AN ANALYSIS OF REPORTED VARIATIONS IN STRIAE OF RETZIUS

by

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INTRODUCTION

Over a century ago, von Retzius studied ground tooth sections and described the lamellated series of thin bands that now bear his name (1). There is general agreement that these bands represent the pattern of enamel development in layered increments. On the other hand their mode of formation remains a question.

Many theories of their formation have been proposed; only a few are given credence today. The main theories include variation of the organic matrix (2)(3)(4), fluctuations in mineralization (5)(6) and periodic bending of the enamel rods (1)(6)(7).

Since the striae of Retzius have not lent themselves well to the experimental process, the theories of their formation have been established on the basis of their morphology. However, a striking characteristic of investigations of striae of Retzius over the years has been the wide divergence of opinion concerning their morphology. Diametrically opposite observations have been made for all enamel structures as they appear in these incremental lines. Thus it is not surprising that the morphologically based theories should vary.

This study was begun with the question of why there have been such conflicting opinions about morphology and the belief that some clarification in this area would be useful in approaching the larger issue of the formation of striae of Retzius.

Generally speaking, the factors leading to the aforementioned diverse findings could fall into either of two broad categories: the methods of preparation and observation, and also the material used for study. A preferred method for the examination of ground sections has not been determined. Such questions as the relative merits of polarized light, non-polarized transmitted light, ideal section thickness, preferred plane of section, and so forth, remain undetermined. Similarly, many factors in the selection of teeth, the selection of areas for study on a given tooth, or even on a given section, might influence the pattern seen.

From among the various potentially contributing factors, it was considered appropriate to select certain specific aspects for this investigation. The specific purposes can be stated to include:

1. A study of the effect of section thickness on the microscopic resolution of striae of Retzius in ground sections of deciduous teeth from minature swine.

2. The utilization of information gained in Part One in order to determine whether variations exist when the following observations are made on transverse sections:

    a. the same stria of Retzius is observed in different regions of tooth.

    b. striae of Retzius close to the surface are compared with those in the middle and those close to the dentino-enamel junction.

    c. striae of Retzius from enamel of one surface region (for example, facial) are compared with those from another region (for example, lingual).

REVIEW OF LITERATURE

General morphological aspects. All dental hard tissues are formed in an incremental pattern. Evidence of this pattern is given by the striae of Retzius in enamel, the incremental lines of von Ebner, the contour lines of Owen in dentine, and by unnamed lines or bands in cementum (8)(9)(10).

Most light microscopic enamel studies have been made using undecalcified, ground sections because the low organic content of enamel provides difficulty during decalcification and subsequent procedures. When ground sections are made either longitudinally or transversely to the tooth's long axis, and viewed under low magnification with the light microscope, the striae of Retzius are seen as dark smoothly curving bands of varying width and intensity. Between them the enamel appears white in color (2)(5)(7)(8)(9)(10). Often, striae of Retzius are not apparent in various places on a given section. The general pattern using a labio-lingual section of an incisor as an example is that showing labial and lingual striae intersecting with the dentino-enamel junction. They curve incisally and toward the tooth surface, their termination depending upon their cervico-incisal position. The more incisally located striae curve over the incisal enamel and intersect at the dentino-enamel junction of the opposite side. Those striae placed closer to the neck intersect with the tooth surface (2)(7)(8)(9)(10). Their surface junction may be seen in the form of shallow grooves encircling the tooth which have been termed perikymata (7)(8)(9)(10)(10)(11)(12). Although the striae of Retzius appear regular on ground section, perikymata sometimes show abrupt but temporary cervical or incisal course deviations.

Since an ameloblast begins to function at the dentino-enamel junction and ends its activity at the enamel surface (13)(14)(15)(16) (17)(18), it can be seen that a given stria of Retzius will always intersect with many enamel rods, some close to the dentino-enamel junction, others close to the surface. They will usually cross the enamel rods obliquely, although in some places, notably the cusp tip, the intersection may be perpendicular. However, on transverse sections, right angle intersections are the prevalent findings.

Transverse tooth sections show the striae of Retzius as concentric bands, parallel to each other and to the dentino-enamel junction, giving an appearance like the growth rings seen on a cut tree-stump. Although the striae themselves are of varying width, the distance from the beginning of one dark band to the beginning of the next has been claimed to be about 16 micra, independent of species (5).

Morphological basis for theories of formation. Three main theories have been formulated by various workers to explain the development of striae of Retzius. Their theories were based on their observations of morphologic changes in the enamel rods and the interprismatic substance of the striae of Retzius. Their observations were made with ground and decalcified sections utilizing transmitted ordinary light, reflected light, transmitted polarized light, micro-hardness test, micro-radiography, phase microscopy and fluorescence microscopy.

The theory of periodic bending: No clear understanding of the morphology or the strücture of the striae of Retzius has yet been established. Retzius (1) in 1837, and more recently, Gosta Gustafson (7), Anna Gustafson (6) and Helmke (18) have related these lines to periodic bending of the enamel prism. Anna Gustafson in contrast to most investigators distinguished between normal and pathologic striae of Retzius. She considered striae of Retzius which are associated with bending of the enamel rods as "normal", whereas those showing other morphological patterns were usually termed "pathologic".

Gosta Gustafson (7) examined longitudinal ground sections with the polarizing microscope and concluded that striae of Retzius are caused by periodic displacements of the enamel rod occurring during the development of the enamel. As a part of his work, he placed the main part of the prisms parallel to one of the polarization planes in an area where he considered the striae of Retzius to be well developed. In this position the main part of the enamel appeared dark, while the striae of Retzius were bright. When the preparation was rotated the incremental lines of the prisms became dark, and the other part of the prisms became bright. He concluded that the prisms change their course at the incremental lines, and then bend back and continue in the same direction as before. In order to support his conclusion that the striae are due to bending of the enamel rods and not to the degree of mineralization, Gustafson applied micro-hardness tests in a row which went obliquely across the striae and the surrounding areas. By comparing the values thus obtained, he could not detect any difference in hardness between the striae of Retzius and the adjacent structure.

Helmke (18) observed bending of enamel rods along striae of Retzius in a study designed primarily to trace the course of enamel rods through a small section of enamel. He utilized decalcified sections of developing monkey dental organs. The sections obtained were approximately .5 micra thick. By marking the position of the enamel rods from each section on a grid, he was able to trace the course of individual enamel rods. He noted bends of the rods as a general widespread feature. However, along a stria of Retzius, the bending occurred regularly, the change in direction being only about one micron, followed by a return to the original course.

The theory of changes in degree of mineralization: The striae of Retzius have also been connected with a variation in the degree of mineralization of different parts of the prism and interprismatic substance. Anna Gustafson (6) referred to striae of Retzius as being due to relative hyper- or hypo-mineralized segments of enamel rods, variation in the interprismatic substance, intersection sheath, intersection substance[1], and compressed enamel matrix, as "pathologic" striae of Retzius. She utilized in her study polarized light, micro-radiography, and transmitted non-polarized light, and observed gradual transitions from hypo- to hyper-mineralized areas along some striae of Retzius. Her conclusion was that it was impossible to tell whether the observed differences were due to variation of mineralization in the striae themselves or in the surrounding areas. Rushton (19)(20)

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[1] The author considered the intersection sheath substance to represent what most workers term the cross striation.

examined sections in ordinary transmitted light and reflected light under high magnification, and found that striae of Retzius were due to increased darkness of the intervals between the prisms. His physiologic explanation was cautious, stating that the interprismatic substance is either more calcified or less highly calcified than the prisms. Rushton (20) in a later study using polarized light concluded that the prisms are more highly mineralized than the interprismatic substance along the striae of Retzius. In addition he found that the prisms themselves do not change in direction.

Schour (5) utilizing ordinary transmitted light connected striae of Retzius with a low degree of calcification of the cross striation in the prism so that the cross striations appear more clearly in the striae of Retzius, and are accompanied by a marked change in the outline of the enamel rods as they traversed the striae of Retzius. He described these particular portions of interprismatic substance and cross striations of successive enamel rods.

The theory of variations in the basic organic structure: The striae of Retzius have also been related by some authors to variation in the basic organic structure. Williams (2) in 1923 observed ground sections and found that striae of Retzius originated from the rhythmic deposition of segments of rods around the entire circumference of a tooth producing the effect of the striae of Retzius. When there is a physiologic disturbance in the metabolism, there is a molecular disturbance in the formation of enamel which changes the action of the light in both reflected and transmitted light. A colored band is produced which is of greater or lesser width (2).

Sognnaes in 1950 (3) studied the morphology of striae of Retzius in the enamel in paraffin sections prepared by a low temperature decalcification procedure. He concluded that the optical appearance of the incremental lines of the enamel is mainly caused by the morphology of the highly organic prism sheath. In his decalcified sections of deciduous teeth, the striae of Retzius and the neonatal lines were found to be composed of a number of heavy dark zones in the prism sheaths along regions of contemporary enamel formation.

Jansen in 1950 (4) studied the pathways of dye penetration into normal enamel. He used trypaflavin, a fluorescent dye, and detected the penetration of this dye by fluorescence microscope. In most cases, even after prolonged application, the dye was shown to be localized exclusively in the prism sheaths. He observed colored Retzius lines composed of rows of thickened parts of the green prism sheaths.

Goland, et al, in 1965 (21) described a new method for preserving the organic components of enamel during the processes of fixation, decalcification and staining, the result being suitable for microscopy. They examined sections of eight micra thickness under phase contrast optics, and observed striae of Retzius in the remaining organic framework. The striae were well preserved and prominent in the cuspal as well as in the gingival areas.

Potential sources of variation. From this review of literature it is apparent that confusion exists concerning the detailed morphology of striae of Retzius. In view of the differing observations, one wonders about the reasons for these differences.

The literature has a notable lack of documentation about potentially critical matters. For example, no studies of the striae of Retzius exist which state the specific thickness of ground sections from which observations were made (2)(5)(6)(7)(19)(20). Yet section thickness may be a very important consideration because of the overlapping of component parts within the tissue. A 100 micron section may have within it 15-20 superimposed enamel rods at varying angles to each other. This situation offers opportunity for optical misrepresentation, primarily through light diffraction. Furthermore, most descriptive studies of striae of Retzius were done decades ago when the successful preparation of thin ground sections of enamel was even less exact than today. It is reasonable to suppose that earlier findings were often based on sections about 100 micra thick. The importance of using thin sections has been pointed out (29). Even so, it is uncertain in what way the changing of section thickness can influence the interpretation of ground sections.

Another technical area receiving poor documentation is that of the role of the light source in influencing the interpretation of ground sections. Most studies have used ordinary transmitted light (1)(2)(5)(19). Polarized light has been used in past enamel studies (20) and has recently experienced a reawakening of interest (6)(7)(29). In addition, reflected light has had some limited use in morphology investigations (2)(7)(19)(20). When more than one optical system has been used, no comparisons of identical regions have been made with each light source. Since the pictures seen under ordinary illumination, as compared with polarized light illumination, depend upon quite different phenomena, their interpretations may vary with the type of illumination.

The material used for observation may also share responsibility for the variety of interpretations seen in the literature. It is possible that the morphology of striae of Retzius may vary depending upon what area of the tooth it is observed.

Gustafson (6) has reported the Retzius lines close to the tooth surface are marked by rod bending while deeper areas show fewer or no lines of Retzius. However, since Gustafson's comments about the deeper layers are not universally true, one wonders whether the closeness of striae of Retzius to the surface or to the dentino-enamel junction may influence their morphological pattern.

Another possible source of morphological variation could be the position of the section with respect to the orientation of enamel rods. Since the tooth has a curved surface, parasagittal sections cut parallel to a midsagittal plane will cut enamel components progressively more obliquely as the distance from the greatest curvature increases. Thus it is possible that the striae of Retzius seen in parasagittal sections could differ with midsagittal sections.

Cervically located striae of Retzius are often more prominent than more occlusally placed growth rings (8)(9)(10). The rate of growth of cervical enamel has been noted to be different than that of occlusal enamel (22)(23). It is an open question whether these differences in accentuation and growth rate might also be expressed in varying morphological forms of the striae of Retzius.

Differences in degree of mineralization between labial and lingual enamel have been suggested in unerupted teeth (24)(25)(26). The appearance of defects and other markings on the enamel surface itself also has a regional difference (28). One wonders if the lines of Retzius may show morphological differences related to their location in facial, lingual, mesial or distal enamel.

Any given striation of Retzius indicates enamel formed at the same time and presumably under the same metabolic influences. The relationship of these factors to the appearance of the striae is not known. For example, it has not been established that the morphology of a certain stria of Retzius is constant in its course around the tooth. Neither is it certain whether that stria would appear the same if studied in longitudinal as it would in transverse section.

Striations of Retzius are said to reflect metabolic changes or variations within the individual. It is generally accepted that, metabolically, the individual lives in a more protected environment during gestation than ever again. Qualitative differences have been seen in enamel formed before as compared with after birth (27)(30). In addition, prenatal enamel is said to have fewer lines of Retzius than postnatal enamel (8)(9)(10). One wonders if the differing environments may also lead to differences in the morphology of the striae of Retzius of prenatal enamel compared with enamel formed after the neonatal period.

The preceding paragraphs pertain to potential sources of variation for striae of Retzius when different areas from the same tooth are studied. However, the possibility remains that the morphology of striae of Retzius may vary within the individual depending on the tooth, between individuals, between the primary and the permanent dentition and between species.

Not every question about the source of variation in morphology of striae of Retzius may need answering. However, many questions do exist about the appearance of a structure whose very description has been used to formulate theories about the mechanism of its elaboration. In order to resolve some of this confusion, this investigator feels it is necessary to start by attempting to identify some of the factors which have contributed to the various and conflicting reports on the morphology of the striae of Retzius.

MATERIALS AND METHODS

Part One

Teeth used in this study were deciduous teeth obtained from young Pittman-Moore miniature swine. The findings are based on photographic records of ground enamel sections taken under standardized conditions. These records were made on each section following successive reductions of thickness.

The deciduous dentitions from two swine were utilized. One section was to be made from each tooth. Four sections were found to meet the requirements for this part of the investigation. A total of 43 teeth were prepared before four satisfactory sections were obtained.

Technical procedures. Handling of teeth prior to sectioning: The teeth were removed from freshly killed swine and placed immediately in saline. The teeth were cleaned, placed in 50%, 70%, 95% and absolute alcohol respectively, and then embedded the same day in a clear embedding plastic (Carolina bioplastic). The sequence of embedding teeth in bioplastic in preparation for sectioning was as follows:

1. The dried specimens were placed in uncatalized bioplastic in order to remove surface air bubbles.

2. One ounce of bioplastic was mixed with five drops of catalyst. A layer about 1/8 inch deep was poured into a small plastic mold and allowed to undergo partial polymerization until it began to harden slightly and was of a tacky consistency.

3. The tooth was pressed into the soft bioplastic at the desired orientation and the mold was filled with a new mixture of bioplastic.

4. After polymerization was complete, the bioplastic block containing the tooth was modified in order to adapt it to the sectioning machine. A lubricated brass ring with the inner diameter tooled to fit the arm of the sectioning machine was placed upon the block. A new bioplastic mixture was poured into the ring. Following polymerization the ring was removed and the block was then ready for sectioning.

Preparation of initial sections. Thick transverse or longitudinal sections (600-1000 micra) were prepared with a sectioning machine manufactured by George F. Motters and sons. Based upon visual judgement, transverse sections were made perpendicular to the long axis of the tooth at about half the cervico-occlusal distance. Longitudinal (mesial-distal) sections were made perpendicular to the transverse axis of the tooth. These thick sections were later to be reduced in stages to appropriate thicknesses (Table 1).

Procedures of measurement. Specific areas on the section were chosen for measurement. A sketch of the section showing the location of the chosen areas was drawn for reference. The areas were measured with a Starrett micrometer calipers before and after the section was cemented to the glass slide. The measurements were repeated during the grinding process until the desired thickness was obtained.

The errors of measurement in sections 32 and 33 were calculated on the basis of groupsing all the thicknesses of each section tegether. Two determinations were made utilizing the formula:

$$\text{S.E. Measurement} = \left(\frac{\text{sum of the differences}^2}{2n} \right)^{\frac{1}{2}}$$

The error of measurement of section 32 is S.D. error = 1.0; that of section 33 is S.D. error = 1.8.

Fixation of section to glass slide. Since multiple records were to be taken from a section at different times, at different thicknesses and always fixed to the same slide, it was necessary to find a mounting media which had both the necessary cementing properties, and also acceptable optical properties. Several kinds of embedding media and cements were tested, including Eastman 910 cement, Canada Balsam (both dry neutral and in xylol solution), and Lakeside (70L) cement in bar form.

Lakeside cement was tested in a number of sections and was shown to be the most satisfactory for the purpose of this part of the study. Several ways to use this cement were tested. The following procedure was used in section 30 and 46:

1. Several scratches were made on a glass slide to aid in retention of the section.

2. The slide with a piece of Lakeside cement on it was placed in an incubation oven. The temperature was raised to 90° C., which is just above the melting point of Lakeside cement. The specimen was placed upon the slide and a lubricated metal weight was placed on the specimen to bring the cement layer to a homogeneous thickness. The temperature was raised to 100° C. and maintained for 15 minutes.

It was found that voids in the cement frequently led to the entrapment of water and eventual loosening of the section or fracture of the enamel. The previous procedure was modified for section 32 and 33 in such a way that the viscosity of the cement was reduced, permitting a more homogeneous cement layer. As a result there was less loss of enamel through fracture and more rapid preparation of thinner sections. The modified procedure is as follows:

1. The temperature of a chromatography oven was raised to about 146° C. The specimen and the slide were placed on a metal plate, put in the oven and kept there for about ten minutes while the temperature fell to and then stabilized at 140° C., which is the flow temperature for Lakeside cement.

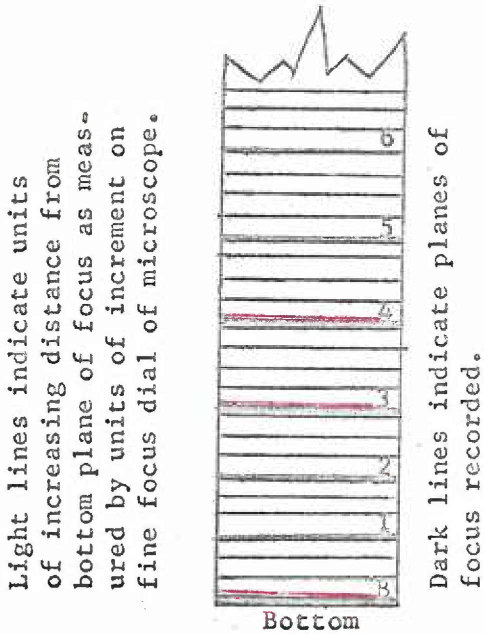
2. The end of the Lakeside cement bar was then touched to the heated section and also to the slide. A small quantity melted immediately and flowed in a thin uniform film. A weight was placed on the section to induce a uniform thickness of cement. The slide was removed from the oven and allowed to cool to room temperature.

Preparation of thin sections. The section mounted on a glass slide with Lakeside cement was reduced with a lapping machine (made by Boodine Electric Company) to about 100 micra at which thickness the first records were obtained. It was then reduced to successive thicknesses, as shown in Table 1, by hand grinding utilizing pumice of the following sizes: 600, 800, 1200 and 3200 mesh. The reduction in thickness was not stopped until the part of the section to be recorded was lost by fracture. The forces and the speed applied in hand grinding were modified as the section was reduced to hopefully avoid any loss of material. However, the maintenance of the area selected for recording at thicknesses below 50 micra was sporadic and unpredictable, even under the best circumstances.

After a section was reduced to any given thickness, it was washed and then run through 50%, 75%, 95% and absolute alcohol. The section was brushed with a paint brush dipped in xylol. A large drop of Zeiss immersion oil of 1.515 refractive index was placed on top of the section. A coverslip was applied and the section was observed.

FIGURE 1

PLAN APPLIED TO EACH SECTION AT EACH THICKNESS
FOR THE RECORDING OF PLANES OF FOCUS



Sections No. 30 and 46. Black. Records were taken at all available planes of focus for each thickness.

Sections No. 32 and 33. Red. Records were made on each thickness only at indicated planes of focus.

Column of Tooth Structure

Reliability of relocating planes of focus. Records of the same plane of focus among different thicknesses were to be compared. Therefore, it was considered important to attempt to substantiate that the available records were actually taken at the same plane of focus. The most effective way to proceed appeared to be to locate and document the lower surface (bottom plane of focus), since this served as the starting point for all subsequent records. The lower surface was considered attained when the outlines of enamel rods first appeared in focus. Since intrinsic landmarks of enamel suitable for documentation were not present in the areas chosen for recording, contaminants at the interface of section and cement were chosen as landmarks. These contaminants appeared to be pumice granules and/or small bubbles. The landmark was carefully drawn and photographed with the intent that it serve as a guide for relocating the bottom plane following reduction of section thickness. The photographs served as objective evidence of the investigator's ability to relocate the bottom plane. These procedures were done for all sections of Part One.

As an additional test of the investigator's ability to relocate a specific plane of focus within and between thicknesses, the following procedure was done on sections 32 and 33. The bottom plane of focus was established, drawn and photographed. The third plane of focus was attained and photographed. The slide was then removed from the stage, replaced, and photographs were taken for a second time of the bottom and the third planes of focus. This procedure was repeated twice for a given thickness, and also for every thickness.

It became apparent that comparisons of landmarks in different thicknesses sometimes showed dissimilarities. An attempt was made to determine if the differences were due to small changes in focal planes. In this phase of Part One, section material was drawn from that prepared as described in Part Two. Thus a section was used which was 39 micra thick and mounted with Eastman 910 cement.

Using this thin section, the bottom plane was established, a landmark chosen, drawn and photographed. The plane of focus was lowered one-half of one fine focus increment; the landmark was drawn again and photographed. This procedure was followed until changes in the appearance of the landmark were minimal. The same procedure was followed during small elevations of the plane of focus beginning at the bottom plane. The photographs were examined to determine the presence and extent of landmark changes as a result of small changes in planes of focus.

Nature and direction of light source: Polarized transmitted light, non-polarized transmitted light and incident light were tested for their usefulness in this study. Only non-polarized transmitted light was found to be suitable for the purposes of this study. The results of experiments with polarized and incident light are presented in Appendix 1.

Procedures of analyzing data. Area of judgement: A specific striation of Retzius in each slide was chosen for judgement from the photograph. The chosen striation and the enamel around it was visible during the evaluation period. All other parts of the photographs were covered with a paper. For example, in section 46, the upper right and left thirds were examined by covering the photograph with a paper cut in such a way as to expose only the area of examination.

Grouping of data according to questions to be asked: The photographs were coded by a second person and grouped in the following way:

1. According to planes of focus from all thicknesses of the same section so that the best thickness at a given plane of focus could be determined.

2. According to section thickness so that the best plane of focus in a given thickness could be established.

TABLE 1

PLANES AND THICKNESSES OF SECTIONS STUDIED IN PART ONE

Slide	Tooth	Plane	Thicknesses in Micra				
30	Lower right first molar	Transverse	106	76	54	40	
32	Lower right third molar	Longitudinal	107	70	52	31	18
33	Upper left second molar	Longitudinal	105	75	50		
46	Upper left lateral incisor	Longitudinal	106	70	53		

Collection of data. Selection of area for observation: All data were collected in the form of photomicrographs taken with a Zeiss photoscope on Agfa IFF black and white film. A suitable area of judgement was determined at the time of the first recording on a given section. Under low magnification a photomicrograph was taken for orientation purposes. The location of the section was determined for future replacements by readings taken from the stage micrometer at the left and the top of the stage. As an additional precaution, a sketch of the area under high magnification was recorded so as to be able to replace the slide at the exact location after reducing its thickness.

Photographic recording of focal planes: Photographs of the first three planes of focus were taken three units of increment apart beginning from the bottom.[1] Subsequent planes of focus were taken four units of increment apart until clear pictures were no longer obtainable (near the upper surface).

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[1] Unit of increment is a graduation interval for fine adjustment which corresponds to a lift of approximately 2 micra as stated by the manufacturers.

Criteria for evaluation: It was originally intended to make evaluations utilizing the morphological features of striae of Retzius reported by various authors. However, it proved impossible to define most of the characteristics sufficiently to be of use in this part of the study where large changes in thickness often resulted in significant changes in morphology. Preliminary work showed three criteria suitable for comparisons of photographs:

1. Continuity.
   Because of some reports of inability to follow the course of enamel rods through the striae of Retzius, rod continuity was chosen as one criterion. Exact determinations of the outline of the enamel rods was difficult in some sections. Therefore, dark bands which could be established to be at least part of enamel rods were examined for continuity, throughout the chosen striae of Retzius.

2. Bending.
   The selected striae of Retzius were examined to detect changes of course of the constituent enamel rods.

3. Outline.
   The enamel rods in the selected striae of Retzius were studied to detect the presence and sharpness of rod outlines.

Evaluation: The photographs which best demonstrated each criteria were credited with (A), the next best with (B) and so on. In each case, the determination was repeated after two days.

Part Two

<u>Source of material.</u> Teeth utilized in the second part of this study were deciduous teeth obtained from one young Pittman-Moore minature swine. Sections from five teeth were used: mandibular right central incisor (section 66), mandibular right third incisor (section 67),

mandibular left lateral incisor (section 65), maxillary left first molar (section 55), and mandibular left central incisor (section 68). A total of 16 teeth were prepared before usable sections were obtained.

Technical procedures. The same procedures were applied as in the first part of this study concerning cleaning and embedding. Transverse sections of 200-300 micra were made between the cervical third and the middle third, approximately perpendicular to the long axis of the tooth.

The sections were mounted to the glass slides with Eastman 910 cement (refractive index = 1.49) after its cementation and optical properties were shown to be acceptable. With this cement it was possible to obtain sections between 30-40 micra without loss in material. It could not have been used in Part One, however, because of the observed tendency for the cement bond to weaken with time, resulting in dislodged, hence non-oriented sections.

The procedures of cementing were as follows:

1. The 200-300 micra section was hand ground with pumice of 3200 mesh to remove surface scratches.

2. Specific areas on the section were measured for thickness determination.

3. Enough Eastman 910 was placed on the glass slide to give a homogeneous film after cementation.

4. The section was then placed on the glass slide and moved circularly for a few seconds until set began so as to induce a thin film of cement. Final set occurred in a very short time, and the section was then ready for the next step.

Before thin sections were prepared, the same areas were remeasured. The section was then reduced by lapping and hand grinding so that areas chosen for observation ranged in thickness between 30-40 micra. Subsequent procedures prior to observation were as presented in Part One.

Collection of data. Striae of Retzius from each section were recorded by means of photomicrographs as in Part One. Locations of selected striae of Retzius were in enamel from each surface region (facial, lingual, mesial, distal) as available. Within each region striae of Retzius intersecting the same group of enamel rods were selected:

1. Close of the tooth surface.
2. Near the dentino-enamel junction.
3. About half the distance from surface to dentino-enamel junction.

Whenever possible a stria of Retzius selected for the aforementioned study was followed for some distance, preferably from one region to the next, and photographed at appropriate intervals.

Procedures of analyzing data. Grouping of data: The photographs from each section were grouped so as to be able to study the following possible morphological differences:

1. Among striae close to the enamel surface, close to the dentino-enamel junction and in the middle third between surface and dentino-enamel junction.
2. Among comparable striae in different surface regions (facial, lingual, mesial, distal).
3. Within specific striae of Retzius in different regions of the tooth.

Criteria for evaluation: The photographs were evaluated by describing the morphological differences in striae of Retzius for the three groups as listed above. Comparisons were made utilizing the characteristics listed below, which were determined following preliminary observations of the records. Each characteristic refers to observed features along the striae of Retzius.

1. Accentuated interprismatic substance.
2. Darkness of segments of enamel rods.
3. Bending of enamel rods.
4. Accentuated cross striations of enamel rods.

FINDINGS

Part One

Section thickness and resolution of striae of Retzius. The results of experiments designed to test the effect of section thickness on the resolution of striae of Retzius are presented in Table 2A, B, and C and Figures 2, 3, and 4.

Comparisons between two successive thicknesses at specific planes of focus were considered first. From Table 2A, B and C it can be seen that reduction of section thickness resulted in improved resolution of the striae in 53 of 78 determinations. This observation was made when all planes of focus for all thicknesses were considered. The sign test was utilized. The hypothesis of having 50 per cent or less plus signs was rejected and the alternative hypothesis of having 50 per cent or more plus signs was accepted at .044 level of significance. This demonstrated that the resolution of striae of Retzius in thinner sections was significantly better than in thicker sections.

Disagreements between the first and second determinations occurred in 28 per cent (17 of 59) of all instances. However, disagreements in the "A" designations were few in situations where at least three thicknesses were available for comparison. Thus of 12 instances of disagreements involving three or more preparations at a given plane of focus, only 2 showed disagreement in the "A" designations.

TABLE 2A

EFFECT OF SECTION THICKNESS ON RESOLUTION OF STRIAE OF RETZIUS

First Determination

Recorded Planes of Focus	106	75	54	40
9th	A	B		
8th	A	A		
7th	A	A		
6th	B	A		
5th	B	B	C	
4th	B	A	D	C
3rd	D	B	C	A
2nd	D	C	B	A
1st	D	B	C	A

Enamel Thickness in Micra
Section 30

Second Determination

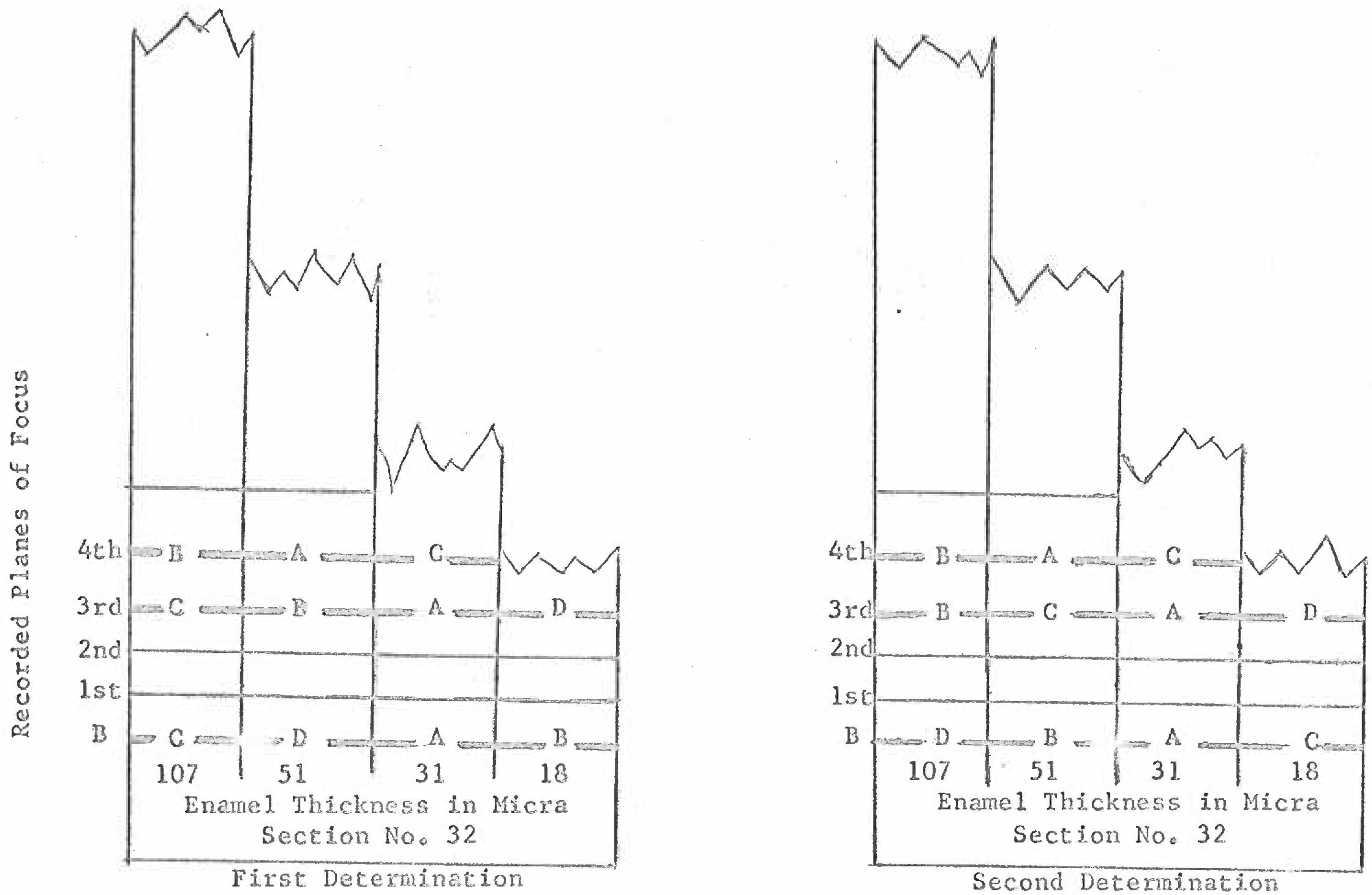
Recorded Planes of Focus	106	75	54	40
9th	A	B		
8th	A	B		
7th	A	B		
6th	A	B		
5th	A	B	C	
4th	B	A	D	C
3rd	D	C	B	A
2nd	D	C	B	A
1st	D	C	B	A

Enamel Thickness in Micra
Section 30

The letter A, B, C and D represent a decreasing order of resolution of the Striae of Retzius for a given plane of focus.

TABLE 2B

EFFECT OF SECTION THICKNESS ON RESOLUTION OF STRIAE OF RETZIUS



The letter A, B, C and D represent a decreasing order of resolution of the Striae of Retzius for a given plane of focus.

TABLE 2C

EFFECT OF SECTION THICKNESS ON RESOLUTION OF STRIAE OF RETZIUS

First Determination

Recorded Planes of Focus	106	70	53
8th	B	A	
7th	A	C	B
6th	B	B	A
5th	C	B	A
4th	C	B	A
3rd	C	B	A
2nd	C	B	A
1st	C	A	B

Enamel Thickness in Micra
Section No. 46

Second Determination

Recorded Planes of Focus	106	70	53
8th	A	B	
7th	A	A	B
6th	C	B	A
5th	C	B	A
4th	C	B	A
3rd	C	B	A
2nd	C	B	A
1st	C	A	B

Enamel Thickness in Micra
Section No. 46

The letter A, B, C and D represent a decreasing order of resolution of the Striae of Retzius for a given plane of focus.

FIGURE 2

SECTION 46. SAME STRIA OF RETZIUS AT 106, 70 AND 53 MICRA.

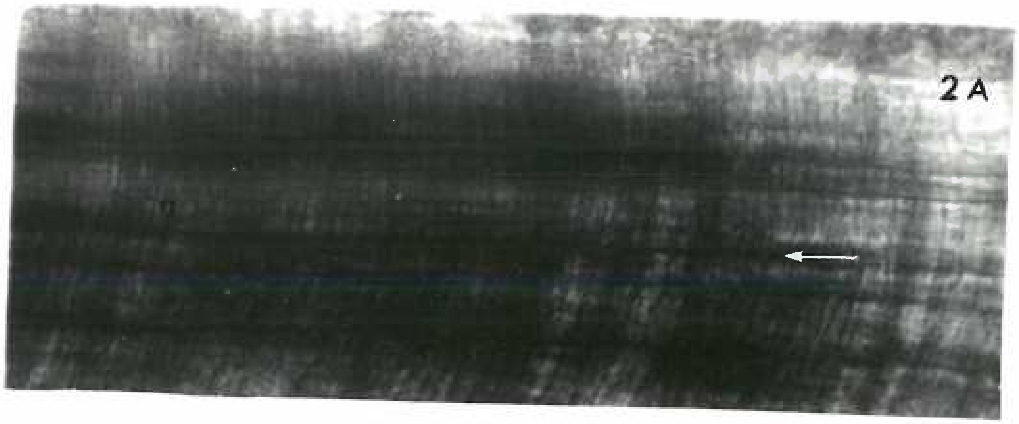
Longitudinal section. Recorded at sixth plane of focus. Photograph was taken using ordinary transmitted light at 176X, enlarged 3.7 times. All photographs presented in this thesis were taken with the same light source, magnification and enlargement factor with the exception of those appearing in Figures 12 and 13.

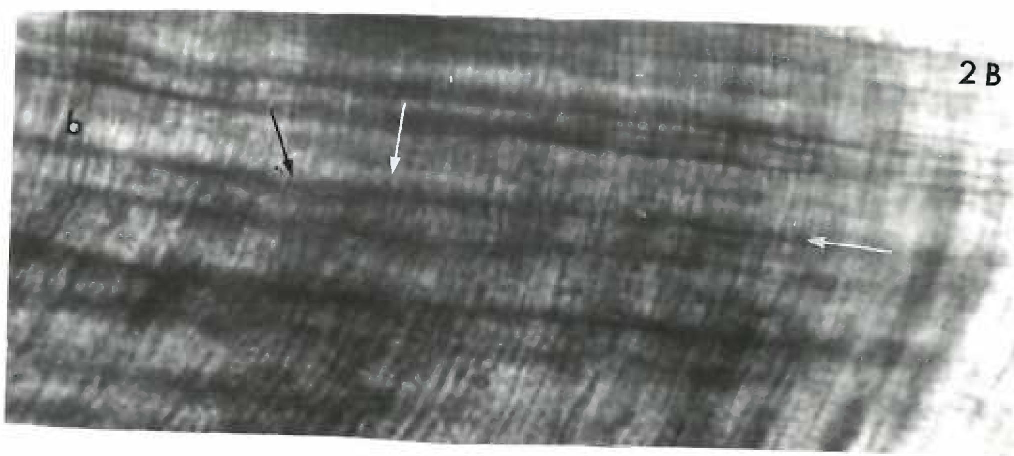
Letters "a", "b" and "c" represent identical morphological components. These are present in an attempt to show the same structure in different thicknesses.

Figure 2A. 106 micra. The selected stria (horizontal arrow) appears as a continuous dark line, giving the impression of being composed of accentuated cross striations.

Figure 2B. 70 micra. The same stria would now be interpreted as showing accentuated interprismatic substance (black arrow) and rod bendings (vertical white arrow).

Figure 2C. 53 micra. Accentuated interprismatic substance and rod bendings are more clearly shown at this thickness.


2 A


2 B
b

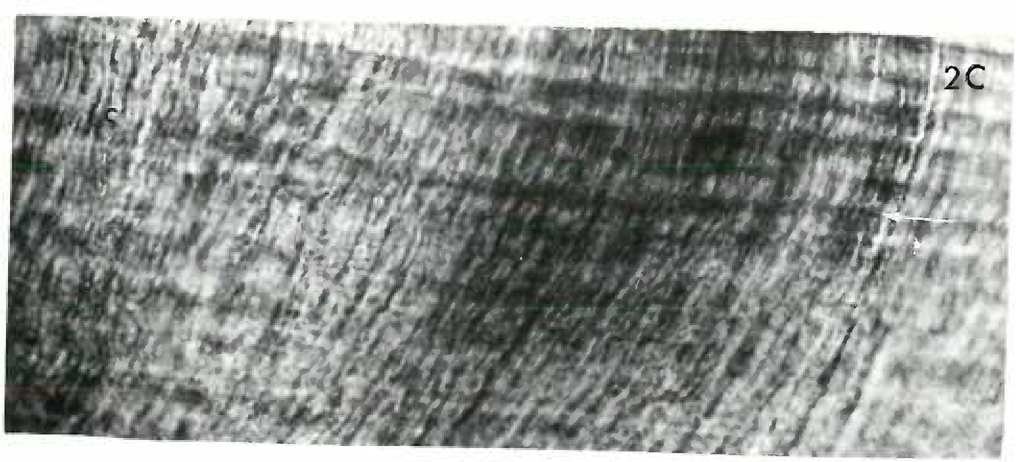

2C

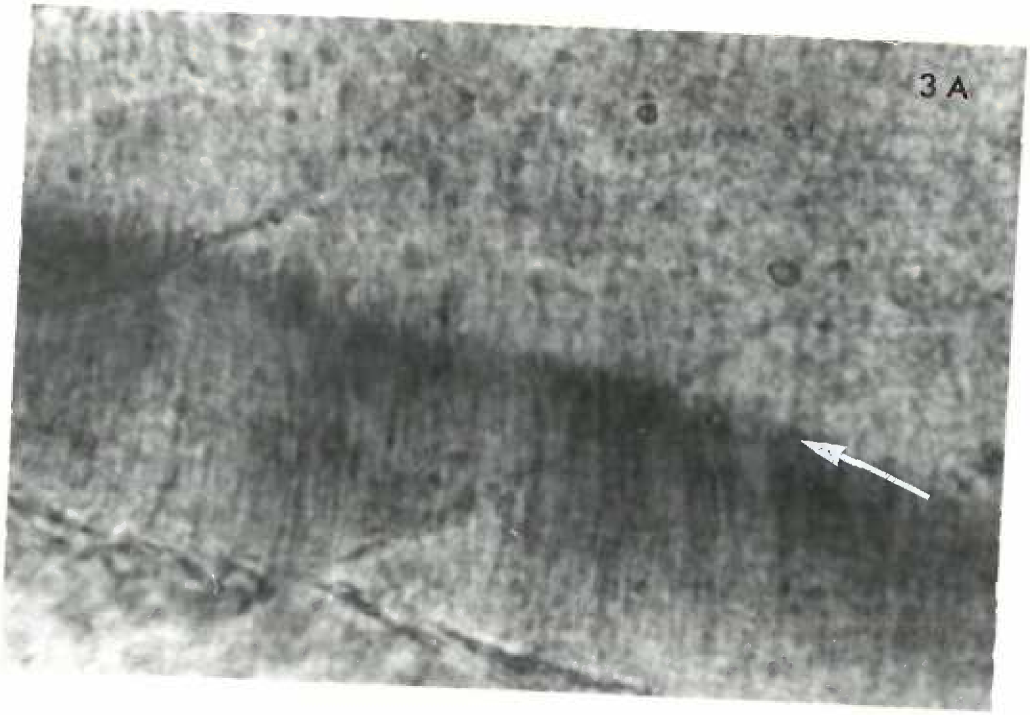
FIGURE 3

SECTION 30. SAME STRIA OF RETZIUS AT 106, 75, 54 AND 40 MICRA.

Transverse section. Recorded at second plane of focus. Large white arrow denotes stria of Retzius selected for study.

Figure 3A. 106 micra. Morphology of selected stria is not clear.

Figure 3B. 75 micra. Accentuated interprismatic substance and rod bending begin to appear.


3 A

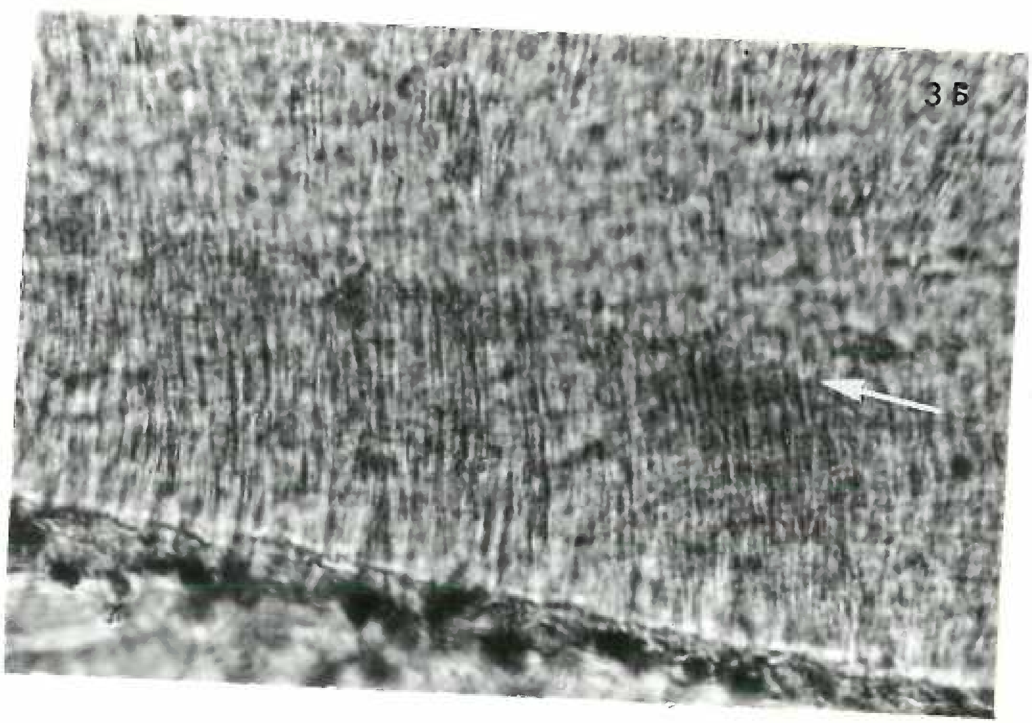
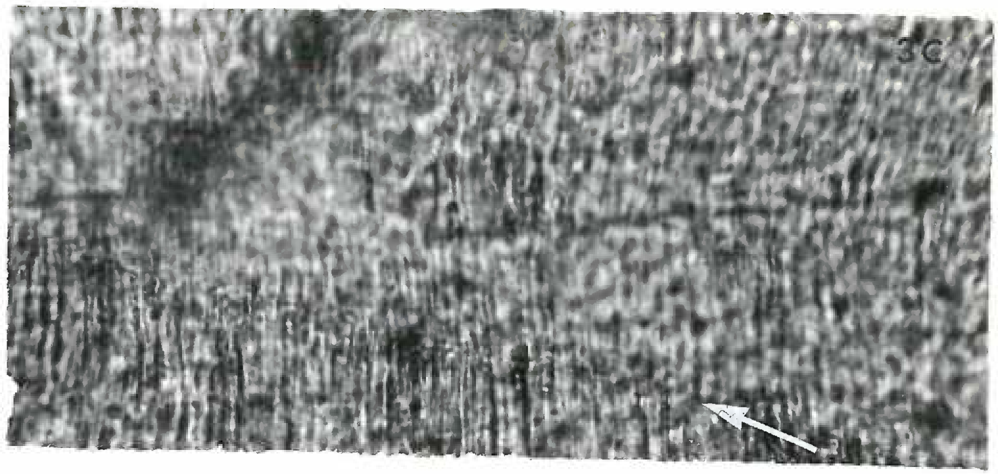

3 Б

Figure 3C. 54 micra. Note the improved detail in morphology as compared with 3A and 3B.

Figure 3D. 40 micra. Note the morphology of the stria is clearest in this photograph.


3C

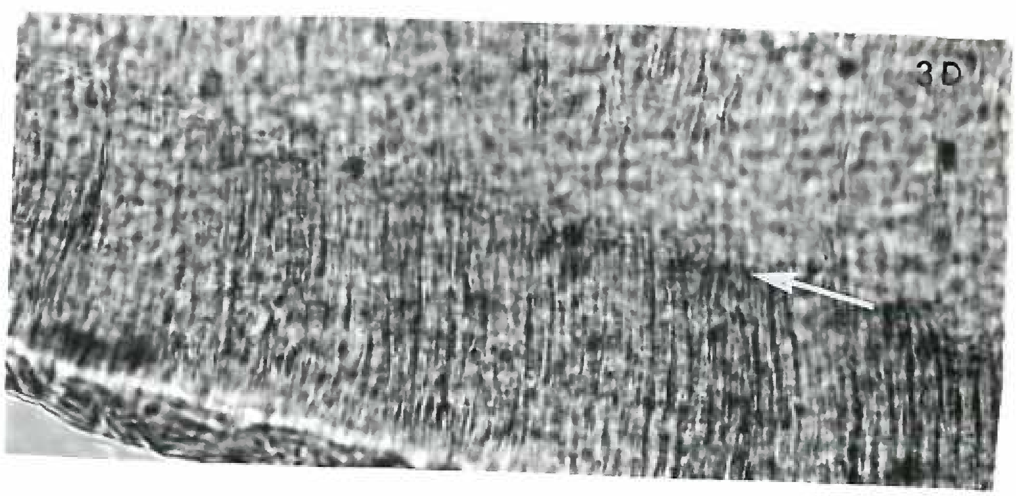

3D

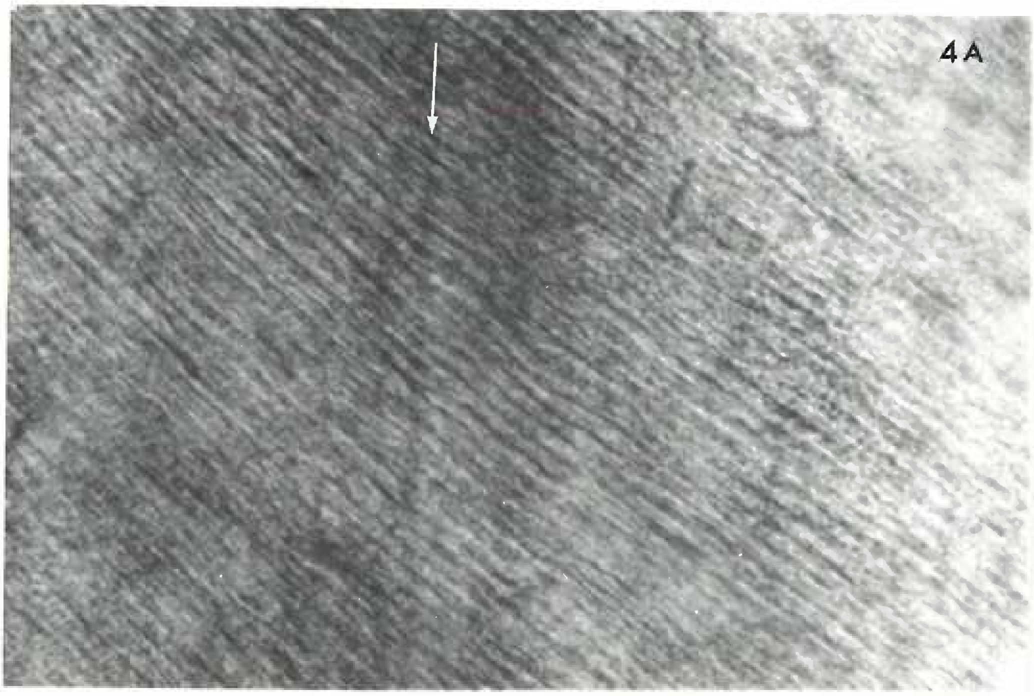
FIGURE 4

SECTION 32. SAME STRIA OF RETZIUS AT 107, 51, 31 AND 18 MICRA.

Longitudinal section. Recorded at third plane of focus. White arrow denotes striae of Retzius selected for study.

Figure 4A. 107 micra. Morphology of selected stria is not clear.

Figure 4B. 51 micra. Note the improvement in the detailed morphology as compared with 4A.


4A

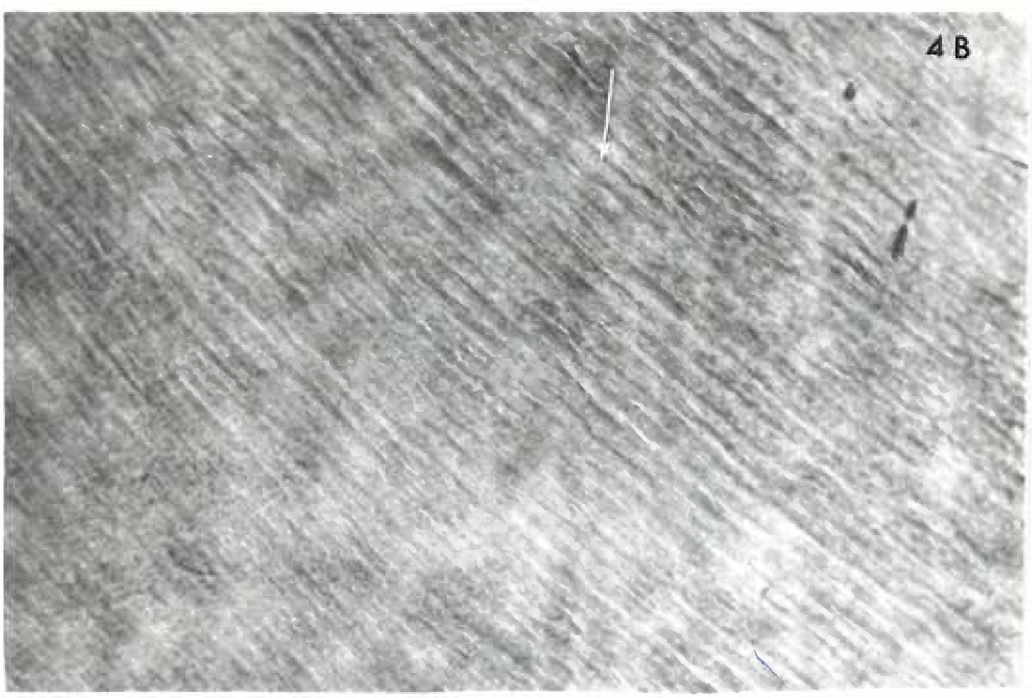
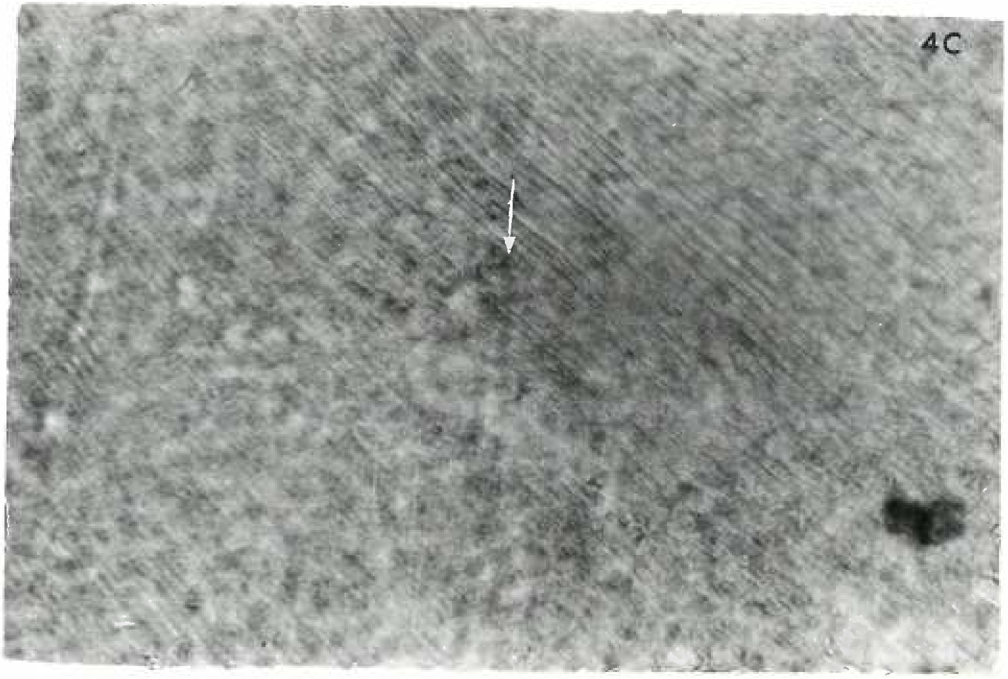
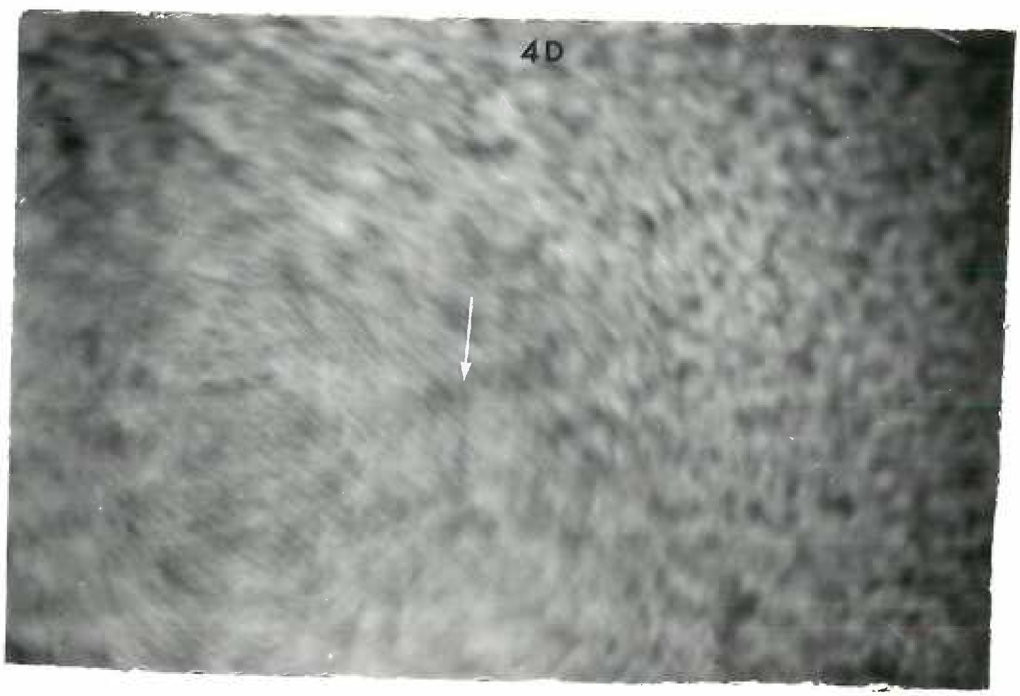

4B

Figure 4C. 31 micra. The morphology of the line is best resolved at this thickness.

Figure 4D. 18 micra. Morphology of selected striae is not clear.


4C


4D

Planes of focus and resolution of striae of Retzius. All photographic records of studied planes of focus within each prepared thickness of sections 46 and 30 were evaluated to determine the best plane of focus for a given thickness. All records of a given section were then compared at the established plane of focus. This was done to help rule out whether the resolution of the striae might depend more upon the plane of focus studied than upon the thickness of the preparation. Table 3 shows that the thinnest preparation gave the best resolution in 6 of 7 available comparisons.

TABLE 3

PLANE OF FOCUS VERSUS THICKNESS IN THE RESOLUTION OF STRIAE OF RETZIUS

Section	Preparation Thickness in Micra	Best Plane of Focus	Preferred Thickness at "Best Plane of Focus"
46	106	4th	53 micra
	70	3rd	53 micra
	53	5th	53 micra
30	106	5th	106 micra
	75	3rd	40 micra
	54	2nd	40 micra
	40	3rd	40 micra

Reliability of establishing specific planes of focus. It was considered important to determine whether records taken at specified planes of focus were actually comparable between thicknesses. The greatest potential source of error appeared to be the location of the preparation's lower surface which had been the starting point for all records. The taking of records from sections 32 and 33 was designed primarily to test the reliability of reaching the same plane of focus within and between preparation thicknesses. Figure 5 shows representative photographs of the bottom landmarks within and between three thicknesses for section 32. It can be seen that the landmark generally appears comparable in all photographs. However, sone differences can be noted.

It was shown that those observed differences in landmark morphology could be due to small differences in the plane of focus. A series of records were made by turning the microscope's fine adjustment one half increment (1 micron) at a time, taking photographs at each of five levels, beginning at the bottom. Differences were always observed between levels in landmark morphology. Sometimes pronounced changes were detected when the plane of focus was changed by only 1 micron. See Figure 6.

In section 33, the re-establishment of bottom landmarks three times at thickness 100 micra showed good inter-landmark comparability. However, the landmarks were lost during the process of reduction to 75 micra. In section 30 the recorded bottom landmarks showed exactly the same morphology among the various preparations. In section 46 the landmarks established at 106 micra thickness were lost in subsequent preparations, most probably by water flushing through an imperfect cement seal.

In preparations where the landmarks were lost during thickness reduction, new landmarks were selected for identification after subsequent reduction. However, the verification of the comparability

FIGURE 5

SECTION 32. CHANGES IN MORPHOLOGY OF LANDMARK WITHIN AND AMONG DIFFERENT PREPARATIONS.

Longitudinal section. Recording at bottom plane of focus. Black arrow denotes landmark selected for study.

Figures 5 A, B, C. 107 micra.
Figure 5 D. 70 micra.
Figures 5 E, F, G. 51 micra.

Figures 5 A, B and C. Note minor changes in landmark within one thickness after it was re-established three times.

Figures 5 E, F and G. Note there are no changes in landmark within one thickness after it was re-established three times.

Figures 5B, 5D and 5F. Note the minor changes in the landmark among these thickness.

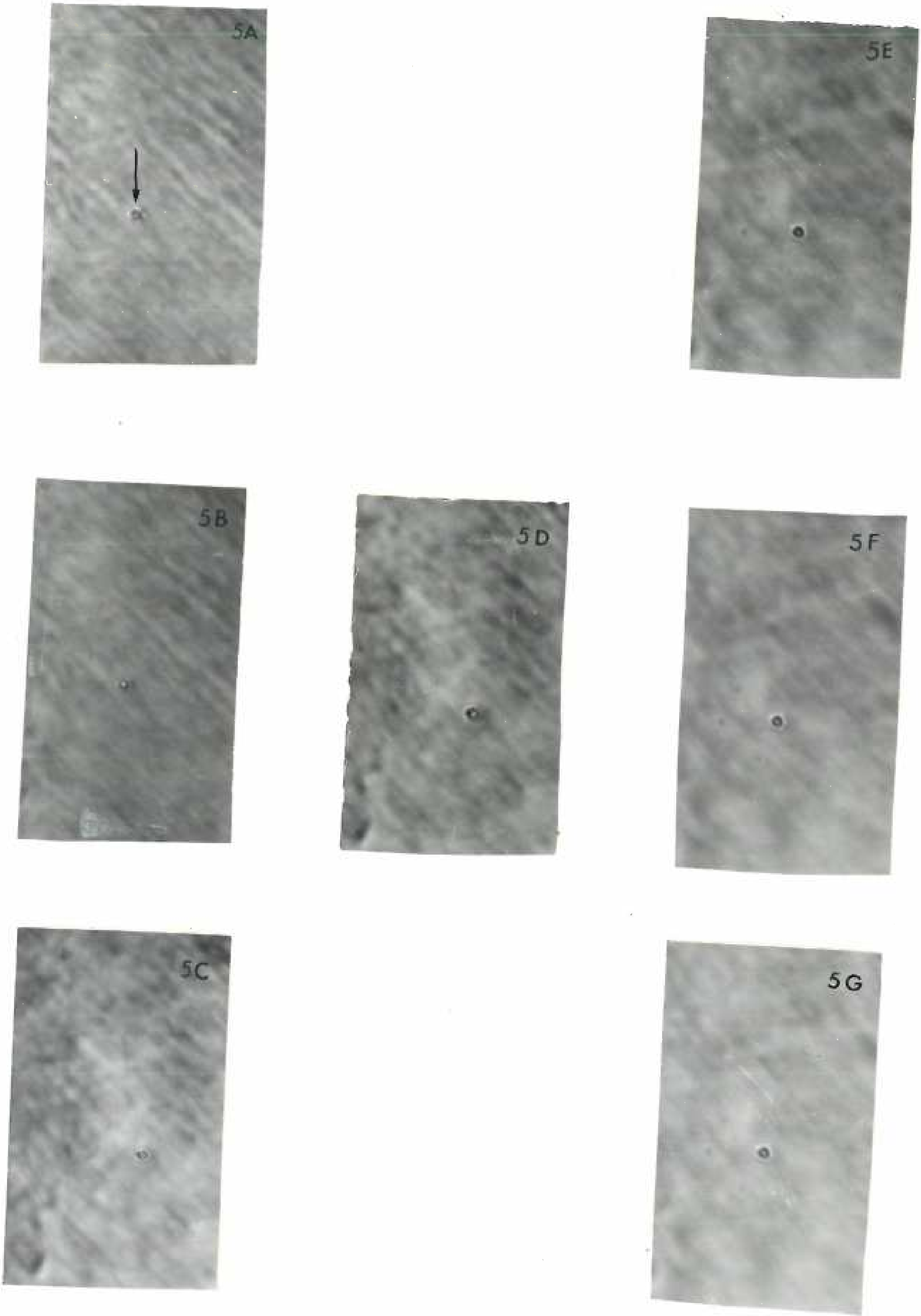
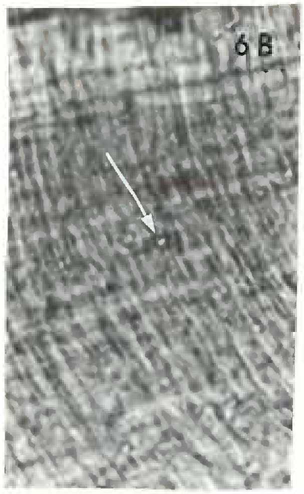

5A
5E
5B
5D
5F
5C
5G

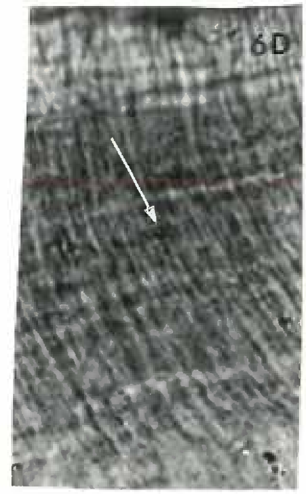
FIGURE 6

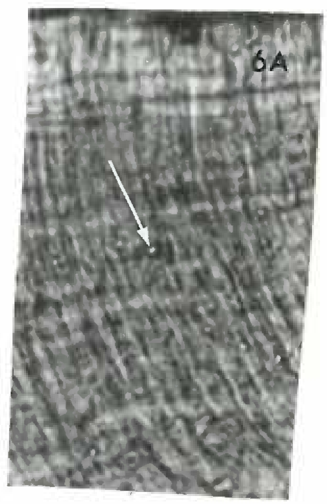
SECTION 55. THE CHANGES IN LANDMARK MORPHOLOGY DUE TO LOWERING THE PLANE OF FOCUS BY 1 MICRON INCREMENTS.

Transverse section. Recorded at bottom plane of focus. White arrow denotes the bottom landmark selected for this study.

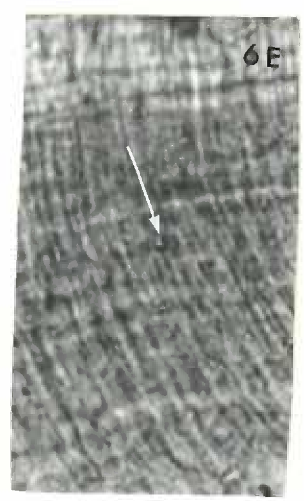
Figure 6A, B, C and D represent successive 1 micron increments. Note that changes are discernible between any two successive increments. Marked changes are seen between B and C.


6B


6D


6A


6C


6E

of the planes of focus could only be obtained by identifying specific enamel components in different thicknesses at the same plane. Figure 2.

Part Two

General findings. Accentuation of the interprismatic substance was shown to be present in every striae of Retzius in each of the five sections examined in this study. (Figures 8, 10 and 11 and Table 4).

Certain other characteristics were present in addition to accentuated interprismatic substance in some records of all groups studied. These characteristics are listed in decreasing order of frequency:

1. Darkness of enamel rods located within the striae studied. (Figures 9 and 11).

2. Bending of the enamel rods along the striae. (Figures 7 and 9).

3. Accentuation of cross striations of enamel rods within the striae of Retzius. (Figures 8 and 9).

Morphology of striae of Retzius in different layers and regions. Striae of Retzius of different layers and of different regions (when the same layer was studied), showed several morphological differences. A complete record of the findings is presented in Table 4. No consistent difference was found in the morphology between layers and between regions. However, when the striae of Retzius in outer and middle layer were compared with the inner layers, all striae showing accentuated cross striations were found in the outer layer (Figures 8, 9, 10 and Table 4).

Morphology of the same stria of Retzius in different regions. A record of the findings is presented in Table 5. Differences in morphology were seen along the course of a single stria. However, the major feature was the varying prominence (as expressed by thickness and intensity of darkness) in some regions as compared with others. For example, in section 65 the selected stria of Retzius was more prominent on the distal and the facial regions than on the lingual (Figure 11). In section 67 the same line showed variation in its morphology from one region to another. This line showed acute bendings and accentuation of cross striations on the facial region close to the distal. However, characteristics could not be detected in the same stria on the façial region closer to the mesial or at the junction between the facial and the distal regions (Figure 10).

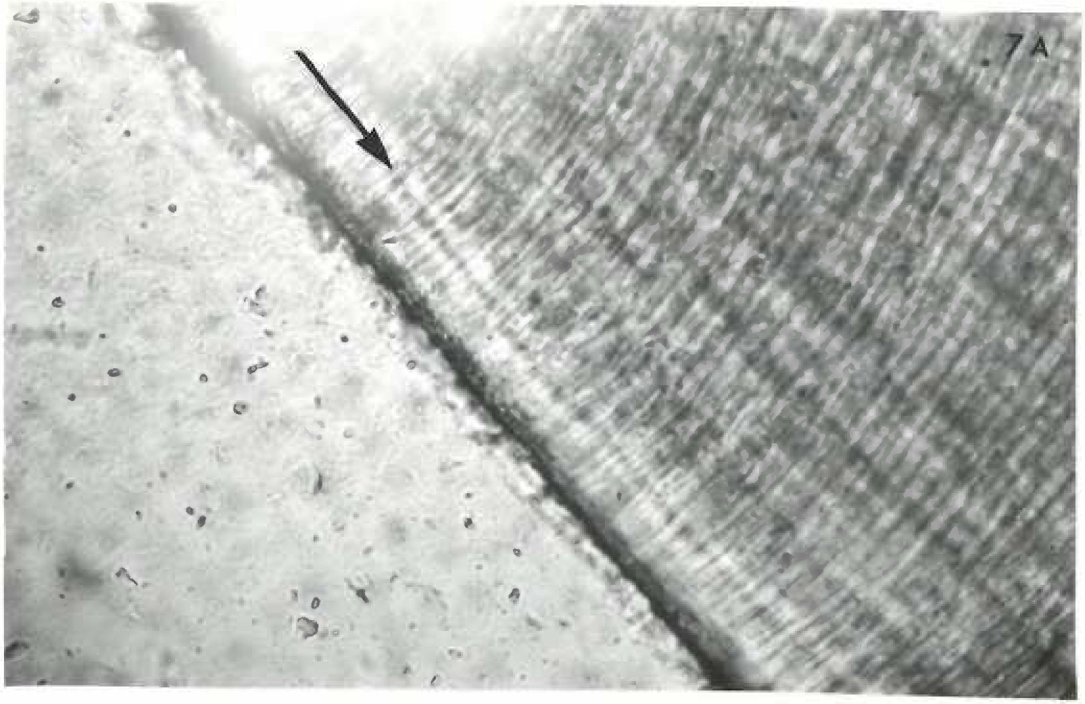
FIGURE 7

SECTION 67. STRIAE OF RETZIUS IN THE OUTER, MIDDLE AND INNER LAYERS.

Transverse section. Facial region, near distal. The large arrow (black and white) denotes selected striae of Retzius.

Figure 7A. Outer layer. Note the accentuation of the interprismatic substance and generalized darkness of a segment of the enamel rod (small black arrow).

Figure 7B. Middle layer. Note the accentuation of interprismatic substance and darkness of rod segments.


7A

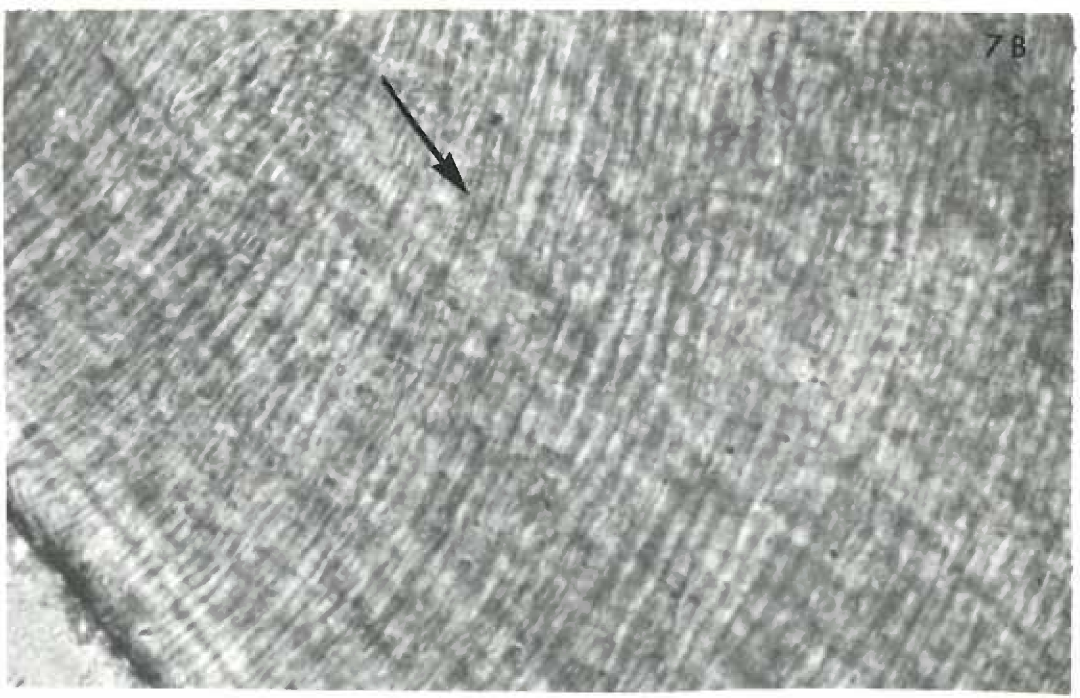

7B

Figure 7C. Inner layer. Note the accentuation of the interprismatic substance and bendings of the enamel rods.

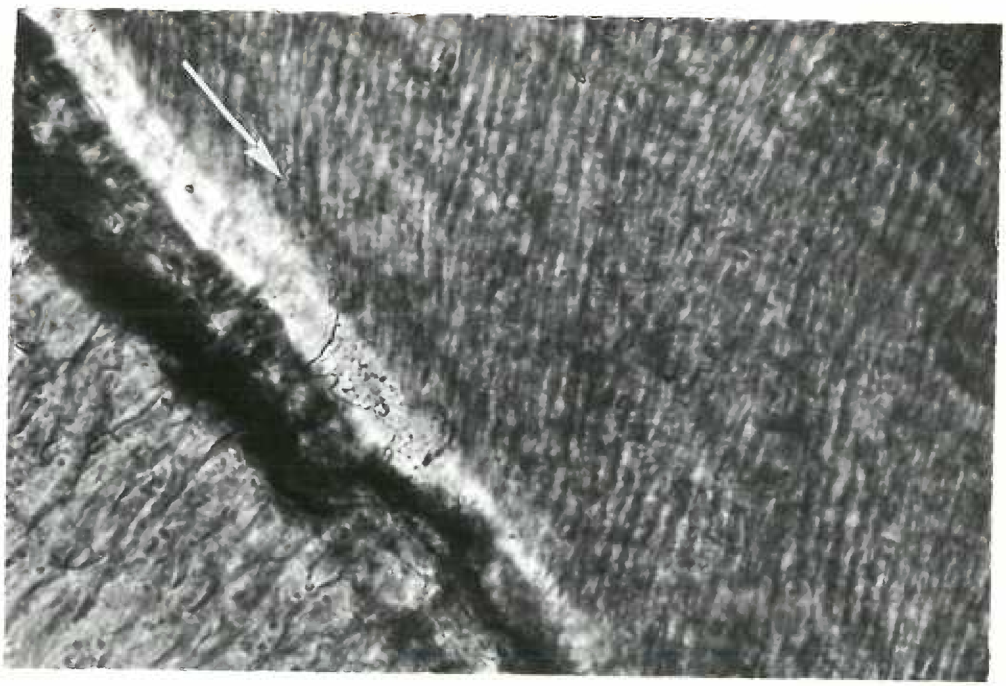


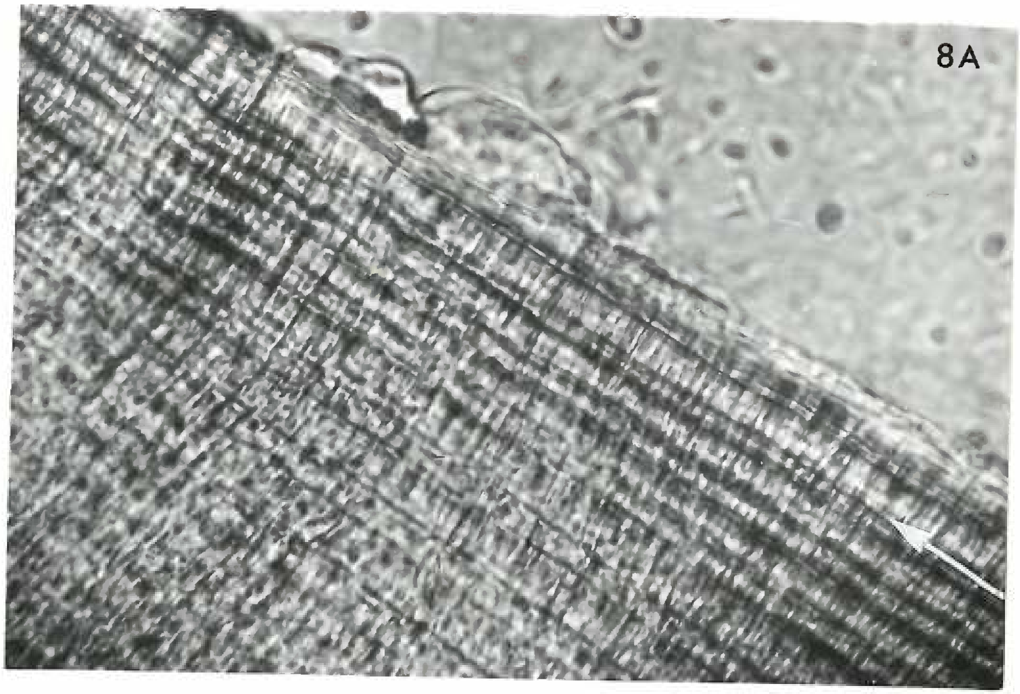
FIGURE 8

SECTION 67. STRIAE OF RETZIUS IN THE OUTER, MIDDLE AND INNER LAYER.

Transverse section. Lingual region. Arrow denotes the selected striae of Retzius.

Figure 8A. Outer layer. Note the striking accentuation of cross striations along the striae of Retzius.

Figure 8B. Middle layer. Note the accentuation of interprismatic substance.


8A

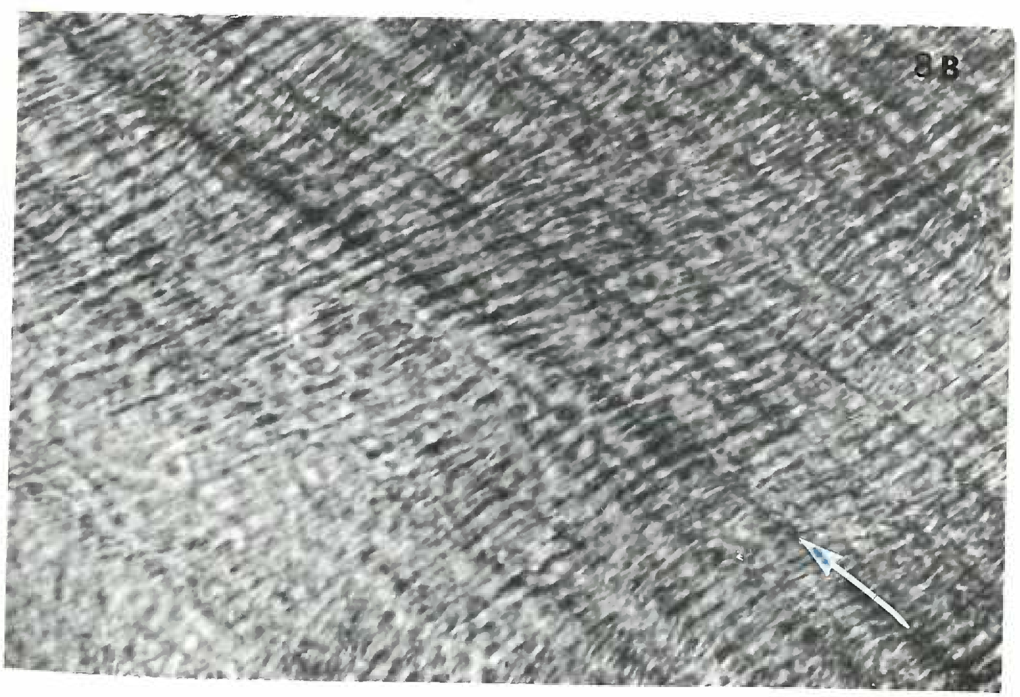

8B

Figure 8C. Inner layer. Note the accentuation of interprismatic substance.

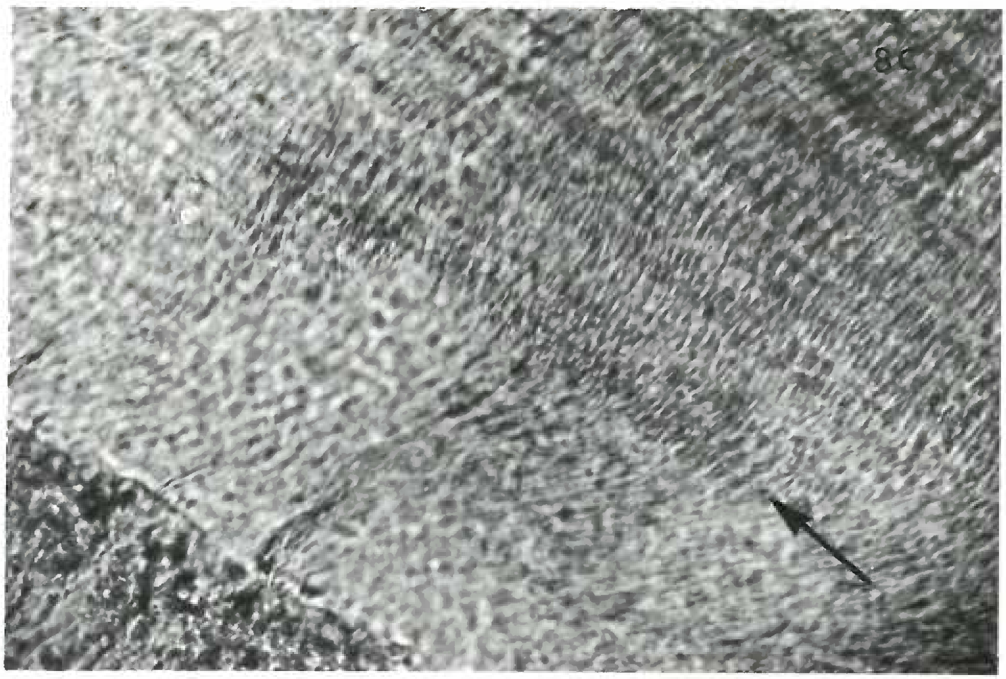


FIGURE 9

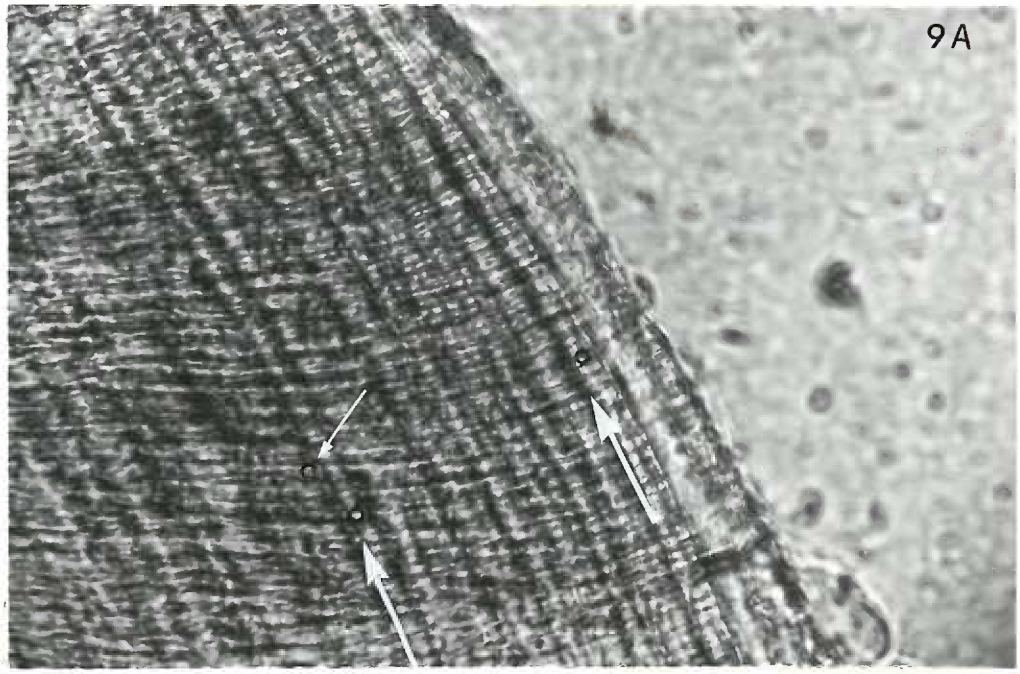
SECTION 67. STRIAE OF RETZIUS IN THE OUTER, MIDDLE AND INNER LAYERS.

Transverse section. Lingual region.

Figure 9A (a). Outer layers. Arrow (a) denotes selected stria of Retzius. Note the accentuation of cross striation, accentuation of the interprismatic substance and acute rod bendings. In addition, darkness of some rod segments.

Figure 9A (b). Middle layer. Large arrow (b) denotes selected stria of Retzius. Small arrow (c) denotes the rod bending which gives the impression of cross striation.

Figure 9B. Inner layer. Arrow denotes selected stria of Retzius. Note general lack of clarity. The stria is not prominent here. Note accentuation of interprismatic substance.


9A

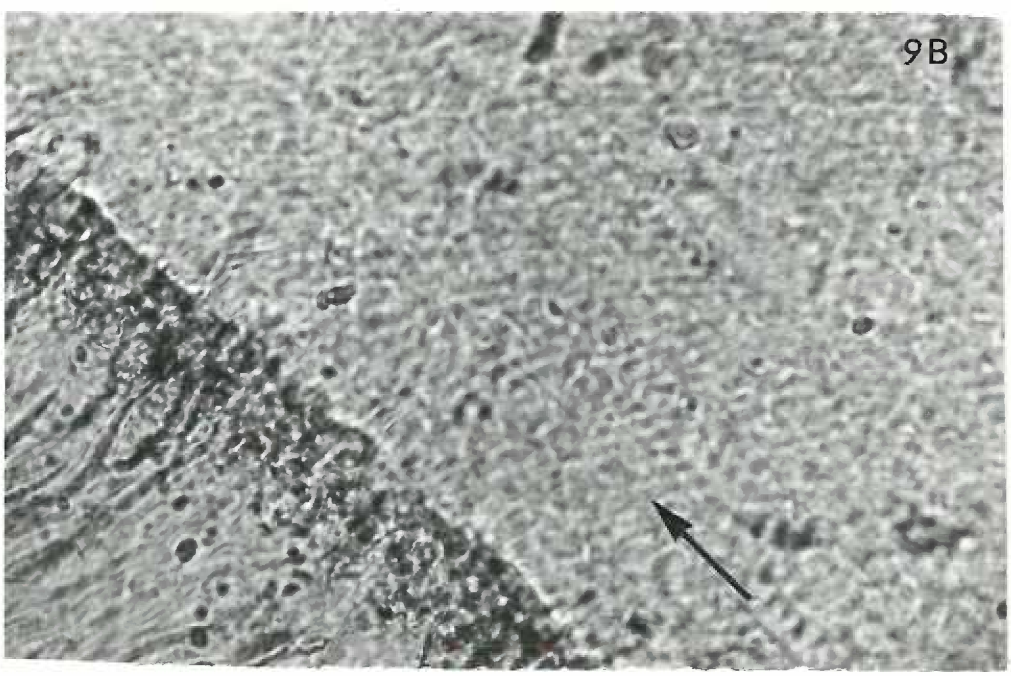

9B

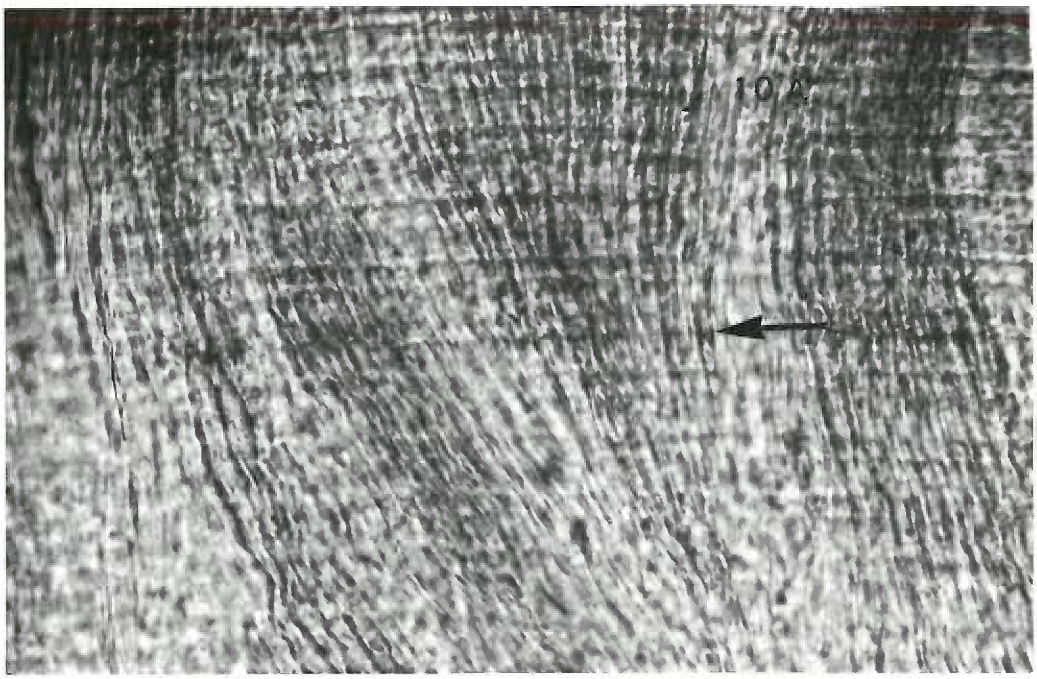
FIGURE 10

SECTION 67. SAME STRIAE OF RETZIUS IN DIFFERENT REGIONS.

Transverse sections. Black arrow denotes the selected stria.

Figure 10A. Facial region close to distal. Note rod bending, accentuation of both cross striations and interprismatic substance.

Figure 10B. Same line facial region near the mesial. Note that there are no accentuated cross striations. The only characteristic observed is the accentuation of the interprismatic substance.


10 A

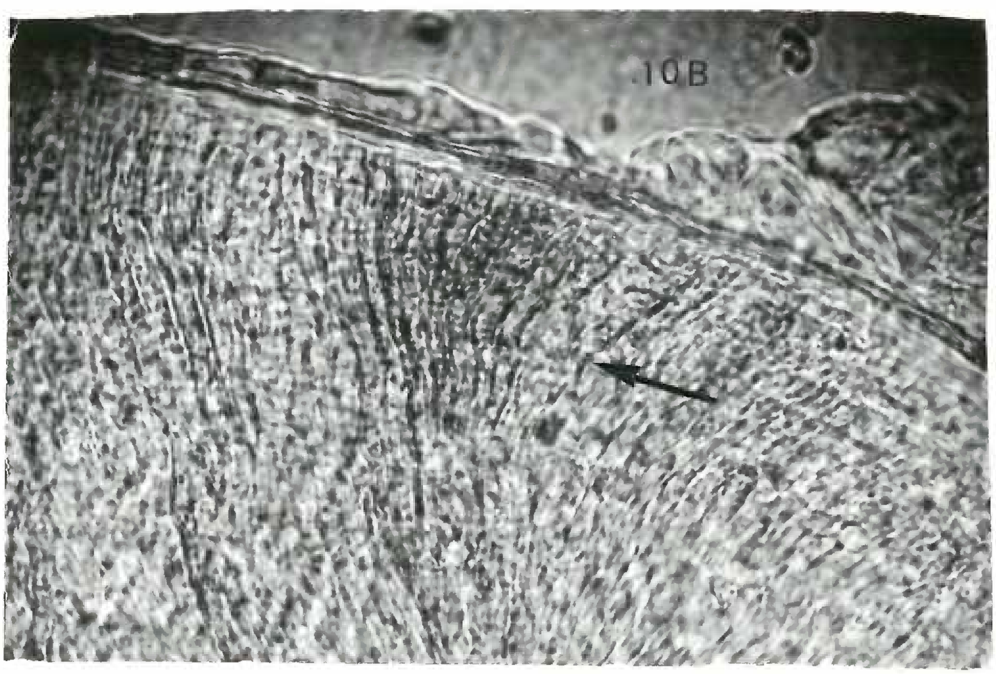

10 B

Figure 10C. The same line at facial-distal junction. Note that the only observed characteristic is the accentuation of interprismatic substance.

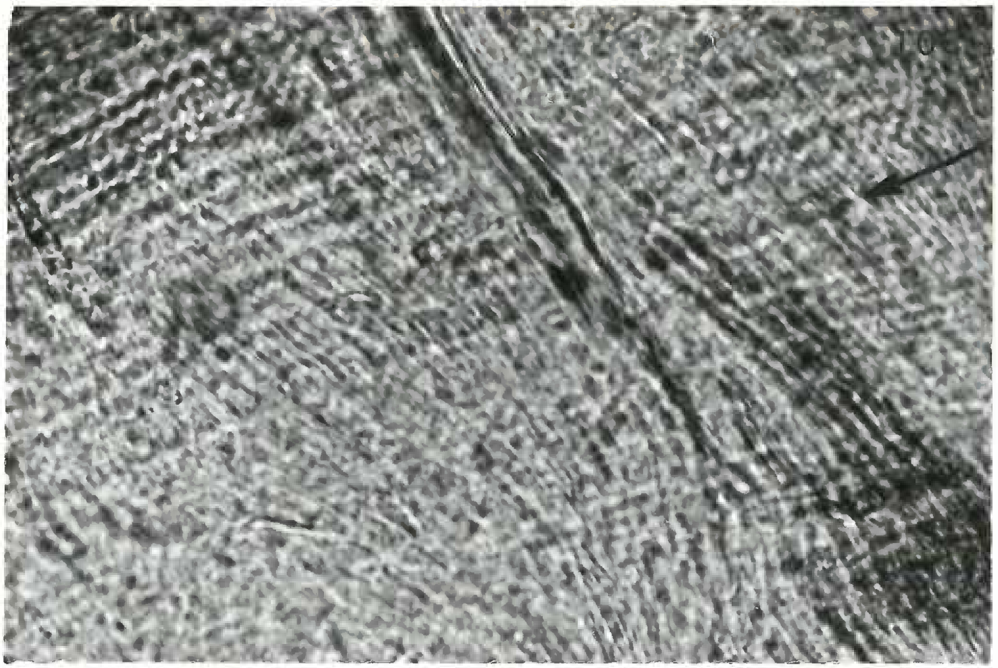


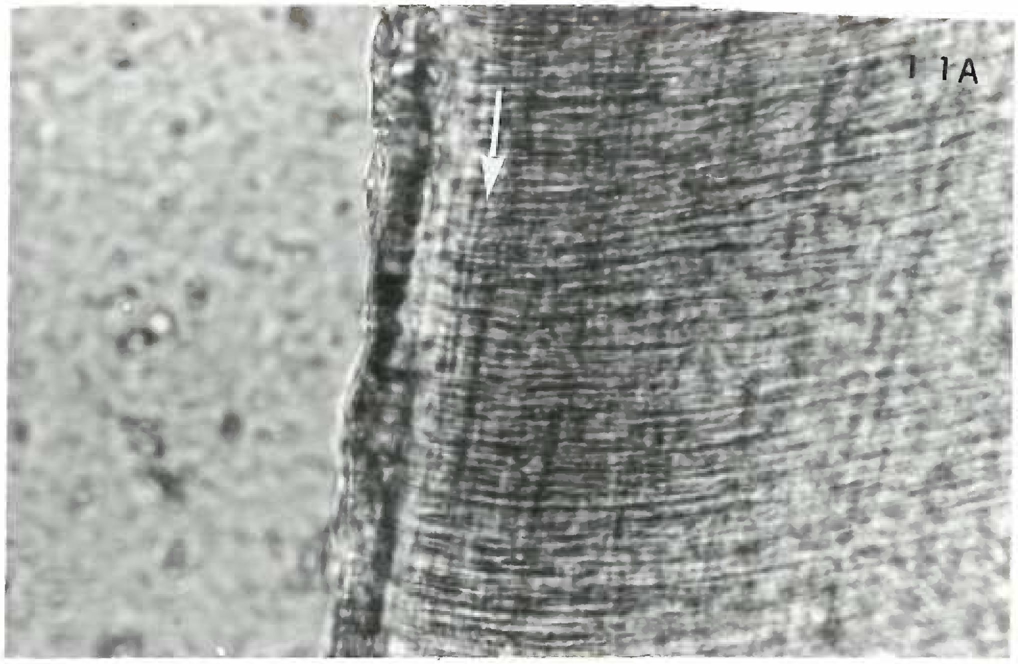
FIGURE 11

SECTION 65. SAME STRIAE OF RETZIUS IN DIFFERENT REGIONS.

Transverse section. Large white arrow denotes the selected striae.

Figure 11A. The selected striae in the outer layer on lingual side near the distal. Note accentuation of the interprismatic substance and generalized darkness along the line.

Figure 11B. The same line on the distal side. Note accentuation of the interprismatic substance and generalized darkness. Small arrow (a) denotes bending which gives the impression of cross striation.


11A

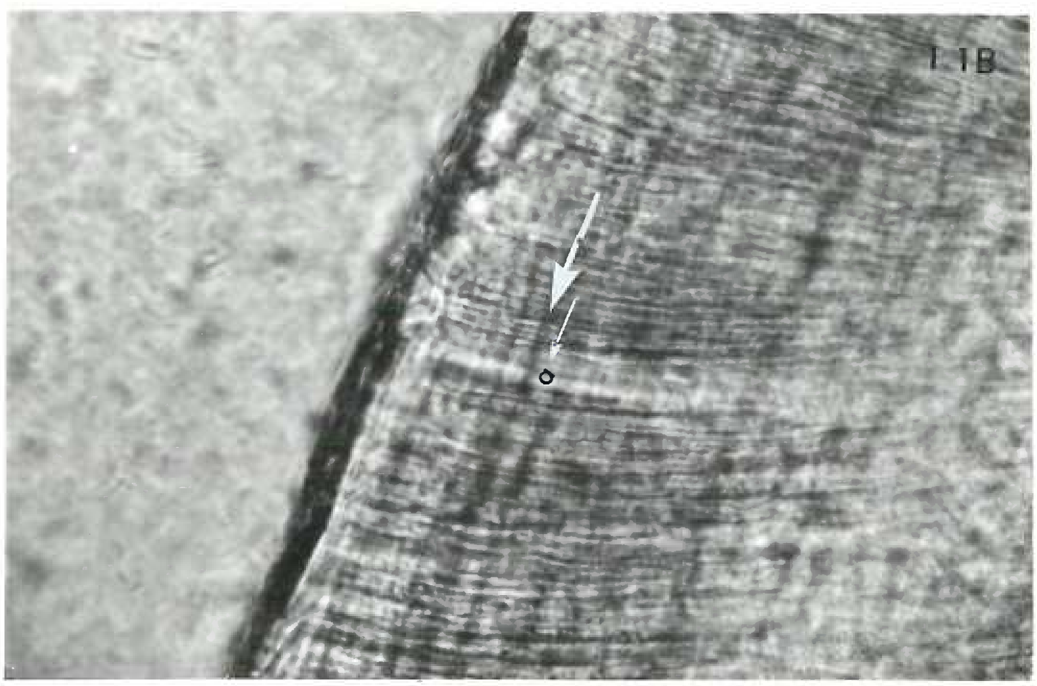
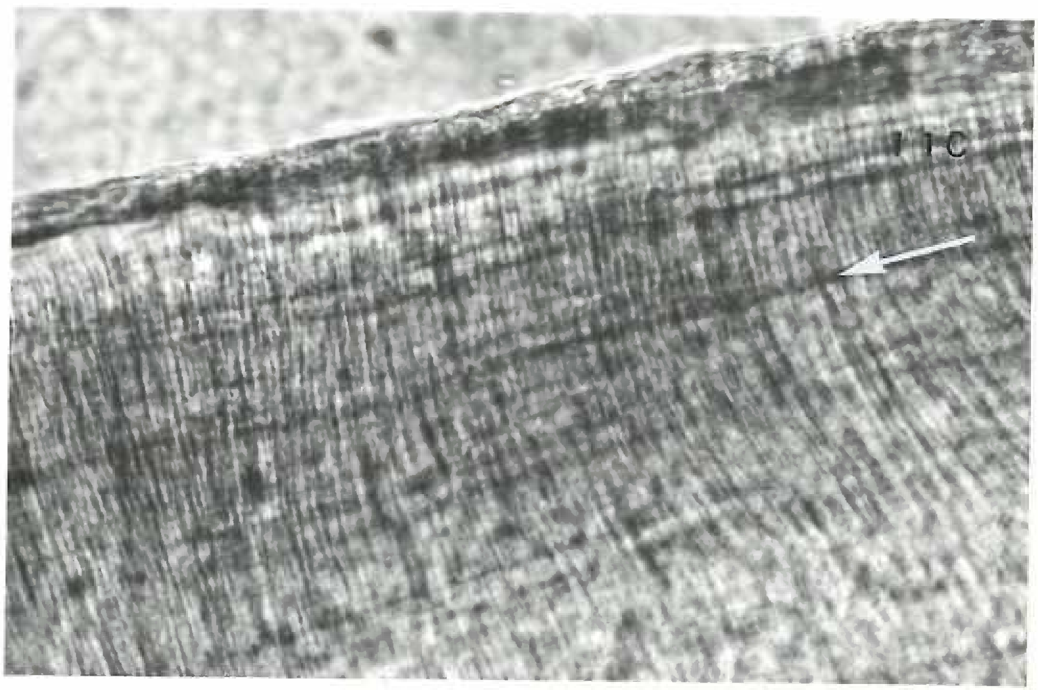

11B
o

Figure 11C. The same line was recorded on the labial side. Note the line here is more prominent than in Figure 11A and B. Note the accentuation of the interprismatic substance and darkness of rod segments.


11C

DISCUSSION

There is no final agreement in the literature on the biological processes involved in the production of striae of Retzius. Some consider that the enamel matrix formation is affected (2)(3). Others consider that the appearance of striae of Retzius reflects variations or disturbances of the mineralization process (5)(6).

The morphological expression of these biological processes has been a source of much more controversy. Some writers have observed an increase in the width of the prism sheath. Jansen and Visser (4) stated that they have observed these changes on ground sections, while Sognnaes claimed to have shown on decalcified preparations that all Retzius lines, as well as the special neonatal lines, were related to thickening of the prism sheaths. On the other hand, Anna Gustafson (6) in her extensive study of striae of Retzius stated that she could not observe any changes in the prism sheath. She said that the changes which other authors related to the prism sheath are actually in the interprismatic substance which increases at the expense of the adjacent prism. This author found it impossible to distinguish on ground sections between the interprismatic substance and prism sheath. Another group of investigators observed that bending along the course of the enamel rod account for the presence of striae of Retzius (1)(6)(7). Schour (5) has connected striae of Retzius with a low degree of mineralization of the cross striation in the prism so that the cross striations appear more clearly in the striae of Retzius.

Other authors have found different morphological patterns in the striae of Retzius. Much of their evidence is however, based on limited numbers of cases, and few have taken into account the great variation which may exist.

The concern in this study centers about the question of how the variety of conflicting reports about the morphology of striae of Retzius can be accounted for. The author's approach has been to investigate first whether technical matters such as section thickness, and second, whether the selection of material for observation may account for some of the confusion.

When all factors concerning the first part of this study are considered, the findings show that the thinner the section, the better the resolution of striae of Retzius down to about 30 micra. These findings did not hold true for one section of 18 micra thickness (Table 2 and Figure 4). The reasons for this difference are not altogether clear. However, the general poor picture presented at 18 micra may be related to changes in the Lakeside cement incurred during the previous thickness reduction. Sufficient problems were encountered in making sections below 30 micra to justify not answering at this time the question whether section thicknesses under 30 micra do not show striae as clearly as 30 micra and above.

Referring to Table 2A, B and C, it can be seen that there were other reversals in which the thicker preparation was credited with better resolution than the thinner preparation. Most of the reversals are between the preparations at the thicker end of the scale, especially at planes of focus above the fifth level.

It was consistently observed that the recorded planes of focus near the upper surface of the section showed poorer clarity than those records taken somewhat deeper in the section. In comparing the resolution of different thicknesses, the uppermost plane in the thinner preparation crosses the thicker preparation somewhat below the surface. This may account for frequent findings that thicker sections gave better resolution when the plane of focus for the thinner preparation was close to the top.

Data in Part One were collected and analyzed on the basis of different thicknesses being recorded at the same plane of focus. Therefore, attempts were made to verify the reliability in locating specific planes of focus. In order to do this, landmarks at the lower surface were determined within one thickness and between different thicknesses. Some changes were observed in the morphology of these landmarks (Figure 5). From an experiment recording small changes in focal planes (Figure 6), it was estimated that differences in the morphology of the bottom landmark seen in Figure 5 could be accounted for by differences in planes of focus within a range of two microns. In some cases the landmarks were lost between thicknesses after reducing the section by hand grinding. When this happened the morphology of the enamel at the planes of focus close to the bottom was compared among the different thicknesses. The morphological structures could be observed in all thicknesses of the same section (Figure 2).

One may question whether the improved resolution obtained during reduction of section thickness brings with it differences in the morphological findings. Observation of the selected stria of Retzius on the longitudinally ground section 46, 107 micra, could be interpreted as showing accentuated cross striations. But when the same stria, at the same plane of focus, was recorded at 70 micra, accentuation of the interprismatic substance and rod bendings began to appear. The thinnest preparation in section 46 (53 micra) showed accentuation of the interprismatic substance and bendings very clearly (Figure 2). From this example, one can see that it may be possible to make a false conclusion if sections of 100 and perhaps 70 micra thicknesses are used. This is probably due to superimposed layers of enamel rods which may result either in an unclear image through the scattering of light rays, or in an altered picture through an additive effect of morphological units.

The findings of the second part of the study showed that there are differences in the morphology of striae of Retzius (some of them striking in nature) in different areas on the same section, or between different sections. The differences, however, were not consistent or characteristic between the groups. These variations were observed on material taken from one animal. The fact that these observed variations do occur indicates that the conclusions of an investigator could be influenced by his selection of sections for observation.

When morphological interpretations of striae of Retzius were made, all characteristics appearing in the striae could also be seen in non-striae enamel. However, these characteristics coincided at particular points in adjacent enamel rods to comprise the striae of

Retzius. It has been previously stated that general darkness of segments of enamel rods were observed in striae of Retzius. An exact interpretation of significance of this darkness was not within the scope of the project. Schour (5) has interpreted darkness in cross striations and interprismatic substance as indicating a lower degree of mineralization than in the brighter areas. However, the author feels that darkness could also be related to a variation in the structural orientation of the tissue or to superimposition of structural component. Because a quantitative approach to the degree of mineralization was not employed, the author prefers not to make any further interpretation which is not based on appropriate data.

Several investigators have attributed striae of Retzius to bending of the enamel rods. This study showed that in every instance where bending occurred, it was accompanied by other features. It must be recalled, however, that most striae of Retzius studies utilized longitudinal sections; Part Two of this study utilized transverse sections. Possible differences or similarities between these two planes of section still await proper documentation.

This investigation showed the importance of studying thin sections in order to obtain the clearest pictures of the morphological pattern of striae of Retzius. The findings of this study pointed out also that biological variations occur in the morphology of striae of Retzius; these variations may account in part for the divergent opinions concerning this subject.

CONCLUSIONS

The findings of this study suggest that the following conclusions may be drawn with respect to deciduous teeth of minature swine:

1. The microscopic resolution of striae of Retzius as seen in longitudinal or transverse ground sections, improves as the section thickness decreases within the range of 100-30 micra.
2. The thickness of the sections may mask the true interpretation of the morphological pattern of striae of Retzius.
3. Different morphological patterns of striae of Retzius were observed among layers, regions and along the same striae of Retzius in different regions when observed in transverse ground sections.
4. An investigator's conclusion regarding the morphology of striae of Retzius could vary, depending upon his selection of material for observation.

The relationship of findings in teeth of miniature swine to those of human teeth await further investigation.

SUMMARY

Variations of morphological patterns and microscopic resolution of striae of Retzius as influenced by section thickness and the selection of the area for observation were studied.

The investigation was divided into two parts. In both parts deciduous teeth from young minature swine were utilized. Ground sections were prepared and all records were obtained in the form of photomicrographs taken with a Zeiss photoscope.

In Part One, records were taken from three longitudinal and one transverse section. Each section was taken from a different tooth. A section mounting method was devised to permit the section's retention on the glass slide during the grinding process when grinding was to be interrupted at certain increments for the purpose of taking records. A stria of Retzius was selected and then recorded from each section at about 100 micra thickness. The same recording was repeated at about 75 and 50 micra levels. When possible, sections were reduced in steps to about 40, 30 and 20 micra and records taken at each thickness. During the recording of each thickness, photographs were taken at various standardized increments from the lower surface of the section. It was therefore possible to make comparisons of the same section between different thickness preparations, each comparison being made at the same increment from the lower surface. The records were analyzed utilizing criteria designed to evaluate microscopic resolution.

The findings of this part of the study showed that the thinner the section, the better the microscopic resolution of striae of Retzius within a range of about 100 to 30 micra. In addition it was found that interpretations of the morphology of striae of Retzius may vary between different thicknesses of the same section.

In Part Two, the morphological pattern of striae of Retzius was observed in 5 transverse sections from 5 teeth, all taken from the same animal. Comparisons were made of striae morphology in the outer enamel layers versus the middle and inner layers on each available surface region of the sections studied. Comparisons of striae morphology were also made in different regions (facial, distal, lingual and mesial as available). These region comparisons were made by grouping the records according to layers. Thirdly, the morphological patterns of single striae of Retzius were compared in different regions.

Differences in the patterns of striae of Retzius were detected in certain areas as compared with others. These differences were observed among different layers, different regions and along the course of single striae in different regions. However, there was no consistent morphological pattern associated with any of the groupings studied.

The findings of this thesis indicate that the morphological pattern of striae of Retzius can vary with the thickness of the section or with the area of the section selected for observation. Therefore an investigator's conclusions regarding striae morphology may be influenced by either of these factors.

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APPENDIX I.

The use of transmitted polarized light and incident light microscopy in the study of striae of Retzius.

Polarized transmitted light. Specific planes of focus of several preparations were recorded under transmitted non-polarized light and under transmitted polarized light. In every case the morphology of striae of Retzius was illustrated better under transmitted non-polarized light. This is demonstrated in Figures 12 and 13.

The literature on polarized light microscopy states that best results are obtained in enamel studies when the analyzer and the polarizer are kept in crossed positions and the stage is rotated until the enamel rods selected for study are at 45° to the crossed polarizer and analyzer.

Preliminary work showed it was difficult to relocate the selected area for the purpose of subsequent recordings, after the stage was rotated. In addition, the initial results showed such consistently better resolution with ordinary transmitted light, that polarized light experiments were discontinued.

The findings of this investigation do not necessarily invalidate the use of polarized light in the study of striae of Retzius. Polarized light has been used frequently for purely descriptive work and in some cases for quantitive determination in the field of dental tissue (6)(7)(20)(24)(29). The rapidity and simplicity of the polarization microscopic technique and its inexpensiveness has perhaps contributed to its popularity among investigators.

Interpretation of the image seen with polarized light can be misleading if not used properly. This is especially true when dealing with thick and highly refractive objects, such as ground sections of dental enamel, in which a great number of morphological units usually are superimposed on each other.

In order to study minute details the section must accordingly be extremely thin, preferably not thicker than the diameter of a single prism. To the knowledge of this investigator, no polarized light studies have ever been made using sections of this dimension. Interpretation of thicker sections must be made with great care and should be compared with the findings obtained from other methods of observation. The difficulty in interpretation in the field of dental enamel is due to the great number of factors contributing to the final image. These include the degree of mineralization, crystallite orientation, orientation of the enamel rod, apatite structure (fluoro- or hydroxy apatite), the fluid in which the ground section is placed for observation, the character of the organic framework, and the spaces between these organic and inorganic elements. All these factors change the rays coming through the specimen and affect the final image (6)(29)(31).

Incident light microscopy. Preliminary work was done concerning the utilization of incident light microscopy in the study of striae of Retzius. First efforts in visualizing striae of Retzius with this method of illumination were unsuccessful. Faculty at the Department of Geology, Oregon State University and the Department of Earth Sciences, Portland State College were consulted regarding the use of this technique. It was decided to discontinue further efforts in developing this method of observation. This decision was made both because of the technical problems encountered and also because of the anticipated difficulties in interpretation related to the translucency of enamel.

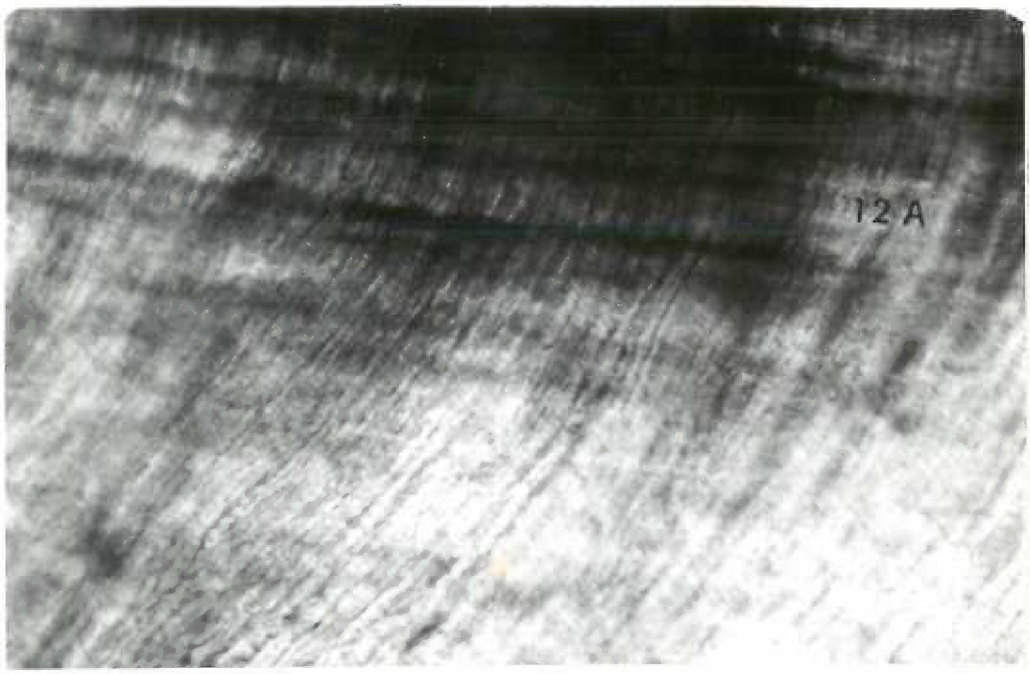
FIGURE 12

SECTION 46. STRIAE OF RETZIUS SEEN UNDER NON-POLARIZED AND POLARIZED LIGHT.

Longitudinal section. '70 micra. Recorded at sixth plane of focus. Both photographs were taken at 176X, enlarged 3.7 times.

Figure 12A. Transmitted polarized light.

Figure 12B. The same lines recorded by transmitted non-polarized light. Note the improved resolution of the striae.


12 A

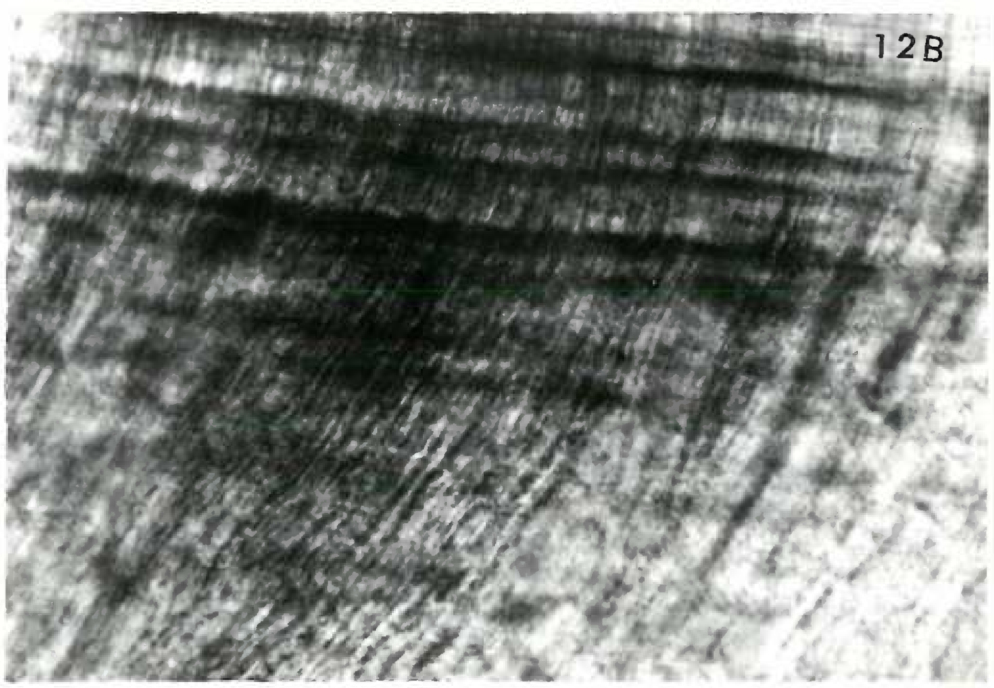

12B

FIGURE 13

SECTION 46. STRIAE OF RETZIUS SEEN UNDER NON-POLARIZED AND POLARIZED LIGHT.

Longitudinal section. 70 micra. Recorded at seventh plane of focus.

Figure 13A. Transmitted polarized light.

Figure 13B. The same lines recorded by transmitted non-polarized light. Note the improved resolution of the lines.


13A

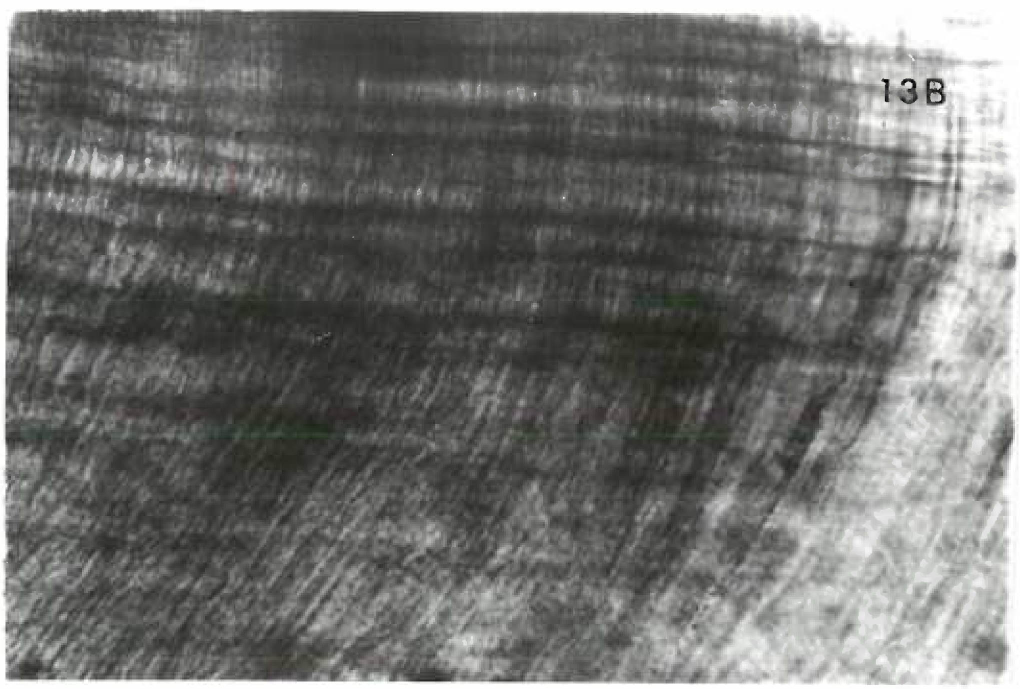

13B

TABLE 4

STRIAE OF RETZIUS IN TRANSVERSE SECTIONS

				Characteristics of Striae of Retzius			
Section	Regions	Layers		Accentuated Interprismatic Substance	Rod Bendings	Rod Darkness	Accentuated Cross Striation
No. 55	Facial	outer	x	x		x	
		inner	x	x		x	
	Facial-Distal	outer	x	x		x	
	Junction	inner	x	x	x		
		outer	x	x			
	Distal	inner	x	x			
		outer	x	x		x	
	Lingual	middle	x	x	x		
		inner	x	x	x		
No. 65	Facial near	outer	x	x		x	
	Distal	inner	x	x			
No. 66	Facial near	outer	x	x		x	
	Mesial	middle	x	x		x	
		inner	x	x	x	x	
		outer	x	x		x	
	Mesial	middle	x	x		x	
		inner	x	x		x	
		outer	x	x			
	Distal	inner	x	x	x		
No. 67	Facial near	outer	x	x	x		
	Distal	middle	x	x			
	Facial-Distal	outer	x	x		x	x
	Junction	middle	x	x	x	x	
		inner	x	x			
		outer	x	x		x	
	Distal	inner	x	x			
	Distal near	outer	x	x		x	
	Lingual	inner	x	x			
	Distal-	outer	x	x	x		x
	Lingual	middle	x	x	x	x	
	Junction	inner	x	x			
		outer	x	x			x
	Lingual	middle	x	x			
		inner	x	x			
No. 68	Mesial near	outer	x	x		x	
	Facial	inner	x	x			
		outer	x	x			x
	Facial	inner	x	x			

TABLE 5

SINGLE STRIAE OF RETZIUS IN DIFFERENT REGIONS

			Characteristics of Striae of Retzius			
Section	Regions	Layers	Accentuated Interprismatic Substance	Rod Bendings	Rod Darkness	Accentuated Cross Striation
No. 55A	Distal	inner	x			
	Lingual	inner	x			
No. 55B	Facial	middle	x	x		
	Facial-Distal Junction	middle	x			x
	Distal	middle	x			
	Distal	inner	x			
	Lingual	middle	x			
No. 65A	Lingual Close to Mesial	outer	x		x	
	Lingual Closer to Mesial	outer	x		x	
No. 65B	Lingual Close to Distal	outer	x		x	
	Distal side	outer	x		x	
	Facial	outer	x		x	
No. 67	Facial Close to Distal	middle	x	x		x
	Facial Close to Mesial	middle	x			
	Facial-Distal Junction	middle	x			
No. 68	Lingual Close to Mesial	outer	x		x	
	Lingual Close to Distal	outer	x		x	