slowly infiltrate the specimen (Stallibrass 1982). EDTA is an organic compound that binds calcium ions together. It acts on the outer layer of hydroxyapatite, gradually decreasing the crystal size (Carson 1990).

The solution of EDTA (Fisher SS412-1) took 1 month and 4 days to complete decalcification. At this point, the roots were decalcified, but the enamel was still hard. The roots were breaking apart which would not allow for proper paraffin infiltration.

Summary

Six protocols were used to gain insight into whether mineralized or demineralized methods provided the best results for modern, prehistoric and historic specimens. Due to the lack of controls surrounding the sample population itself, this step of the research had to be carefully followed and documented to minimize the biasing as much as possible.

Chapter 6

Results

Each slide was examined using two blind tests. First, an interpretation of the animal's age and season of death was made without looking at the known information. The chart in Appendix B is an example of how these results were recorded. After this was complete, a second blind analysis was performed a week later to determine if the subjectivity of a single observer would pose problems. Often more than one age was gleaned from a tooth. If this occurred the oldest age was recorded as being the hypothesized age. This maintained a consistent pattern of analysis. Prior to the blind tests, a number of practice deer teeth of unknown age were sectioned and analyzed to become familiar with the procedure.

Seasonality Assessment

The season of death information was correctly estimated for all but three of the fallow deer. Specimen numbers 10, 11, and 14 all contained an incorrect assessment of this phenomenon during at least one of the blind tests. Seasonality for the white-tailed deer was correctly determined, except in cases where cementum annuli could not be seen at all. At least one analysis for the following specimens contained inconclusive seasonality information: 22, 24, 26, 27, 29, 211, 214, 215, 219, 221, 222, 223, 225, 229, 235, 236, 239, 240, 30, 31, 32, 33.

Aging Assessment

Estimating the age of the fallow deer and white-tailed deer deserves a more in-depth explanation than does the seasonality assessment. This process was complicated and highly variable. Therefore, a description of what was viewed under the microscope for each specimen is given. Following the descriptions are four tables. The first is an overview of the outcome of the blind tests, the second shows the percentage that were accurate in the tests, the third demonstrates the effects of age on accuracy and the fourth shows the variation between cement area and correct age assessment.

Fallow Deer - Group 1 - First Protocol

10 - This fallow deer first molar was difficult to interpret. The only clear area of cementum was at the cemento-enamel junction (CEJ). The first examination showed an age of 2 years old. The second examination produced varied results of both 1.5 and 2.5 years old. The animal actually died at 1 year 9 months of age, which should show the beginning of a spring/summer increment.

11 - This tooth was sectioned with the alveolus intact. The first examination showed ages of both 2 years and 1.5 years. The second examination was interpreted as the animal dying at 4 years old with the only clear area being at the CEJ. Both exams put the animal dying in the spring; however, the deer actually died in November at age 3.5.

12 - This deer died at 6 months of age, which was clearly shown throughout the cementum of this molar. There was a distinct dark line around the root, except at the apex which is the area of cellular cementum.

13 - This was another good example of a deer that died at 6 months old and was interpreted accurately. The single dark line could be seen in most areas of the root. However, as one looked away from the CEJ the increment became less distinct.

14 - The first and second analyses placed this fallow deer's death at 1.5 years old, which was correct. The increments were fairly clear throughout the acellular portions of the root.

15 - Only a limited area proved distinct for this specimen, which was at the CEJ. The first examination placed the animal's age of death at 2.5 years old. The second examination showed the animal dying at 1.5 years of age. The actual age at death corresponds to the second examination.

16 - Most of the tooth was unreadable, but the definitive CEJ gave the correct age of 1.5 years old for both analyses.

17 - This tooth produced a clear and accurate reading during both analyses with the age at death being 6 months old. This molar was sectioned within the alveolar portion of the mandible.

White-tailed Deer - Groups 2 and 3 - First Protocol

20 - This deer molar was sectioned with the alveolus intact. The first and second examinations produced results of 6 months and 1.5 years. These results were each found at the CEJ. The true age of death for this deer was 1.5 years old.

21 - The results of the first examination was 1.5 years old and the second examination showed both 6 months and 1.5 years. The actual age of the animal was 2.5 years old. This tooth was still in the alveolus. The cementum was poor throughout most of the root.

22 - This tooth, which was in the alveolus, produced inconclusive results in the first examination. The cementum had no true definition to it. During the second analysis an age of 6 months was reported due to a single dark line. However, this age was doubted because the apex of the roots were fully formed, which has not yet occurred by 6 months of age (see Figure 6.1). The animal actually died at 1.5 years old.

23 - There was a clear, single, dark band in all but one area of the tooth. Both examinations accurately predicted 6 months old as the age of death.

24 - The initial analysis was inconclusive, reporting unclear, splitting cementum. The second interpretation did admit to unclear cementum as well, but concluded that the CEJ showed signs of the animal being 6 months old. This was the age of death for this white-tailed deer.

25 - Despite faint, blurry cementum, the animal was correctly aged to 6 months of age.

26 - This tooth remained in the alveolar bone during embedding and sectioning. Both analyses produced inconclusive results, whereby the cementum was either blurry or could not be seen. The deer's age at death was 3.5 years old.

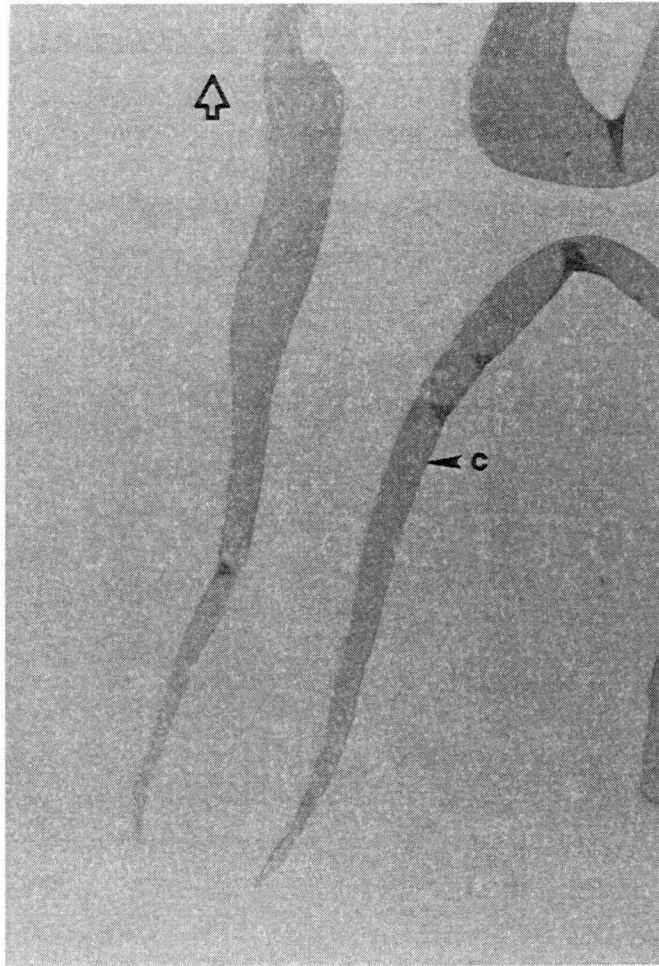


Figure 6.1: A six month old white-tailed deer tooth whose roots are not fully formed (at x25)

C = Cementum

The arrow is pointing towards the crown of the tooth.

27 - The first examination of this tooth was uninterpretable and the second examination showed signs at the CEJ of the animal being 1.5 years old. This was the accurate age. The alveolus was still present. In another trial, a second section of this molar was stained with Toluidine Blue. The first analysis concluded that the animal was 1.5 years old at its death and the second analysis showed areas pointing to either 6 months or 1.5 years. The only interpretable area was at the CEJ.

28 - Annuli could only be seen in a limited area at the CEJ. In both analyses the deer was correctly aged to 1.5 years old. The alveolus was still present.

29 - This molar was still encased by the mandible during sectioning. The first examination demonstrated an age of 1.5 years old at the CEJ and the cement pad with the rest of the root remaining unclear. The second examination reported uninterpretable cementum. The animal actually died at 3.5 years of age (see Figure 6.2 and Figure 6.3).

210 - This molar was sectioned within the alveolus and was stained using Toluidine Blue. The first analysis interpreted the animal to have died at 1.5 years old. Despite the use of a stain, the annuli were still unclear throughout most of the tooth. The second exam saw signs of a single dark increment, which would point to the animal having died at 6 months old. However, this tooth was similar to tooth 22 in that the roots were fully formed, creating doubt as to its age being 6 months old. This doubt proved accurate because the animal actually died at 1.5 years old.

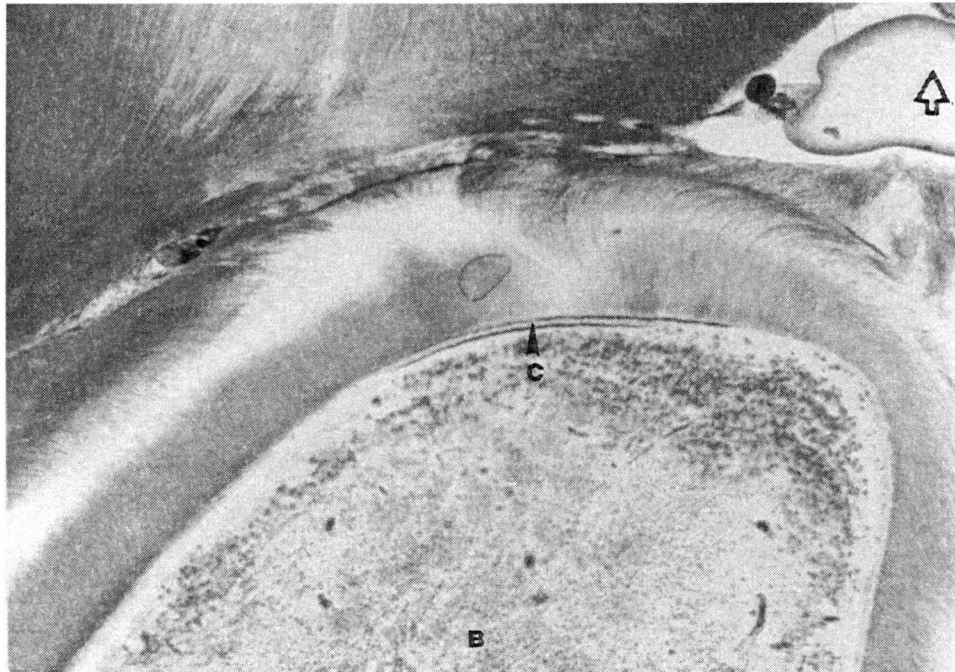


Figure 6.2: Specimen 29 - Aged to 1.5 years old at the cement pad (at x50)
B = Alveolar bone
C = Cementum
The arrow is pointing towards the crown of the tooth.

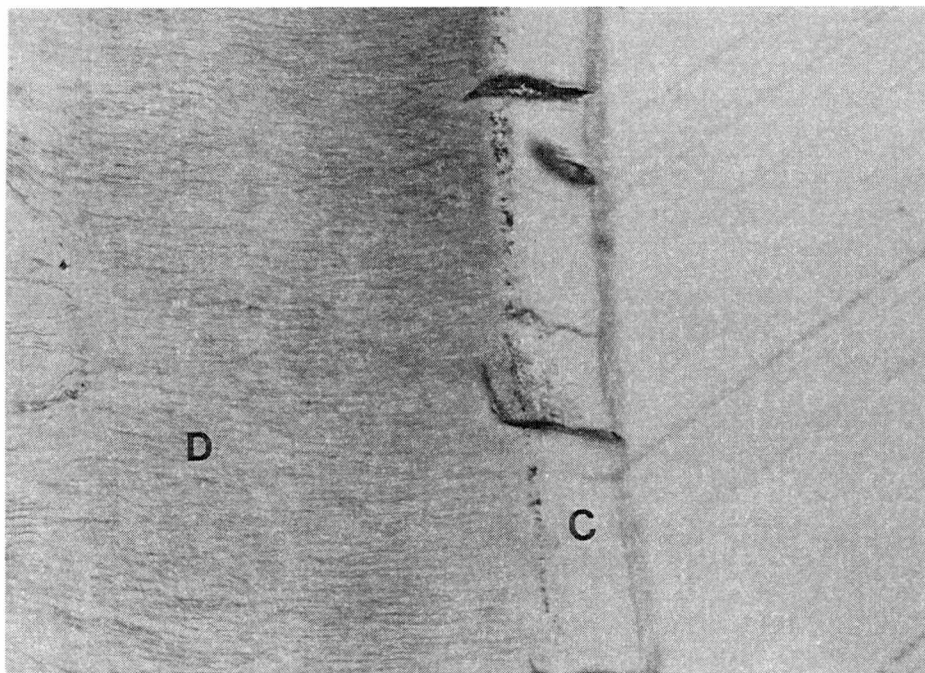


Figure 6.3: Specimen 29 - Unclear cementum (at x100)
C = Cementum
D = Dentine

211 - The tooth was sectioned within the alveolus. Both examinations reported inconclusive results due to poor cementum. The animal died at 1.5 years of age.

212 - The first inspection of the molar within the alveolus reported an age of 2.5 years old. There was some splitting reported, but one could see where the lines converged. The second inspection showed clear cementum at the CEJ, but it was blurry elsewhere. The age of death was determined to be 1.5 years old. The actual age of death was 1.5 years old.

213 - The first examination provided two interpretations of 2.5 years old and 1.5 years old. The former age was seen at the CEJ and the latter age was seen at the inter-radicular area. The second examination resulted in an age of 2.5 years. The deer died at 1.5 years old and the alveolus was present.

214 - Both examinations reported unclear and uninterpretable cementum. The tooth had been sectioned within the mandible. The actual age was 1.5 years old.

215 - The tooth was cut within the alveolus. The first analysis placed the animal at 6 months old. There is one dark increment at the CEJ. The second examination reported inconclusive results. The first analysis was correct.

216 - The age of death for this white-tailed deer was interpreted correctly as 6 months old during both examinations.

217 - This molar remained in the alveolus for the analysis. The first and second exams reported identical findings of three different ages. The CEJ showed a single dark increment, while directly below that area were three incremental lines in a dark, light, dark pattern (see Figure 6.4). Lastly, the cement pad placed the animal at 2.5 years of age (see Figure 6.5). The deer's actual age at death was 1.5 years old, which corresponds to the outer-radicular area.

218 - This tooth was sectioned within the mandible. The first exam placed this deer's age of death at 2.5 years of age with the clearest point being at the inter-radicular area below the cement pad. The second exam reported an age of 1.5 years, which was the animal's actual age at death.

219 - The first examination of this tooth was inconclusive and the second examination placed the animal's death at 1.5 years of age with the CEJ being the only interpretable area. The accurate age as was determined by the eruption/wear method was 1.5 years old. This tooth was still encased in the alveolus upon sectioning. A second method was employed once again to see if staining improved the ability to interpret the increments. This time the first examination showed two ages, one being 6 months and the other being 2.5 years. Each age was seen at the CEJ but at opposite sides of the tooth. The second examination of the stained slide showed the animal having died at 6 months of age.

220 - The CEJ was fairly clear and reported an age of 1.5 years. The rest of the cementum was very unclear. The second analysis showed two ages, one being 1.5

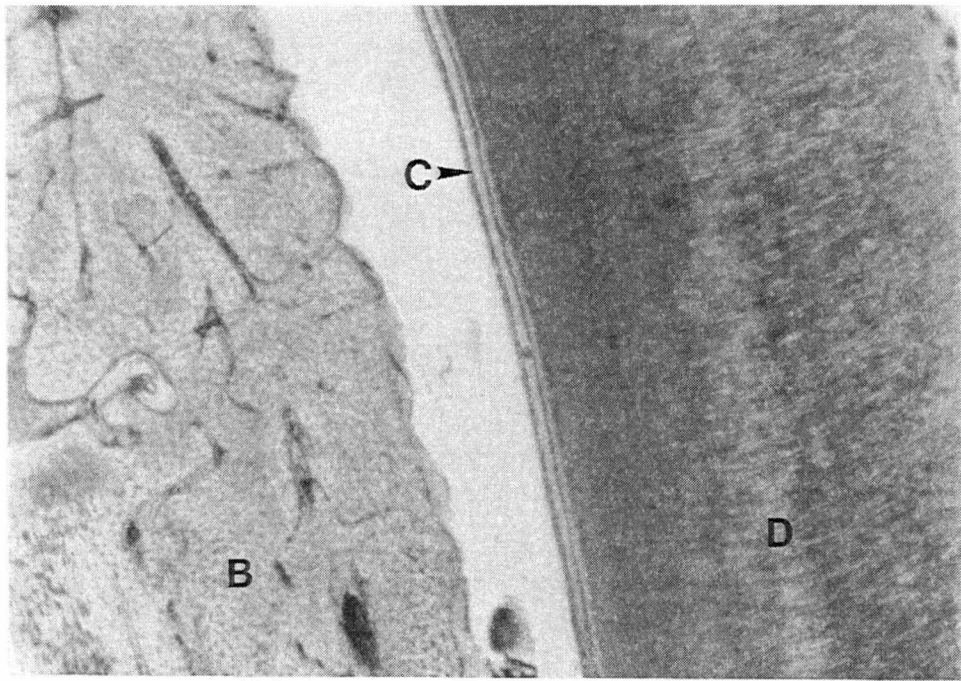


Figure 6.4: Specimen 217 - Aged to 1.5 years old at the outer-radicular area (at x50)
B = Alveolar bone
C = Cementum
D = Dentine

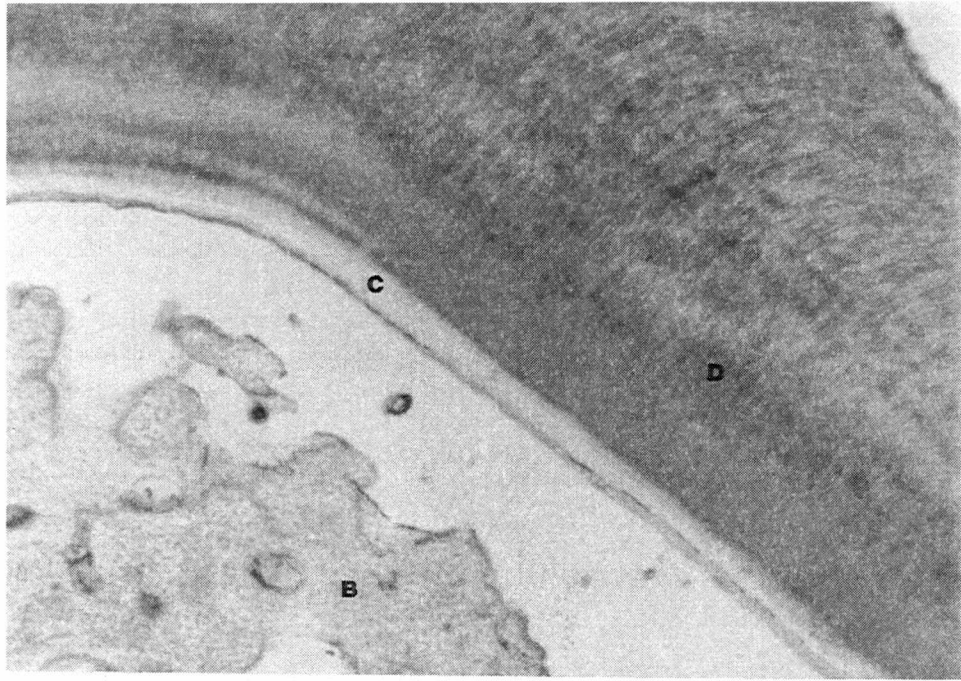


Figure 6.5: Specimen 217 - Aged to 2.5 years at the cement pad (at x50)
B = Alveolar bone
C = Cementum
D = Dentine

years. These incremental lines were at the same area as seen in the prior analysis. The inter-radicular area of the root demonstrated an age of 2.5 years old. The animal died at 2.5 years old. This tooth was also sectioned within the alveolus.

221 - This tooth was examined with the mandible still present. The first analysis proved to be inconclusive due to unclear cementum. The second examination showed signs of three distinct ages: 6 months old, 1.5 years old, and 2.5 years old. The animal's true age was 1.5 years old. During the second exam the accurate age was seen at the CEJ.

222 - The alveolus was intact for the thin sectioning of this white-tailed deer molar. The first inspection produced an age of 1.5 years old. The increments could be seen at the CEJ and the inter-radicular portion of the root, below the cement pad and above the cellular cementum. The second analysis was inconclusive. The first interpretation proved to be accurate.

223 - The CEJ did not show any clear increments during the first analysis; however, the area below it did show an age of 6 months old. This increment was very faint. The second examination was inconclusive. The animal's true age at death was 1.5 years old.

224 - This white-tailed deer tooth was correctly aged to 1.5 years old in both examinations at the inter-radicular area. The remainder of the cementum was very unclear.

226 - This animal was correctly aged in both analyses. The deer died at 6 months old. The interpretable increments were located only at the CEJ.

227 - The alveolus was maintained for this sectioning process. The first examination gave an age of 1.5 years old at the CEJ. The rest of the root was uninterpretable. The second analysis interpreted the same area as being 2.5 years old. The animal did indeed die at 2.5 years old.

228 - This animal was aged to 1.5 years and 6 months during the first analysis. The CEJ showed the most increments which the areas below it pointed to a younger deer. The second analysis aged the deer to both 2.5 years old and 1.5 years old. The correct age at death for this deer is 1.5 years old.

229 - The first examination found the deer to be 1.5 years old at the inter-radicular portion of the root. The CEJ and cement pad were unclear. The second examination was inconclusive. The animal was correctly aged during the primary analysis.

231 - The first examination gave an age of 1.5 years at the CEJ and the second examination followed suit. The age of this white-tailed deer is actually 3.5 years old.

232 - Both examinations gave more than one ages, the oldest being 2.5 years. The correct age of 1.5 years was found at the outer-radicular area during both assessments.

233 - This deer was incorrectly aged. The first examination placed it at 3.5 years old,

while the second examination placed it at 4.5 years old. The deer died at 5.5 years of age.

235 - The cementum was unclear during both analyses, resulting in no age estimations. The deer died when it was 4.5 years old.

236 - The results of both analyses were inconclusive. Cementum was present at the CEJ, but no incremental lines could be discerned. Elsewhere, the cement areas were obscure. The animal's true age was 2.5 years old.

237 - This first exam placed the animal's death at two different ages. The CEJ showed 2.5 years of increments, while only one dark annulus could be seen directly below this area. The second exam placed the animal at 3.5 years at the CEJ with the rest of the root being relatively unclear. The actual age of death was 1.5 years old.

239 - The alveolus was present for the embedding and sectioning of this first molar. The cementum could be seen in the first examination but no increments were identified. The second inspection of the thin section pointed to an age of 1.5 years old at the CEJ with the rest of the tooth being indistinct. The animal's true age was 2.5 years old at the time of its death.

240 - This deer's age or season was not assessed due to a lack of cement clarity. Its age at death was 5.5 years old.

242 - This tooth was also sectioned with the alveolus still present. The majority of the cementum was vaguely present. A single dark line was seen, but due to completely formed roots the animal had to be older than 6 months at death. The second analysis resulted in an age of 1.5 years old with the majority of the cementum being unreadable. Both analyses were wrong with the white-tailed deer being 4.5 years old.

243 - The majority of this tooth was unclear; however, the first examination did find two areas with fairly lucid results, each being at the CEJ. One age was 1.5 years old and the other was 6 months old. The next analysis could only age the animal to 6 months of age. The deer's age of death was at 6 months.

244 - During the first analysis, the cementum was reported to be the clearest at the CEJ and at the outer-radicular area. The second analysis concluded that the aforementioned areas along the root plus the inter-radicular area below the cement pad were clearest. In both instances the deer was correctly aged to 1.5 years old.

White-tailed Deer - Group 3 - Second Protocol

225 - The majority of cementum during the first analysis was reported as being present yet unclear. The inter-radicular area did show an age of 1.5 years old. The second inspection of the slide was inconclusive. The animal's actual age was 3.5 years old.

230 - There was a dark, distinct line throughout the acellular area. This correctly placed the animal's death at 6 months of age.

234 - Both inspections reported one area of the CEJ showing the deer to be 6 months old, while the other area of CEJ was unclear. The animal's age was 6 months old at the time of its death.

238 - The first analysis placed the white-tailed deer at 6 months old, reporting a fairly distinct dark increment. The second examination did age the animal to 6 months old at the CEJ with the opposite area pointing to an age of 1.5 years old. The animal was correctly aged during the initial analysis.

241 - Both analyses placed the animal appropriately at 6 months old, despite the majority of the cementum being indistinct.

White-tailed Deer - Group 2 - Third Protocol

25 - This tooth was decalcified with hydrochloric acid. Both analyses correctly aged the animal to 6 months old. The dark increment was much more distinct than seen with the non-decalcified method.

210 - This tooth was also decalcified with hydrochloric acid. Each analysis showed the same precise interpretation of the animal being 1.5 years old at death. This produced better results than the plastic and stained thin section for this specimen (see Figure 6.6).

White-tailed Deer - Archaeological - Fourth Protocol

31 - This tooth was unsectionable. The roots decalcified properly but the enamel was too firm to be cut with the microtome.

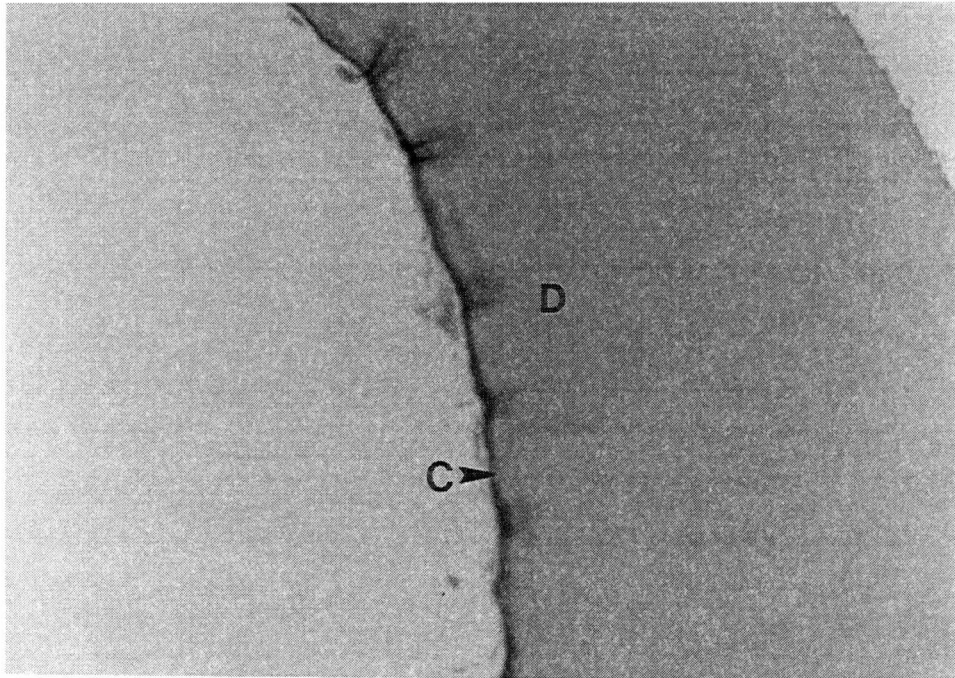


Figure 6.6: Specimen 210 - A modern decalcified tooth (at x50)
C = Cementum
D = Dentine

White-tailed Deer - Archaeological - Fifth Protocol

32 - This tooth was fully decalcified and it was properly sectioned and stained. However, the integrity of the cementum was completely gone (see Figure 6.7).

33 - The same phenomenon occurred as with the previous specimen (see Figure 6.8).

40 - The historic tooth fared better than did the prehistoric teeth. In both examinations the cementum could be seen throughout the tooth; however increments could be seen at the CEJ which aged the deer to 2 years with a spring/summer death. From an eruption/wear examination of the tooth, the animal was thought to be between 2 and 3 years old at the time of its death (see Figure 6.9).

White-tailed deer - Archaeological - Sixth Protocol

30 - This tooth was decalcified with EDTA. Embedding was never attempted for two reasons. First, the enamel was still too hard to be sectioned. Second, the roots were breaking apart from the slightest touch.

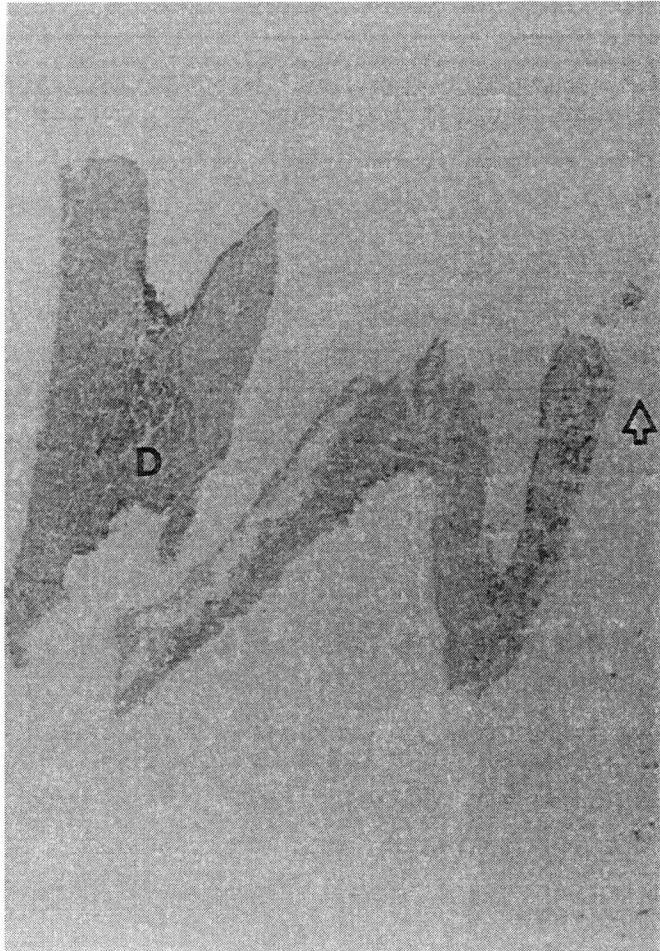


Figure 6.7: Specimen 32 - A prehistoric decalcified tooth (at x25)
D = Dentine
The arrow is pointing towards the crown of the tooth.

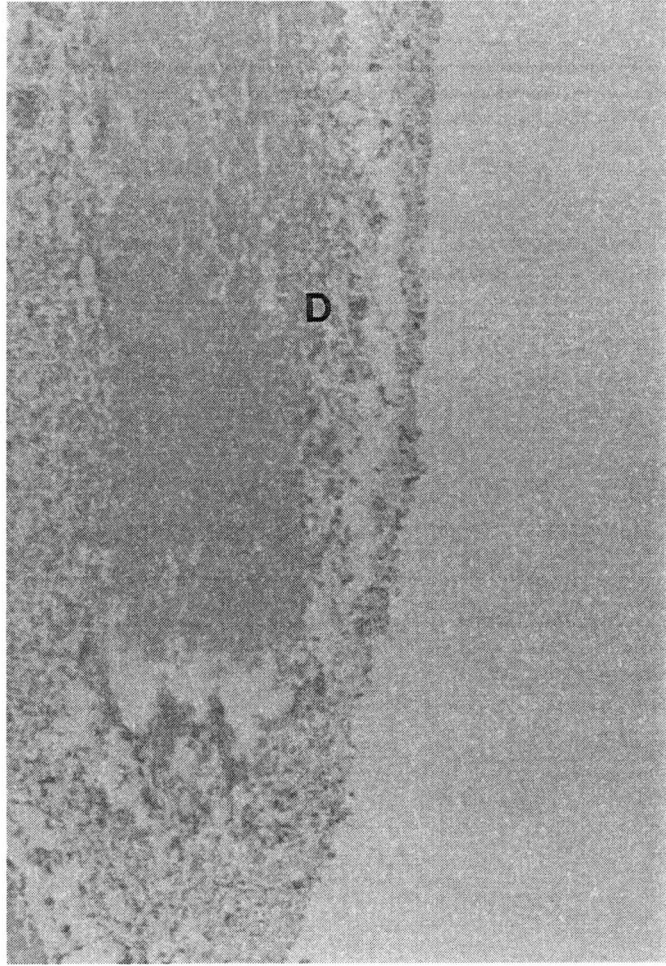


Figure 6.8: Specimen 33 - A prehistoric decalcified tooth (at x50)
D = Dentine

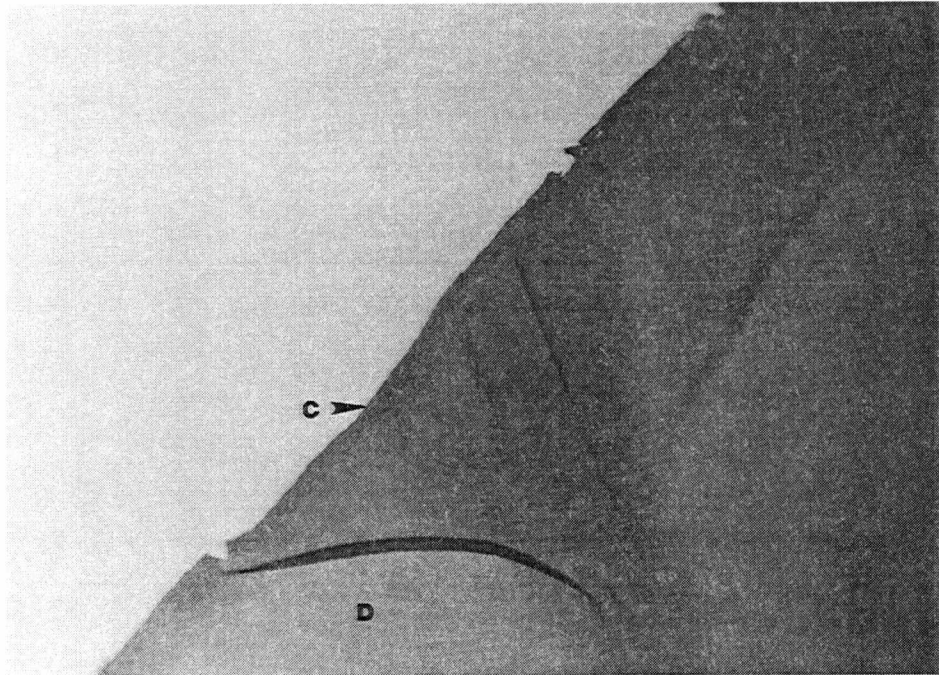


Figure 6.9: Specimen 40 - A historic decalcified tooth (at x100)
C = Cementum
D = Dentine

Table 6.1: Results of Blind Tests

ID	Sex	Method	1st Exam	2nd Exam	Actual Age and Season
10	F	min/unstain	2 y spr/sum	2.5 y fall/wint	1 y 9 m spring
11	M	min/unstain	2 y spr/sum	4 y spr/sum	3.5 y fall/wint
12	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
13	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
14	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	1 y 10 m spring
15	F	min/unstain	2.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
16	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
17	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
20	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
21	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	2.5 y fall/wint
22	F	min/unstain	-	6 m fall/wint	1.5 y fall/wint
23	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
24	F	min/unstain	-	6 m fall/wint	6 m fall/wint
25	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
26	F	min/unstain	-	-	3.5 y fall/wint
27	F	min/unstain	-	1.5 y fall/wint	1.5 y fall/wint
27	F	min/stain	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
28	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
29	F	min/unstain	1.5 y fall/wint	-	3.5 y fall/wint
210	F	min/stain	1.5 y fall/wint	6 m fall/wint	1.5 y fall/wint
210	F	decalcified	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
211	F	min/unstain	-	-	1.5 y fall/wint
212	F	min/unstain	2.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
213	M	min/unstain	2.5 y fall/wint	2.5 y fall/wint	1.5 y fall/wint
214	M	min/unstain	-	-	1.5 y fall.wint
215	M	min/unstain	6 m fall/wint	-	6 m fall/wint
216	M	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint

Table 6.1: Continued

ID	Sex	Method	1st Exam	2nd Exam	Actual Age and Season
217	M	min/unstain	2.5 y fall/wint	2.5 y fall/wint	1.5 y fall/wint
218	M	min/unstain	2.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
219	M	min/unstain	-	1.5 y fall/wint	1.5 y fall/wint
219	M	decalcified	2.5 y fall/wint	6 m fall/wint	1.5 y fall/wint
220	M	min/unstain	1.5 y fall/wint	2.5 y fall/wint	2.5 y fall/wint
221	M	min/unstain	-	2.5 y fall/wint	1.5 y fall/wint
222	M	min/unstain	1.5 y fall/wint	-	1.5 y fall/wint
223	F	min/unstain	6 m fall/wint	-	1.5 y fall/wint
224	M	min/unstain	1.5 y fall/wint	1.5 y fall/wint	1.5 y fall/wint
225	F	min/unstain	1.5 y fall/wint	-	3.5 y fall/wint
226	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
227	M	min/unstain	1.5 y fall/wint	2.5 y fall/wint	2.5 y fall/wint
228	F	min/unstain	1.5 y fall/wint	2.5 y fall/wint	1.5 y fall/wint
229	F	min/unstain	1.5 y fall/wint	-	1.5 y fall/wint
230	M	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
231	F	min/unstain	1.5 y fall/wint	1.5 y fall/wint	3.5 y fall/wint
232	F	min/unstain	2.5 y fall/wint	2.5 y fall/wint	1.5 y fall/wint
233	F	min/unstain	3.5 y fall/wint	4.5 y fall/wint	5.5 y fall/wint
234	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
235	F	min/unstain	-	-	4.5 y fall/wint
236	M	min/unstain	-	-	2.5 y fall/wint
237	F	min/unstain	2.5 y fall/wint	3.5 y fall/wint	1.5 y fall/wint
238	F	min/unstain	6 m fall/wint	1.5 y fall/wint	6 m fall/wint
239	F	min/unstain		1.5 y fall/wint	2.5 y fall/wint
240	F	min/unstain	-	-	5.5 y fall/wint
241	F	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
242	F	min/unstain	6 m fall/wint	1.5 y fall/wint	4.5 y fall/wint

Table 6.1: Continued

ID	Sex	Method	1st Exam	2nd Exam	Actual Age and Season
243	F	min/unstain	1.5 y fall/wint	6 m fall/wint	6 m fall/wint
244	M	min/unstain	6 m fall/wint	6 m fall/wint	6 m fall/wint
30	-	decalcified	-	-	-
31	-	decalcified	-	-	-
32	-	decalcified	-	-	-
33	-	decalcified	-	-	-
40	-	decalcified	2 y spr/sum	2 y spr/sum	2 to 3 y

Table 6.2: Percentage of Results for Protocols 1 and 2, Mineralized and Unstained

Species	Protocol	Test	Percentage
<i>Dama dama</i>	1	A	50%
<i>Dama dama</i>	1	B	62.5%
<i>Odocoileus virginianus</i>	1	A	16%
<i>Odocoileus virginianus</i>	1	B	42%
<i>Odocoileus virginianus</i>	2	A	60%
<i>Odocoileus virginianus</i>	2	B	80%
<i>Dama dama</i> and <i>Odocoileus virginianus</i>	1 and 2	A	25%
<i>Dama dama</i> and <i>Odocoileus virginianus</i>	1 and 2	B	49%
<i>Dama dama</i> and <i>Odocoileus virginianus</i>	1 and 2	C	8%
<i>Dama dama</i> and <i>Odocoileus virginianus</i>	1 and 2	D	30%

A = The percentage of animals that were accurately aged in both blind tests with only one answer given for each analysis.

B = The percentage of animals that were accurately aged in at least one blind test with only one answer given in the correct analysis.

C = The percentage of animals, minus the 6 month olds, that were accurately aged in both blind tests with only one answer given for each analysis.

D = The percentage of animals, minus the 6 month olds, that were accurately aged in at least one blind test with only one answer given in the correct analysis.

Table 6.3: Analysis of White-tailed Deer (Mineralized/Unstained) by Age Categories

Category	6 months	1.5 years	2.5 years	3.5 years	4.5 years
1	64%	11%	0%	0%	0%
2	100%	42%	1%	0%	0%

Category 1 = The percentage of animals that were accurately aged in both blind tests with only one answer given for each analysis

Category 2 = The percentage of animals that were accurately aged in at least one blind test with only one answer in the correct analysis

Table 6.4: Analysis of Cementum Area that Demonstrated a Correct Age Assessment

Area	Percentage for Fallow	Percentage for White-tailed	Percentage for Fallow and White-tailed
A	80%	76%	77%
B	20%	2%	6%
C	30%	33%	33%
D	60%	57%	58%

Area A = cemento-enamel junction (cej)

Area B = cement pad

Area C = inter-radicular area

Area D = outer-radicular area

Summary

The results of this project were numerous and detailed. The particulars of the results and their ramifications for archaeologists will be discussed in the following chapter. However, as a summary point, an emphasis must be placed on the fact that the resin methods provided poor results for modern specimens. Demineralization of modern teeth worked well using hydrochloric acid. Prehistoric teeth were destroyed during demineralization, yet the cementum of historic teeth fared well when fixed and decalcified.

Chapter 7

Discussion

First Goal: Methodologies

Numerous aspects of the methods were explored. With six protocols, each facet of the research had to be carefully explored, making sure that the results were not due to a lack of controls but rather to valid interpretations.

Inconsistencies

One immediate problem concerns the fact that the cementum is never seen continuously around the root (see Figure 7.1). This is worrisome because one cannot accurately age a tooth due to the possibility of resorption areas or split lines without a convergence point. Cementum behaving in this manner could be because of two possibilities. The first is destruction. The cement covers the root and would be exposed to post-mortem diagenesis, especially in archaeological teeth. This would also make it nearly impossible to say with certainty that the outermost layer represents the last season of the animal's life. This last layer could easily be worn away. The fallow deer molars, which were examined first, were removed from the mandibles with pliers by cracking the bone away from the tooth. It was thought that the lack of visual continuity was possibly due to destruction of the cement by the pliers. Therefore, the group of white-tailed deer that were examined next were thin sectioned with the mandibles intact. Despite this precaution, in most instances this still demonstrated either incongruous annulations or a complete lack of lines.

The second theory of variable incremental lines concerns the nature of cement

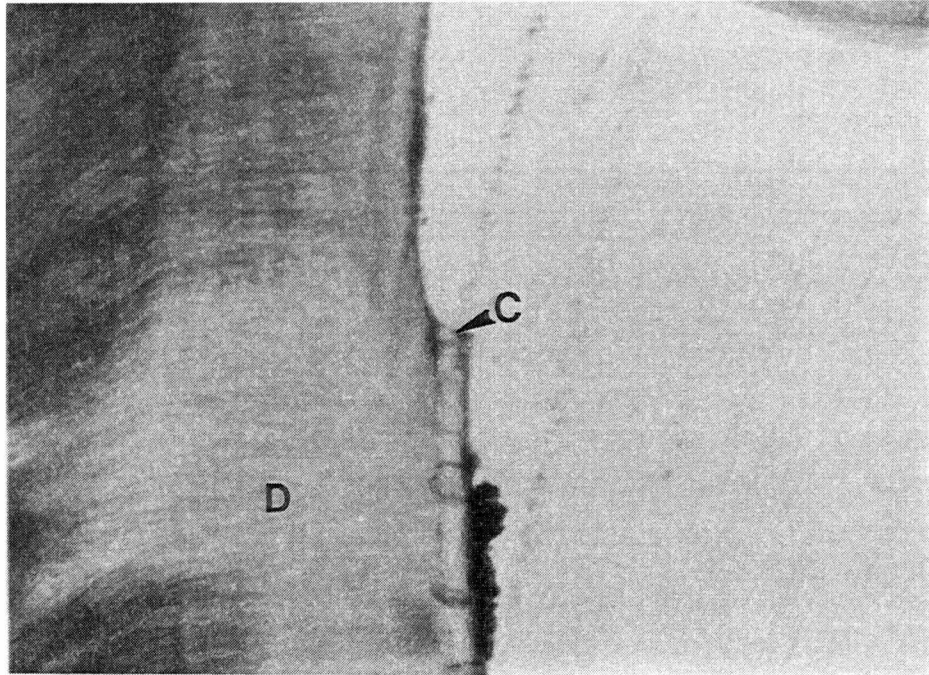


Figure 7.1: Broken cementum (at x50)
C = Cementum
D = Dentine

itself. One must first remember that cement is laid down throughout a mammal's life to offset wear. Its other function is to attach the periodontal ligament to the root surface of the tooth. Since the periodontal ligament is highly mobile and reacts to stress and mechanical changes, as seen in orthodontics, it is possible that cement reacts in a similar manner. Cement is not turned over constantly as bone is, but it is remodelled by cementoblasts (Hillson 1996). If an excess of stress is placed upon one area of the tooth, the cement may build up in that area more rapidly and definitively; therefore, providing the best area for visualization. Since the teeth are hypsodont, continuing to erupt throughout the animal's life to compensate for occlusal attrition, stress from wear may or may not be constant due to food type and availability (Chapman and Chapman 1970). Along these same lines, Oetelaar (1981) notes that resorption of the cementum also can occur as a result of mesial drift or dental traumas. Hillson (1996) states that because of these incongruencies it is difficult to discern what features are being counted in annulation studies.

These ideas could explain why cementum is seen more often in some areas than others and why certain areas are more apt to be correct (see Table 6.4). By examining the data concerning the best area of the root to observe cementum annuli, the percentages show that the cemento-enamel junction (CEJ) was by far the most accurate inspection point (see Figure 7.2). This holds true for both species of deer. This fact correlates with what Buie (1986) and Zanon (1996) found in their analyses of white-tailed deer. Similarly, Burke and Castanet (1995) asserted that the best place for visualization of horse cementum was directly below the gumline. Other researchers (Lockard 1972; Koike and Ohtaishi 1985) claim that the best place to examine the cementum is at the cement pad. However, for this analysis, the cement pad was the

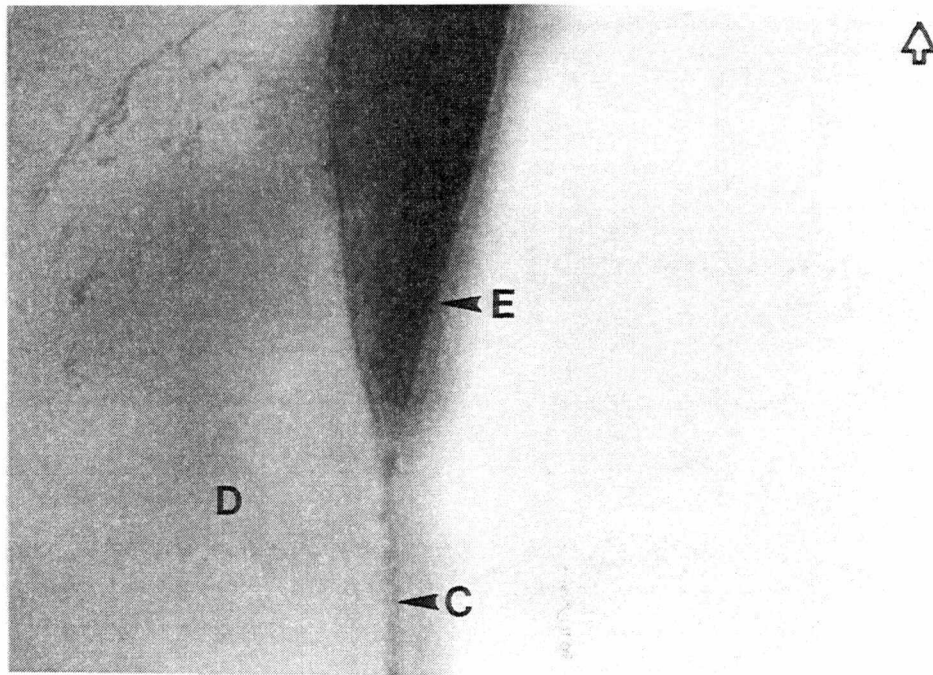


Figure 7.2: The cemento-enamel junction (at x50)

C = Cementum

D = Dentine

E = Enamel

The arrow is pointing towards the crown of the tooth.

worst place for identifying clear, visible increments.

At the beginning of the blind tests it was unknown that the CEJ and outer-radicular surface would produce the best results. Therefore, a decision had to be made concerning how to determine age and season of death for the deer. For consistency the oldest age was chosen when there was a within root discrepancy. An initial choice had to be made to either risk over-aging from split lines or under-aging because of resorption areas. In retrospect, the best way to handle this would be to accept the age interpreted at the cemento-enamel junction whenever possible.

There are also problems with interpreting the annuli that *can* be seen. This process is highly variable and is subject to inter-observer biases. Other people who had prior experience with cementum increment studies examined the slides. There were always inconsistencies with these examinations, although the results were not mentioned in this paper due to the non-systematic nature of the second and third party analyses. Landon and co-workers (n.d.) also noted this type of problem in a study of gray wolves (*Canis lupus*). Three observers counted the cementum increments in a sample of 12 known-age wolves. They found that observer 1, the least experienced with this technique, produced the best results while observer 3, the most experienced, was consistently inaccurate.

During blind tests performed solely by the author, the results often varied between observations (see Table 6.1). Even when incremental lines are present, it is easy for the same area to produce variable conclusions. As mentioned previously, Owens (1980) asserts that the area which lies at the dentinal junction may in actuality be a layer of unmineralized dentine, not cementum. This, along with the uncertainty of when cementum begins the formation process can confuse season and age at death

analyses (Frederick 1966; Grue and Jensen 1979). Cementogenesis commences before eruption, which in turn would throw off the starting point that one uses to age the deer.

Mineralized Technique

The percentage of a completely accurate interpretation for both fallow and white-tailed deer using the mineralized/unstained method is very low at 25%. When taking out the 6 month old deer, the clearest and easiest to decipher, the percentage drops to only 8% (see Table 6.2). This is not acceptable for aging animals in this manner. The lack of clarity and consistency in the increments leads one to believe that the method is far from foolproof.

Less than satisfactory results were also found by Buie (1986). He reported a 30% accuracy rate for the white-tailed deer that he examined. Not only did he experience difficulties in interpreting the date of death due to conflicting increments, he also found that finding a true age for when formation begins was over-generalized. This simplification of cementogenesis could distort the entire estimation of age and season at death.

These results are much different than the results reported by Lockard (1972). He found that bands could be interpreted in 88% of his sample group of white-tailed deer molars. However, he also postulates that the increments are more readily seen in teeth of animals from cold climates than those from warm areas. This could be one explanation for the difficulties in discerning true and accurate annuli in this study.

It is also obvious, during an examination of the white-tailed deer, that the older an animal gets, the more difficult it is to discern annulations (see Table 6.3). Similar assessments were also found in studies performed by Buie (1986), De Young (1989),

and Weinand (1997). For this study, deer placed in the 6 month old category were completely unerring in 64% of the animals and accurate in at least one test 100% of the time (see Figure 7.3). The accuracy rate begins to quickly fall as an animal's age increases with a 0% accuracy rate for animals aged 4.5 years and older. Over time, cementogenesis must become highly variable and complicated due to factors of formation that must be scientifically explored.

There did not seem to be much advantage to using one mineralized thin sectioning technique over another, referring to the first and second protocols. There were pros and cons to both methods. The saw used in the first protocol (Leitz) was much speedier than the second saw, yet the second saw (Buehler) allowed one to see exactly where the cut was going to be made. If cost is a factor in buying equipment, then purchasing the Buehler saw would be appropriate. Neither saw could cut a section thin enough where grinding and polishing would not be a necessity.

Staining the slides with Toluidine Blue did slightly enhance the cementum. However, it did not bring forth any areas of interpretable increments that were not visible prior to staining. Spinage (1976) and Koike and Ohtaishi (1985) found that staining could alter the slides in a negative manner. They asserted that this process could create unstable images or falsely change the appearance of the annuli. Therefore, this is a step that can be avoided if one desires.

Similarly, polarized light did make the annuli slightly more contrasting as to whether they appeared as light or dark lines. However, this did not enhance the cementum enough to where it made a difference in results. Both transmitted and polarized light produced the same end result for each interpretation.

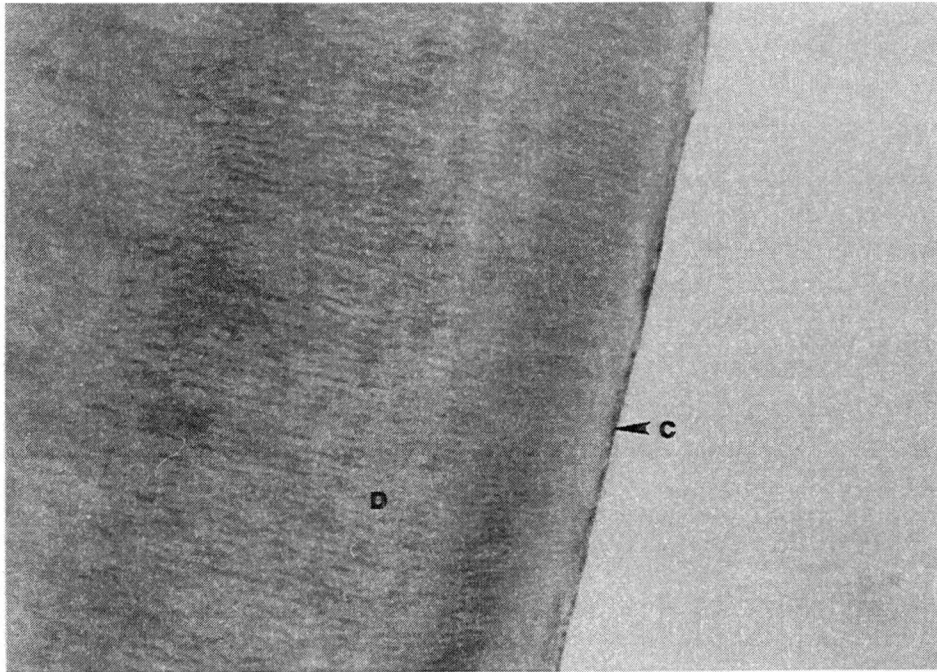


Figure 7.3: The first cemental increment in a six month old deer (at x100)
C = Cementum
D = Dentine

Decalcified Technique

Although only a few modern specimens were examined using the decalcification method, the results appear to be as accurate as reported in much of the wildlife biology literature (Fancy 1980; Bookhout 1994). For modern samples, decalcifying and embedding in hydrochloric acid is an acceptable, rapid, and precise method of aging a deer with cementum (see Figure 6.6). Contrasting to what Fancy (1980) and Naylor et al., (1985) assert, this method produces results more quickly than the plastic, nondecalcified protocol. One can examine a slide under the microscope in approximately 52 hours using this technique, whereas a section made using the plastic method can easily take over a week or two to get to the microscope stage.

The fixative/decalcifying Solution F from Baxter performed best when decalcifying archaeological teeth. Fixation did seem to somewhat protect the structure of the archaeological teeth as Fancy (1980) and Oetelaar (1981) asserted in their studies. Although the annulation results were poor for prehistoric specimens, this was the foremost solution for preparing the tooth for the embedding process without destruction.

The white-tailed deer tooth from the Gibbs site produced a rather promising outcome. Although only one historic tooth was examined using this technique, the integrity of the cement was still intact and increments could be seen in a small portion of the root. This may point to the decalcifying technique being the most appropriate for historic specimens. Similarly, Savelle and Beattie (1982) found this to be the case. Decalcification produced dependable results for historic muskoxen teeth, but it destroyed the prehistoric ones.

Second Goal: Applicability to Archaeology

This section of the discussion is truly the crux of the analysis. Taking known phenomenon from the present and applying it to occurrences in the past is the basis for processual archaeological theory. Without legitimate modern collections, credible interpretations about the past cannot be made.

Seasonality

Seasonality assessments seem to be more readily interpretable than aging evaluations; however, many concerns should still be addressed. If cementum increments could be seen in the thin sections, the correct season of death was usually ascertained. Upon occasion the last increment visually appeared different in opposite areas of the root. However, if a specimen clearly showed either an opaque or translucent increment at death, without conflicting interpretations, the analysis most likely proved to be accurate.

One must exercise caution when doing a seasonality assessment because of the lack of knowledge concerning formation processes. As Lockard mentions (1972), it is possible that the beginning and ending times for growth and arrest lines may differ between and among species. To compound the problem, this may also present obstacles in interpreting increments from like species in a similar environment (Buie 1986). It is also very likely that the increment formation rate could vary from year to year and as the animal ages (Frederick 1966). This could readily confuse an interpretation of seasonality and question the validity of using modern collections as a comparative basis for archaeological teeth.

The majority of this sample contained white-tailed deer and fallow deer that died

in the fall/winter. When the cementum was clear, this could be interpreted rather easily, with the exception of one fallow deer. However, two of the fallow deer died in the spring, yet three out of four blind tests interpreted the sections as being fall/winter deaths. Although March and April (their months of death) are considered to be spring months, the increments may not enter a growth phase until late spring/early summer for this species in this environment. The phenomenon may be so varied from one case to the next that the uniformitarian assumption may not accurately apply to cementum annuli seasonality studies in archaeology.

Aging

The cementum increment method for aging should only be used under certain circumstances by archaeologists. There are too many problems, especially when dealing with teeth that could be thousands of years old. No archaeological conclusions concerning age at death should be drawn unless the cementum can be seen around the majority of the acellular root area. The next imperative is that more than one observer perform blind tests and that each researcher involved interprets the annulations in the same manner. Until the time a method is devised where there is a very high accuracy rate for comparative collections, prehistoric archaeologists should not use mineralized tissue histology in this manner to draw conclusions about past cultures.

If archaeologists can efficiently use the decalcified technique as wildlife biologists have, this form of analysis would be the most feasible. This study points to this protocol as being the most likely scenario for historic teeth. However, the mineralized method is less than consistent. Information from this study shows that it is possible to see cement lines in a 200 year old tooth from Eastern Tennessee; however,

one must remember that increments could not be gleaned from this specimen throughout the cement. Only a small area produced visible lines and this creates room for doubt and inconsistencies. Although increments could be seen in the historic tooth, it was not quite as clear as the modern decalcified teeth.

Mediocre aging methods should not warrant the destruction of an artifact. If the cementum annuli method is used as a possible verification of evidence that is already present, it is suggested that the tooth be longitudinally cut in half and first decalcified. If that proves to be ineffective, the other half of the tooth could be embedded in plastic and thin sectioned. If the decalcification method produced reliable results, at least part of the tooth would be preserved.

If archaeologists want to gain the respect that they deserve from science, they should not borrow other disciplines' methodologies only to warp them and force them to fit an archaeological agenda. Archaeologists must completely understand the nature behind formation processes and how to interpret this knowledge before any attempts are made at assessing seasonality or age analyses. Unless this method provides one with the confidence to say, within the boundaries of scientific knowledge, these results point to a probable occurrence in the past, it should not be employed. However, if a cementum increment analysis only partially works in the realm of anthropology, researchers should accept that fact and definitive interpretations should not be published.

Third Goal: Fallow Deer Versus White-tailed Deer

As for using white-tailed deer as a comparative tool at the family level for fallow deer, nothing in this study can support that hypothesis. If the annuli cannot be counted with more confidence than was done here, it would be impossible to make such

conjectures.

As mentioned throughout this paper, there are many technologically and analytically tedious aspects of a cementum increment investigation that a researcher must contend with. Before attempting to compare and contrast species, the first and main concern is to understand how to read and interpret the slides. It is difficult to visualize what other people have written about until actually looking at thin sections for one's self. Even published photographs can be deceiving because the best sections at the clearest cementum area are usually the only ones shown. This is deceptive in that it does not represent an accurate picture of someone's research if another person wants to replicate his/her methods of analysis.

Matson (1981) compiled a workbook for analyzing thin sections from various mammals. This is helpful in that it tells researchers where difficulties may lie. However, the problems and formation patterns for each population of animals may differ within species due to the complicated variables behind formation itself. Because of these inconsistencies, it is doubtful that white-tailed deer teeth could be used in lieu of fallow deer teeth.

Summary

The three goals of this project can be easily summarized despite the myriad of inter-related discussions surrounding each topic. First, as for the best protocol to use, decalcification has proven to be promising for modern and historic specimens. Second, archaeologists should first concentrate on understanding formation. Until that time, prehistoric teeth should not be analyzed and historic teeth should only be studied using extreme caution with regard to interpretation. Third, no conclusions can be drawn for

using deer within the same family to expedite research.

The author must insist that archaeologists would produce more reliable site interpretations by focusing their energies into other forms of seasonality and age assessments. By weighing the lack of credible results with the inevitable destruction of faunal artifacts, cementum increment research is obviously not the best route for gaining knowledge about the past.

Chapter 8

Conclusions

Theoretically, using cementum annuli to aid archaeological analyses of age and season at death is feasible. Practically, for fallow deer and white-tailed deer from Tennessee, the mineralized method does not provide confident results. The decalcified method destroys the prehistoric teeth, yet there is room for hope and further exploration for historic specimens. Where does this leave archaeologists? It leaves archaeologists with the knowledge that the decalcification protocol, which is the most accurate method, can *possibly* work for our discipline under specific, yet unknown, circumstances. With regard to historic teeth, it is emphasized that the decalcification method be the protocol most extensively explored.

The author can only say that the methods investigated for this project work, to the extent that they do or do not, for these two species in this environment. This must be made apparent due to the likelihood that the environment and nature of each species influences cementum formation. Before cementum annuli methods can be applied to the past with confidence, knowledge must first be gained in the realm of cementum formation. As Morey (1982:144) stated, "Ideas pertaining to seasonality are only as good as empirical testing demonstrates." It must be stressed that cementum increments are very precarious and that seasonality and aging analyses be made with caution.

Future Research

Although many methods were used in this study, there are still a few possible analyses that could be performed in the future to shed light upon this subject. This type

of research, especially in the realm of archaeology, has a long way to go before it can be completely substantiated. Some ideas on this subject are discussed in the following sections.

Scanning Electron Microscopy

It is a possibility that light or polarized microscopy, which are the standard microscopic tools, may not provide the necessary perspective to confirm or discount the use of annulations as a viable aging and seasonality agent. In order to provide a more thorough analysis, scanning electron microscopy (SEM) could be used. This produces a topographical view of the cementum for determining whether or not the outer portion of the roots are manifested reliably under a microscope.

SEM would expose the teeth in a structural, multi-dimensional manner. The scanning electron microscope contains a narrow electron beam that moves sequentially across the surface of the tooth. It reflects or emits electrons which produces a three dimensional image (Junqueira et. al., 1995). SEM does not require an in depth pre-treatment. Therefore, it could show the annulations without interruptions if that is the actual nature of the cementum. If there are still cementum areas with missing or resorbed annulations, this would prove that the inconsistencies are not the result of excessive manipulation or processing, but rather a manifestation of biological mechanisms within the tooth itself.

For this type of research Environmental Scanning Electron Microscopy (ESEM) would be the best to use. This method, which was first introduced in the late 1980s, allows for the visualization of hydrated specimens. The advantage of today's ESEM is that specimens can be observed in their native state without fixation, dehydration, or

drying (Dykstra 1992). Prior to this, the examination of bone or teeth required in depth treatments such as dissection, fixation, freezing, drying, coating, demineralization, and acid etching (Boyde and Jones 1974).

Determining the Boundaries of Archaeological Age

The decalcified technique seems to produce the clearest results for modern specimens in this study and throughout the literature. The one historic tooth examined in this study also points to the feasibility of this technique for archaeological specimens that are 200 years old. An obvious step would be to examine a series of archaeological teeth that range in age to determine how old a tooth is when the integrity of the cement and the organic component decline. If the method provides clues to the age and season of death for an animal that is 200 years old, what will a 300 year old specimen look like under a microscope? If someone inspects a series of teeth in this manner, taking depositional environment into consideration, the boundaries of a satisfactory cementum increment examination may be more clearly understood.

Fixing and Embedding Fresh Mandibles

Another beneficial analysis could come from fixing a fresh deer mandible and embedding the hard and soft tissue in plastic resin before thin sectioning. This would maintain the position of the periodontal ligament, which is in direct correlation to the areas of cementogenesis and resorption. If the cementum appears clear at this stage, then some of the interpretive problems most likely would lie during decay or manipulation, in which case archaeological interpretations would be even more arduous.

Labelling Cementogenesis

There is much more to cementum formation than simply comparing like species in a like environment. The main point of concern is to first determine *why* these increments form or do not form as they do. One needs to do a comprehensive study on the biological and environmental hypotheses concerning formation. Once formation has truly been understood, then middle range theory can be explored for using modern samples as comparative tools for archaeological fauna.

Since cementum reacts similarly to bone (Hillson 1996), it may be feasible to label the cementum increments as they are being laid down by cementocytes in a living population of animals. By alternating doses of labelling agents such as calcein and tetracycline, one could determine the growth and resorption rates for particular species.

Summary

Much information was gathered from this project and can be succinctly summarized:

- Undecalcified thin sections produced divergent and unreliable results.
- As the deer age, their increments are less discernable.
- Often, one tooth would exhibit more than one age.
- The cementum was never continuously seen around the root.
- The cemento-enamel junction is the best area to glean correct age and seasonality assessments.
- Decalcified thin sections worked well for modern and historic specimens, but destroyed prehistoric teeth.
- No correlations could be drawn for using a white-tailed deer comparative collection to

interpret archaeological fallow deer.

The inconsistencies found within this analysis could be explained in three ways. First, this researcher interpreted her thin sections wrong. This is doubtful because both her preparation and analytical techniques were monitored by researchers who are well-trained in histology. Second, the environment of Tennessee is such that cementum increments in this sample of fallow and white-tailed deer did not form in a predictable manner. This could be an integral aspect of the problem. However, without understanding all of the influences on formation, cementum increment analyses cannot be completely controlled despite the environmental conditions. Third, this method of assessing age and seasonality profiles is unreliable, inconsistent, and problematic. Unfortunately for scientists who have dedicated time to studying cementum increments, this is where the research stands.

The main priority in this type of research must be to understand formation processes. Until these processes can be more fully comprehended, archaeologists should not publish material concerning the interpretation of sites in this manner. If formation processes are discerned, future research may best be conducted on historic specimens using the decalcified technique. This scenario is one that many prehistoric archaeologists do not want to face, especially for those numerous researchers who already have their names attached to this form of interpretation. It must again be emphatically stressed that archaeologists should not use cementum increment analyses at this time. The method simply does not work well enough to engage in valid interpretations concerning the past. There are too many unknowns associated with cementum that can no longer be ignored. It is very important that archaeological conjectures be based on scientific facts, not partial truths.

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Appendices

Appendix A

Materials

Table A.1: Materials and Equipment Used in the First Protocol

Process	Material/Equipment
Dehydration and Polymerization	Alcohol
	Acetone
	Cyclohexene dioxide
	Diglycidyl ether of polypropylene glycol
	Nonenyl succinic anhydride
	Dimethylaminoethanol
	Plastic or aluminum cups that withstand low grade heat
	American Scientific drying oven DX-58
Cutting	Leitz 1600 diamond blade saw
Mounting	Plastic or glass slides
	Clear epoxy
Grinding and Polishing	Mark V Lab grinder and polisher
	1500 and 600 grit sandpaper
	velvet
	Liquid aluminum

Table A.2: Materials and Equipment Used in the Staining Process of the First Protocol

Process	Material/Equipment
Creating the staining solution	Toludine Blue
	Distilled water
	Paper filter
	Sodium carbonate
Staining the slide	Corning PC 351 stirrer/hot plate

Table A.3: Materials and Equipment Used in the Second Protocol

Process	Material/Equipment
Cutting	Buehler Isomet Slow Speed Saw
	Buehler diamond wafering blade
	Buehler isocut fluid
Mounting	Glass slides
	Cyanoacrylate adhesive (Superglue)
	Alcohol

Table A.4: Materials and Equipment Used in the Third Protocol

Process	Material/Equipment
Decalcification	Hydrochloric acid
	Plastic or glass container
	Corning PC 351 stirrer/hot plate
	Needle
Embedding	Alcohol (varying percentages)
	Xylene
	Paraffin
	Shandon Citadel 2000
	Embedding cassette
	Shandon Embedding Center
Sectioning and Mounting	American Optical 82D Spencer Microtome
	Fisher TissuePrep Flotation Bath
	Glass slides
Paraffin Removal and Staining	Alcohol
	Water
	Hematoxylin
	Ammonia water
	Eosin-y
	Xylene

Appendix B

Datasheet for Blind Tests

ID	Condition of Slide	Interpretive Comments	Drawing of Root	Age	Season

Species _____ Date of Analysis _____

Method of Slide Preparation _____

Appendix C

Nomenclature

Acellular cementum - The area of cementum that begins at the cemento-enamel junction and extends approximately halfway down the root until it comes into contact with the cellular cementum. It is the portion of cementum used in aging and seasonality research (Avery 1992).

Acidophilic - Tissues that easily stain with acidic compounds. Eosin-y is one such stain (Junqueira et. al., 1995).

Alveolus - The part of the maxilla and mandible that contains the tooth sockets.

Ameloblast - Cells responsible for enamel production. Produced by the Shield of Hertwig (Avery 1992).

Basophilic - Tissues that easily stain with basic compounds. Hematoxylin is one such stain (Junqueira et. al., 1995).

Canaliculi - The area of cementocytes that contains cytoplasmic projections. They allow for nutrient flow (Avery 1992).

Cellular cementum - The cementum that covers the apical portion of the root and provides the necessary support due to stress, wear, and mastication. It is not appropriate for aging or seasonality studies (Lieberman 1994).

Cementoblast - A large cube-shaped cell that actively forms the cementum (Avery 1992).

Cementocyte - A cell located in the lacuna of cellular cementum whose purpose is to maintain the cementum (Avery 1992).

Decalcified sectioning - See *demineralized sectioning*.

Demineralized sectioning - This may also be referred to as decalcified, paraffin, or standard histological sectioning. In this process the mineral component of the tooth is removed, leaving a “rubber-like” substance that easily conforms to the paraffin.

Dentin - The area of the tooth surrounding the pulp chamber and underlying the enamel and cementum. It is 70% inorganic, consisting of hydroxyapatite, carbonate, magnesium, and fluoride. It contains 20% organic material and 10% water (Avery 1992).

Enamel - The hardest tissue in mammalian bodies. It gains its strength from interlocking rods. It covers the exposed tooth surface and is composed of 96% inorganic material (hydroxyapatite) and 4% water and the protein enamelin (Avery 1992).

Eosin-Y - A counter stain that tints acidophilic tissues. It is used in decalcified

sectioning (Junqueira et. al., 1995).

Fixation - A process using formalin and/or formaldehyde that preserves the tissue morphology and molecular composition of said tissues (Junqueira et. al., 1995).

Granular layer of tomes - A zone underlying the cementum that appears grainy under a microscope. It is likely caused by a merging of the dentinal tubules (Avery 1992).

Ground sectioning - See *mineralized sectioning*.

Hematoxylin - A nuclear stain that tints basophilic tissues. It is used in decalcified sectioning (Junqueira et. al., 1995).

Intermediate cementum - Also referred to as cementoid. Deposited by the shield of Hertwig during root formation. After this process it disintegrates. A premineralized substance that occurs before the actual cementum is laid down (Avery 1992).

Lacuna - A small cavity within the cementum that contains a cementocyte (Avery 1992).

Light microscopy - Allows for visualization based on an interaction of photons and tissues (Junqueira et. al., 1995).

Mineralized sectioning - This may also be referred to as ground or plastic sectioning. It is a thin sectioning method, whereby the tooth is dehydrated, embedded in plastic, and cut. The tooth is fully calcified during this process.

Odontoblast - Cells that produce dentin. They are formed by the Shield of Hertwig (Avery 1992).

Paraffin sectioning - See *demineralized sectioning*.

Periodontal ligament - A connective tissue that adheres the tooth to the alveolus by way of Sharpey's fibers. The periodontal ligament is composed of collagenous fiber bundles (Avery 1992).

Plastic sectioning - See *mineralized sectioning*.

Polarized microscopy - Using two filters that normally would not allow light to pass. However, when oriented tissue structures are located between the filters, the axis of the light is rotated. This produces bright structures against a dark background (Junqueira et. al., 1995).

Pulp chamber - The area that contains the "living" constituents of the tooth. The pulp consists of blood vessels, nerves, connective tissue, and various cells (Avery 1992).

Scanning electron microscopy - (a.k.a. SEM) A narrow electron beam is moved

sequentially across the surface to be examined. It reflects or emits electrons which produces a three dimensional image (Junqueira et. al., 1995).

Sharpey's fibers - Perforating fibers found in the alveolar bone. They are an intermediary attachment point between the alveolus and the tooth (Junqueira et. al., 1995).

Shield of Hertwig - (a.k.a. Hertwig's sheath) An epithelial root sheath that begins at the cemento-enamel junction in the root. It is where odontoblast production occurs. It is also found in the inner cell layer of the crown where it forms ameloblasts. After these physiological occurrences, the shield disintegrates and cementum formation begins (Avery 1992).

Standard histological sectioning - See *demineralized sectioning*.

Toluidine Blue - A stain used in plastic, mineralized sectioning.

Vita

Mary Benedix was born in Memphis, Tennessee on December 31, 1971. While in Memphis, she attended Saint Louis School and Saint Agnes Academy. She lived there until she graduated from high school in 1990. At that time she moved to Knoxville, Tennessee and attended college at the University of Tennessee, majoring in anthropology. During her undergraduate career, Mary emphasized studies in archaeology. She received her bachelor of arts in anthropology in May of 1994.

Mary began graduate school the following August at the University of Tennessee, Knoxville, where she continued her education in anthropology. She excavated at Grotte XVI, a prehistoric site in southwest France, during the summers. This fostered an interest in prehistoric archaeology and zooarchaeology. Mary also held a graduate assistant position from 1996 to 1998 at the Electron Microscopy Laboratory and the Oral and Maxillofacial Surgery Department at the University of Tennessee Medical Center. There she acquired the necessary skills to carry out her research in mineralized tissue histology. In May of 1998 Mary received the degree of master of arts in anthropology.