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Aging pronghorn antelope by the incisor cementum

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AGING PRONGHORN ANTELOPE BY THE INCISOR CEMENTUM

by

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INTRODUCTION

A reliable technique for aging pronghorn antelope (Antilocapra americana) would be valuable for their effective management. Two previous studies of aging have been made on pronghorn. Dow and Wright (1962), using known-age animals, developed a method based upon the sequence of tooth succession and the rate of wear of mandibular dentition. Because of a lack of known-age animals over 6 years of age this method was uncertain for the older animals. There was also some doubt on the accuracy of aging the 3 1/3 and 4 1/3 year age classes. Koleonsky and Miller (1962) described a technique for aging pronghorns by the growth of the lens but lacked known-age animals with which to verify the method.

For the past several years studies on aging mammals have been based on changes in the microscopic structure of the dentine and cementum of the teeth. Scheffer (1950) examined the skull of a known-age Alaskan fur seal (Callorhinus ursinus) and noticed "concentric ridges" around the roots of the teeth. The number of ridges observed corresponded to the age of this seal in years. Further examination showed that the growth ridges were formed by the pattern of winter dentine deposition. A thin layer of cementum covered the ridges. An accurate method for aging was developed using the growth ridges of dentine in the canine teeth for fur seals up to 4 years of age. To establish the technique 75 known-age animals were used. Other teeth of the jaw were found to have dentinal ridges. The teeth of other Pinnipeds were also noted as having growth ridges.

Laws (1952, 1953), concurrently with and independently of Scheffer, developed a reliable method of aging the elephant seal (Mirounga leonina) by examination of ground and polished sections of the canines. The dentine was observed to have a banding of alternating zones of light and dark deposits. It was established that these bands had an annular and regular pattern and could be correlated with age. Laws (1952, 1953) later examined the teeth of seals, whales and a number of other vertebrates, living and fossil, and found internal rings or external ridges which could possibly be correlated with age.

A technique for aging moose (Alces alces) by the cementum of the first incisor teeth was developed by Sergeant and Pimlott (1959). The incisors were cut longitudinally and polished. Although dentine layers could be seen, the boundaries of these layers were indistinct and thus could not be counted with accuracy. In the area of the root where the cement was thickest, alternating opaque and translucent zones of the cementum could be readily counted. The number of cementum zones and the maximum thickness of the cementum was correlated with age in years. This method was verified by a single known-age animal.

Hewer (1960) in an aging study of grey seals (Halichoerus grypus) correlated the rings in the cementum in the canine teeth with age in known-age animals.

A study of aging mule and black-tailed deer (Odocoileus hemionus) by the use of the incisors was described by Low and Cowan (1963). The first incisor teeth were sectioned and stained with hematoxilyn using standard histological technique. This method resulted in a more detailed knowledge of the structure of the annual layers in the cementum. There was found, in the cementum, a pale-staining zone of rapid increment

that was added during the spring and summer and a dark-staining zone formed by the reduction in increment rate during the fall and winter. The deer were aged by a count of these zones. Each pair of contrasting zones represented one year. The reliability of this technique was established by correctly aging 20 known-age animals.

A number of other studies have been made using the cementum of the teeth to age various animals. McEwan (1963) developed a microtechnique for examining the cemental increment lines to age caribou (Rangifer tarandus). Mitchell (1963), in an investigation of over 500 red deer (Cervus elaphus), found annular increment patterns in the cement pad of the lower first molar. This pattern was correlated with the age of two known-age animals. Van Nostrand, et al. (1964) used the cementum layers on the mandibular molars of the beaver (Castor canadensis) as one of the criteria for aging individuals. Mundy and Fuller (1964) detected annual layers in the cementum of the third lower molar of grizzly bears (Ursus horribilis). These layers were subsequently used for aging the bears. Novakowski (1965) used the cementum of bison (Bison bison) teeth as one of the criteria for age. Ransom (1966) aged white-tailed deer (Odocoileus virginianus) by the structure of dental cementum in the mandibular first molars using a technique similar to that of Mitchell's (1963). Gilbert (1966), by using ten known-age specimens and a microtechnique modified after McEwan (1963), confirmed the ages of white-tailed deer by counting the number of cementum annuli in the first incisors. This survey of the literature shows that a number of artiodactyls can be aged by the annual growth pattern of the structures of the teeth. It would be reasonable to assume that pronghorn antelope (Antilocapra americana) could be aged by comparable methods.

The first purpose of this study is to develop a reliable aging technique for pronghorn antelope using variations in the pattern of deposition in the cementum or dentine of the incisiform or molariform teeth using known or established-age animals. The second purpose is to see if this technique can then be used to check the age estimates made by the more easily-used field technique of aging pronghorns by tooth succession and wear as developed by Dow and Wright (1962).

MATERIALS

Approximately 20 known-age and established-age lower jaws from the University of Montana Zoological Museum were available for this study. All but two of these were the same jaws collected and used by Dow and Wright (1962) to establish the pronghorn wear age classes of their study. Dow (1952) collected over 1300 hunter-killed pronghorn jaws. These were also available for examination. In addition to the above, about 100 jaws of pronghorns from Yellowstone National Park, Wyoming, and the National Bison Range, Moiese, Montana, were used. These jaws were obtained from the pronghorn material collected by University of Montana graduate student Bartholomew O'Gara in 1965-1966 for a doctoral study. A 4-year-old known-age castrated buck and a female known to be over 12 years of age were also studied.

The term established-age refers to those pronghorns whose exact date of birth is unknown but whose year of birth is known.

METHODS

A rapid and simple technique was used to prepare the sections of pronghorn teeth for examination. This technique consisted of cutting thin sections of the teeth with a tooth saw, grinding the sections down by hand on carborundum paper and, if necessary, staining the sections.

The tooth saw was borrowed from its inventor, Dr. R. W. Fields of the Department of Geology, University of Montana. It consists of a base to which is attached a mounted Dremel Moto-Tool, basically a small electric drill. In the chuck of the Moto-Tool is a shaft which holds the small, thin, circular saw blade. Also attached to the base and adjacent to the Moto-Tool is a movable, adjustable mount for the tooth. A third mount holds a water can with a squeeze bulb.

The grinding process was done as suggested by Frost (1958). An 8-inch square glass plate was used as a base. Upon this was placed a sheet of carborundum wet or dry paper grit side up. Carborundum paper #320 or #400 is satisfactory. A microscope slide with a strip of carborundum paper wrapped around it, grit side out, was used as the tooth section holder. The unground section of tooth was placed between the holder carborundum and the glass base carborundum. Water was amply applied to the carborundum paper and the holder carborundum was rotated slowly thus grinding the section.

To remove the incisors from the mandible the following technique was used. The mandible was split along the symphysis resulting in a

right and left half, in which the teeth were in a more exposed position for easy removal. A tooth was removed by chipping away the surrounding alveolar bone with a pen knife. The peridontal membrane was removed by wetting the tooth and then rubbing the membrane away. If not done the membrane would roll up into strings and disrupt the grinding process. The tooth was then placed in the adjustable saw mount and, after cutting off the crown, cross or sagittal sections of from 150 to 250 microns thick were cut from the root. The resulting sections were ground as previously described. From time to time the section was checked for thickness with a 30X binocular dissecting microscope with an ocular micrometer. Occasionally, the cementum annulations of the section would be so distinct that a count could be made without staining. In most cases, however, the section had to be ground to about 16 microns and then stained. The grinding process left a lot of debris on the section and it was washed for 4 or 5 minutes in a detergent and water solution and then rinsed in tap water.

After rinsing the section was decalcified by placing it in 2.5% nitric acid for 2 to 3 minutes. Next, removed from the acid, the section was neutralized in a saturated solution of lithium carbonate and 70% ethyl alcohol for 15 or more minutes. Next the section was rinsed in distilled water and finally fixed to a microscope slide with egg albumen fixative. The section was then stained from 5 to 30 minutes with Erlich's or Delafield's hematoxylin, being examined every 5 minutes until the desired staining was reached. Following staining, the section was rinsed and blued in tap water and counterstained in Eosin for 2 to 5 minutes. The section was then dehydrated in two changes

each of 70% ethyl alcohol, 95% ethyl alcohol, 100% ethyl alcohol, a 1:1 solution of ethyl alcohol and xylene, and xylene, with a time of about 2 minutes in each change. A cover glass was mounted over the section using a 60% synthetic resin mounting medium. The unstained sections in which the annulations could be seen were suitably mounted by placing the dry section on a microscope slide and covering it with a cover glass, the edges of which had been covered with a narrow band of Duco cement. Care was taken not to get the cement on the section.

Measurements

Three quantitative measurements were taken on each incisor and recorded. First, before removal from the mandible, the distance from the top of the jaw line on the lingual side of the tooth to the apex of the tooth was measured in millimeters (Figure 1, a.). This measurement would give some idea of incisor wear. Secondly, to determine incisor growth and wear, the length of the tooth was taken, in millimeters, around the outside curve. The third measurement was that of the maximum thickness of the cementum in microns.

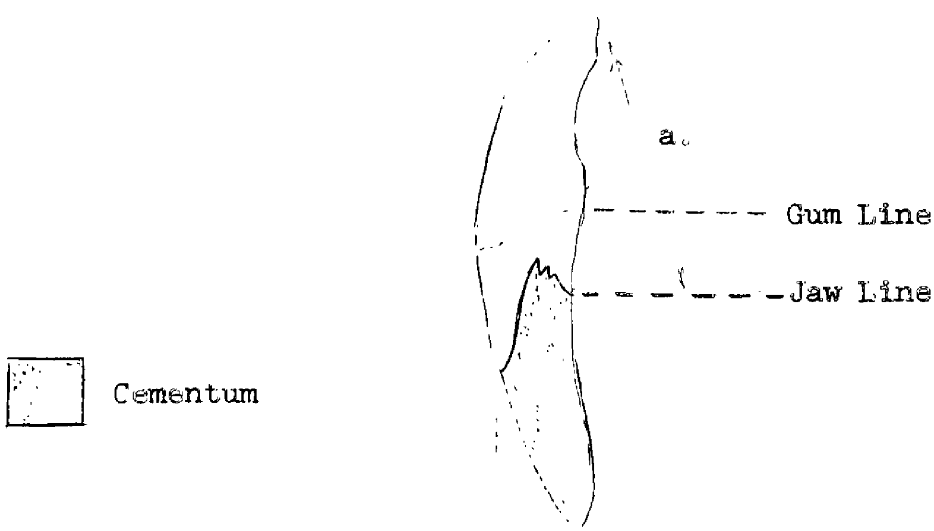

Figure 1. Incisor relationship to gum line and jaw line and cementum covering the root. a., Jaw line to tooth apex measurement.

RESULTS AND DISCUSSION

Preliminary Examination

As the first permanent incisor is easy to remove and is also the first permanent incisiform tooth to erupt (Dow, 1952), it was chosen for the initial examination. A series of first permanent incisors were removed from the jaws of various animals which had been aged by tooth wear. Cross sections were cut from the roots, polished and examined under a microscope using transmitted and reflected light. No definite alterations could be seen in the structure of the enamel or dentine. The cementum in some of the 6 1/3 year and older wear age class incisors had alternating opaque and translucent zones as described for moose incisor cementum by Sergeant and Pimlott (1959). However, no distinct bands were observed in the incisors of the younger age classes. Sections of the first incisors from various age classes of animals were then prepared for staining. The stained sections revealed, under transmitted light, much more detail than unstained sections. The cement and the dentine were observed to have alternating bands. After a brief and unsuccessful effort to correlate the dentinal bands with age, the dentine was by-passed in favor of the obviously more consistent bands in the cementum.

The bands in the cementum of the first incisors could be approximately correlated with the age of both the young and the old age classes. I decided that the best method to prepare the established-age pronghorn teeth for examination would be by staining. I also decided

to continue using the first permanent incisor because a preliminary examination revealed that, although the other mandibular teeth had cementum bands, they appeared to be no more distinct than those of the first incisor. As mentioned above, the first incisor was easy to remove and erupted early. Thus, it was more likely to give a more complete record of annulations in the cementum than the other incisiform teeth. The results of the sectioning of the mandibular series of teeth will be discussed in more detail later.

An overall examination of the first permanent incisor shows that the cementum covers all of the root (Figure 1). The cementum is thicker on the lingual side of the tooth and thinner on the labial side. It is generally thickest on the terminal portion of the root and tapers thinly as it approaches the crown and overlaps the enamel (Figure 2).

Structure of Stained Cementum and Aging Technique

Observation of the cementum in stained cross and sagittal sections of the incisors showed a pattern of pale-staining and dark-staining bands in its structure that could be correlated with periodic events of the year. The pale-staining band is wide and, presumably, is formed during the spring and summer. The dark-staining band is narrow and is presumed to be formed during the fall and winter. This staining pattern in the cementum was noted by Low and Cowan (1963) for mule and black-tailed deer, by McEwan (1963) in caribou, and by Gilbert (1966) for white-tailed deer. A pair of these alternating bands make up one annulation.

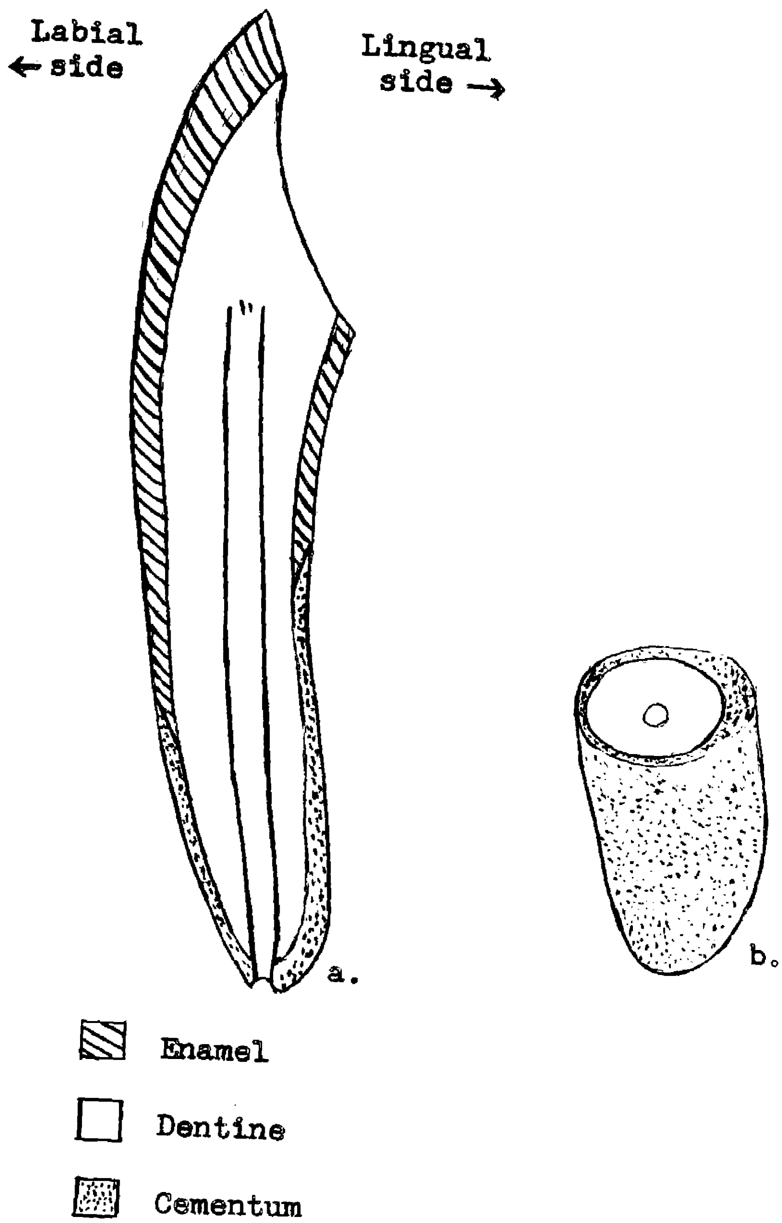

Figure 2. Diagram of a sagittal section (a) and a cross section (b) of a pronghorn incisor. 4X.

The appearance of the annulations and the method for aging by them is illustrated in a photomicrograph of the cementum of a 3 1/3-year-old established-age pronghorn incisor (Figure 3).

Figure 3. Cementum annulations in the incisor of a 3 1/3-year-old pronghorn. Cross section. 440X. X, dentino-cemental interface; A, narrow summer band; B, first fall-winter band; C, wide spring-summer band; D, second fall-winter band; E, spring-summer band; F, third fall-winter band.

The dentino-cemental interface is marked X. The cementum is laid down on the dentino-cemental interface and the tooth begins to erupt at approximately 14 months of age in the pronghorn. This would be in the second summer of the animal's life. The narrow, pale-staining band, A, represents the period between eruption and the end of this second summer. Zone B is the dark-staining fall-winter band which begins forming when the animal is about 1 1/3 years of age. Band C represents the third spring and summer of life and the second fall-winter band seen,

D, begins forming when the animal is approximately 2 1/3 years old. The wide, pale-staining spring-summer zone, E, precedes the fall-winter line, F, which was just beginning to form at 3 1/3 years when the animal was killed. Assuming that the pronghorn fawns are born around June 1 (Dow and Wright, 1962) at this latitude and knowing that this particular animal was killed on September 7, it can be calculated that this animal was about 3 1/3 years of age. This calculation agrees with the 3 1/3 year established-age of the animal.

From records obtained from the Montana Fish and Game Department, Dow (1952) noted that most pronghorn fawns were dropped from May 20 to June 20. For the present study the average fawning date of June 1 will be used for aging pronghorns by the cementum of the first incisors. Established-age animals, therefore, can be used with almost the same confidence as known-age animals because the birth date is known to within about 15 days and the date of death is known to the day.

Discussion of Established-age Pronghorn Incisor Cementum

The incisors from established-age animals of 1 1/2, 2 1/3, 4 1/3, 5 1/3 and 6 1/3 years were sectioned and stained. The cementum of these animals shows the same general pattern of annulations as observed in the animal known to be 3 1/3 years old.

Figure 4 shows the cementum of the first incisor of a 1 1/2-year-old established-age animal killed in the winter. From the dentino-cemental interface (X), there is a light-staining band of summer increment (A). Adjacent to this and on the outside surface is the dark-staining fall-winter band (B), which began forming at about 1 1/3 years of age.

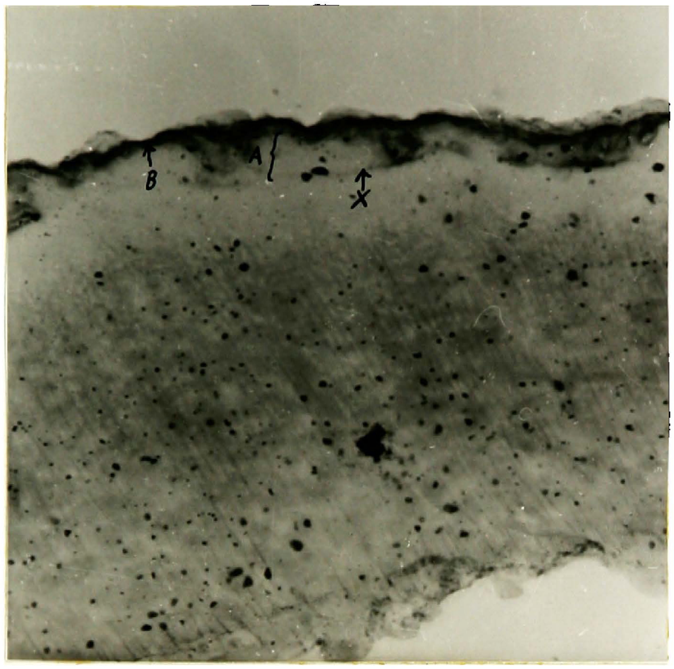

Figure 4. Cementum annulation in the incisor of a 1 1/2-year-old pronghorn. Cross section. 230X. X, dentino-cemental interface; A, narrow summer band; B, first fall-winter band.

The cementum of a 2 1/3-year-old pronghorn killed in the fall is shown in Figure 5. From the dentino-cemental interface there is, in order, the narrow summer band (A), the fall-winter band (B), the wide, spring-summer band (C), and on the outside the fall-winter band (D) which began forming at about 2 1/3 years.

The cementum of a 4 1/3-year-old established-age animal killed in the fall is shown in Figure 6. In this particular incisor the dentino-cemental interface and the narrow summer band could not be observed with certainty. The first band appearing outward from the light-colored, granular, outer layer of dentine was the fall-winter band which was then followed by a wide spring-summer band, then the 2 1/3 year fall-winter band, another spring-summer band, the 3 1/3 year fall-

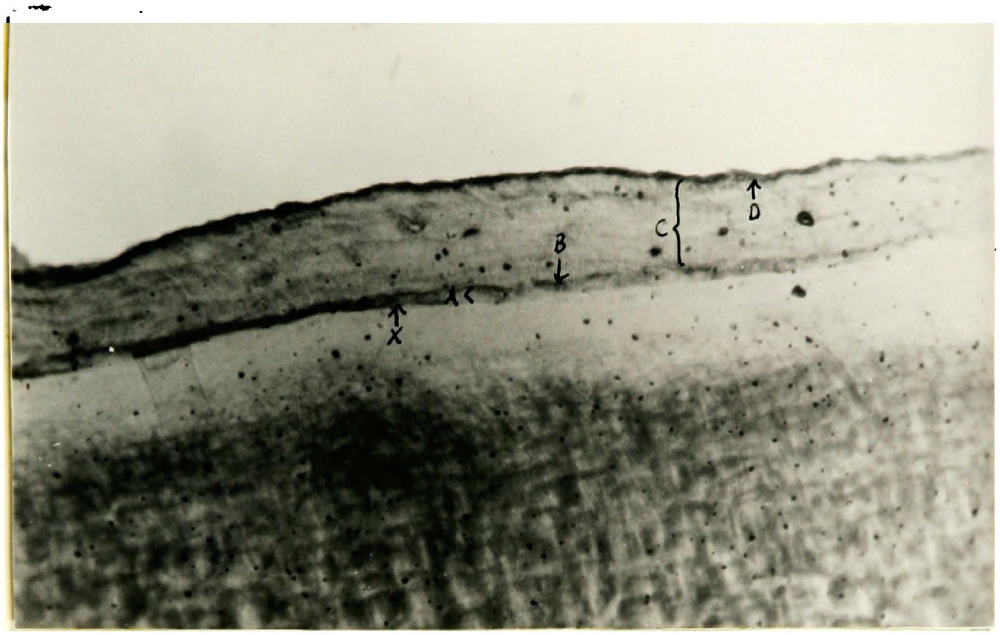

Figure 5. Cementum annulations in the incisor of a 2 1/3-year-old pronghorn. Sagittal section. 214X. X, dentino-cemental interface; A, narrow summer band, B, first fall-winter band; C, wide spring-summer band; D, second fall-winter band.

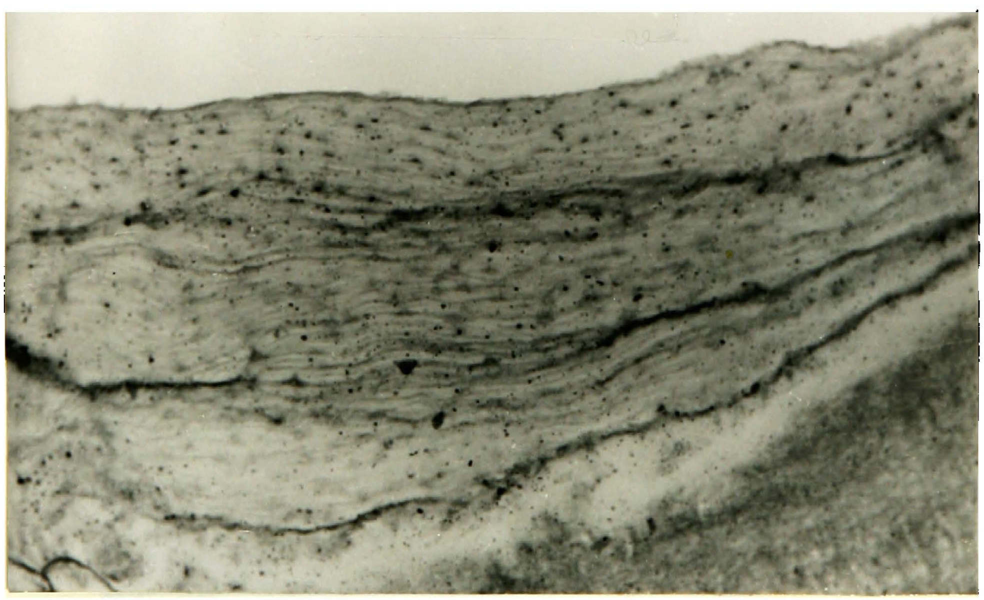

Figure 6. Cementum annulations in the incisor of a 4 1/3-year-old pronghorn. Sagittal section. 100X.

winter band and finally a wide spring-summer band which was on the outside. The 4 1/3 year fall-winter band may have just been forming on the outside but this could not be definitely seen.

Figure 7 shows a section of a 5 1/3-year-old established-age pronghorn first incisor. This animal was killed in the fall. In addition to the dentino-cemental interface and the following extremely narrow summer band there are four wide spring-summer bands and four fall-winter bands with a fifth fall-winter band possibly forming on the outside.

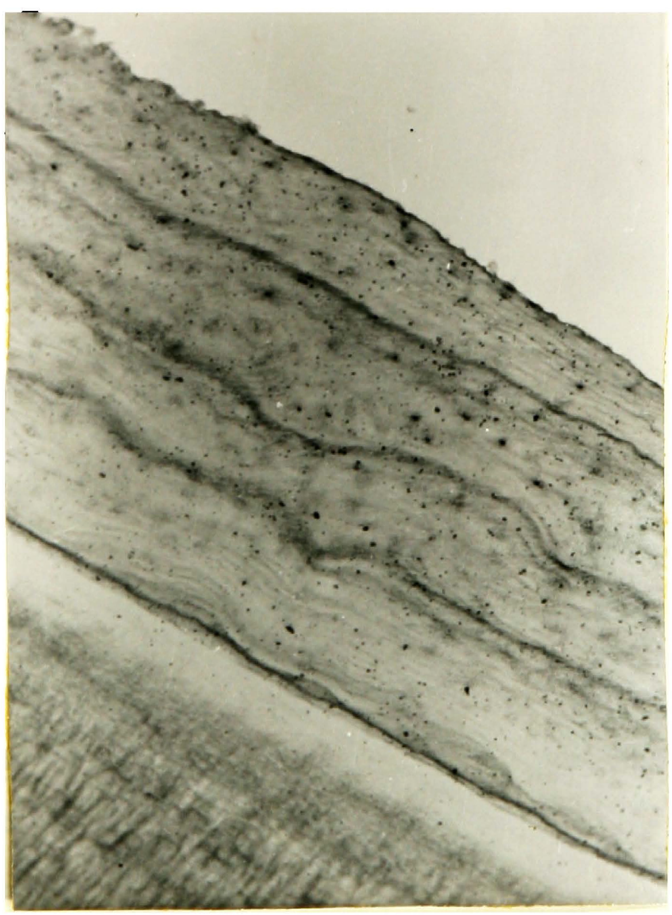

Figure 7. Cementum annulations in the incisor of a 5 1/3-year-old pronghorn. Sagittal section. 130X.

Figure 8 shows an incisor section of a 6 1/3-year-old established-age pronghorn killed in the fall. From the dentino-cemental interface and narrow summer band that follows it there are five wide spring-summer bands and five fall-winter bands with the sixth fall-winter band apparently forming on the outside.

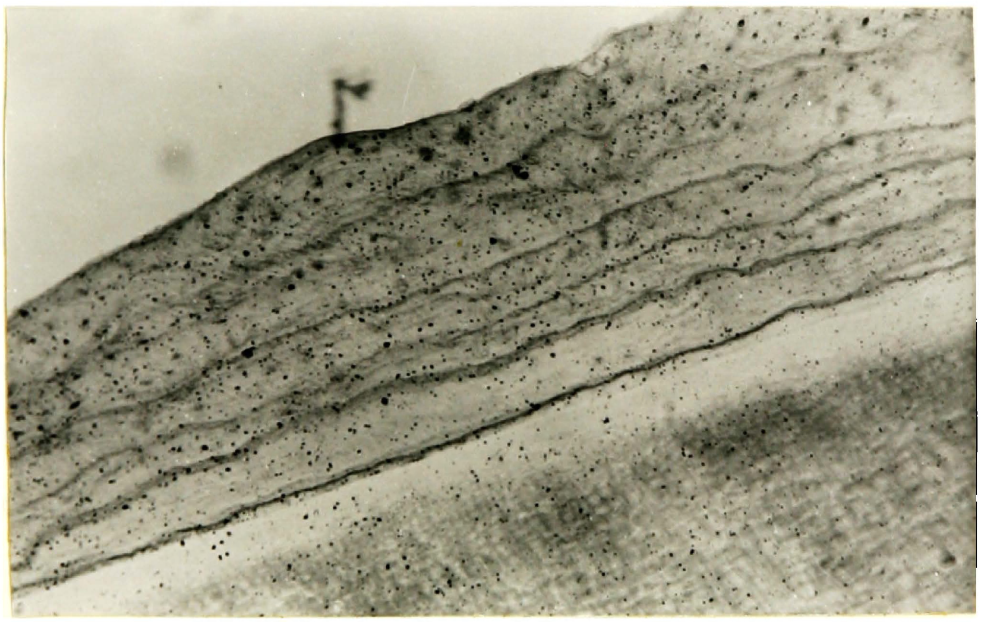

Figure 8. Cementum annulations in the incisor of a 6 1/3-year-old pronghorn. Sagittal section. 100X.

It is obvious that up to and including the 6 1/3-year-old established-age animal that there is a 100 per cent correlation between the number of cementum annulations and the pronghorn's age. It is also apparent that an increase of one year in a pronghorn's age results in the addition of one annulation, consisting of a spring-summer band and a fall-winter band, to the outside of the previous year's cementum of the first permanent incisor. A total of ten established-age pronghorns were used to determine the reliability of the cementum aging technique.

Of these, one was 1 1/2, two were 2 1/3, two were 3 1/3, one was 4, two were 4 1/3, one was 5 1/3, and one was 6 1/3 years old.

The cementum of a pronghorn tagged as an adult and known to be over 12 years old was examined. Only three to five annulations could be seen in the first incisor. It was noticed when the tooth was removed from the jaw that it was about to fall out and the root was exposed to wear. Perhaps the cementum was worn away or resorbed. The inability to age this animal represented a major loss to the study because there were no other known-age or established-age animals over 6 1/3 years available for study. This suggests that animals over 12 years old cannot be aged by the cementum annulation method. It seems likely that some of these older animals can be aged by this method because Table 4C shows two animals that were aged by cementum annulations to be 12±1 years of age. The cementum annulations of the first incisor of a pronghorn estimated to be 9 1/3 years old by the cementum is shown in Figure 9.

One easy way to age by cementum annulations is to count the number of fall-winter bands. If a fall-winter band is present on the outside of the section the actual count of these bands will equal the age of the animal in years, providing the first band is counted as 1 1/3 years. If a spring-summer band is observed on the outside, count the number of fall-winter bands and then add one year to obtain the age of the animal. Again count the first dark band as 1 1/3 years. This method is for an animal killed in the fall.

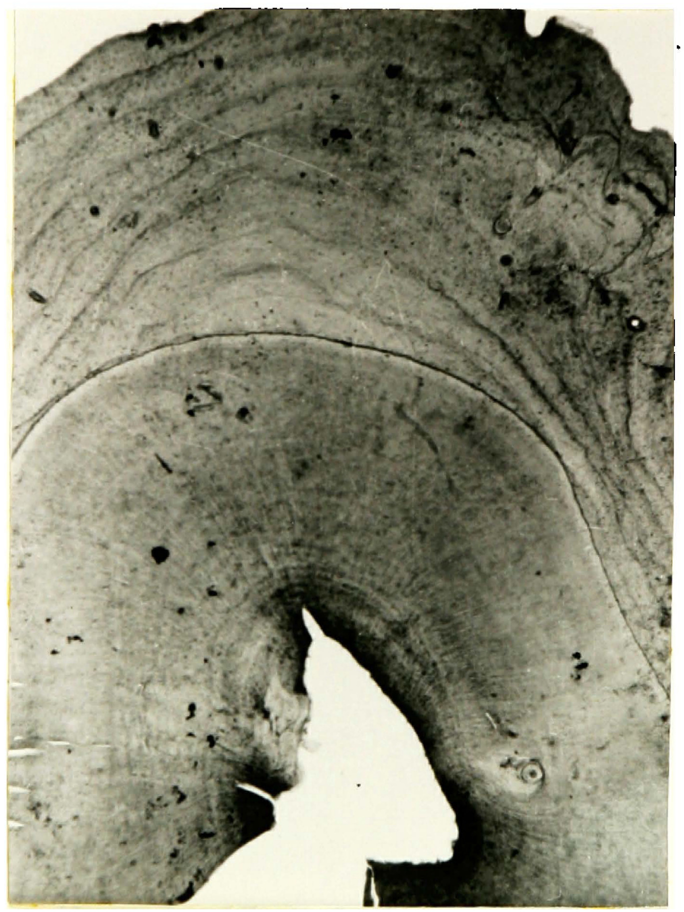

Figure 9. Cementum annulations in the incisor of a pronghorn estimated to be 9 1/3 years old. Cross section. 27X.

A Comparison of the Tooth Wear Aging Technique with the Cementum Aging Technique

A method for aging the pronghorn antelope by the sequence of tooth eruption and the rate of wear of the mandibular dentition was developed by Dow and Wright (1962). Using this technique, over 1300 pronghorn mandibles were placed in age classes. Known and established-age animals from a few days old to 6 1/3 years were used to establish the age classes. They constructed a life table, using statistics, to indicate the hypothetical population structure of the 1300 pronghorns as it may have occurred in the wild in the year 1951. They compared the hypothetical population structure with the age-classes they established by tooth wear and found significant agreement in the age classes of 1 1/3, 2 1/3, 5 1/3 and 6 1/3 years. Their sample of 3 1/3 and 4 1/3-year-olds was not as predicted. Instead they found that the 4 1/3 year age class was larger than the 3 1/3 year age class.

It was also found by Dow and Wright (1962) that aging animals over 6 1/3 years of age by tooth wear was uncertain due to a lack of known-age specimens with which to compare. They assumed that the tooth wear of these older animals would follow the general pattern shown by the 3 1/3 to 6 1/3 year olds. That is, from 3 1/3 years of age, two infundibula would disappear from the molariform dentition from each side of the jaw per year up to the age of 9 years when all 12 infundibula per side would be missing. Animals over 9 years of age could not be aged further by tooth wear.

The first part of this study thus shows that pronghorns can be reliably aged by the annulations in the cementum of the first incisors

using established-age pronghorns up to 6 1/3 years of age.

The second part of the study will be to: first, collect evidence to show that the number of annulations in the cementum of the first incisors continue to be correlated with age in pronghorns over 6 1/3 years of age as they are in those under 6 1/3 years of age, and second, to show that the cementum aging technique can be used to age tooth samples of each age class established by Dow and Wright (1962). From this sample it was hoped that some idea could be obtained of the validity of the 7 1/3 year and older wear age classes and that some conclusions could be drawn as to why Dow and Wright (1962) found more 4 1/3 year olds than 3 1/3 year olds. Third, each wear age class will be sampled by the cementum aging technique to show the amount of agreement between the two techniques. It can be assumed that the nearer the amount of agreement is toward 100 per cent the more reliable the two techniques are.

First incisor tooth samples, for cementum aging, were selected from the jaws within each wear age class. These were chosen to include as many infundibular patterns as possible in an age class rather than in a random manner. I used no formula in selecting the size of the sample from a wear age class.

In order to compare the cementum aging technique with the tooth wear technique and to record the data for future reference, Tables 1 through 4 were constructed. In each table the section left of the double line was taken, in part, from the wear age class tables published by Dow and Wright (1962). The pronghorn tooth formula is $I\frac{0}{3}$, $C\frac{0}{1}$, $P\frac{3}{3}$, and $M\frac{3}{3}$. The incisors are abbreviated (i), canines (c), premolars (p),

and molars (m). The prefix D stands for a deciduous tooth and the prefix P stands for a permanent tooth. The suffix 1 stands for the first tooth of that series, 2 means the second tooth of that series, and 3 means the third tooth of that series. For example, Pi1 means first permanent incisor, m3 means third molar and so on. On the left side of the tables under the heading of molariform teeth is the column which lists the presence (P) or absence (A) of the infundibula of each tooth in a mandible. Before the molariform teeth are worn down each tooth has a set number of infundibula. There are 12 infundibula per side on the mandibular molariform teeth. In aging by tooth wear Dow and Wright (1962) suggested generally that pronghorns with a full set of permanent molariform teeth and no or one infundibulum missing would be classed as a 3 1/3-year-old. If two or three infundibula were missing per side the animal would be classed as a 4 1/3-year-old and so on up to the time when the animal had all the infundibula missing and was placed in the 9 1/3 year and older class.

Within each particular age class there are jaws that have the same number of infundibula missing but which have the infundibula missing from different teeth. Thus, in an age class the jaws that have the same infundibula missing from the same teeth are grouped together in a sub-class. In Tables 1 and 2 the top section (A) records incisiform tooth data. The bottom section of Tables 1 and 2 (B) records and tabulates the data for the molariform teeth.

On the right side of the double line in the tables are two sets of columns which were derived from the results of the incisor cementum aging samples of the present study. Animals whose ages were believed

TABLE 1

SAMPLE OF CEMENTUM AGES FROM 3 1/3-YEAR-OLD WEAR AGE CLASS

Status of Dentition		Number in Cementum Age Sample				
		Observed Age		± 1 Year		
		3	4	3	4	5
A) Incisiform Teeth						
Pil,2,3,Pcl		1	1	2		
Pil,2,3,Dcl		3				1
Pil,2,3,Pcl erupting		2	1		1	
Pil,2,3,Dcl one side Pcl erupting other		1				
	Total	7	2	2	1	1
B) Molariform Teeth						
Pp2 Pp3 Pp4 m1 m2 m3						
P P PPP PP PP PPP P P PPP PP PP PPP		4	1		1	
P P PPP AP PP PPP P P PPP AP PP PPP		2		2		
P P PPP AP PP PPP P P PPP PP PP PPP			1			1
P P PAA PP PP PPP		1				
	Total	7	2	2	1	1
	Per cent	78	22			

TABLE 2

SAMPLE OF CEMENTUM AGES FROM 4 1/3-YEAR-OLD WEAR AGE CLASS

Status of Dentition		Number in Cementum Age Sample: Observed Age 3	4	5	± 1 year 3	4	5
A) Incisiform Teeth							
Pi1,2,3,Pc1			6	5		2	
Pi1,2,3,Pc1 erupting			1		2		
Pi1,2,3,Dc1			2		3	1	1
Pi1,2,3,Dc1 one side Pc1 erupting other		1					
	Total	1	9	5	5	3	1
B) Molariform Teeth							
Pp2 Pp3 Pp4 m1 m2 m3							
P P PPP AA PP PPP P P PPP AA PP PPP			6		2	3	
P P PPP AA PP PPA P P PPP AA PP PPA			3	3	3		1
Other combinations		1		2			
	Total	1	9	5	5	3	1
	Per cent	7	60	33			

TABLE 3

SAMPLE OF CEMENTUM AGES FROM 5 1/3 AND 6 1/3-YEAR-OLD WEAR AGE CLASSES

Status of Dentition	Number in Cementum Age Sample: Observed Age 4	5	6	7	± 1 Year 5
A) 5 1/3 year wear age class					
Molariform Teeth: Pp2 Pp3 Pp4 m1 m2 m3					
P P PPP AA AP PPA	1				
P P PPA AA PP PPA	1				
Other combinations	1	1	3		
Total	3	1	3		
Per cent	43	14	43		
B) 6 1/3 year wear age class					
Molariform Teeth: Pp2 Pp3 Pp4 m1 m2 m3					
P A PAA AA AP PPA P A PPA AA AP PPA	1				
P A PAP AA AA PPA P A PAP AA AP PPA			1		
P A PPP AA AA PPA P A APA AA AP PPA					1
P A PAA AA PP PPA P A PAA AA PP PPA					1
P P PAA AA AA PPA P P PAA AA AA PPA			1		
P P PPA AA AA PPA P A PPA AA AA APA				1	
X A PAP AA AP PPA P A PAA AA AP PPA					1
Total	1	0	2	1	3
Per cent	25		50	25	

TABLE 4

SAMPLE OF CEMENTUM AGES FROM PROBABLE 7 1/3, 8 1/3 AND 9 1/3 YEAR AND OLDER TOOTH WEAR AGE CLASSES

Status of Dentition of Molariform Teeth	Number in Cementum Age Sample: Observed Age					± 1 Year				
	5	6	7	8	9	5	7	8	9	12
A) 7 1/3 year wear age class										
Pp2 Pp3 Pp4 m1 m2 m3										
P A PAP AA AA APA			1							
P A PAA AA AA APA					1					
P A AAA AA AA PPA		1		1						
P A AAA AA AA PPA										
P A PAA AA AA PPA			1			1				
A A PAA AA AA PPA	1									
A A PAP AA AA PPA										
A A PAP AA AA PPA			1							
P A APA AA AA PPA										
Total	1	1	3	1	1	1				
Per cent	14	14	43	14	14					
B) 8 1/3 year wear age class										
A A AAA AA AA APA							2	1	1	
A A AAA AA AA APA										
P A AAA AA AA APA							1			
A A AAA AA AA APA										
A A PAA AA AA APA					1					
A A PAA AA AA APA										
A A PAA AA AA PPA								1		
A A PAA AA AA APA										
Total					1		3	2	1	
Per cent					14		43	29	14	
C) 9 1/3 year and older wear age class										
All infundibula missing-Total					1				1	2
D) Total number of 6-year-plus wear class aged by cementum	1	1	3	1	3	1	3	2	2	2

to be observed to the year because the cementum annulations stained well and could be clearly seen and counted were placed in the "observed" column of each table. Animals whose ages were probably observed but due to poor staining or minor cemental variations there was some doubt on their ages were placed in the ± 1 year columns. As the "observed" columns are the ones I believe to be most reliable they will be the ones primarily discussed. The ± 1 year columns are included to aid the discussion when enough data could not be obtained from the first columns.

In Table 1, the 3 1/3-year-old wear age class, a sample of nine pronghorns were placed in the "observed" columns by the cementum aging technique. Using these columns it can be seen that there is a 78 per cent agreement between the two aging techniques. The 22 per cent remaining were classed as 4 1/3-year-olds by the cementum aging technique and as 3 1/3-year-olds by the tooth wear aging technique.

In Table 2, the 4 1/3-year-old wear age class, there were 14 animals placed in the "observed" columns by cementum aging. There is a 60 per cent agreement between the two techniques.

Dow and Wright (1962) found that the 4 1/3-year wear age class they established had more animals placed in it than the 3 1/3-year-old wear age class. According to their hypothetical population structure, which showed a decline in numbers as the animals became older, there should have been more 3 1/3-year-olds than 4 1/3-year-olds. They assumed that there were errors in assignment of animals to the 4 1/3-year age class rather than that the population of pronghorns was not increasing at a constant rate. An examination of the "observed" columns in Table 2 suggests that Dow and Wright's (1962) assumption was correct.

A number of animals aged 4 1/3 years by the tooth wear technique were assigned to other age classes according to the cementum aging technique.

Dow and Wright (1962) assumed that a number of animals classed as 4 1/3 years old were actually 3 1/3 years old. The found that 75 of the 136 animals they aged as 3 1/3 years by molariform tooth wear had Pcl in place and 44 of the 136 animals in this class had Dcl in place. One hundred and twenty-one of the 148 animals placed in the 4 1/3-year age class by the wear on the molariform teeth had Pcl in place and 20 had Dcl present. They suggested that as the presence of Dcl was more common in the 3 1/3-year class those animals in the 4 1/3-year class that had Dcl present were actually 3 1/3-year-olds that may have had more than normal molariform wear and were thus misclassified. These animals would account, in large part, for the larger number of animals placed in the 4 1/3-year class. They alternately suggested that there may have been a 1-year delay in the shedding of Dcl and that there are 4 1/3-year-olds with Dcl present. Aging a sample of the 4 1/3-year-old wear age class by the incisor cementum technique shows that two of the animals tooth wear aged as 4 1/3 years old were also cementum aged as 4 1/3 years old. It seems unlikely that all of the 4 1/3-year-old wear aged animals, with Dcl present, are misclassified. This further suggests that there may actually be a year's delay in the shedding of Dcl. From the examination of Table 2 it appears likely that the reason why there are more 4 1/3-year-old animals wear-aged than 3 1/3-year-old animals is because some 3 1/3-year-old animals with more than usual molariform tooth wear were misclassified as 4 1/3 years old and some 5 1/3-year-old animals with less than usual molariform tooth wear were misclassified as 4 1/3 year olds.

Table 3 A, the 5 1/3-year wear age class, shows only a 14 per cent agreement between the two aging techniques. This agreement is believed to be too low, perhaps because of the small sample size of only seven animals. By interpolating between the 4 1/3-year agreement in Table 2 and the 6 1/3-year agreement in Table 3 B there should have been about a 55 per cent agreement between the two methods in the 5 1/3-year age class.

In the 6 1/3-year class (Table 3 B) there was a 50 per cent agreement between the two aging techniques. As only four animals were placed in the "observed" columns, this agreement is probably distorted due to the small sample size.

In the probable 7 1/3-year wear age class (Table 4 A) there is a 43 per cent agreement between the two aging techniques. Although this agreement is a low figure it can be seen that the majority of animals that were "wear aged" and placed in this class were also placed in this class by incisor cementum aging. This suggests that, even though Dow and Wright (1962) did not have known-age animals to confirm their criteria for this age class, this class as established by tooth wear age is probably a somewhat valid one. It can also be surmised that the cementum aging criteria for this class are probably valid.

In Table 4 B, the probable 8 1/3-year wear age class, there is only one animal placed in the "observed" columns established by the incisor cementum technique so these columns and the ± 1 year columns were combined to show a 25 per cent agreement between the two aging techniques.

Table 4 C, the probable 9 1/3 year and older wear age class shows a 100 per cent agreement between the two aging techniques in that all four animals wear aged at 9 1/3 years and older were also cementum aged at 9 1/3 years and older. Two of these animals were aged by the cementum technique at 12±1 years.

When the data of Table 4 A, B, and C are totaled Table 4 D is the result. This shows that of the 19 animals aged at over 6 1/3 years by the tooth wear method, 16 were aged at over 6 1/3 years by the cementum. This is including the ± 1 year columns. From this it seems logical to assume that the number of first incisor cementum bands increase with age in the 7-year and older pronghorns. It is realized that an examination of the first incisor cementum of 7 1/3 year and older known-age animals will be necessary to check this assumption.

A total of nine animals that were placed in various age classes by the tooth wear aging technique could not be aged or could be aged only to ± 2 years by the cementum aging method because of poor staining or cementum abnormalities. These were not included in the data of Tables 1-4.

The possibility that the variation in cementum deposition stops or increases at some particular stage in the life of the pronghorn can not be precluded until a number of known-age pronghorn are studied. The literature on artiodactyl cementum annulations shows that this is unlikely. In red deer, a 17-year-old known-age stag was found to have a corresponding number of cementum annulations on the first molar (Mitchell, 1963). Gilbert (1966) found in white-tailed deer that the cementum annulations of the first incisors of four known-age animals

from 7 1/2 to 11 years old corresponded to their respective ages. Ransom (1966) found this to be the case in white-tailed deer molar cementum in known-age animals up to 11 years old. In the above cases older known-age animals were not available for study.

The close correlation between the two techniques in the pronghorns over 6 1/3 years of age indicates that the wear ages for the older animals are possibly valid. The overall per cent agreement of the two techniques is low. This suggests that one or both techniques are somewhat inaccurate. As the age classes become older there was less agreement between the two techniques. This possibly indicates that it is more difficult to place the older animals in the correct class. From the present study only generalizations can be made concerning the older age classes. More and older known-age animals are needed to establish these classes and to determine the reliability of the two techniques for aging.

Incisor Length and Tooth Wear Measurements

In order to determine if incisor wear would be a good criterion for age the length of the incisors was measured and the distance from the inside jaw line to the incisor apex was measured. Figure 10 shows these measurements for each age group. The age groups were established by the cementum annulation aging technique. In an effort to provide adequate data, the measurements of the animals whose ages were "observed" and those animals aged to ± 1 year were averaged together.

The range of incisor lengths tended to overlap considerably between the age groups making these measurements of little use for reliable aging. The averages of the jaw line to incisor apex measurements

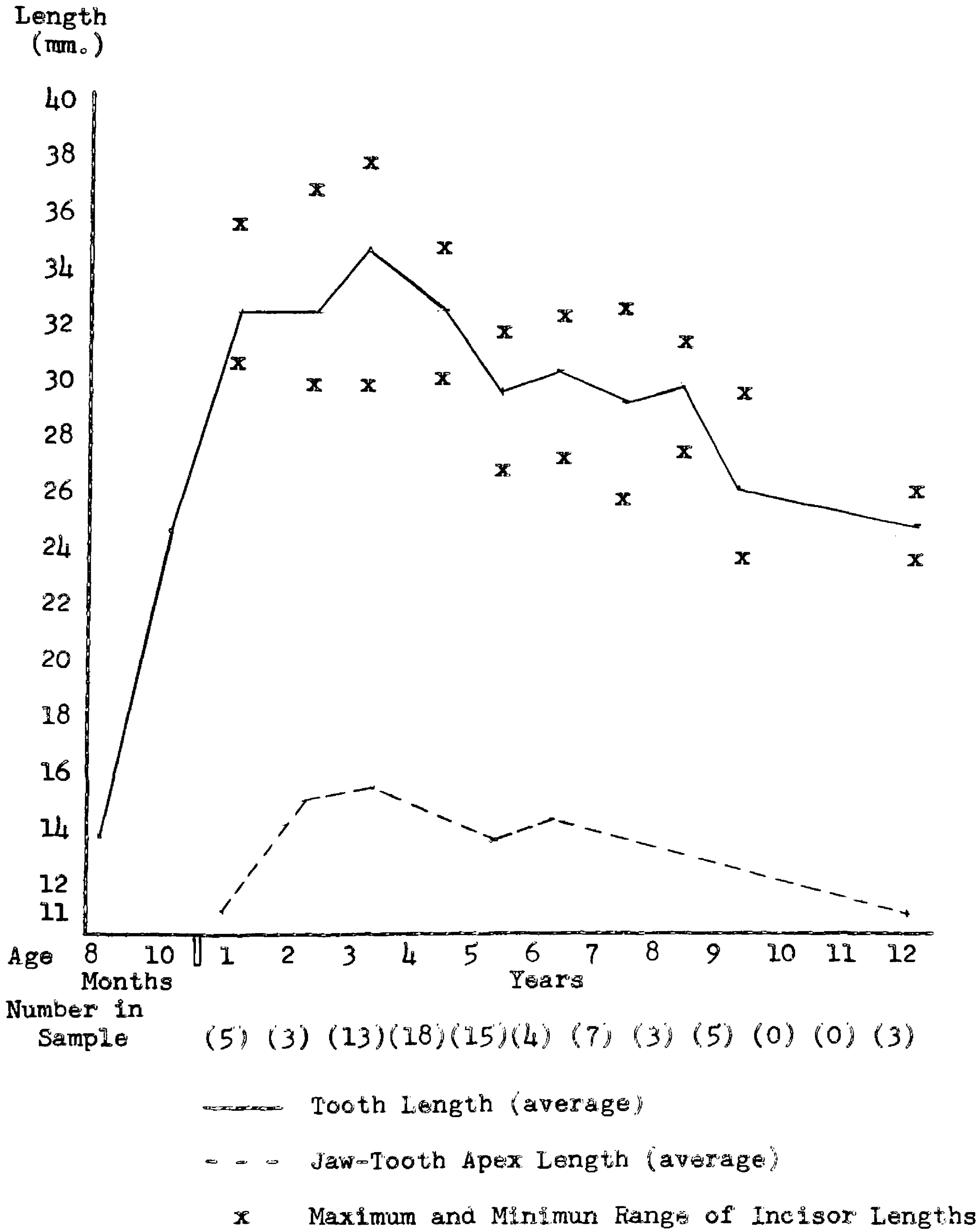

Figure 10. Graph of permanent incisor length and wear in relation to cementum age.

followed the same pattern as the averages of the incisor lengths. The graphs of the averages showed a general trend. It appears that the first incisor does not reach full length until the animal is about 3 years of age. From 3 years of age to 12 years the incisor is worn down at an almost constant rate. It is assumed that once the tooth erupts it is subject to the same rate of wear. It is interesting to note that the regular decline in the incisor lengths in the animals aged at under 6 1/3 years of age by the cementum method continues in the animals aged at over 6 1/3 years of age. This suggests that the cementum annulation aging method is perhaps placing animals over 6 1/3 years of age in the correct age group.

Cementum Thickness Measurements

The maximum cementum thickness was obtained from incisors of pronghorns whose ages were "observed" by the cementum aging method. These measurements were not extremely accurate because most of the sections were cut somewhat obliquely causing an increase in the maximum thickness. In order to obtain the maximum cementum thickness from the cross sections of a tooth it was necessary to cut a series of cross sections from each tooth and then measure the section with the thickest cementum. A 30X binocular dissecting microscope with an ocular micrometer was used to take the measurements. Because the measurements were of a preliminary nature they were not treated statistically. Figure 1? illustrates the range and averages of these measurements.

It can be seen that the ranges in each age group have some overlap with adjacent ranges. Maximum cementum thickness does not appear to be a reliable criterion for aging. It can be, however, a valuable

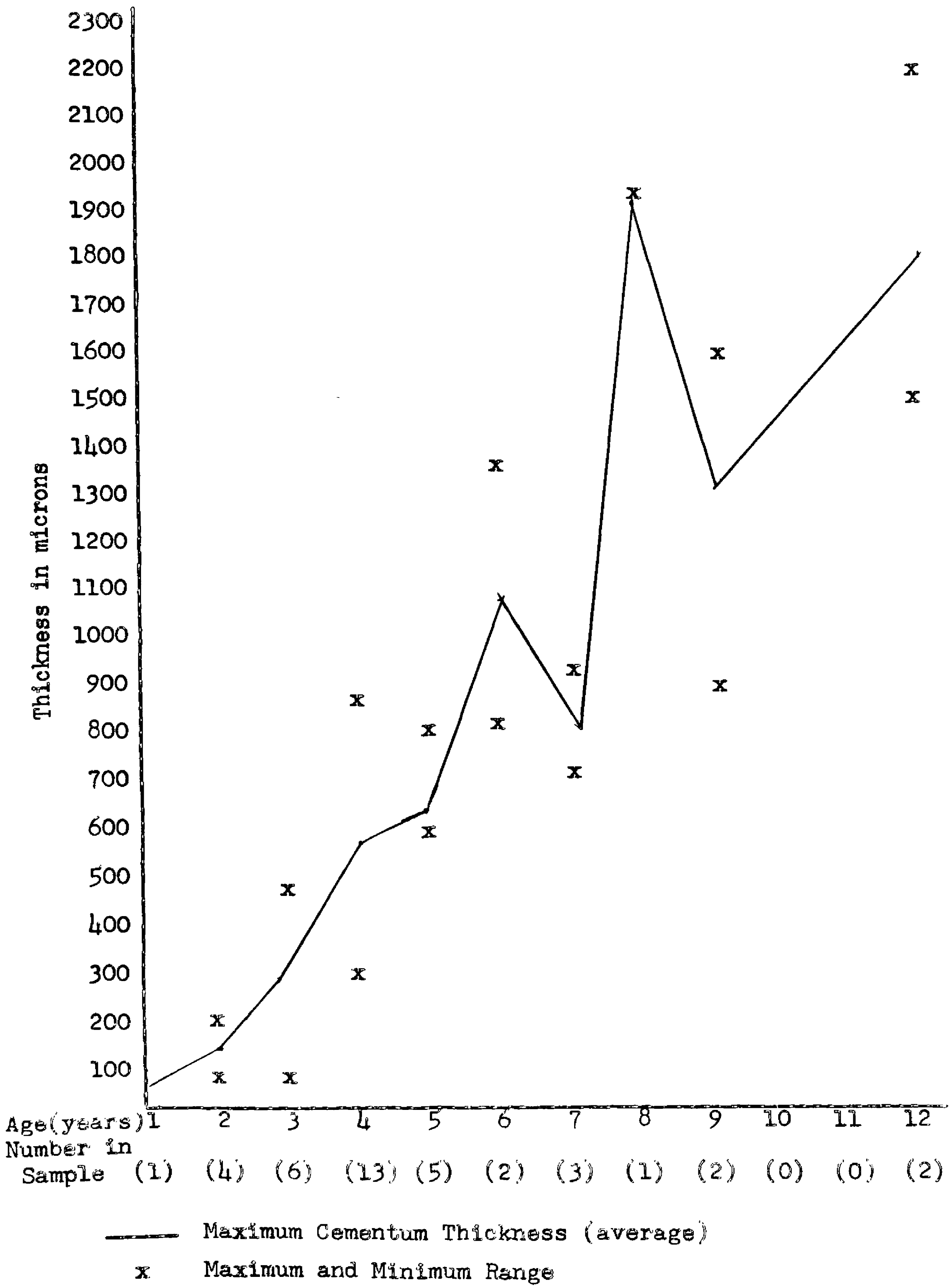

Figure 11. Graph of maximum cementum thickness and age.

clue as to relative age when aging pronghorns by the annulations. In general, the constant increase in cementum thickness is apparent in the incisors of animals over 6 1/3 years of age as it is in the incisors of animals classed as 6 1/3 years and younger by the cementum aging method. This is further evidence to suggest that the technique is perhaps placing the 7-year and older animals in the correct age class. The exception to this generalization is the incisor with 3-5 annulations from the animals known to be over 12 years of age. The thickness of the cementum was 560 microns. This tooth was not included in the graph.

Causes of Difficulty in Aging by Cementum Annulations

From Tables 1-4 a count of the animals placed in the "observed" columns by cementum aging is 44 as compared to 26 animals aged to ± 1 year. This shows that in the majority of sections the cementum annulations could be clearly seen and counted. Among those sections in which the annulations could not be seen clearly were some that did not stain well due to poor microtechnique. In another group the annulations could not be seen or counted reliably because of cementum resorption and repair. Because of this all of the cementum in any one section should be examined carefully for the number of annulations. Figure 12 shows a section of incisor which has undergone extensive resorption and repair and the annulations could not be counted accurately. In this section even a portion of the dentine was resorbed and then repaired with new cementum. Other sections of this tooth indicated that this resorption occurred 3 to 4 years after eruption. This phenomenon appears to occur at any age in the pronghorn teeth.

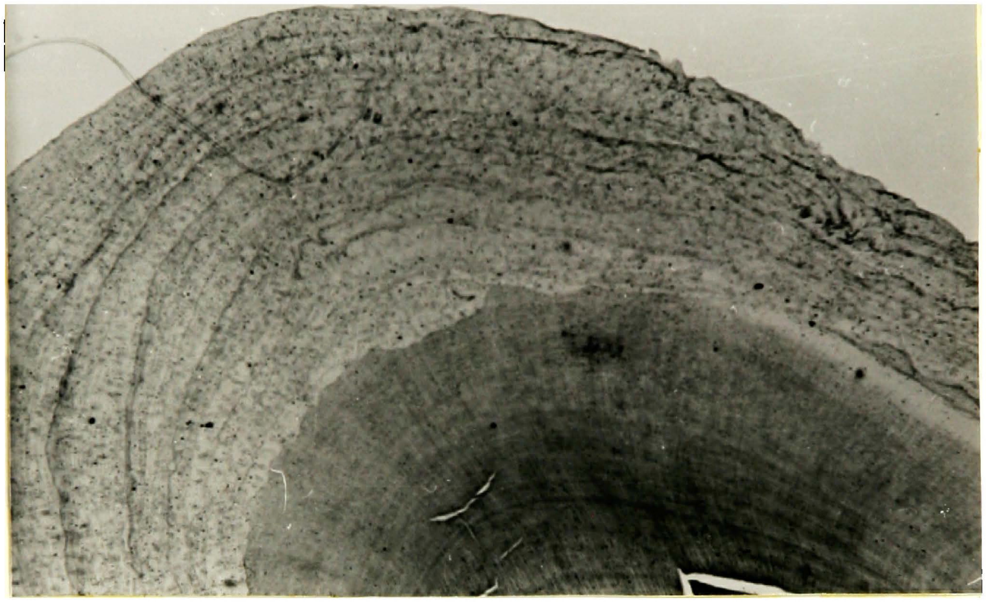

Figure 12. Cross section of an incisor showing resorption and repair of the cementum. 40X.

Usually the resorbed and repaired areas are local spots on the teeth and the other sections of the cementum remain normal. These areas can often be identified in the cementum of the pronghorn and are similar to areas of resorption and repair in human teeth as illustrated by Henry and Weinmann (1951). The causes of resorption and repair in human teeth are trauma and systemic or idiopathic factors and age (Henry and Weinmann, 1951). Perhaps some of these factors are causes of resorption in pronghorn teeth. The section in Figure 9 shows an area of resorption and repair.

Lamellae

The cementum of pronghorns exhibit fainter staining increment lines, called lamellae, within the annulations. These can be seen in

Figures 6 and 7. Low and Cowan (1963) noted lamellae in their examination of mule and black-tailed deer cementum. They also stated that similar lamellae were described by Nishiwaki et al. (1958) for the teeth of whales. Gilbert (1966) found lamellae in the cementum of white-tailed deer.

Occurrence of Rut Lines

Some of the stained sections of the pronghorn incisors showed an additional dark-staining band next to the fall-winter band. As the rut occurs in Montana pronghorn in September (Wright, et al., 1962) it was assumed that this additional band, the innermost of the two bands, was a rut line as described for mule and black-tailed deer by Low and Cowan (1963). The rut and fall-winter bands can be seen in the dark band areas of Figures 7 and 8. The appearance of the rut and fall-winter bands do not ordinarily cause any difficulty in aging by cementum annulations because the two structures are close together. Rarely it occurred that a certain section showed what appeared to be rut and fall-winter bands far enough apart to be classified as two separate fall-winter bands. This, however, is a matter of judgment. When this appeared to happen the animal was given a maximum and minimum age and not included in the data. Usually when this is suspected the maximum thickness of the cementum can be used as a clue to age.

Cementum Annulations in Teeth Other Than the First Incisor

Mitchell (1963) determined in red deer that the number of cementum annulations in the individual teeth of one side were the same as the corresponding teeth of the other side. Also, the number of annulations in the teeth of a side corresponded to the time of eruption of the teeth.

In the present study one entire set of ten teeth from a side of the mandible was sectioned in two animals. In one animal, wear aged to be 8 1/3 years old, cross sections were made of the incisiform teeth and sagittal sections were made of the molariform teeth. The sections were examined unstained. In the second animal, wear aged to be 7 1/3 years old, the incisiform teeth were cut in cross section and the molariform teeth were cut in frontal section. These sections were stained and examined. In both animals the cementum of all the teeth had alternating bands in its structure which may have been annulations. There was so much difficulty in counting the cementum bands, because some were not distinct, that the inadequate results were not included in the study. Whether or not the other incisiform and molariform teeth could be used for aging was thus not determined with certainty by this limited study. It was found later that the left and right first permanent incisors of many animals had the same number of cementum annulations. It is probable that the pronghorn teeth have cementum annulations equal in number in corresponding teeth and in an eruption sequence but this needs further study.

Width of Cementum Annulations

Sergeant and Pimlott (1959) found in moose that the thickness of cementum added from year to year was about the same from the ages of 1 to 10 years. McEwan (1963) noted that the thickness of annulations increased with age in caribou. Gilbert (1966) found in white-tailed deer that the annual bands tended to decrease as the animals approached old age. In pronghorns it appears, generally, that the individual cementum annulations tend to increase in thickness as the animals became

older. This is a general impression obtained from examining a large number of undecalcified sections. Unfortunately, the thickness of individual annulations were not measured in unstained sections. Table 5 shows the relative width of the annual increments of some of the established-age animals.

TABLE 5

RELATIVE THICKNESS OF ANNUAL BANDS OF CEMENTUM IN ESTABLISHED-AGE PRONGHORNS

Animal		Thickness of Band (microns					
		Band Number					
No.	Age (years)	1	2	3	4	5	6
E17	2 1/2	13	68				
E18	3 1/3	8	26	44			
E19	3 1/3	18	86	78			
E20	4 1/3	0	149	198	168		
E21	4 1/3	0	129	99	109		
E22	5 1/3	19	229	158	130	68	
E23	6 1/3	19	80	198	197	396	117

The primary purpose of Table 5 is to quantify and record the annual cementum added per year in a selected group of pronghorn first incisors. The measurements are not comparable between the animals because most were not taken from the area of maximum cementum thickness. There is considerable variation in the thickness of the annual bands in any one age among the animals. The measurements of band Number one were taken from the dentino-cemental interface to the end of

the first fall-winter band; band 2 was from the end of the first fall-winter band to the end of the second fall-winter band, and so on to the sixth fall-winter band. The measurements are somewhat distorted because they were taken on stained, decalcified sections and I believe some stretching and shrinking of the annulations occurs in sections so prepared.

Comparison of Cross and Sagittal Sections

At the beginning of the study I found that cross sections were easier to cut, more sections could be made, and were easier to grind without destroying them than sagittal sections. Later in the study enough experience was developed to prepare good sagittal sections. Sagittal sections were found to be preferable to cross sections because they showed the continuity of the annulations better, thus were more reliable for aging. It is believed that if sagittal sections had been used from the beginning of the study more animals would have been placed in the "observed" columns of Tables 1-4, providing that the cementum stained well.

Time of Formation of Fall-Winter Band and Rut Band

The exact time of the formation of the fall-winter band was difficult to determine because the outside edge of the section had to be examined on an animal killed at a particular date. It was difficult to determine if the outside edge of the section showed a true band or merely showed an artifact formed by lighting conditions. By examining unstained sections of incisors it was found that the fall-winter band was already forming by December 6. The opaque spring-summer band was

apparently forming as late as October 26. In stained sections, in the males, a dark-staining rut or winter line is forming on the outside by September 7. It is difficult to determine if a dark band forming on the outside of a section is a rut or a fall-winter band. In the females a stained section showed that the spring-summer zone was still forming by October 26. From these data it can be assumed that the rut line begins forming around August and September and the fall-winter line begins forming around November or possibly as early as September. Some animals were collected in the spring but were collected too late to be studied and included here. Therefore, the time when the spring-summer band begins to form is not known.

Time of Formation of the First Layer of Cementum

The time of the formation of the first layer of cementum has been subject to some discussion. Sergeant and Pimlott (1959) noted that on moose calf incisors the cementum was about .12 mm. thick around the time of eruption and it was suggested that a portion of the cementum was laid down before eruption. Low and Cowan (1963), in aging deer by the cementum, dated the first incisor's eruption at 12 months from the dentino-cemental interface. The time of the formation of the cementum was discussed but was not determined. Gilbert (1966) found that in white-tailed deer the cementum was formed prior to the eruption of the first incisor.

It was necessary to determine when the first layer of cementum began to form in the incisors of pronghorn antelope in order to age by the cementum annulations. A series of pronghorn first incisors, ranging from several months before eruption to when the incisor was fully in

place, was examined. Table 6 shows the results of this examination. It can be seen from the table that, in the pronghorn, the cementum of the first incisor begins forming just prior to eruption, between 12 and 15 months.

TABLE 6

EXAMINATION OF FIRST INCISORS TO DETERMINE WHEN THE CEMENTUM FIRST FORMS IN THE PRONGHORN

Age (Months)	Aging Method	Status of Eruption	Tooth Length (mm.)	Cementum Thickness (microns)
8	eruption	unerupted	13	0
11	known	unerupted	24	0
12	known	unerupted	25	0
15	eruption	unerupted	32	10
15	eruption	unerupted	32	13
15	eruption	P 1/4	32	13
15½	eruption	just erupting	28	13
16½	eruption	P 1/4-1	36	52
20	eruption	fully in	35	41

The Causes of the Alternating Structure of the Cementum

The causes of the formation of the alternating structure of the cementum have been speculated upon by a number of researchers. Sergeant and Pimlott (1959) suggested that the alternating sequence may be related to seasonal variations in feeding conditions. McEwan (196[illegible])

surmised that seasonal differences in the amount of sunlight and the quantities of summer and winter forage may be factors. Low and Cowan (1963) noted that food may be of primary or secondary importance and that the behavior of the annulations may be due to cyclic hormonal changes or other related photoperiodic responses.

In pronghorns, Wright, et al. (1962) mentioned that the bucks loose weight during the rut. Perhaps the rut band is caused by factors leading to this loss of weight. A 4-year-old pronghorn buck that had been castrated at birth was available for study. An examination of the first incisor cementum showed that the animal could be accurately aged by the annulations. There seemed to be no particular difference between the cementum of this animal (Figure 13) and that of a normal 4 1/3-year-old male (Figure 6) except that there appeared to be fewer lamellae per annulation. It could also be seen that the dark-staining band was well formed on the outside of the cementum by the time of the castrated buck's death on August 11. Unfortunately, no other adult male had been collected this early so a comparison of the time of formation of the outside bands could not be made. The outside dark-staining band appears to be formed, in the castrated buck, earlier than those found in the normal bucks. No other discussion can be made on the causes of the alternating structure of the cementum from the present study.

Figure 13 Annulations in the incisor cementum of a 4-year-old castrated buck pronghorn. Sagittal section. 220X.

SUMMARY

A new technique for aging pronghorn antelope was developed. The first permanent incisor of the pronghorn was sectioned, decalcified, stained and the cementum was examined. The cementum showed alternating light and dark-staining bands. Each pair of alternating bands represented one annual layer of cementum. Some unstained sections of incisors showed cementum annulations. A series of ten established-age pronghorns up to 6 1/3 years of age was accurately aged by the cementum annulation technique. The incisor of a pronghorn known to be over 12 years old was found to have only three to five annulations. The root of this tooth may have been damaged or resorbed. This was a major loss to the study because no other known-age pronghorns over 6 1/3 years old were available.

A sample of pronghorns originally aged by the tooth eruption and wear technique was subsequently aged by the cementum annulation technique. There was up to 78 per cent agreement between these two aging techniques in pronghorns aged at 6 1/3 years and younger. There was a high degree of agreement (16 of 19) between the two methods in aging pronghorns to be over 6 1/3 years of age. Incisor length and wear and maximum cementum thickness are not good criteria for aging pronghorns. It appears that incisor length declines because of wear as the pronghorn becomes older. It appears that the maximum cementum thickness of the incisor increases with age. The comparison of the number of cementum annulations with animals aged by tooth replacement and wear, with incisor length and wear, and with cementum thickness suggests that aging pronghorns by the number of

annulations is possible in pronghorns older than 6 1/13 years of age, as it is in pronghorns 6 1/3 years old and younger.

Resorption and repair occurs occasionally in pronghorn cementum. In addition to the dark-staining fall-winter bands and the light-staining spring-summer bands, there appear to be dark-staining rut lines present in the cementum annulations. Somewhat less than half of the pronghorns aged by cementum annulations were difficult to age because of poor staining technique, resorption and repair and rut lines. The other incisiform teeth and the molariform teeth of the pronghorn have bands of alternating structure in their cementum. Sagittal sections are preferable to cross sections. The fall-winter bands begin to form around November or possibly as early as September. The rut band appears to form around August or September. The time when the spring-summer band begins to form is not known.

The first layer of cementum in the first permanent incisor forms just prior to the eruption of the tooth. The incisor cementum of a 4-year-old castrated buck was found to have annulations which could be correlated with age. Although the cementum aging technique is established in pronghorns up to 6 1/3 years of age, a series of known-age pronghorns over 6 1/3 years old are needed to verify this new aging technique for the older animals. When this is done it can then be determined whether or not this technique is more accurate and desirable to use than other techniques for aging pronghorns.

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