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Incremental cementum lines in the teeth of tropical African mammals

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(With 3 plates in the text)

The correlation of series of two darkly-staining lines in the tooth cementum of tropical African mammals from areas of bimodal equatorial rainfall is briefly reviewed, and contrasted with the findings in southern Africa where bi-annual lines are claimed for areas of unimodal rainfall. A sample of African buffalo teeth from a unimodal rainfall area in southern Tanzania was examined and the conclusion reached that, in general, one dark line per year was formed. A miscellaneous selection of mainly known age animals was also examined with varied results. Anomalies in the apparent formation of cementum lines are discussed.

Contents

Introduction

In a recent review attention was drawn to the uncertainty of the correlation of the series of alternating dark and light bands, in the cementum of the teeth of tropical African mammals, with rhythmic annual incidence (Spinage, 1973). An average of two bold dark lines per year was found in the cementum of the first permanent incisor of waterbuck *Kobus defassa* Rüppel at the equator in western Uganda: latitude 05′S, longitude 30°E (Spinage, 1967); this was also true for buffalo *Syncerus caffer* Sparrman from the same area (Grimsdell, 1973); and from the Serengeti, latitude 2°30′S, longitude 35°E (Sinclair, 1970). A relationship of two cementum lines per year was also suggested for the Thomson's gazelle *Gazella thomsonii* Günther at latitude 3°S, longitude 37°E, in northern Tanzania (Robinette & Archer, 1971): and for the Grant's gazelle *Gazella granti* Brooke from approximately the same area, latitude 2°45′S, longitude 36°55′E (Spinage, in press). It was postulated that the two bold dark lines formed each year were the result of the bimodality of the rainfall in the equatorial zone. But Simpson & Elder (1969) also found a relationship of two cementum lines per year on average in Greater kudu *Tragelaphus strepsiceros*

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Pallas from southwest Rhodesia, an area subject to a unimodal annual rainfall. They postulated that the two lines must be due to "hormonal balance on the one hand and nutritional stress on the other". In a later paper Simpson (1973) regarded two lines as probably equalling one year of age in bushbuck *Tragelaphus scriptus ornatus* Pocock mainly collected in Rhodesia in an area also of unimodal rainfall. Grimsdell (1973) also found two lines per year in a captive buffalo of known age from the South African Lombard Nature Reserve, Transvaal. This area experiences a wet summer and a dry winter; but there is a marked dry period in the middle of the summer, so that we have effectively a bimodal rainfall.

The findings south of the equatorial zone suggest there may be an inherent rhythm approximating to two lines each year, irrespective of seasonal changes, and that the lines are not the result of nutritional variation. To clarify the significance of the relationship of the lines to age, a selection of buffalo teeth from a unimodal rainfall area in southern Tanzania, the Rungwa Game Reserve, latitude 7°20′S, longitude 31°40′E, was examined: and in addition a number of teeth from miscellaneous species, captive and wild, some of which were of known age.

Materials and methods

(a) Twenty-two buffalo first incisors from the Rungwa Game Reserve, Tanzania, were obtained from dried mandibles some four weeks after death.

(b) The first incisor of a Defassa waterbuck from the same area was similarly obtained. This animal was in Spinage's category 'H': an estimated age of about 10 years (Spinage, 1967).

(c) The cementum pad which forms in the interradicular arch of mandibular M1, was obtained from a wild Black rhinoceros *Diceros bicornis* L., 'Pixie', of the Amboseli National Reserve, Kenya, latitude 2°30′S, longitude 37°E. This animal was known to have been born about November 1952 (Taberer, 1959) and was killed in September 1973, aged 21 years.

(d) The maxillary canine was obtained from a captive lion *Leo leo* L., which died aged approximately 24 years. This animal, 'Brutus', had spent most of its life at Rumuruti, Kenya, latitude 20°N, longitude 36°30′E.

(e) The first incisor was obtained from a captive giraffe *Giraffa camelopardalis* L., which died in the London Zoo aged 23·5 years. This animal, 'Maud', originated from Kitale, Kenya, but had been in captivity in London since before the permanent incisors should have erupted.

(f) The cementum covering an African elephant *Loxodonta africana* Blumenbach fifth molar, was also examined for comparative purposes.

All tooth specimens were decalcified in EDTA (Drury & Wallington, 1967), embedded in paraffin wax m.p. 58°C; and stained in Ehrlich's haematoxylin after sectioning at 10 μm. All teeth were sectioned medianly through the sagittal plane as cementum growth is not uniform over the whole tooth root, and transverse sections may miss the zone of maximum increase in thickness. Two sections from each specimen were prepared, and each counted on two separate occasions under the light microscope: the mean of the four counts was taken. In the larger sections of rhinoceros and lion, photographs were also taken of the gross sections, and used to assist in interpretation of the counts.

Results

Buffalo

The permanent first incisor, I1, erupts by 2 years 9 months (Grimsdell, 1973): thus age in years of the specimen was first calculated as the number of cementum lines plus three, and secondly as half the number of cementum lines plus three. To determine which might be the correct annual relationship, ages calculated in these ways were compared with estimated ages of the mandibles derived from M1 enamel height, and from visual appraisal of wear, according to the criteria of Grimsdell (1973) (Table I), in which cementum line age was related to declining height with wear of MI, and also to changes in the crown patterns of the molariform teeth.

Correlation between ages derived from M1 enamel height and those derived from visual appraisal was found to be significant (r=0·755, P=$\leqslant$·001). Agreement of cementum

TABLE I

Cementum line counts in buffalo first incisor teeth with estimated ages of the animals in random order of collection

Specimen no.	$\bar{x}$	Range	s.d.	Estimated age* (years) M1 height	Visual appraisal
1	5	—	0	10	6
2	8	7—10	1·5	12	14
3	8	7—9	1·2	11	8
4	5	4—6	0·8	10	6
5	6·5	6—7	0·6	12·5	11
6	3·5	3—4	0·6	7	6
7	7	6—10	1·9	12	15
8	5	4—5	0·5	8·5	7
10	5	4—6	0·8	12	13
11	8	—	0	10	9
12	8·5	8—9	0·6	11·5	7
13	3	3—4	0·5	8·5	6
14	5	4—6	0·8	9	8
15	6	—	0	7·5	6
17	10	8—12	1·6	13	17
18	4	3—4	1·0	7	8
19	4	4—5	0·6	8	8
20	5	—	0	9·5	7
21	13·5	12—16	1·9	11	14
22	4	3—5	0·8	10·5	9
23	5	4—5	0·5	10	7
24	4	—	0	12·5	14

* According to the criteria of Grimsdell, 1973.

ages was tested by chi-square: the null hypothesis being that cementum line ages would not agree with the estimated ages. The lines generally appeared diffuse and difficult to interpret, probably a reflection of the broad areas of cementum shown by such large teeth. Although agreement was almost significant at the 95% level of probability, the comparison of ages derived from one cementum line equalling one year, with ages derived from M1 enamel height, suggested the null hypothesis be accepted. Agreement was less so when comparison was made with age derived from visual appraisal. When two cementum lines were equated with one year of age, the null hypothesis was highly significant at the 95% level of probability (Table II).

TABLE II

Comparison of ages in years derived from cementum line counts with ages estimated from M1 enamel height and visual appraisal in the buffalo, when 1 line equals 1 year, and 2 lines equal 1 year

	Age derived from M*1* enamel height			Age derived from visual appraisal		
	χ^2	d.f.	*P*	χ^2	d.f.	*P*
1 line =1 year	11·707	20	>·05<·100	17·501	20	>·25<·50
2 lines =1 year	39·196	20	>·99<·995	34·221	20	>·975<·99

TABLE III

Agreement of ages of buffalo derived from cementum line counts with ages derived from M1 enamel height and visual appraisal, expressed as percentages

	Age derived from M1 enamel height				Age derived from visual appraisal			
	Correct	Within 1 year	Within 2 years	>2 years	Correct	Within 1 year	Within 2 years	2> years
1 line =1 year	18	27·3	27·3	27·3	9·1	27·3	22·7	40·9
2 lines =1 year	0	0	18·2	81·8	9·5	19	19	52·4

In Table III comparison is shown expressed as percentage of occurrences of agreement within the sample. This shows that 45·3% agreed to within one year of age, when age derived from one cementum line equated with one year was compared with age derived from M1 enamel height, and 36·4% when compared with age derived from visual appraisal. When two lines were equated with one year, there was no agreement to within one year, and 28·5% agreement with ages derived from M1 enamel height and visual appraisal respectively. The mean age of the sample derived from one cementum line equalling one year, and two cementum lines equalling one year, was 9 and 6 years respectively. This compared with the mean age of the sample derived from M1 enamel height and visual

TABLE IV

Cementum line counts of the teeth of miscellaneous mammals, compared with the expected number of lines

Species	Mean line count	*n**	Range	s.d.	Expected number at: 1 line/year	2 lines/year
Waterbuck	9·3	18	7—11	0·9	7·5	15
Rhinoceros†	33·2	13	27—45	6·7	19·5	39
Lion†	20·1	14	16—25	3·3	22	44
Giraffe†	21	7	19—22	1·2	20	40

* *n*=the number of counts on each individual animal.
†=known age animals.

appraisal of 10·1 and 9·4 years respectively. But one specimen (R21) was anomalous, showing 16 clear lines in one area giving an age of 11 or 19 years: which compared with 11 or 14 years derived from M1 enamel height and visual appraisal. Thus this specimen appeared to be laying down two lines per year in this portion of the tooth. However, the remarkable clarity and regularity of these lines was not typical of buffalo cementum lines.

Other species

A summary of the results is given in Table IV.

Waterbuck

The first permanent incisor erupts at about 2·5 years (Spinage, 1967) giving an expected cementum line count of 7, assuming one line per year is formed in this unimodal rainfall area. The observed mean was 9·6.

Rhinoceros

Assuming mandibular M1 to erupt at 1·5 years (Goddard, 1970), the expected number of cementum lines would be 39 for this bimodal rainfall area; compared with the observed mean of 33·2. But major bands number only 20–21, compared with an expected value of 19 if only one bold cementum line is formed each year.

Lion

Although lions in some areas are subject to seasonal fluctuations in prey abundance (Schaller, 1972) there is no overt environmental factor to affect a captive lion at the equator, and an expected number of lines can not be deduced from the assumptions pertaining to seasonal nutritional changes. The permanent canines erupt at 1·25 years (Schaller, 1972), and should there exist an annual incremental rhythm the expected number of lines would be 22 (Plate I(a)). The observed mean of 20·1 was close to this value.

Giraffe

A temperate zone mammal would be expected to form one cementum line per year (Klevezal' & Kleinenberg, 1967); but in captivity seasonal factors other than food quality could be the inductive factor causing alteration in the rate of cementum formation. For example, the seasonal change in ultra-violet light and its effects upon vitamin D metabolism (Laws, 1962). Eruption of the first permanent incisor was put at 3·5 years (Singer & Boné, 1960), giving an expected number of 20 lines, compared with an observed mean of 21. Plate I(b) shows 21 possible major lines.

Comparison of the observed mean number of lines of each of the four species, with the expected number of lines, the latter being regarded as synonymous with a sample mean, resulted in the null hypothesis being accepted at the 95% level of probability ($\chi^2 = 1{\cdot}67$, d.f. $= 2$, $P = < {\cdot}75 > {\cdot}5$).

Discussion

The results of the buffalo cementum line counts strongly support the hypothesis that the formation of the lines is influenced by the environment, and that an annual unimodal rainfall pattern results in one distinct darkly-staining line per year, compared to two lines in the bimodal rainfall patterns of the equatorial zone. Differences found by other workers in southern Africa could suggest that cementum line formation is very susceptible to climatic change.

The results from the miscellaneous species do not unequivocally support an annual relationship of cementum lines with age; but the test applied is an extremely rigorous one when single specimens of different species are treated as a homogeneous species sample. Despite lack of agreement at the 95% probability level the means are of reasonable orders of magnitude when compared with the expected results, agreement being highly non-significant when alternative values based on one or two lines per year are used for comparison ($\chi^2=32{\cdot}378$, d.f.$=2$, $P= << {\cdot}995$): and the range of values does not embrace both possibilities.

For the waterbuck sample, expected age was estimated from tooth wear, which may have a 76% possibility of agreement with cementum line age to within one year in a bimodal rainfall situation (Spinage, 1967). Thus differences between expected and observed values may be due to underestimation of actual age: but equally age could be overestimated. In the rhinoceros sample microscopic examination suggested the formation of two cementum lines per year, but in macroscopic view it can be seen that only half of these are prominent (Plate I(c)). In this area the generally dry months of June to August had an average of 12·8 mm of rain during a ten-year period; while the other generally dry part of the year from December to February, experienced 280·8 mm over a seven-year period. Thus if cementum lines are sensitive indicators of the presence or absence of rain, we would expect to find a marked difference in line intensity. This was not borne out by studies on the Grant's gazelle in an area 50 km distant (Spinage, in press), in which the animals showed two distinct lines per year. The answer to this anomaly may simply be that in the large tooth the effects are spread over a broader zone and therefore more difficult to detect when slight. The rhinoceros cementum shows two different rates of growth, the lines being at first crowded together, and then spaced more widely (Plate I(c), (d)). The first cementum line can be assumed to have been laid down in 1954, during an increasingly dry phase which terminated with excessively heavy rainfall during November–December 1961 and January 1962. Rainfall then remained 23% above its previous average for the next five years. This suggests that, in the region of slow cementum growth, there should be eight annual lines of marked intensity, and in the distal portion of rapid growth, 12 annual lines if this change in rate of cementum growth is a reflection of the environmental change. The reverse appears to be shown, with a possible 12 lines in the proximal portion, and seven lines in the distal. The structure of the cementum does not therefore accurately reflect the possible environmental influences. Indeed the causal factor initiating an increased rate of cementum growth may be the alveolar and mesial shift of the tooth with increasing age.

PLATE I. (a) Cementum lines in the canine tooth of a 24 years old captive lion. Twenty-two possible annual lines are indicated. 10 μm, Ehrlich's haematoxylin. (b) Cementum lines in the first incisor of a 23·5 years old captive giraffe which died in the London Zoo. Twenty-one possible annual lines are indicated. 10 μm, Ehrlich's haematoxylin. (c) Cementum lines in the mandibular M1 cementum pad of a 21 years old wild Black rhinoceros. Nineteen possible annual lines are indicated. a=zone of slow growth, b=zone of rapid growth, ac=acellular cementum. 10 μm, Ehrlich's haematoxylin. (d) From another part of the same tooth as in (c), showing the very distinct line between the two zones of cellular cementum. Data as in (c).

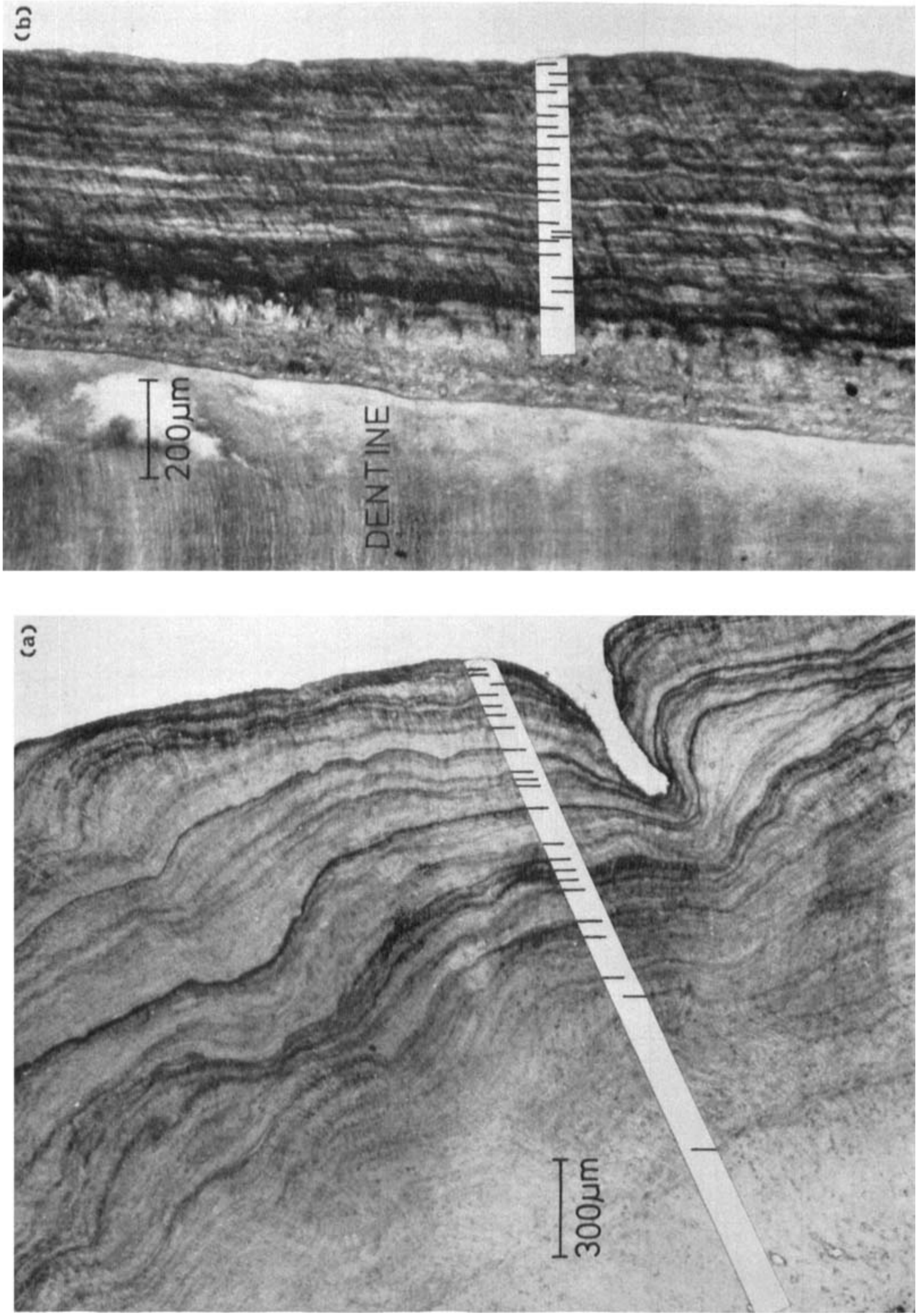

PLATE 1 (a), (b).

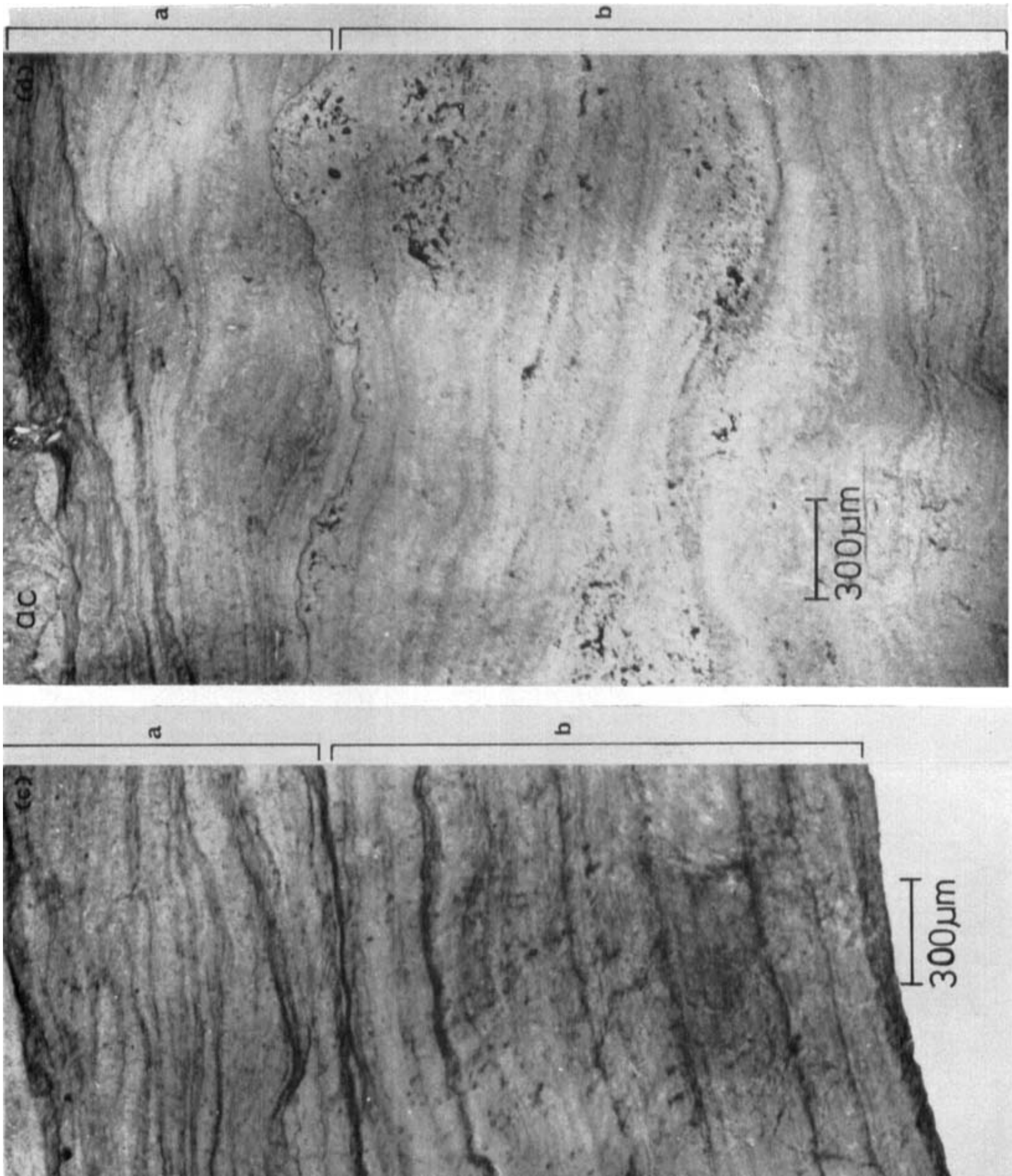

PLATE I (c), (d).

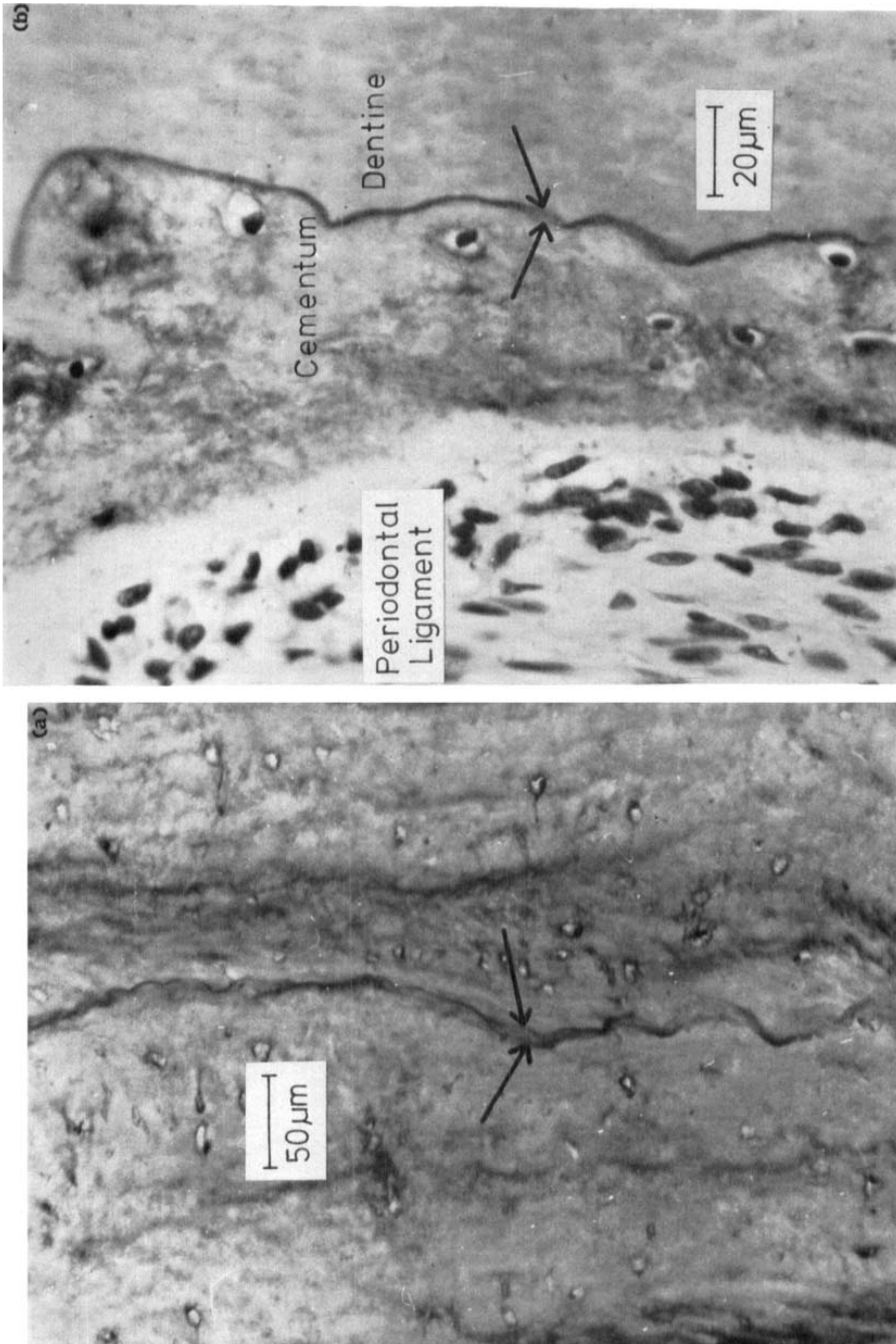

PLATE II (a), (b).

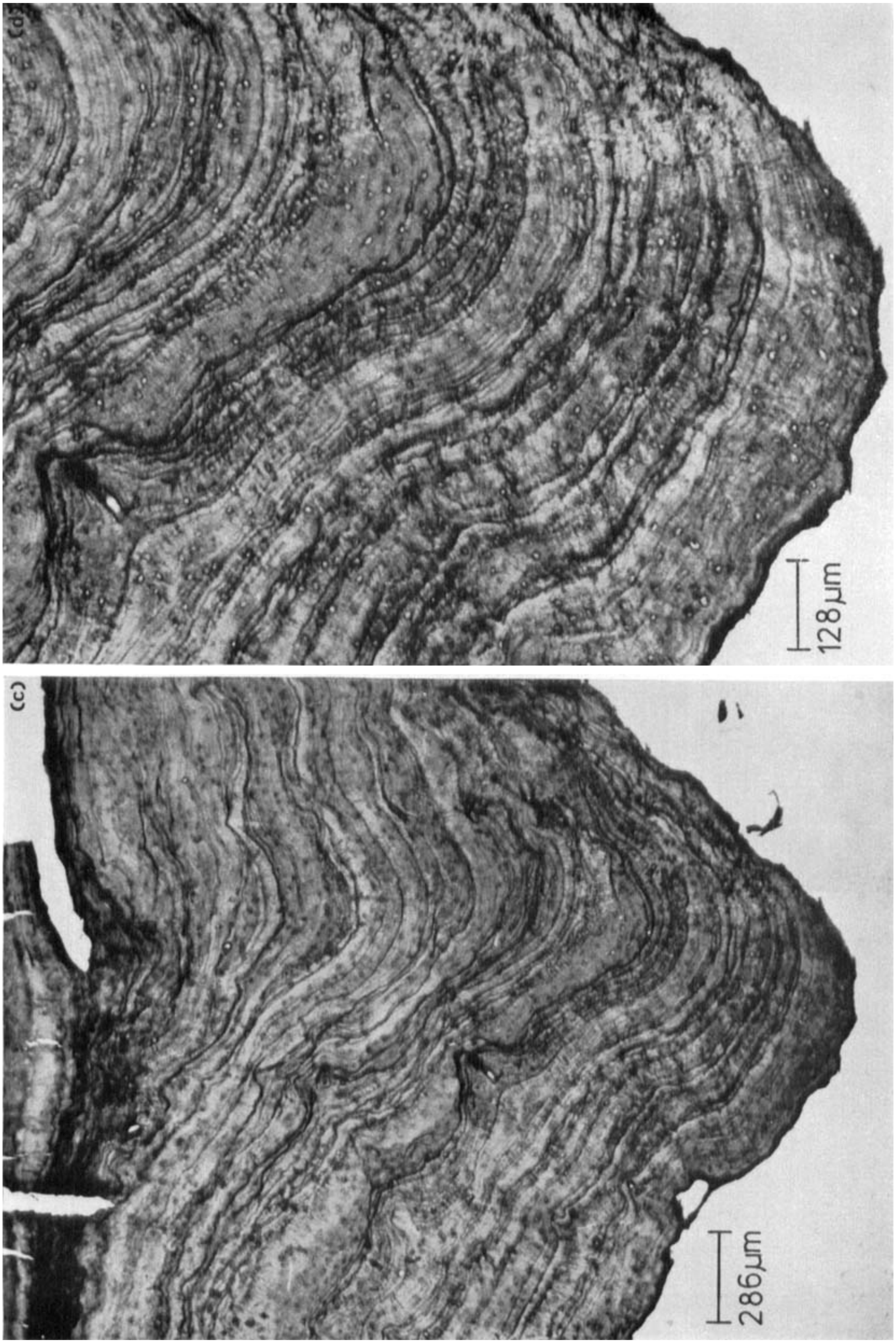

PLATE II (c), (d).

The giraffe sample showed extremely compressed cementum compared to wild specimens but appeared to be following the annual rhythm expected of temperate zone animals. The lion results were anomalous, suggesting an inherent annual rhythm in spite of a presumed lack of nutritional change. There are no other studies on tropical carnivores to confirm this.

The data have been presented as objectively as possible, but it is suggested that the major source of variation between the observed and expected numbers of lines is due to observer error, variation in the staining intensity of lines leading to differences of interpretation: rather than a departure from the suggested theory of seasonally-related increments. For example, Fullmer (1967) notes: "interlamellar regions may stain blue with haematoxylin, as they frequently do in bone."

Simpson & Elder (1969) and Simpson (1973) interpret the results of two lines per year at the equator (Spinage, 1967) as "double lines", but there is a difference between "double" lines and two lines per year. Cementum lines sometimes give the appearance of being paired resulting from the thickness of the section and the convoluted nature of the cementum face. Such a "double" line is shown in the rhinoceros specimen (Plate II(a)); and its nature is confirmed by a carious intrusion found in a buffalo's tooth, in which the cementum-dentine interface is shown to have the same double appearance (Plate II(b)). This differs again from coalescing lines, which result from the irregular nature of the advancing cementum. Observation suggests that the cementum grows intermittently, seasonal variation imposing more distinct lines; but sometimes the basic pattern of growth shows up as in the M1 maxillary cementum pad of a Grant's gazelle. This gave a count of more than 61 background lines, and a count of 12 to 14 prominent lines (Plate II(c), (d)). The incisor cementum gave a count of 12 lines and an estimated age of 7·5 years (Spinage, in press). A highly improbable age of more than 30 years demonstrates the fallacy of counting the background lines; but when such lines stain well they can be confused with seasonal lines. The lion canine cementum also showed background lines, with a probable number of more than 20 between two possible annual lines (Plate III(a)). Examination of the cementum covering the elephant molar confirmed the basic growth pattern of cementum, no matter what its function and the extent of deposition. The age of the cementum in the elephant's mouth would have been of the order of six to eight years in the specimen examined (Plate III(b)).

Grue & Jensen (1973) found that in the Red fox *Vulpes vulpes* L. in Denmark, the dark lines were formed mainly in the early summer. Comparison with the varied observations of workers elsewhere suggested that there was no specific time of the year when these lines were formed in the Canidae. In the buffalo sample examined here only five animals could confidently be said to show dark lines forming, or just formed, although all specimens were collected at the very end of the dry season. It is possible that this zone may come

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PLATE II. (a) A "double" line in the cementum of the tooth shown in Plate I(c). The arrows indicate the apparent two lines. 10 μm, Ehrlich's haematoxylin. (b) A "double" line at the cementum-dentine interface of a carious intrusion in a buffalo incisor. The arrows indicate the apparent two lines. 10 μm, Ehrlich's haematoxylin. (c) Section through the maxillary M1 cementum pad of a Grant's gazelle of estimated age 7·5 years, showing a possible 14 major growth zones, and more than 61 background cementum lines. 10 μm, Ehrlich's haematoxylin. (d) A 2·2× enlargement of part of (c) showing the numerous background lines. Data as for (c).

away during tooth extraction or processing, unless the tooth is fixed with its periodontal membrane intact. One specimen (R22) had an intensely blue-staining line which split during processing (Plate III(c)), supporting the concept that the darkly-staining lines are regions of limited protein matrix, and which therefore could remain adhering to the periodontal membrane during tooth extraction when forming the outer surface of the tooth. The formation of the intense line in this specimen appeared to be attributable to pathological causes, as the acellular cementum layer was unusually thick and also separated from the dentine in processing. As other animals of the same, or older, age did not show a similar line it was unlikely to be the reflection of a very dry season.

If however Grue & Jensen's hypothesis (1973) holds true, unless the difference in timing can be related to differences in food supply or other environmental factors, the causal factor of regular bold cementum line formation, and its relationship to an annual rhythm, must still remain in doubt. Miller (1974), whilst confident that the dark lines in Barren ground caribou *Rangifer tarandus groenlandicus* are formed during winter periods of little or no growth, nevertheless points to some recorded departures from the relationship and differences among other workers in interpreting them. The anomalous buffalo specimen with twice the expected number of lines, quoted above, suggests there is still a need for caution in interpreting annual relationships. I endorse Miller's conclusion that the assumptions need to be verified by controlled studies.

Summary

Some uncertainty exists concerning the correlation of two dark lines in the cementum of the teeth of equatorial African mammals with the annual bimodal rainfall, workers in southern Africa claiming a similar relationship outside of the equatorial zone. Incisor teeth from a sample of 22 African buffalo from a unimodal rainfall area in southern Tanzania were therefore examined; together with one specimen of a waterbuck from the same area, a wild Black rhinoceros of known age from the equatorial zone, a captive lion of known age from the equatorial zone, and a captive known age equatorial giraffe from the temperate zone (the London Zoo). The average number of dark lines counted in the buffalo sample supported the hypothesis that one dark line each year is formed in a unimodal rainfall area. The miscellaneous mammals showed some anomalies compared with the expected results, but in general also supported the theory of dark line formation. Attention is drawn to differences between "double lines", coalescing lines, and two lines per year. Reference to temperate zone workers shows that some doubt still exists concerning cementum line causation and its interpretation elsewhere.

I am most grateful to members of the staff of the College of African Wildlife Management, Mweka, who collected buffalo mandibles for me; to the donors of the miscellaneous mammal specimens; and to the Principal of the College for providing facilities to carry out this woik.

PLATE III. (a) Enlargement (6×) of the lion cementum shown in Plate I(a), showing the numerous background lines, in this case more than 20 between the two apparently annual lines. 10 µm, Ehrlich's haematoxylin. (b) The pattern of cementum growth shown in the cellular cementum covering an elephant fifth molar, of estimated age six to eight years in the mouth. 10 µm, Ehrlich's haematoxylin. (c) Sagittal section of a buffalo first incisor showing a pathologically broad zone of acellular cementum with splitting during processing along the dentine-cementum interface; and splitting also along an intensely-staining cementum line in the cellular cementum. 10 µm, Ehrlich's haematoxylin.

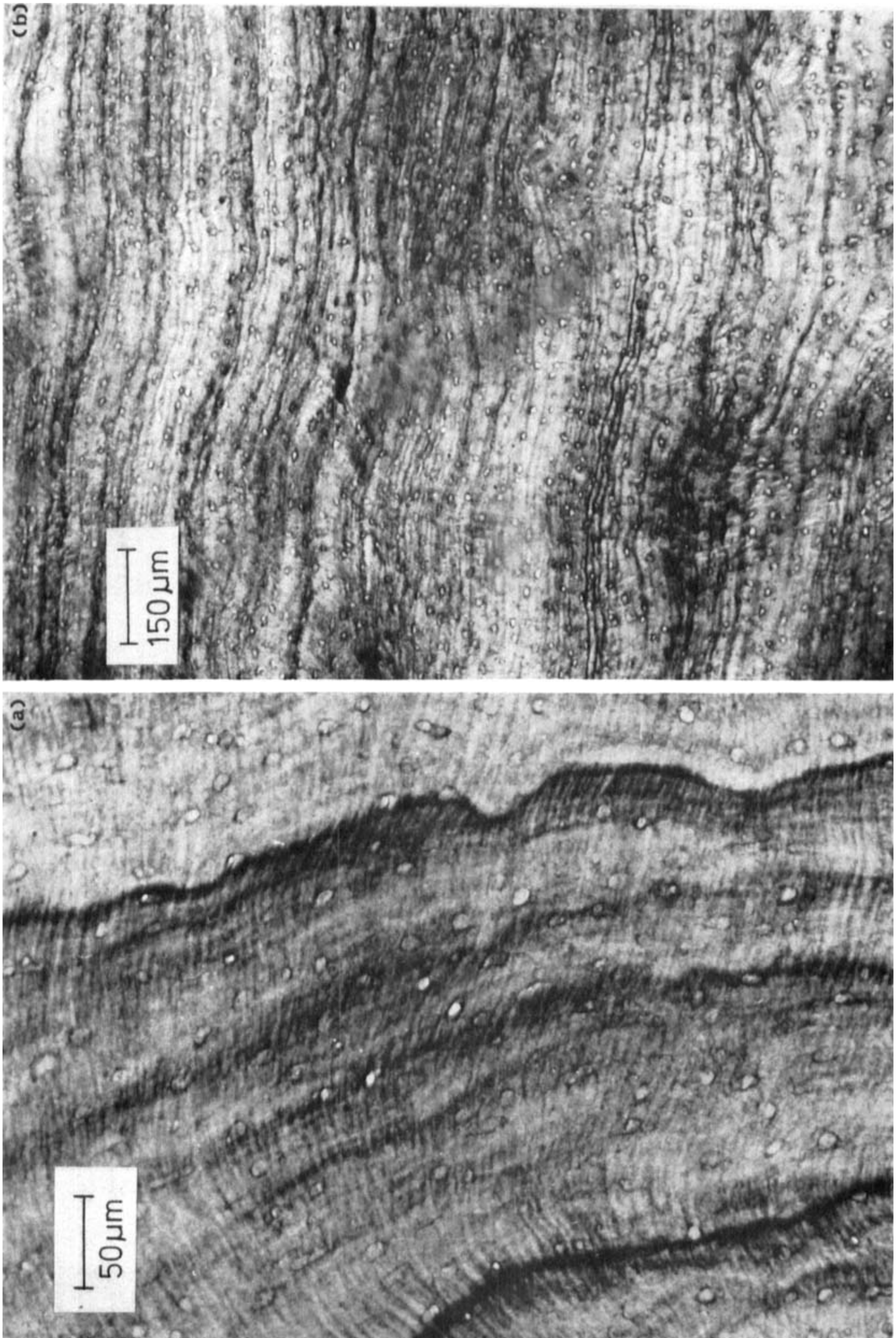

PLATE III (a), (b).

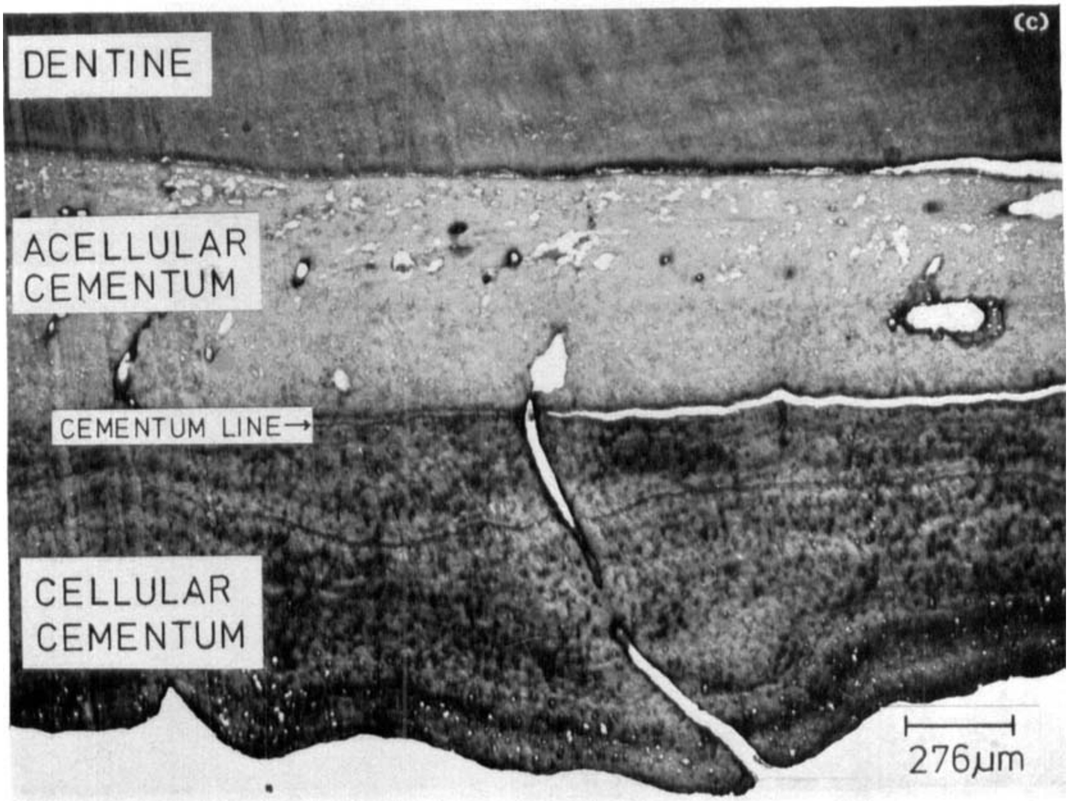

PLATE III (c).

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