PERFORATING FIBRES IN THE HUMAN MANDIBLE

A project report submitted in partial fulfilment for the degree of Master of Dental Surgery

by

RICHARD JOHN EDWARDS, B.D.S., B.Sc. Dent.(Hons).

Department of Dental Health,
The University of Adelaide,
Adelaide, South Australia.

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SUMMARY.

Serial sections were cut mesiodistally, horizontally and buccolingually through the molar, premolar and incisor regions of the human mandible for histological examination. The specimens of mandible were obtained from males aged 19, 20, 21, 46 and 68 years, and females aged 11 and 19 years. Mayer's haematoxylin and eosin, Mallory's phosphotungstic acid and haematoxylin, Heidenhain's aniline blue, Humason's modification of the Mallory stain, and the Gordon and Sweet silver stain counterstained with Van Gieson, were used to determine the most suitable stain for the demonstration of perforating (Sharpey) fibres. The Gordon and Sweet silver with Van Gieson counterstain suitably demonstrated perforating fibre bundles traversing the crestal third of the interdental and interradicular bone in the buccal and incisor periodontal areas of the 11 to 21 year old specimens. Perforating fibre bundles were also observed traversing the labial and buccal alveolar bone in the incisor, premolar and molar areas of the 11 to 21 year age group.

The implications of these findings are discussed in relation to other histological studies of perforating fibre bundles in man and the mouse. The possible role of the perforating fibre bundles in orthodontic relapse is also considered.

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SIGNED STATEMENT.

This project report is submitted in partial fulfilment of the requirements of the Degree of Master of Dental Surgery in the University of Adelaide.

This report contains no material which has been accepted for the award of any degree or diploma in any University. To the best of my knowledge and belief, it contains no material previously published or written by another person except when due reference is made in the text of the report.

RICHARD J. EDWARDS.

INTRODUCTION.

In 1975 Cohn reported that human transalveolar (Sharpey) fibre bundles traversed the interdental and interradicular bone wherever lamellated bone was found without Haversian systems. He also stated that the collagen (transalveolar) fibre bundles passed through the bone on the buccal and lingual aspects in the crestal, middle, and apical third of the alveolar wall to unite with the collagen bundles of the overlying periosteum and lamina propria. Cohn suggested that this arrangement represented a functional adaptation of the transalveolar fibre bundles to occlusal and muscular forces which permitted the maximum support to the tooth in its alveolus. He also proposed that, as a result of his findings, a reassessment of Sharpey fibre bundles was warranted in relation to the maintenance, repair, and aging of the periodontium.

In a preliminary histological study by the present author (R. J. Edwards 1974) the human mandibular premolar region of three males aged between 19 and 21 years was examined. The findings differed from those reported by Cohn (1975) who did not state the age or sex of his human material. Therefore, the present author has endeavoured to study the perforating (Sharpey) fibres in a group of human mandibles of differing ages and sex.

The influence of collagen fibres on orthodontic tooth movement was first suggested by Oppenheim in 1911 and subsequently re-emphasized by Skillen and Reitan in 1940. Reitan (1959, 1969) has shown histologically that the free gingival fibres and transseptal fibres in dogs remained stretched and displaced for at least 232 days after orthodontic rotation of the teeth. He suggested that the free gingival and transseptal fibres were more active in maintaining the tooth in its stable relationship than the periodontal fibres. On the basis of these and other observations, numerous surgical techniques have been proposed to reduce post-orthodontic tooth movement. Boese (1969), J. G. Edwards (1970), Pinson and Strahn (1974) and Walsh (1975) described various methods which involved the incision of the supracrestal gingival fibres to prevent relapse. However, relapse, which J. G. Edwards in 1970 defined as "that tendency of orthodontically moved teeth to partially return to their original pretreatment position and alignment" has not been eliminated from orthodontic treatment.

Based on the observations of the present author, the surgical techniques currently employed to prevent relapse may not be acceptable.

AIMS OF THE INVESTIGATION.

The purpose of this project was to study the perforating (Sharpey) fibre bundle pattern in the anterior and posterior region of human mandibles, obtained from motor vehicle accident victims, with reference to:

(1) the findings by Cohn (1975),

(2) possible differences in the fibre bundle pattern in human specimens of varying ages,

(3) observations of the anatomical arrangements which may have implications in the surgical treatment to reduce or eliminate orthodontic relapse.

CHAPTER 1.

REVIEW OF THE LITERATURE.

ALVEOLAR BONE.

There is a widespread variation in the terminology used in the macroscopic and microscopic description of the various types of bone. For the purpose of clarification, the following definitions used by Bloom and Fawcett (1975) will be used in this report.

1. Macroscopic.

   There are two forms of bone observable with the naked eye;

   (a) cancellous or spongy bone which "consists of a three dimensional lattice of branching bony spicules or trabeculae delimiting a labyrinthine system of intercommunicating spaces that are occupied by bone marrow."

   (b) compact bone which "appears as a solid mass, in which spaces can be seen only with the aid of the microscope. The two forms of bone grade into one another without a sharp boundary".

2. Microscopic.

   Compact bone is mainly composed of "the mineralized interstitial substance, bone matrix, deposited in layers or lamellae, that is "lamellated bone". Bloom and Fawcett stated that these lamellae were in three common patterns;

   (a) the great majority were "arranged concentrically around longitudinal vascular channels within the bone to form cylindrical units of structure called haversian systems."

(b) between the haversian systems were "angular fragments of lamellar bone of varying size and shape" called "interstitial systems".

(c) "immediately beneath the periosteum on the external surface of the cortical (compact) bone and on the internal surface subjacent to the endosteum, there may be several lamellae that extend uninterruptedly around much of the bony circumference".

The histogenesis of mandibular (membrane) bone where constant changes are occurring during its development has resulted in the term "woven bone". Bloom and Fawcett described this as "early intramembraneous bone in which the collagen fibres run in all directions", to distinguish it from the lamellar bone formed in subsequent remodeling, which contains collagen in highly ordered parallel arrays.

A term not used by Bloom and Fawcett, but defined by Weinmann and Sicher (1955), was "bundle bone". Weinmann and Sicher stated that it "contained a great number of Sharpey's fibres". They noted that the main difference between "bundle bone" and "simple mature bone" was a scarcity of fibrils in the "bundle bone" and that those fibrils which were present were "arranged at right angles to the Sharpey's fibres".

Bloom and Fawcett described "alveolar bone" as the bone which surrounded and supported the teeth and to which the principal fibres of the periodontal ligament attached. They stated that the "alveolar bone" consisted of "cancellous bone between two layers of cortical bone". The outer layer was a continuation of the cortical layer of the maxilla or mandible; the inner cortical plate was adjacent to the periodontal ligament of the teeth.

The present author has restricted the description of the bone observed in his findings to the terms: alveolar, cancellous and lamellated (compact).

HISTOLOGY OF THE PERFORATING (SHARPEY) FIBRES OF THE PERIODONTIUM.

In 1856, Sharpey described connective tissue fibre bundles which "penetrated" the circumferential lamellae on the surface of bone. Since the pioneer studies of Tomes (1859) and Black (1887) there has been almost complete acceptance of their concept that the fibre bundles of the periodontal ligament were embedded as Sharpey fibre bundles in the alveolar bone and cementum. The cemental ends of the periodontal ligament penetrated the cementum and terminated at the cemento-dentinal junction (Zwarych and Quigley 1965, Kraw and Enlow 1967, and Gianelly and Goldman 1971). Zwarych and Quigley (1965) histologically examined the jaws of white mice and found more fibre bundles inserting into the cementum than alveolar bone. By contrast, the cementum-inserted bundles were thicker than those which inserted into the alveolar bone.

Eccles (1959) studied the histological structure of the periodontal ligament of albino rats. He mentioned that the Sharpey fibre bundles could be seen passing through the alveolar bone, but he did not discuss their course.

Pedler (1956) in a histological study of human bone, observed that the Sharpey fibre bundles were embedded in the alveolar bone. He discussed the possibility of the attachment being either a mechanical or an adhesive bond by means of the cement substance. Pedler did not describe the degree of fibre bundle penetration.

In 1960, Smith studied the collagen fibre patterns in mammalian bone. He observed that woven-fibre bone consisted of (1) a calcified matrix permeated by collagen fibres, and (2) lacunae which contained ostoecytes. The collagen fibres were of two types which differed in appearance and development. Firstly, the calcified matrix was always penetrated by a mesh-work of collagen fibres which bore no relationship to either the lacunae or to the vascular spaces of the tissues. He stated that these fibres did not lie parallel to one another as was the tendency in all other types of bone. However, they did show some degree of preferential orientation in a direction parallel to the long axis of the bone. These types of fibres were a constant feature of woven fibre bone and he referred to these fibres as the "intrinsic fibres" of the tissues.

Jones and Boyde(1974) also discussed the arrangement of the intrinsic fibres of the human alveolar process. They noticed that the orientation of the intrinsic fibres was not consistent and appeared to be related more to the available space for fibre tracts than to the line of insertion of the extrinsic (Sharpey) fibres. Jones and Boyd suggested that this arrangement favoured the proposal that these intrinsic fibre tracts might actually indicate the amount of space available for osteoblastic movement between the extrinsic fibres.

Smith (1960), apart from the intrinsic fibre bundles, observed Sharpey or perforating fibres in many areas of woven fibre bone. These fibres were larger than the intrinsic fibre bundles and had a mean diameter of 10-12 µm. Smith stated that Sharpey fibres represented originally extraosseous collagen fibres which had been included within the bone during its growth. He suggested that this arrangement provided continuity between the intraosseous bundles and the collagen bundles situated outside the bone and in the vascular spaces within the bone. Smith noted that Sharpey fibres in bone invariably stained deeply with the Weidenreich method, but their continuations both outside the bone and into its vascular spaces were unstained. Yet his conclusions of Sharpey fibre continuity were based on the Weidenreich stain.

Cohn (1970), using Van Gieson and colloidal iron stains, reported that cemento-alveolar fibre bundles of the periodontal ligament could pass without interruption through the full thickness of the surrounding tooth alveolus in the mouse mandible. Quigley

(1970) studied histologically the Sharpey fibres of the mouse periodontal ligament. He used a bacterial collagenase which was claimed to preferentially remove those collagen fibre bundles which were calcified during life, while preserving the non-calcified collagen bundles. This technique was employed to study the mode of attachment of tooth to bone. Quigley observed that the perforating (Sharpey) fibre bundles behaved as non-calcified collagen and were not digested by the enzyme. These fibre bundles were essentially continuous and passed through the interdental septum and thus extended from tooth to tooth. He noticed that the perforating fibre bundles were spiral in nature and frequently branched and anastomosed with each other. Gianelly and Goldman (1971) referred to the work of Quigley (1970) and Cohn (1970) but did not comment or elaborate on their findings.

Cohn (1972a) studied the Sharpey fibre bundles in the alveolar bone of the mouse with serial sections cut mesiodistally or buccolingually at 8 micrometres through the long axis of the roots. The sections were stained with the Van Gieson stain or by the colloidal iron stain of Bohacek and Gupta (1968). Cohn observed that the Sharpey fibre bundles in cementum were smaller in diameter and more numerous than those in the alveolar bone and they appeared to be embedded through the full thickness of the cementum. Jones and Boyde (1974) studied the mineralization pattern of Sharpey fibres in the human alveolar process with a scanning electron microscope. They observed that the diameter of these fibres was greater at their insertion into the alveolar bone (mean 7.15 µm) than at the cementum (mean 6.8 µm). In the interdental region, Cohn (1972a) observed that some periodontal fibre bundles of the alveolar crest group curved downwards from the cemento-enamel junction of the first molar to pass through the coronal portion of the alveolar crest and become continuous with similarly orientated fibre bundles of the second molar. In the small group of horizontal fibres, he noticed a similar continuity with the equivalent group of fibre bundles of the adjacent tooth. He further stated that on the buccal and lingual aspects of the first molar, the alveolar crest group of fibre bundles passed apically from the cementum through the alveolar crest and then emerged to intermingle with the periosteum or connective tissue fibres of the overlying gingivae.

Cohn (1972a) also examined the oblique group of fibre bundles in the interdental region and noticed that these fibre bundles did not terminate as Sharpey fibres at varying depths beneath the alveolar surface. The oblique group of fibres passed coronally from the cementum of the first molar to reach and penetrate the septum. Cohn observed that these fibre bundles arched, turned obliquely toward the apex, and became continuous with similarly orientated fibre bundles which were attached to the cementum at approximately the same level of the second molar tooth. He demonstrated the collagen fibre bundle continuity of the oblique group with the overlying periosteal fibres on the mesial, buccal and lingual aspects. Cohn observed a similar orientation and continuity of the oblique fibre bundles in the interradicular region.

In another study of the Sharpey fibre bundles in the alveolar process of the marmoset Cohn (1972b), using Van Gieson and colloidal iron stains, observed transalveolar fibre bundles wherever compact, lamellated bone predominated in the alveolus. He could not trace the Sharpey fibre bundles through the cancellous bone but hypothesized that with better techniques and thicker sections it might be possible to demonstrate transalveolar fibre bundles.

In 1973 Cohn investigated the transalveolar fibre bundles in the jaws of Macaca mulatta in histological sections. He observed that a type of compact bone which lacked Haversian systems prevailed in the region of the alveolar crest. In the interradicular and interdental regions the transalveolar fibre bundles were attached to adjacent tooth roots at approximately the same level. He noted that these fibre bundles joined with the collagenous fibre bundles of the overlying periosteum on the mucosal surfaces. He further observed parallel bundles of fibres within the bone, interweaving to a slight extent. However, the bundles generally retained their individuality and formed a series of flattened arches placed coronally one above the other.

Bernick, Grant, Levy and Dreizen (1974) investigated the periodontal ligament of the marmoset using Mallory and silver nitrate stains. They did not observe any morphological evidence of collagen fibre bundles traversing the alveolar bone as intact groups which inserted into the cementum of adjoining teeth.

Frank, Lindemann, and Vedrine (1958) sectioned small blocks of human alveolar bone at a thickness of 400 µm and studied decalcified and undecalcified sections with the scanning electron microscope. They described two patterns in which the collagen fibres of the periodontal ligament entered the alveolar bone: (1) in the form of Sharpey fibres which were usually unmineralized, and (2) collagen fibres which entered the bone in a haphazard manner. The latter fibres contributed to the collagen matrix of the bone and were impregnated with bone salts. A less mineralized zone of the alveolar bone adjacent to the periodontal ligament, and which contained Sharpey fibres, was called the preosseous area.

Cohn (1975), using a modified Mallory stain (Humason, 1967), examined various regions of the human maxillary and mandibular alveolar process. The jaws were obtained from 16 adult cadavers although he stated that "a few fresh specimens were also examined". He observed that many cemento-alveolar fibre bundles of the periodontal ligament, instead of being anchored in bone as conventional Sharpey fibre bundles, traversed the entire thickness of the alveolus. These transalveolar fibre bundles were observed only in regions of non-cancellous, lamellated bone lacking Haversian systems. He noted that the alveolar crest between adjacent anterior teeth frequently consisted of lamellated bone. Cohn observed that some fibre bundles of the alveolar crest group passed apically from each adjacent tooth to penetrate the crest, and unite within bone in the shape of a letter V.

Several horizontally orientated periodontal fibre bundles of one tooth, after entering the lamellated bone of the crest traversed the bone in a slightly coronal path and joined similarly orientated fibre bundles of the neighbouring tooth to form transalveolar fibre bundles. Cohn stated that the union of these collagen fibre bundles from adjacent teeth resulted in a series of overlapping arches within the crest, with the apices of the arches pointed occlusally and meeting at the vertical interface between two bony lamellae. The vertical interface varied in its position from the middle to either side of the alveolar wall. Cohn suggested that this arrangement represented a functional adaptation of trans-alveolar fibre bundles to occlusal and muscular forces permitting maximum support of the tooth in its alveolus. He postulated that the interweaving of adjacent bundles within the alveolar bone, where the transalveolar fibre bundles were relatively parallel, was also a functional adaptation to masticatory forces.

Cohn (1975) also investigated the vestibular and lingual aspects of human anterior teeth and observed that periodontal fibre bundles comprising the alveolar crest, horizontal, and oblique groups frequently continued into bone as transalveolar fibre bundles. Cohn did not observe transalveolar fibre bundles in similar areas of premolars and molars. He considered this was due to the lack of lamellated bone in those sites. The transalveolar fibre bundles in the incisor and canine region passed into the labial and lingual crest and then turned sharply in an apical direction. After some interweaving of adjacent bundles, the fibres penetrated through the crest. He reported that after these fibre bundles emerged from the bone, they "fused" either with the collagenous bundles of the overlying periosteum or with the lamina propria of the gingiva.

Lamellated bone without Haversian systems was also seen by Cohn (1975) at the interradicular crest. He stated that transalveolar fibre bundles formed a series of arches within the bone in this region. He further said "because of technical difficulties, evidence for the periodontal continuity of these arches was lacking. It was therefore assumed that the ends of each arch were attached at approximately the same level on adjacent teeth".

Cohn (1975) reported that the transalveolar fibre bundles in man and the marmoset differed from those in the mouse. In the two primates, adjacent fibre bundles showed a moderate amount of interweaving, although they retained their individuality as they coursed through the bone. He reported little or no interweaving in the mouse transalveolar fibres. Cohn (1975) discussed the study of Bernick, Grant, Levy and Driezen (1974) and stated that their study involved alternate histological sections in the marmoset. He proposed that they did not observe transalveolar fibres because they had not used serial sections in their study.

ORTHODONTIC TOOTH MOVEMENT.

J. G. Edwards (1971) stated that the position of the teeth was influenced in the alveolar arches by three principal groups of fibres;

I. The gingival fibres which originated in the cementum and terminated in the connective tissues of the gingivae,

II. The transseptal (interdental) fibres which passed superiorly to the alveolar crest and connected adjacent teeth,

III. The alveolo-dental (periodontal ligament) fibres which connected alveolar bone to the cementum.

He suggested that the fibrous elements of the periodontal ligament adapted to tooth movement in three possible ways; (1) progressive osteogenic and, to a lesser extent, cementogenic activity aided in the shortening of the extended fibres and in the reattachment of new fibres developed during tooth movement, (2) the stretching of the wavy collagen fibres and the reorientation of their directional morphology permitted a small amount of tooth movement, and (3) the existence of a type of intermediate plexus might allow an elongation of fibre bundles by a form of slippage of the fibres over one another and a subsequent reorientation of the fibres in the new tooth position.

Kraw and Enlow (1967) observed in the human that the fibre bundles attached to the resorptive surface of the alveolar bone were not detached or destroyed during tooth movement, but maintained a direct continuity with those fibre bundles within the bony matrix. Those resorptive areas, not directly associated with the periodontal vascular bundles, were characterized by a retention of the principal collagen fibre bundles freed from the bone matrix as a result of bone resorption. As they were uncovered, the perforating collagen fibre bundles then became actual periodontal ligament fibre bundles which inserted into the remaining bone and retained their direct continuity within the bone. Kraw and Enlow suggested that these unsevered fibres underwent a process of shortening and relinkage with precollagenous fibrils in the existing periodontal ligament. They stated that fibres of moving teeth were characterised by three separate zones. The first, an inner zone, contained mature collagen bundles which were relatively stable and inserted directly into the cementum. The second zone of similar arrangement, but less stable, was embedded in the alveolar wall. The third zone was an intermediate area which was highly unstable and composed of immature collagen fibres that underwent considerable reconstruction. Hindle (1967), as a result of a study of the intermediate plexus using polarised light, concluded that the collagen in the zone of the intermediate area was less mature.

Both Eccles (1959) and Trott (1962) studied histologically the developing principal fibres in albino rats and found no evidence of a permanent intermediate plexus. Trott, however, noted the presence of an intermediate plexus during the period of active eruption. Zwarych and Quigley (1965), using white mice molars, were able to trace many fibre bundles passing without interruption from the alveolar bone across the periodontal ligament to the cementum. They did not observe an intermediate plexus in white mice.

Sicher (1942, 1959, 1962 and 1970), however, maintained that orthodontic tooth movement with respect to the alveolar socket did not occur by the new attachment of fibres in bone and cementum, but rather by the formation of new links between the alveolar and cemental fibres in the intermediate plexus. Sicher (1970) was also convinced of the presence of an intermediate plexus in the transseptal group of fibres. Gianelly and Goldman (1971) suggested that some mechanism for ligament adjustment probably had to be present because bone and cementum were distinct, independent structures. They maintained that the important principal was not the exact location of this adjustment or linkage zone, but the need for its presence.

Ten Cate (1976) stated that he had used the transmission electron microscope to study the mechanism of remodelling and turnover of the periodontal ligament during physiological tooth movement. His studies indicated that the cellular basis for remodelling and turnover of the collagen in the normal functioning ligament was the fibroblast. He also stated that the fibroblast was capable of synthesizing and degrading collagen simultaneously. Ten Cate suggested that all the fibroblasts in the ligament had this property, and therefore there was no need to postulate the existence of an intermediate plexus. He concluded by stating that this cellular mechanism would explain the remodelling of the ligament associated with orthodontic tooth movement.

ORTHODONTIC RELAPSE.

J. G. Edwards (1970) defined relapse as that tendency of orthodontically moved teeth to partially return to their original position and alignment. He stated that relapse should not be regarded as a pathological or abnormal phenomenon but as an unwanted or undesirable symptom of oral physiology.

Gianelly and Goldman (1971) suggested that when a tooth was in a stable position, it was assumed that the sum of all the forces acting on the tooth must be below the hypothetical level of a tooth moving force. They stated that a tooth which demonstrated a relapse tendency probably had a net positive force applied to it. This force might be directly or indirectly exerted by various habits, tooth position, ligament and supraalveolar tissues, muscles and bone.

Reitan and Skillen (1940) histologically demonstrated a marked displacement and stretching of the supporting structures of the periodontium during orthodontic rotation of dog teeth. Their investigations indicated that relapse after orthodontic movement of teeth might not be as closely related to elastic forces in the periodontal ligament itself as was previously thought. On the basis of their histological study, they suggested that after orthodontically rotated teeth were retained for only 28 days, most of the oblique and apical periodontal ligament fibres had resumed their normal, wavy, pretreatment appearance. However, in the same article, Reitan and Skillen described a very prolonged directional reorientation of collagen fibres in the gingival and transseptal groups above the alveolar crest. Reitan (1959, 1967, and 1969) in studies of the effects of orthodontic movement on dog incisors and molars, stated that the collagen fibres in the supracrestal group were the cause of orthodontic relapse since they remained distorted for at least 232 days.

J. G. Edwards (1968) studied the periodontium after the orthodontic rotation of teeth in dogs and considered that the fibres of the gingivae remained attached to the tooth during rotation. This attachment resulted in a displacement of the gingivae in the direction of the tooth movement. He observed that the periodontal ligament fibres attached to the alveolar wall were rapidly reorganized after rotation of a tooth. After five months of retention it was noted that the transseptal and gingival fibre bundles were still taut and orientated in the direction of rotation.

Erikson, Kaplan and Aisenberg (1945) reported a persistence of the transseptal fibre bundles after teeth had been approximated in an extraction space of monkeys. These teeth were held in approximation for almost a year, and their histologic sections showed that the transseptal fibre bundles were compressed and the excess interproximal tissue had not been removed. It was concluded that there seemed to be no process which shortened these fibres or removed them.

Over the years many biological and surgical methods have been devised for preventing relapse.

BIOLOGICALLY BASED METHODS.

In 1919 Hawley proposed and illustrated a removable appliance incorporating a labial bow for use as a retainer after active orthodontic treatment. Many other appliances such as cuspid to cuspid and molar to molar retainers, cemented staples (Case 1921), the bite plate and extra-oral traction (Hahn 1944), and the positioner of Kesling (1945) have been suggested as aids to reduce orthodontic relapse. All these appliances utilized the principle that holding the teeth in their corrected positions for a period of time, ranging from two years (Case 1921) to five years (Tweed 1944), would allow the readjustment of the collagen fibre bundles of the periodontal ligament.

Rogers in 1936 advocated the use of myo-functional therapy during the retention period to strengthen weak muscles and re-establish their proper function. He believed that this would reduce the relapse tendency. In 1944, Hahn discussed current retention methods and suggested that careful attention to the final interdigitation of the teeth into an ideal occlusion would reduce the tendency for relapse to occur. He proposed that the reduction of relapse would be achieved by the simple locking of the cusps which occurred during tooth contact. The occlusal equilibration of teeth after orthodontic treatment was suggested by Rothner (1952) and Blume (1958) as a method for preventing occlusal trauma and as an aid in the prevention of relapse.

Early rotation of malposed teeth before they erupted into occlusion was suggested by Riedel (1960), and Furstman and Bernick (1972). These three authors stated that this procedure would prevent rotational relapse as the fibres apical to the alveolar crest did not become well organised until the tooth was in occlusion. Begg (1962) and Riedel (1969) proposed the use of overrotation and overcorrection, respectively, to prevent relapse. They based their proposals on the concept that any relapse which did occur would only return the tooth or teeth to the desired posttreatment position.

SURGICAL METHODS.

Talbot (1896) was the first person to describe a surgical method for easier tooth movement and the reduction of relapse. His method involved the removal of the entire alveolar process in the line of travel of the tooth without damaging the periodontal ligament. In 1932 Skogsberg advocated a technique which he called alveolar septotomy to ensure posttreatment stability of his orthodontic cases. He used a fissure bur to remove a segment of bone between the orthodontically repositioned teeth. This left intact the compact bone of each alveolus, the crest of the septum, the tissues of the interdental papilla, and the interdental septum of bone below the middle third of the root. He did not perform this operation with the aim of neutralizing the forces within the soft tissues, but to neutralize those forces which, he considered, were due to the elasticity of the cancellous portion of the bone. An alternative method was investigated by Hallett (1956) who proposed the method of immediate torsion and claimed greater stability of the rotated teeth. However, he mentioned that de-vitalization and root resorption were undesirable side effects.

Thompson (1959) succeeded in lessening the relapse of approximated teeth in monkeys by surgical gingivectomy after dental extractions. Boese (1969) in a study of the stability of rotated teeth in monkeys identified two phases of orthodontic relapse. Firstly, during the initial four weeks following orthodontic rotation of teeth, a significant proportion of the relapse was caused by the stretched principal fibre bundles. This phase was terminated before eight weeks, when the remodelling of the alveolar bone provided new attachments for the principal fibre bundles. Secondly, after eight weeks, the relapse was caused by the supra-alveolar fibre bundles. This latter phase, according to Boese, continued until almost complete relapse had occurred, since the cemental attachments of the transseptal fibre bundles remained unchanged. Boese further observed that a gingivectomy of the rotated tooth, followed by a minimum of eight weeks' retention, significantly reduced relapse to one tenth the amount of the amount of the control.

J. G. Edwards (1970) implicated the gingival (supra-alveolar) fibres as the cause of rotational relapse on the basis of gingival distortion of rotated human mandibular premolars. He suggested that severing of these fibres by gingival slice cuts to a depth of 3 mm below the alveolar crest would prevent relapse. J. G. Edwards (1971) also examined a surgical technique for the prevention of relapse in extraction cases for teenage orthodontic patients. He found by surgically excising the excess tissue present after the teeth were approximated that opening of the extraction space was eliminated or diminished in all the cases treated.

Nielson (1971) showed that transsection of the supraalveolar fibre bundles on orthodontically rotated teeth in monkeys significantly reduced the relapse tendency without causing any observable damage of a permanent nature to the soft tissues or bone. Billberg (1971) described a similar surgical technique to that of Edwards (1970) which effectively reduced relapse after orthodontically correcting rotated incisor teeth in humans. Pinson and Strahn (1974) studied the effects of surgical division of the gingival fibres of orthodontically rotated teeth in humans. Their technique, which they called pericision, involved surgery followed by a sixteen to twentyeight week retention period. They noticed a measurable amount of relapse even after the pericision and the retention period.

Walsh (1975) clinically assessed the pericision technique of Pinson and Strahn (1974) and observed a relapse in 27% of the teeth which had been retained for 15 weeks compared with the control group. He stated that retention for at least 12 weeks following surgical division of the gingival fibres resulted in a greater than two thirds reduction in relapse.

FUNCTIONAL AND AGE CHANGES OF THE PERIODONTIUM.

Eccles (1959) used albino rats ranging in ages from 10 to 37 days to study the stages of development of the collagen fibres of the periodontal ligament in different regions of the jaws. His investigations supported the theory that function played an important part in stimulating development and orientation of the fibre bundles of the periodontal ligament. Ramfjord (1959) selectively extracted teeth from human patients whose treatment plans involved complete exodontia. He was thus able to study the periodontal changes associated with an increase or decrease in occlusal stress. Ramfjord histologically demonstrated that the most functionally stable periodontal structures were the Sharpey fibres entering the cementum and the periodontal fibres coronal to the alveolar crest. He stated that most of the adjustments of the principal fibres to changes in the functional demand appeared to occur at the surface of the alveolar bone and in the middle zone of the periodontal ligament.

Begg (1971) stated that the continual loss of tooth substance by attrition was a normal functional process and that the mesial migration of the teeth compensated for this tooth reduction.

Grant, Chase, and Bernick (1973) stated that the alveolar bone in the Galago primate species became denser with age. They observed that the osteophytic trabeculae were sequentially replaced by anastomosing trabeculae and finally by Haversian systems. The alveolar medullary spaces became smaller with the progressive encroachment of the bone. These findings contrasted with the observation by Weinmann and Sicher (1955) of osteoporosis in aged humans. Grant and Bernick (1972) did not observe signs of osteoporosis in their study of aged humans. They did however, describe other changes which they attributed to age. For example, Sharpey fibres were not demonstrable with silver nitrate stains in the alveolar bone but were stained in the cementum and the principal fibre bundles were noticeably thicker with lessened cellularity. Grant and Bernick also noted the lack of recent physiologic tooth movement in the specimens which were studied. The human material examined was obtained from three males aged 55, 72 and 76 years, and a 92 year old female. Both the mesial and distal, as well as the oral, vestibular, and apical alveolar surfaces showed minimal evidence of resorption and negligible apposition. Grant and Bernick (1972) suggested that physiological tooth migration was slowed or even halted in old age as remodelling of the bone slowed or ceased. Weinmann and Sicher (1955) stated that resorption on the alveolar wall towards which the teeth moved was not a continuous process. They reported that it occurred in waves, each of which was followed by a period of repair. This, they suggested, was in accordance with a rather general rule that resorption of bone was excessive during growth and when subjected to pressure. The over-resorption of the bone allowed the immediate reconstruction of the bone by apposition. Weinmann and Sicher proposed that during tooth movement, the period of repair was of special importance as it permitted the reattachment of the suspensory fibres of the periodontal ligament.

Moss (1975) postulated that the transseptal fibre system was of considerable importance in causing approximal drift. He suggested that the contractile force present in the transseptal fibres, which were attached to adjacent teeth and not to the interdental or interradicular bone, maintained tooth contact. The observations made by Cohn of the continuity of the periodontal ligament fibres of adjacent teeth by means of perforating (transalveolar) fibres might also aid in the understanding of approximal drift in humans.

CHAPTER 2.

MATERIALS AND METHODS.

MATERIALS.

Sixteen segments of human mandible were obtained from either the buccal or anterior regions of thirteen motor vehicle accident autopsy subjects. Fourteen segments showed no (negligible) periodontal involvement while the 46 and 68 year old specimens showed evidence of chronic periodontal disease. The segments were examined histologically as indicated in Table I, page 2.6.

METHODS.

SURGICAL REMOVAL OF THE SPECIMENS.

The surgical removal of the specimens of mandible was complicated by the need to restore the facial appearance of the autopsy subjects. Commencing at the routine "T" incision made at autopsy, two incisions were made approximately parallel to the sternomastoid muscles and terminating behind the ears. The soft tissues were dissected away to expose the lower border of the mandible in the area required for removal. The segments were then removed with a Desouter Necropsy Saw (Desouter Bros. Ltd., London, N.W.9). Initially bilateral removal of the mandibular buccal segments was performed, but the resultant lack of support for the anterior segment prevented complete restoration of the facial appearance with papier mache packing. It was therefore decided to carry out only unilateral removal of mandibular segments. The remaining buccal segment, together with papier mache packing in the missing segment, provided sufficient support for the symphysis region to restore the subject's facial appearance after suturing.

HISTOLOGICAL PROCESSING AND SECTIONING.

Fixation -

The gross specimens (fig. 1 p.2.7) were placed in 10 per cent buffered neutral formalin solution (Appendix I p.6.1) for seven days after removal at autopsy.

Decalcification -

Prior to decalcification, the gross specimens were cut into the smaller segments required for examination (fig. 2 p.2.8) with a small water cooled diamond saw (W. E. Niclas, New York). This division of the gross specimens hastened the decalcification process. The specimens were decalcified in the commercial decalcifying solution, Decal (Omega Chemical Corporation, Cold Springs, New York). After five days, the specimens were checked for decalcification with X-ray films taken on the subsequent days. Decalcification was completed in most cases within 8 days.

Neutralization -

After decalcification was complete, the specimens were placed in a solution of 5 per cent sodium sulphate (Na_2SO_4) for one day prior to processing.

Processing -

Before dehydration (Appendix I.2 p.6.1), the specimens were cut with a safety edge razor blade as indicated in fig. 2 p.2.8. This reduced the size of the specimen for blocking and enabled the cut face to be orientated in the desired plane for sectioning.

Paraffin Embedding -

The specimens were infiltrated with paraffin wax (Appendix I.2, p.6.1). Depending on their size, the specimens were then blocked in plastic or metal moulds using the "Tissue Tek II Tissue Embedding Centre" (Lab-Tek Products, Division Miles Laboratories Inc., Naperville, Illinois). The processed specimens were blocked with an orientation to allow sectioning in the required plane.

Sectioning -

Serial sections were cut on a Rotary Microtome Model 820 (American Optical Corporation, Scientific Instrument Division, Buffalo N.Y.). Sections cut at 8 um proved to be the most suitable for staining and examination of the perforating fibre bundles. Each section was flattened by floating on a warm water bath, transferred to a gelatinized slide (Appendix II.2 p.6.3) and then placed in a warm air oven at 60C for 2 hours. In total, 3,149 sections were cut and mounted on 2,439 slides.

STAINING.

Five histological staining methods were used to determine the most suitable stain for the demonstration and subsequent examination of perforating (Sharpey) fibre bundles. The stains were:

I. Mayer's haematoxylin and eosin,

II. Heidenhain's aniline blue,

III. Mallory's phosphotungstic acid and haematoxylin,

IV. Modified Mallory stain (Humason 1967),

V. Gordon and Sweet silver stain counterstained with Van Gieson.

The staining techniques are detailed in Appendix III, p.6.4.

The use of Gordon and Sweet silver stain caused some of the sections to become detached from the glass slides. Lynch, Raphael, Mellor, Spare and Inwood (1969) suggested that the lifting of the sections was due to the high alkalinity of the silver solutions. Initially, albuminized slides (Appendix II.1, p.6.3) were used to prevent this occurrence. However, some lifting of the sections continued to occur. The sections were then mounted on gelatinized slides (Appendix II.2, p.6.3) and no further problems of section loss were encountered.

PHOTOGRAPHY.

Photographic records of the gross specimens were made with a Minolta SRT 101 and an Auto Bellows 1 (Minolta Camera Company, Osaka, Japan). Diagrams for inclusion in this report were prepared from X-ray and photographic records.

The low power photomicrographs were taken with a Polaroid MP4 Camera System (Polaroid Corporation, Cambridge, Massachusets, U.S.A.) and a green filter. All the other photomicrographs were taken with either an Axiomat N.D.C. Microscope (Zeiss, West Germany) using Ilford FP4 cut pack 5" x 4" film, together with a green filter, or an Olympus EHT Microscope and a C35 A camera body (Olympus, Tokyo) with Ilford Pan F 35 mm film.

Units used in this report -

All units used in this report follow the recommendations of the Standards Association of Australia (1974).

LOCATION KEY.

A location key (fig.3 p.2.9) was used for the purpose of identifying the various areas of the mandibular interdental and interradicular septa which were histologically examined.

For the purpose of setting out the findings, the areas described were not listed in ascending numerical order.

TABLE I.

Identification of the 16 individual specimens and a description of the area and the plane of section in which they were examined.

NUMBER	SEX	AGE	AREA	PLANE OF SECTION
3 1 1	male female male	19 11 68	mandibular right first and second premolars	
1	male	19	mandibular left first and second premolars	all sections cut mesiodistally
1	male	19	mandibular right central and lateral incisor	
1	male	46	mandibular right first and second molars	
1 1 1	male female male	19 19 21	mandibular right first and second premolars	
1	male	20	mandibular right first and second molars	all sections cut horizontally
1	male	19	mandibular left central and lateral incisor	
2	male	21	mandibular right and left first and second premolars	all sections cut buccolingually
1	male	19	mandibular right first molar	

Fig. 1. Gross specimen of a human mandible from the first premolar to the first molar. 19 year old male, right side, lingual aspect.

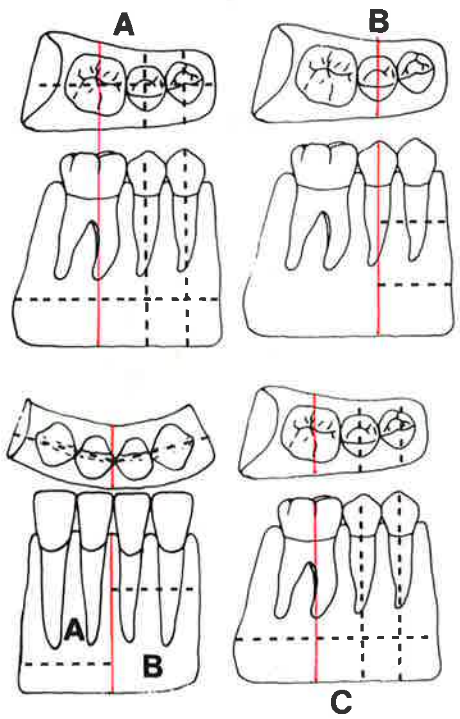


Fig. 2. The gross specimens were sectioned along the red line to hasten decalcification. The remaining dotted lines indicate the division after decalcification and prior to blocking for orientation in the three planes, A, mesio-distal; B, horizontal; C, buccolingual.

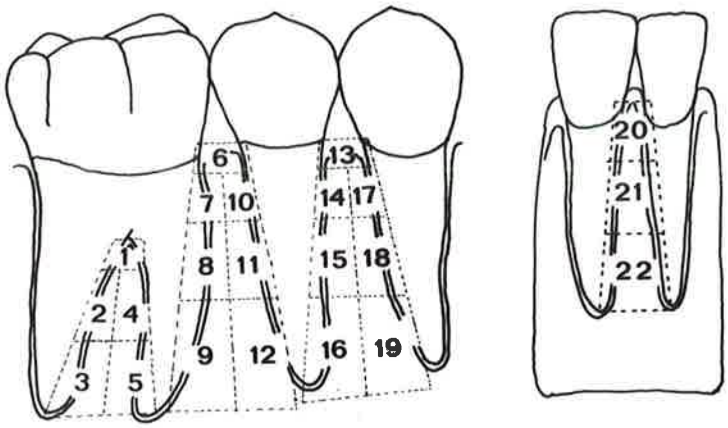


Fig. 3. Location key used to correlate the histological sections being described with their anatomical location in the mandibular posterior and incisor segments.

CHAPTER 3.

FINDINGS.

STAINING REACTIONS OF THE FIVE TRIAL STAINS.

The five histological stains used in this project varied in their suitability for the histological visualization and the black and white photographic demonstration of the perforating fibres. Haematoxylin and eosin stained the periodontal fibres, the perforating fibre bundles, and the interdental alveolar bone various shades from pink to red. The stain did not enable the perforating fibres to be traced easily with the light microscope or readily recorded with black and white photography (fig. 4, p.3.3). The Mallory phosphotungstic acid haematoxylin stain, although a trichrome stain, did not provide sufficient contrast between the yellow to pink colour of the perforating collagen fibre bundles and the pink ground substance of the alveolar bone for recording with black and white photography (fig. 4, p.3.3). Heidenhain's aniline blue stain enabled the perforating fibre bundles to be traced in the alveolar bone far more easily than the haematoxylin and eosin or the Mallory stain. The blue-stained collagen fibre bundles contrasted with the red-stained alveolar bone when viewed with the light microscope. However, the black and white photographic result was unsatisfactory (fig. 4, p.3.3).

The modified Mallory stain (Humason 1967) used by Cohn (1975) enabled the perforating fibres, which stained red, to be easily traced in the pale pink stained alveolar bone. The black and white photographic result (fig. 4, p.3.3) was not satisfactory.

Gordon and Sweet's silver stain, counterstained with Van Gieson, proved to be the most suitable method for the demonstration of perforating fibre bundles in alveolar bone. The dark brown collagen of the perforating fibre bundles contrasted with the red ground substance of the alveolar bone to provide good visual detail and satisfactory black and white photographs (fig. 4, p.3.3).

1

2

3

4

5

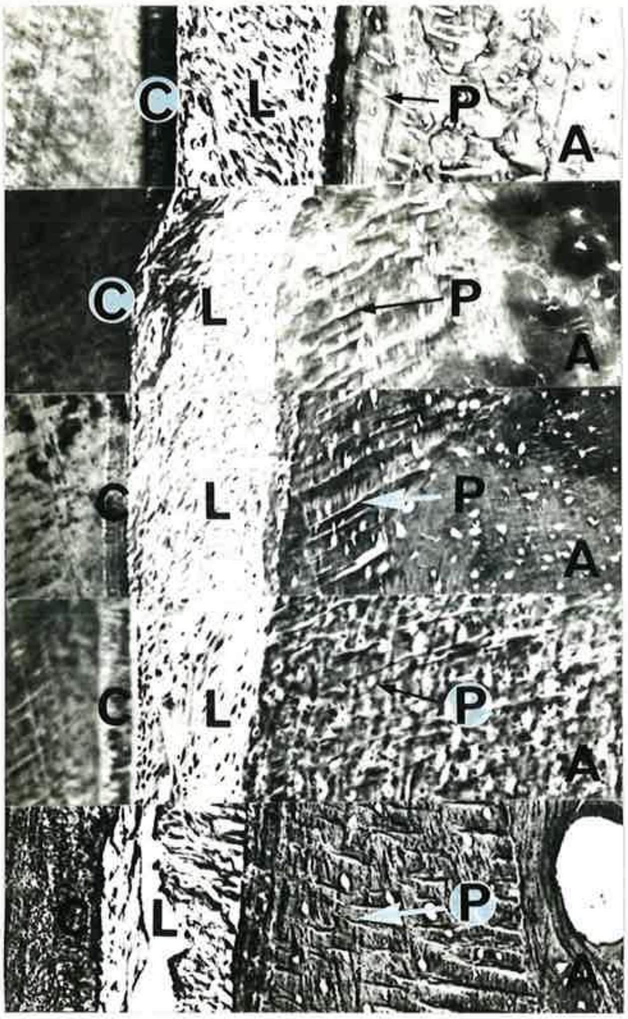


Fig. 4. Black and white photographic record of the staining reaction with

1. Mayer's haematoxylin and eosin
2. Mallory's phosphotungstic acid and haematoxylin
3. Heidenhain's aniline blue
4. Modified Mallory stain
5. Gordon and Sweet silver counter-stained with Van Gieson.

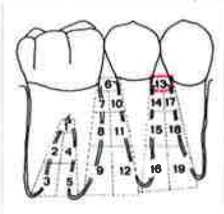
Area 18; 19 year old male; mesiodistal section. A, alveolar bone; C, cementum; L, periodontal ligament; P, perforating fibre bundles. X 100.

PREMOLAR REGION.

Mesiodistal sections of the interdental bone between the mandibular right first and second premolars. (Four 19 year old males.)

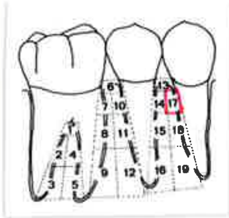
The interdental crest consisted of lamellated bone and below the crest the bone divided into mesial and distal cortical zones separated by cancellous trabecullae (fig. 5, p.3.9).

Area 13.



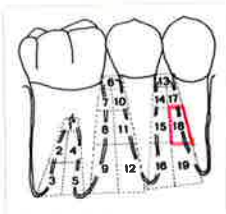
Perforating fibre bundles with an orientation parallel to the occlusal plane, were seen penetrating the alveolar bone crest to varying depths. These fibre bundles divided into fibres which penetrated the bone in a spiral path and interweaved with other perforating fibres. Some of these fibres terminated at the reversal line as divergent finger-like projections. A few of the perforating fibre bundles were observed penetrating the crestal bone and then dividing into fibres which traversed the bone. These fibres anastomosed in the midline of the septum with similar fibres originating from other perforating fibres which penetrated the crest from the opposite surface (fig. 6. p.3.10).

Area 17.



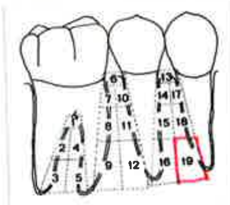
The perforating fibre bundles were seen pentrating the bone at a slight angulation upwards towards the occlusal plane (fig. 7 p.3.11). The fibre bundles divided into fibres which traversed the first reversal line and then terminated at the innermost reversal line as divergent fingerlike projections. The angulation of the fibres towards the occlusal plane decreased after they had penetrated the bone. Minor interweaving of the perforating fibres was observed as they traversed the alveolar bone. Some intrinsic bone matrix fibres were seen orientated at right angles to the perforating fibres while other bone matrix fibres were randomly arranged within the bone (fig. 8 p.3.12).

Area 18.



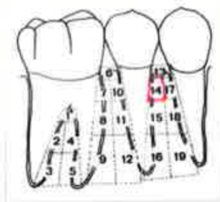
The angulation of the perforating fibre bundles was similar to that observed in Area 17 and they also reduced their angulation after entering the interdental septum (fig. 9 p.3.13). The perforating fibre bundles divided into fibres after penetrating the bone but did not interweave with the other fibre groups as occurred in Area 13 and 17. The fibres traversed the first reversal line and terminated at the innermost reversal line as divergent fingerlike projections. Intrinsic bone matrix fibres seen in this area were orientated parallel to the long axis of the tooth and not parallel to the perforating fibres (fig. 10 p.3.14). In some sections, perforating fibre bundles were noted penetrating the lamellated bone to terminate at a reversal line and then were observed for a short distance adjacent to an intervening marrow space (fig.11 p.3.15).

Area 19.



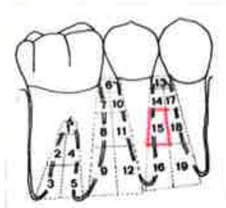
The alveolar bone was more densely lamellated than in Areas 18 and 17. However, reversal lines were not observed. The perforating fibre bundles were fewer in number but penetrated the bone to a similar depth to that observed in Areas 17 and 18. The angulation of the fibre bundles within the bone was more apical and they showed less division into fibres (fig.12 p.3.16). although they again terminated as divergent fingerlike projections. Intrinsic bone matrix fibres were observed orientated parallel to the long axis of the tooth and not perpendicular to the perforating fibre bundles (fig. 13 p.3.17).

Area 14.



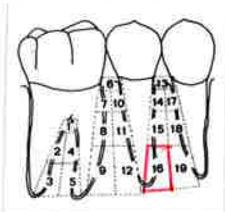
The perforating fibre bundle arrangement was similar in the basic pattern to that observed in Area 17. The alveolar bone was penetrated by the fibre bundles at a slight inclination towards the occlusal plane (fig. 14 p.3.18). The perforating fibre bundles divided into fibres which interweaved with other perforating fibres to a greater extent than that seen in Area 17 (fig.15 p.3.19). The angulation of the perforating fibres was reduced after penetration of the alveolar bone. The pattern of bone deposition and resorption, indicated by the reversal lines, was more irregular than that observed in the corresponding Area 17. The intrinsic bone matrix fibre arrangement was similar to that observed in Area 17.

Area 15.



The depth of penetration by the perforating fibre bundles into the alveolar bone (fig.16 p.3.20) was much less than that observed in Area 18 (fig. 9 p.3.13). The bundles divided into fibres after entering the alveolar bone and terminated at the only reversal line as divergent fingerlike projections. The perforating fibre bundles penetrated the bone at a greater inclination towards the occlusal plane than in Area 14 although less than that in Area 18. Intrinsic bone matrix fibres were observed orientated parallel to the long axis of the teeth (fig. 17 p.3.21).

Area 16.



The alveolar bone was densely lamellated (fig. 18 p.3.22) with only minimal surface penetration by the perforating fibre bundles. These fibres did not terminate as divergent fingerlike projections. The surface of the bone was characterized by Howship's lacunae which indicated that resorption had occurred in this area (fig.19 p.3.23). Some intrinsic bone matrix fibres were observed which were orientated parallel to the long axis of the interdental bone.

/3.24

Fig. 5. Interdental alveolar bone consisting of cancellous trabeculae (C) separating the outer layers of cortical bone (B) between the mandibular right first and second premolars.
D, dentine; L, periodontal ligament.
Areas 13-19; 19 year old male (d); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 8.

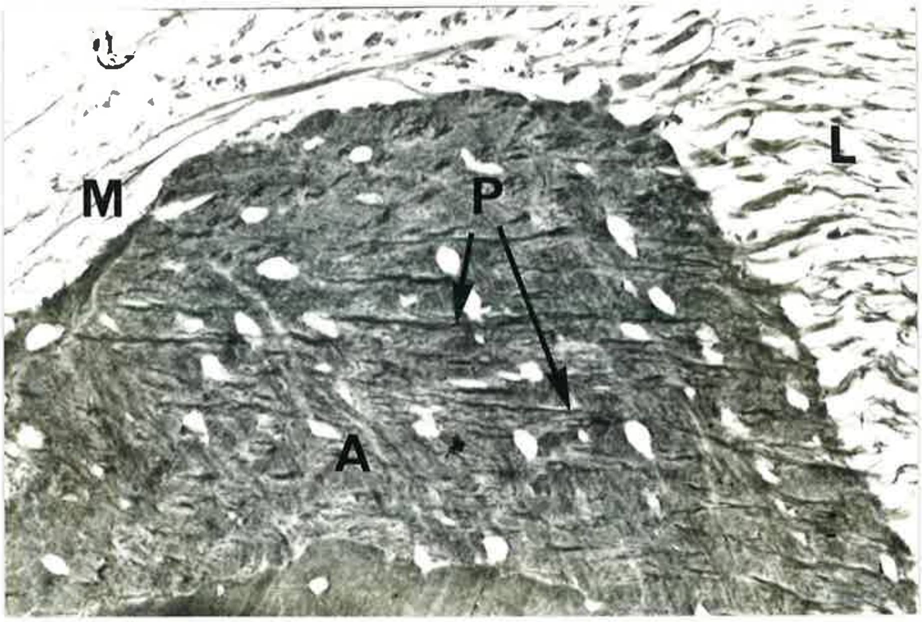


Fig. 6. Perforating fibre bundles (P) enter the alveolar bone (A) of the interdental crest from the mesial (M) and distal to anastomose near the midline. L, periodontal ligament. Area 13; 19 year old male (a); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 200.

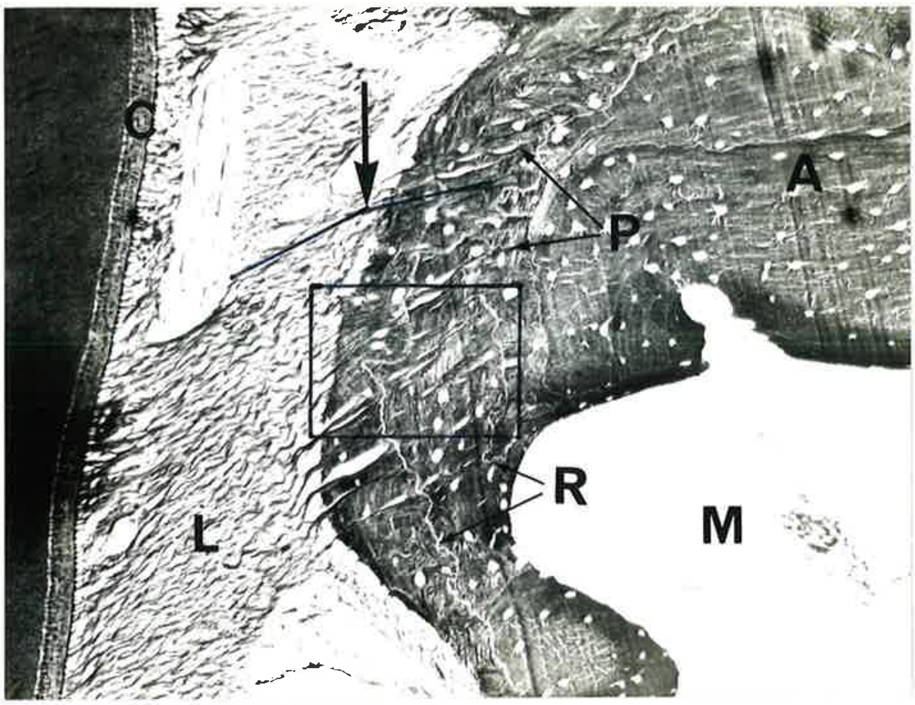


Fig. 7. Perforating fibre bundles (P) enter the alveolar bone (A), reduce their inclination (arrow) and then terminate at the innermost reversal line (R).
C, cementum; L, periodontal ligament; M, marrow space.
Area 17; 19 year old male (a); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

Fig. 8. Higher magnification of Fig. 7 (inset). Perforating fibre bundles (P) divide into fibres after entering the alveolar bone (A) and terminate at the innermost reversal line (R) as divergent fingerlike projections.
I, intrinsic bone matrix fibres; L, periodontal ligament.
X 400.

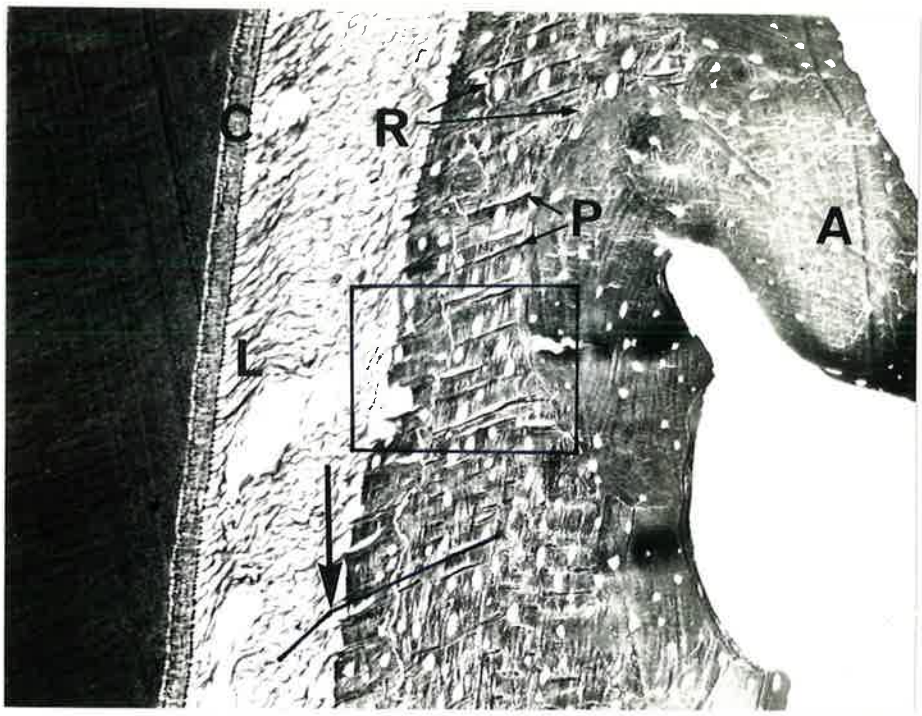


Fig. 9. Perforating fibre bundles (P) change their angulation (arrow) after entering the alveolar bone (A) and terminate at the innermost reversal line (R). C, cementum; L, periodontal ligament.
Area 18; 19 year ole male (c); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

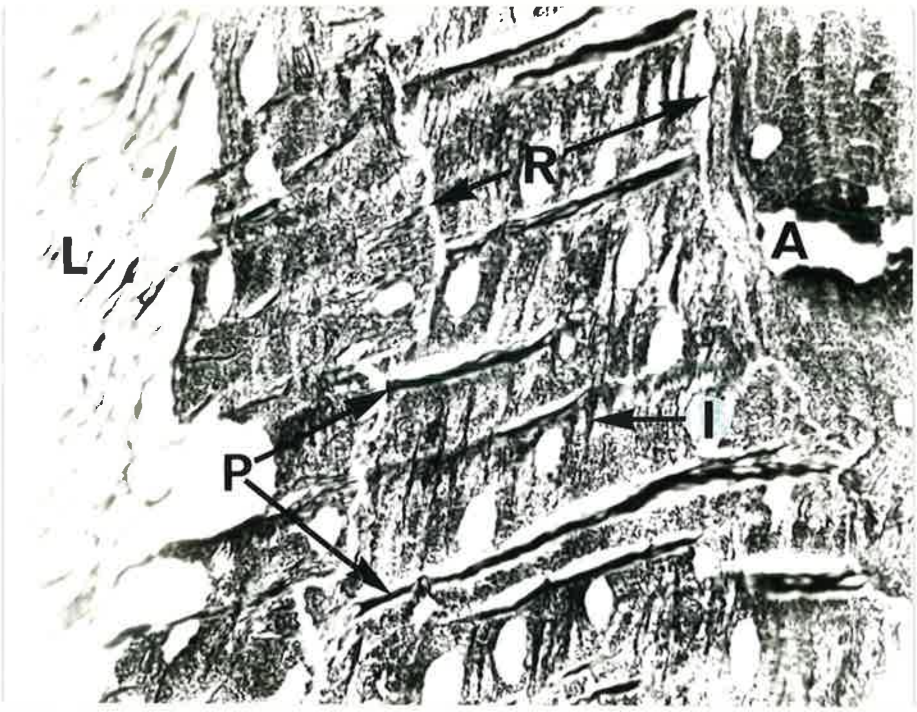


Fig.10. Higher magnification of Fig. 9 (inset). Perforating fibre bundles (P) divide into fibres after entering the bone (A) and terminate at the innermost reversal line (R). I, intrinsic bone matrix fibres; L, periodontal ligament.
X 400.

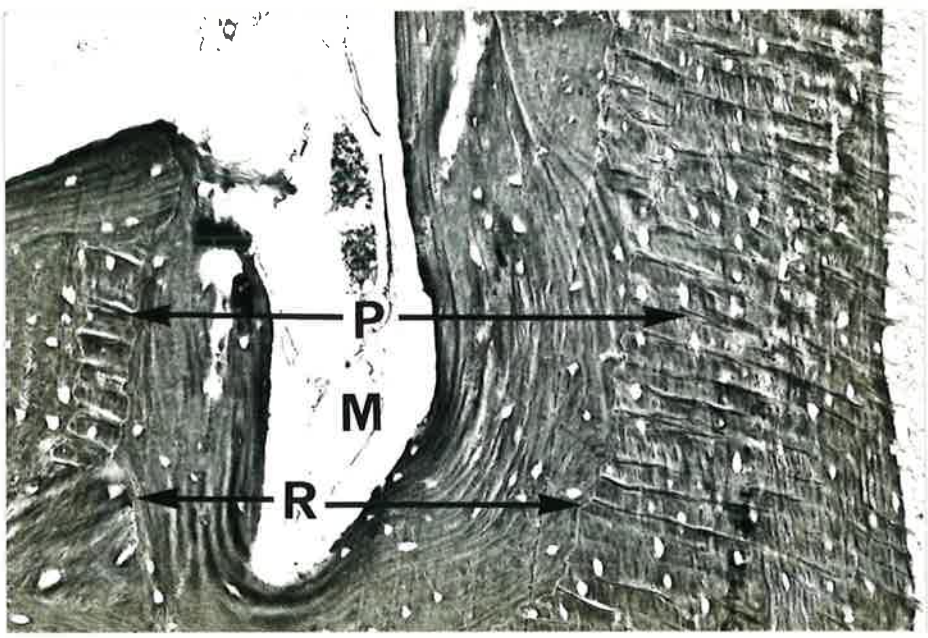


Fig.11. Perforating fibre bundles (P) penetrate the alveolar bone (A) to the reversal line (R) and then are apparent for a short distance between two more reversal lines adjacent to the marrow space (M).
Area 18; 19 year old male (b); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

Fig.12. Fewer perforating fibre bundles (P) penetrate the dense lamellated alveolar bone (A) to terminate at the reversal line (R).
C, cementum.
Area 19; 19 year old male (a); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

Fig.13. Higher magnification of Fig.12 (inset). Fewer and larger perforating fibre bundles (P) penetrate the dense lamellated bone (A) with the intrinsic bone matrix fibres (I) orientated at right angles to the bone surface.
X 400.

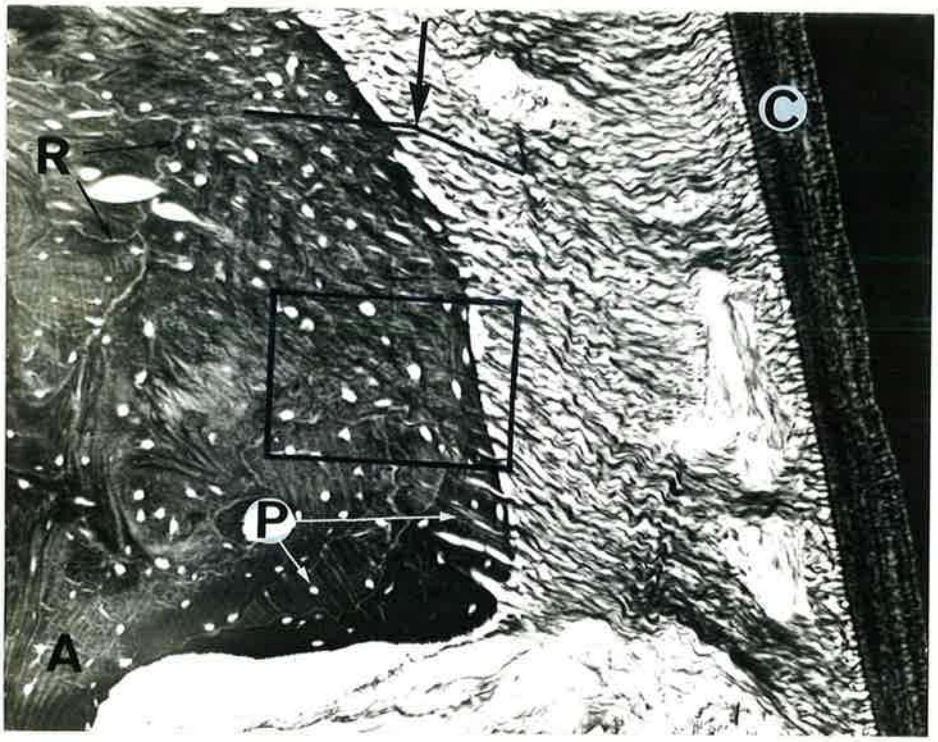


Fig.14. Perforating fibre bundles (P) traverse the bone (A) at a reduced angulation (arrow) after penetration. The reversal lines (R) were more irregular than in Area 17.
Area 14; 19 year old male (c); mesiodistal section.
C, cementum; L, periodontal ligament.
Gordon and Sweet silver and Van Gieson.
X 100.

Fig.15. Higher magnification of Fig.14 (inset). Perforating fibre bundles (P) divide into fibres which interweave as they traverse the alveolar bone (A). Random arrangement of the intrinsic bone matrix fibres (I). L, periodontal ligament; R, reversal lines. X 400.

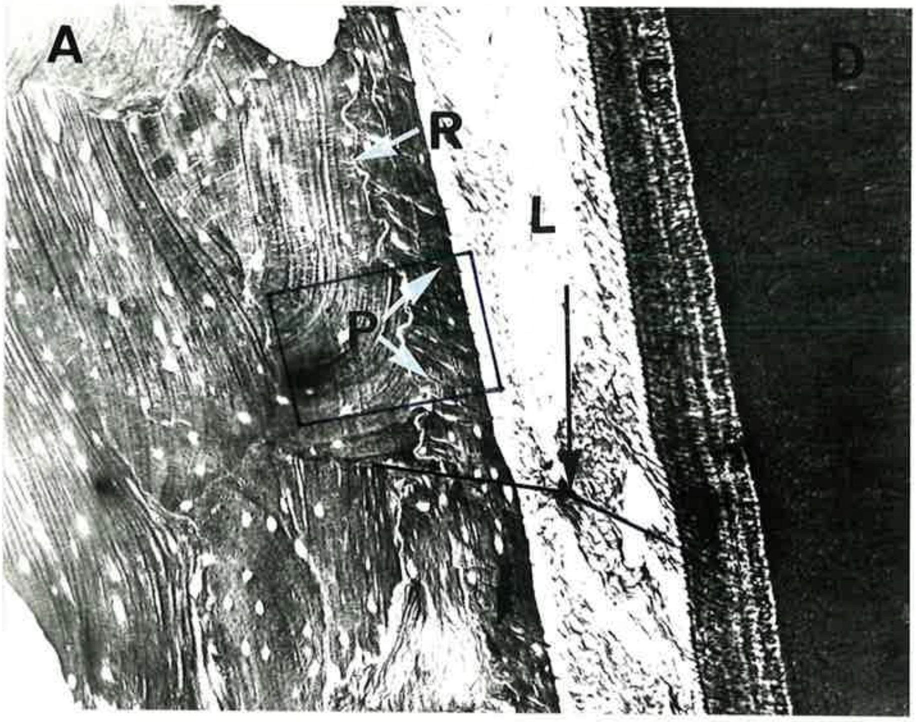


Fig.16. Reduced penetration of the alveolar bone (A) by the perforating fibre bundles (P) and at a reduced angulation (arrow) to terminate at the reversal line (R).
C, cementum; L, periodontal ligament.
Area 15; 19 year old male (b); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

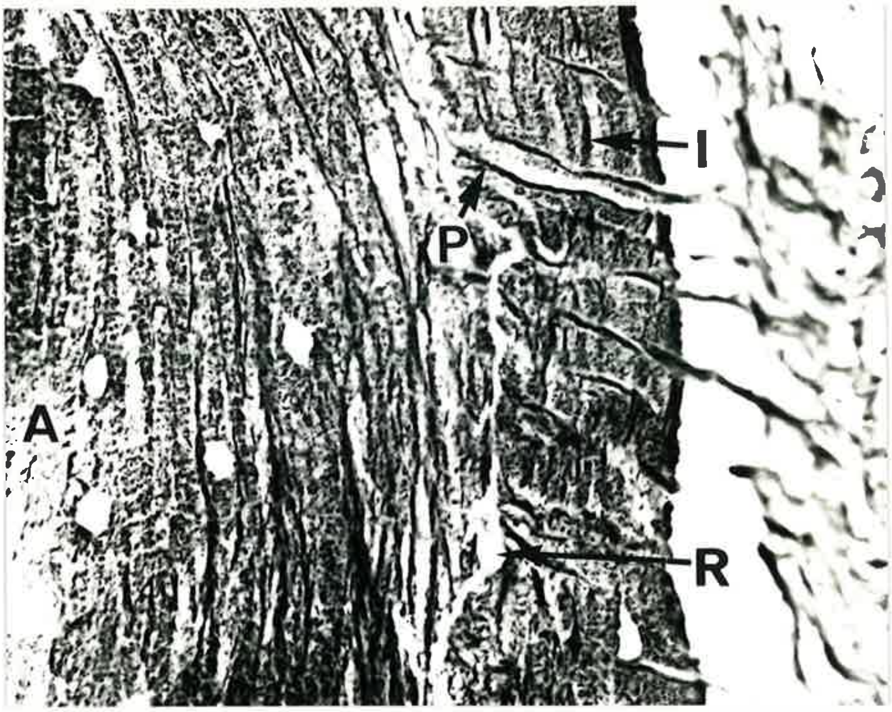


Fig.17. Higher magnification of Fig.16 (inset). Perforating fibre bundles (P) penetrate the alveolar bone (A) to terminate at the inner-most reversal line (R). Intrinsic bone matrix fibres (I) orientated parallel to the bone surface.
L, periodontal ligament.
X 400.

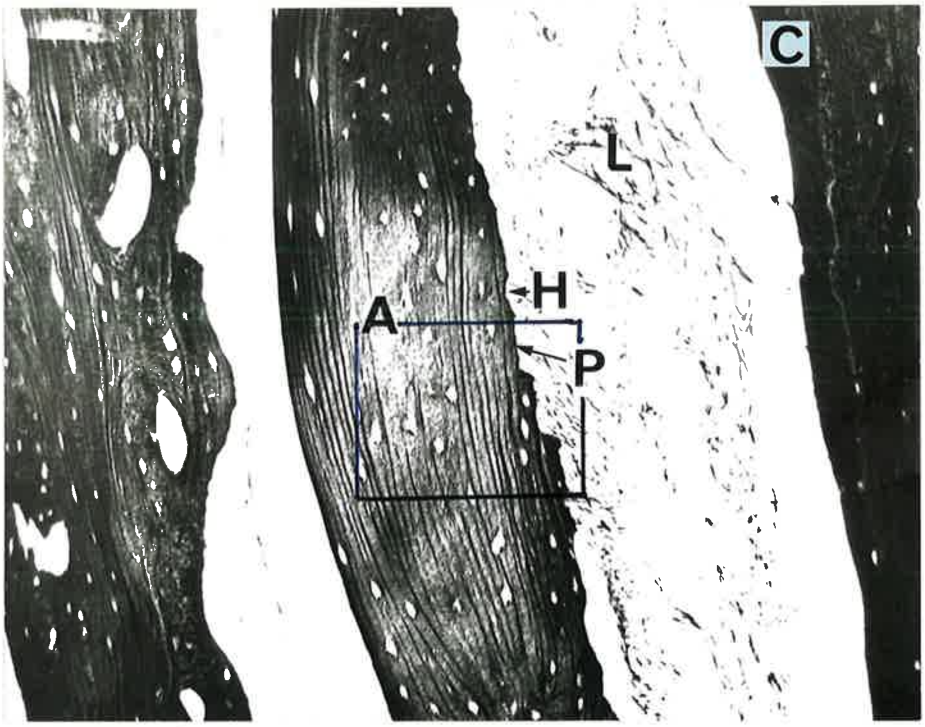
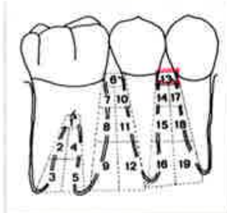


Fig. 18. Dense lamellated bone (A) with minimal perforating fibre bundle (P) penetration and Howship's lacunae (H). C, cementum; L, periodontal ligament.
Area 16; 19 year old male (d); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

Fig. 19. Higher magnification of Fig. 18 (inset). Minimal penetration of the perforating fibre bundles (P) into the alveolar bone (A). R, resting line. H, Howship's lacunae; L, periodontal ligament.
X 400.

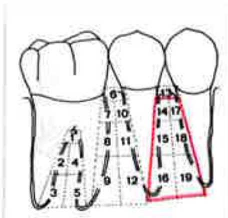
Horizontal sections of the interdental bone between the mandibular right first and second premolars (19 year old female, 19 and 21 year old males).

Area 13.



At the crest of the interdental bone, perforating fibre bundles entered the dense lamellated bone from the mesial and distal surface and anastomosed in the midline of the bony septum (fig.20 p.3.26).

Areas 14-19.



Perforating fibre bundles were observed penetrating the interdental bone to varying depths. In Areas 17, 18 and 19, the fibre bundles entered the bone at right angles to its surface and divided into fibres which penetrated the first reversal line to terminate at the deeper reversal line as fingerlike projections (fig. 21 p.3.27). The perforating fibres were inclined occlusally since the fibres were cut partially in a traverse plane.

In Areas 14, 15 and 16, the degree of perforating fibre bundle penetration was less than that observed in Areas 17, 18 and 19. The bundles penetrated the interdental bone and terminated at the first reversal line as fingerlike projections (fig.22 p.3.27). The angulation of the fibre bundles was approximately at right angles to the long axis of the tooth compared to the oblique angulation in Areas 17, 18 and 19.

Lingual cortical plate.

The perforating fibre bundles penetrated the dense lamellated bone and terminated as fingerlike projections. The bundles divided into fibres which traversed the bone in a spiral path (fig. 23 p.3.28). Complete penetration of the bone was not observed.

Buccal cortical plate.

Crestal third area.

The perforating fibre bundles penetrated the thin lamellated bone, divided into fibres, and then traversed the entire thickness of the bone, to emerge on the buccal surface. These fibres anastomosed with the collagen fibres of the periosteum. Individual fibres were traced through the full thickness of the buccal cortical plate (fig. 24 p.3.29).

Middle and apical third areas.

The lamellated bone on the buccal aspect was thicker in the middle and apical areas and complete fibre traversal of the bone was not observed. The fibre bundles superficially penetrated the bone and then divided into fibres which terminated in finger-like projections at the reversal line similar to the pattern observed in the lingual cortical plate.

/3.30

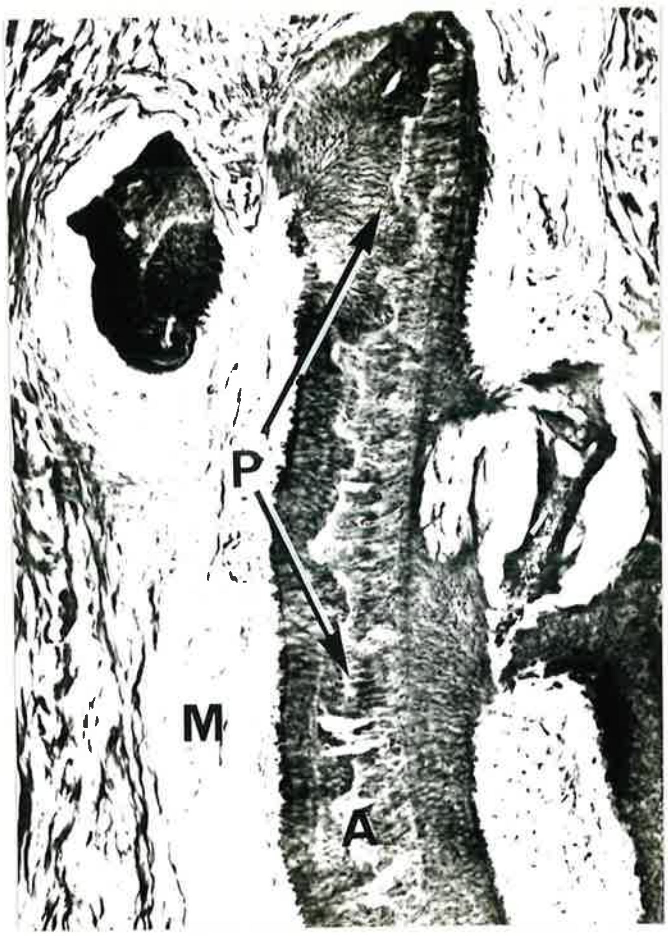


Fig.20. Perforating fibre bundles (P) enter the alveolar bone (A) from the mesial (M) and distal surface and anastomose within the bone.
Area 13; 19 year old female; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 250.

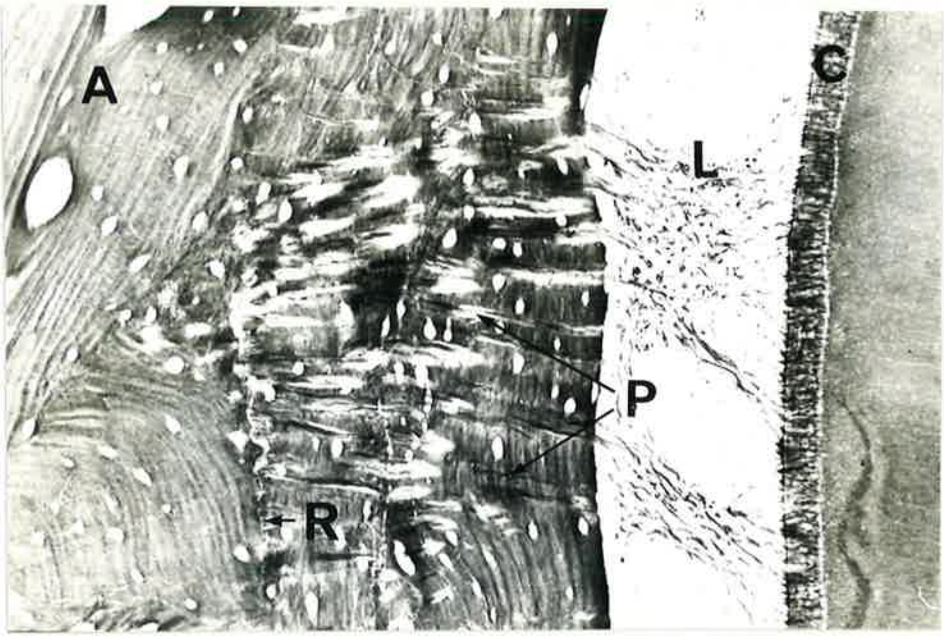


Fig. 21

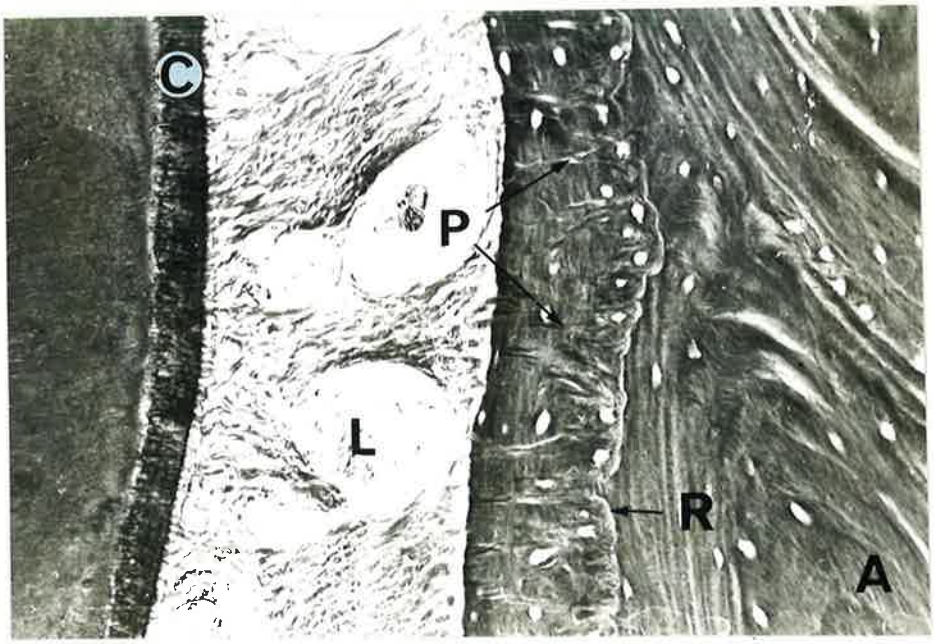


Fig. 22

Figs. 21, 22. Greater penetration of the perforating fibre bundles (P) into the alveolar bone (A) on the mesial surface Fig. 21 than on the distal surface of the same interdental septum, Fig. 22. The bundles terminated at the innermost reversal line (R) as fingerlike projections. C, cementum; L, periodontal ligament.
Fig. 21, Area 14; Fig. 22, Area 17; 19 year old male; horizontal sections.
Gordon and Sweet silver and Van Gieson.
X 100.

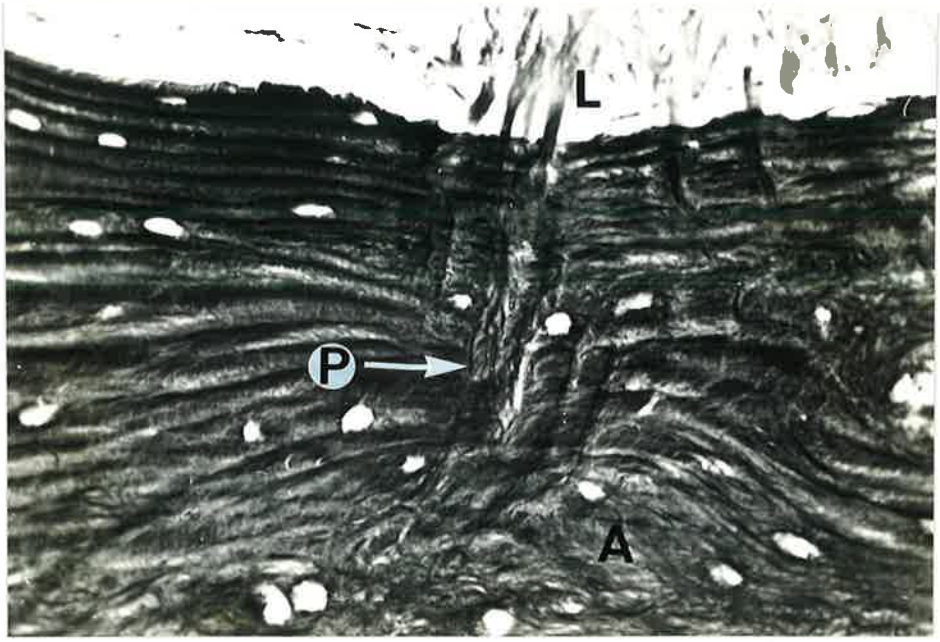


Fig.23. Perforating fibre bundles (P) penetrate the dense lamellated bone (A) of the lingual cortical plate to terminate as fingerlike projections.
L, periodontal ligament.
Middle third area; 19 year old female; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 250.

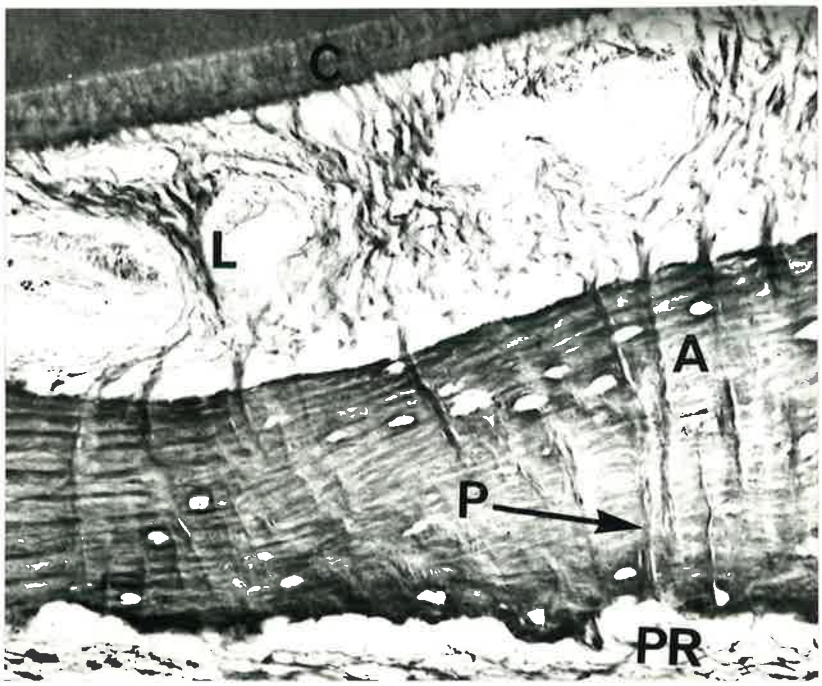


Fig.24. Perforating fibre bundles (P) traverse the full thickness of the buccal lamellated bone (A).
C, cementum; L, periodontal ligament; PR, periosteum.
Crestal third area; 21 year old male; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 275.

<u>Buccolingual sections of the mandibular right first and second premolars (21 year old male)</u>.

At the crest of the alveolar bone on the buccal surface, perforating fibre bundles were seen penetrating to varying depths. A small number of these bundles penetrated the crest from the periodontal ligament side and curved apically through the bone (fig.25 p.3.31). The perforating fibre bundles were traced on serial sections and were noted emerging and anastomosing with the collagen fibre bundles of the overlying periosteum. Evidence of complete penetration of the lingual alveolar crest was not obtained nor in the middle and apical third areas.

Perforating fibre bundles were observed traversing the middle third of the buccal bone (fig.26 p.3.32). The bundles penetrated the lamellated bone at an apical inclination and emerged to anastomose with the collagen bundles of the overlying periosteum.

In the apical third, the perforating fibre bundles penetrated the bone and terminated at the inner-most reversal line as fingerlike projections.

/3.33

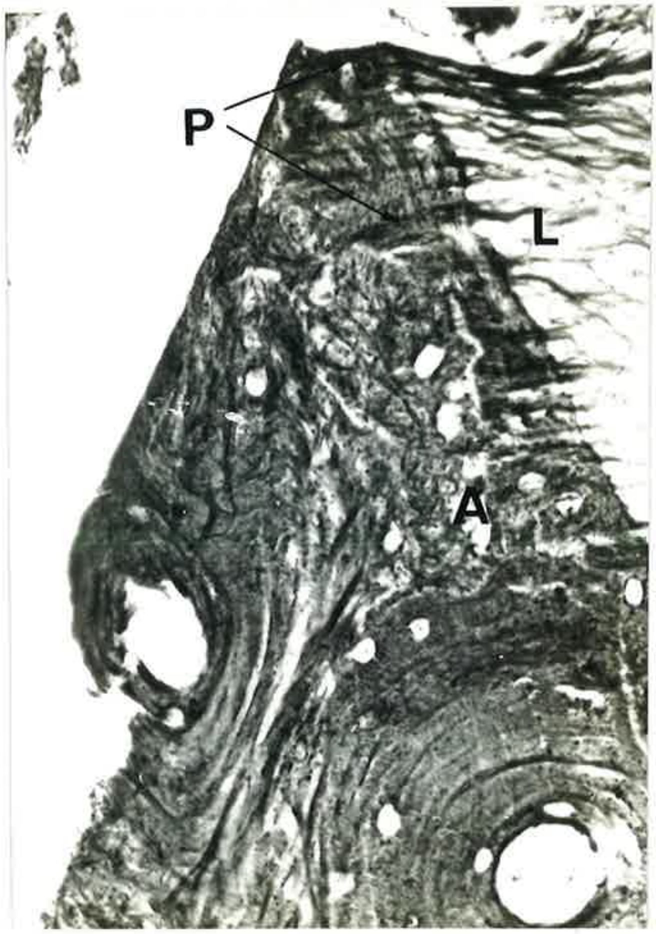


Fig.25. Perforating fibre bundles (P) penetrate the crest of the buccal bone (A) overlying the mandibular right first premolar and then curve downwards.
L, periodontal ligament.
19 year old male; buccolingual section.
Gordon and Sweet silver and Van Gieson.
X 200.

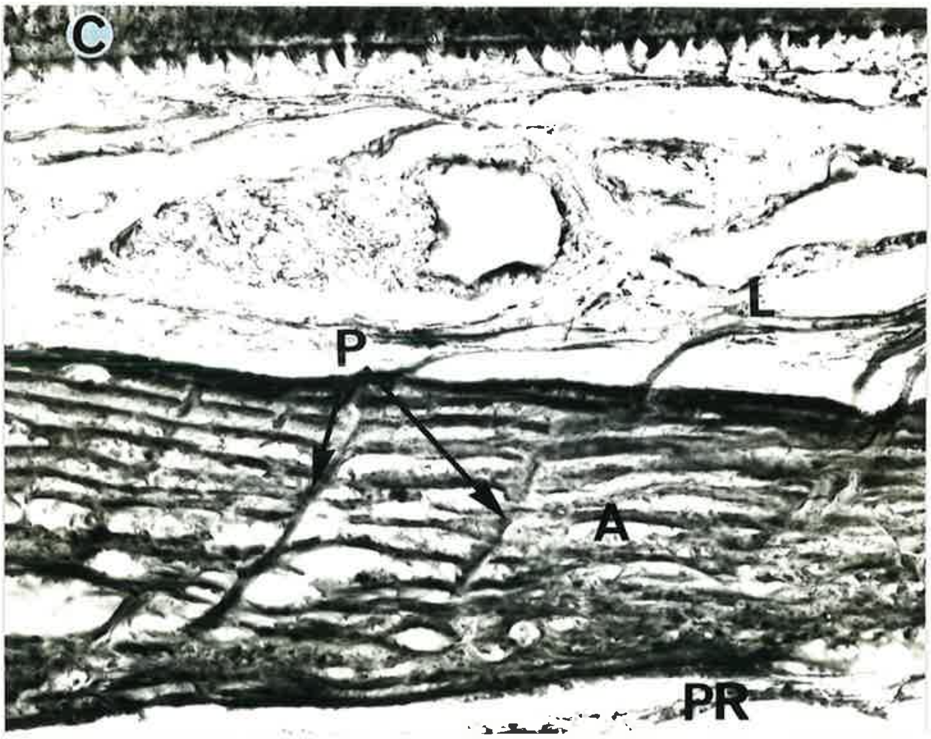
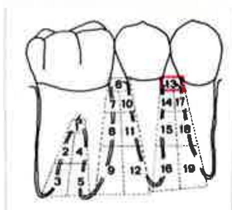


Fig.26. Perforating fibre bundles (P) traverse the entire thickness of the buccal bone (A).
C, cementum; L, periodontal ligament; PR, periosteum.
Middle third area; 19 year old male; bucco-lingual section.
Gordon and Sweet silver and Van Gieson.
X 450.

Mesiodistal sections of the interdental area between the mandibular right first and second premolars (68 year old male).

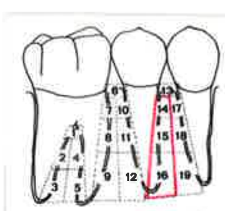
The interdental bone was more cancellous than that observed in the 19 to 21 year old specimens and similar to the interradicular bone of the 46 year old specimen (fig. 40 p.3.58). There were fewer large marrow spaces (fig. 27. p.3.35) and more trabeculae than in the 19 to 21 year old specimens (fig.5, p.3.9). The intensity of the staining reaction and also the number of the perforating fibre bundles was less than that observed in the younger specimens.

Area 13.



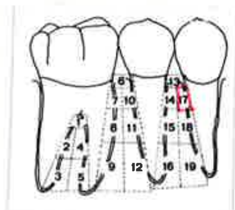
The perforating fibre bundles entered the alveolar bone, penetrated to a minimal depth and terminated at the innermost reversal line (fig.28 p.3.36). Perforating fibre bundles were not observed completely traversing the alveolar crest as seen in the 19 to 21 year old specimens. The crest of the interdental bone showed evidence of inflammation and bone resorption in sections stained with haematoxylin and eosin.

Area 14, 15, and 16.



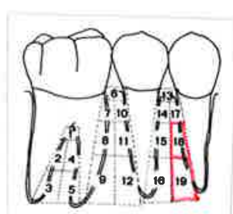
These areas were similar in their histological arrangement of the perforating fibre bundles to those observed in the 19 to 21 year old specimens. The perforating fibre bundles penetrated to the innermost reversal line in Areas 14 and 15 and to a minimal depth in Area 16. Howship's lacunae were also observed in Area 16.

Area 17.



The fibre bundles of the periodontal ligament were smaller in diameter and less numerous than those observed in the 19 to 21 year old specimens. The perforating fibre bundles, unlike those observed in the 19 to 21 year old specimens, entered the alveolar bone and then divided into fibres which dramatically changed their angulation (fig. 29 p.3.37). The fibres curved upwards to terminate at a reversal line as divergent finger-like projections.

Area 18 and 19.



The general histological arrangement of the perforating fibre bundles was similar to that in the 19 to 21 year old specimens although the change in angulation after entering the bone was again greater.

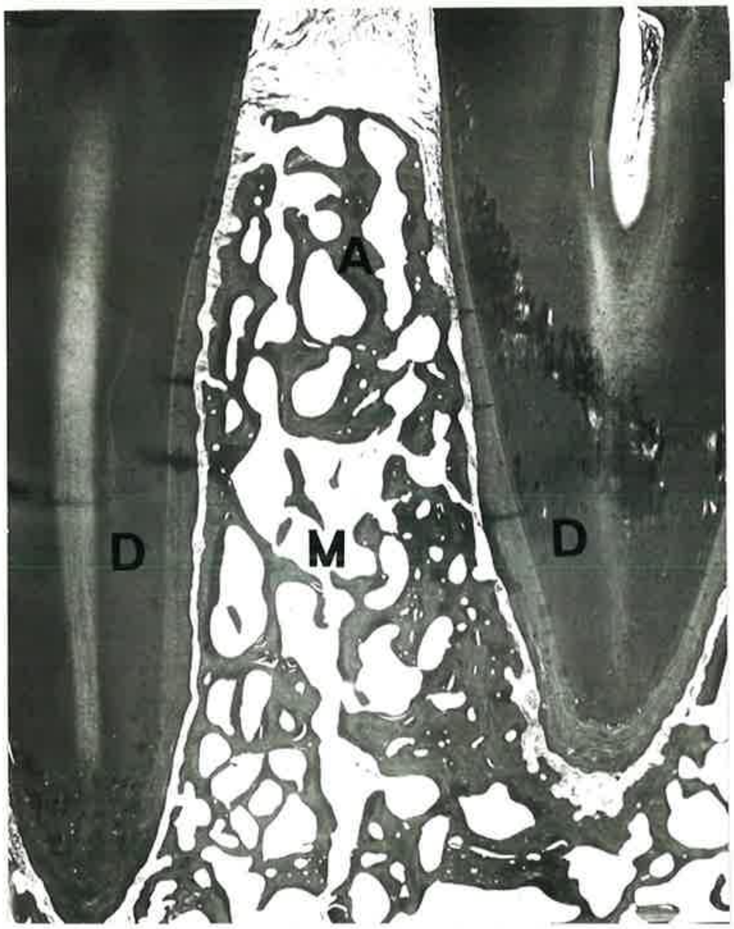


Fig.27. Cancellous nature of the interdental bone between the mandibular right first and second premolars. A, alveolar bone; D, dentine; M, marrow spaces. Areas 13-19; 68 year old male; mesiodistal section. Gordon and Sweet silver and Van Gieson. X 10.

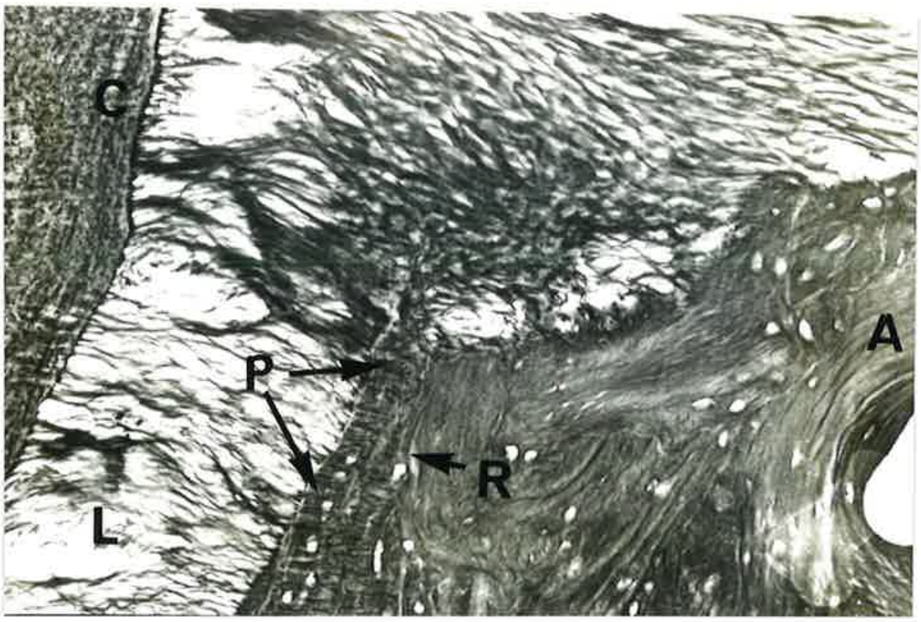


Fig.28 Minimal penetration of the perforating fibre bundles (P) into the alveolar bone (A) which terminate at the innermost reversal line (R).
C, cementum; L, periodontal ligament.
Area 13; 68 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

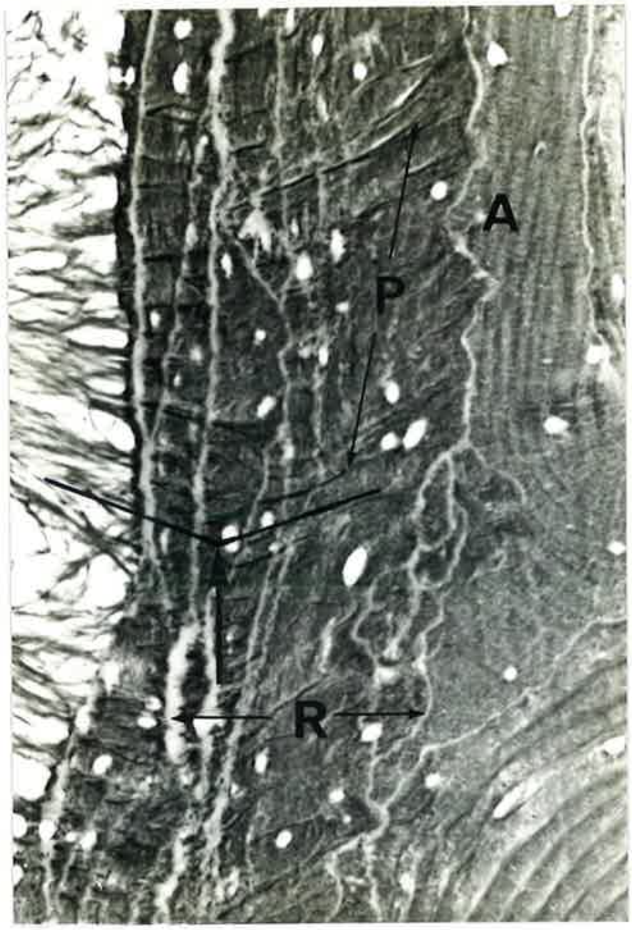
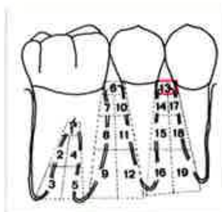


Fig.29. Perforating fibre bundles (P) change their angulation (arrow) after entering the alveolar bone (A) and terminate at a reversal line (R). Area 17; 68 year old male; mesiodistal section. Gordon and Sweet silver and Van Gieson. X 200.

Mesiodistal sections of the interdental area between the mandibular right first and second premolars (11 year old female).

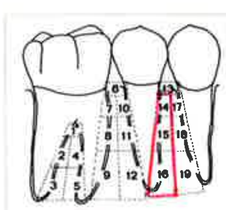
Lamellated bone formed the mesial, distal and crestal border of the interdental area surrounding a large marrow space with few trabeculae (fig.30 p.3.40).

Area 13.



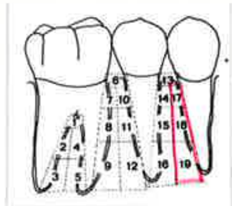
Perforating fibre bundles entered the alveolar bone on the distal surface and terminated at the reversal line which followed the bony outline of the mesial surface (fig. 31 p.3.41). Slightly below the crest, the fibre bundles followed a similar occlusally inclined path as the more crestal bundles, but completely traversed the reversal line to emerge and continue as the principal fibre bundles of the periodontal ligament (fig.31 p.3.41). on the mesial aspect.

Area 14, 15 and 16.



Minimal perforating fibre bundle penetration was observed in these areas. The bundles entered the bone and terminated at the innermost reversal line. The fibre bundle pattern was similar to that observed in the 19 to 21 year old specimens. The bone surface in these areas was characterized by Howship's lacunae which indicated that bone resorption had occurred.

Area 17, 18 and 19.



The perforating fibre bundles entered the alveolar bone at a slight occlusal inclination and completely traversed the bone to terminate at the large marrow space (fig.32 p.3.42). The arrangement of the principal fibre bundles of the periodontal ligament was irregular and less organized than in the 19 to 21 year age specimens.

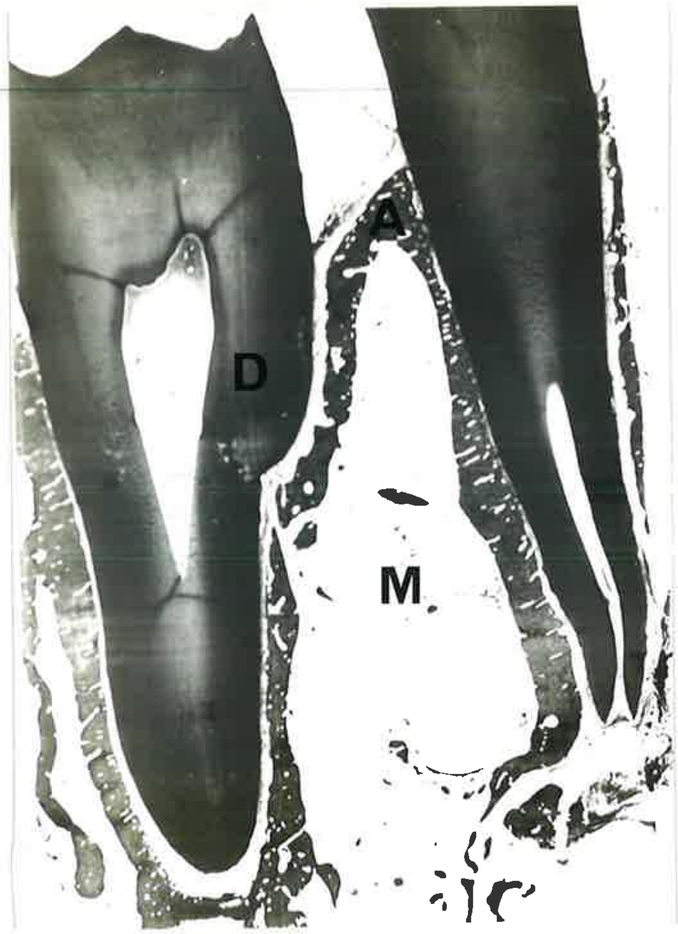


Fig.30. Large marrow space (M) surrounded by alveolar bone (A) forming the interdental area of the erupted mandibular right first and second premolars. D, distal.
Areas 13-19; 11 year old female; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 8.

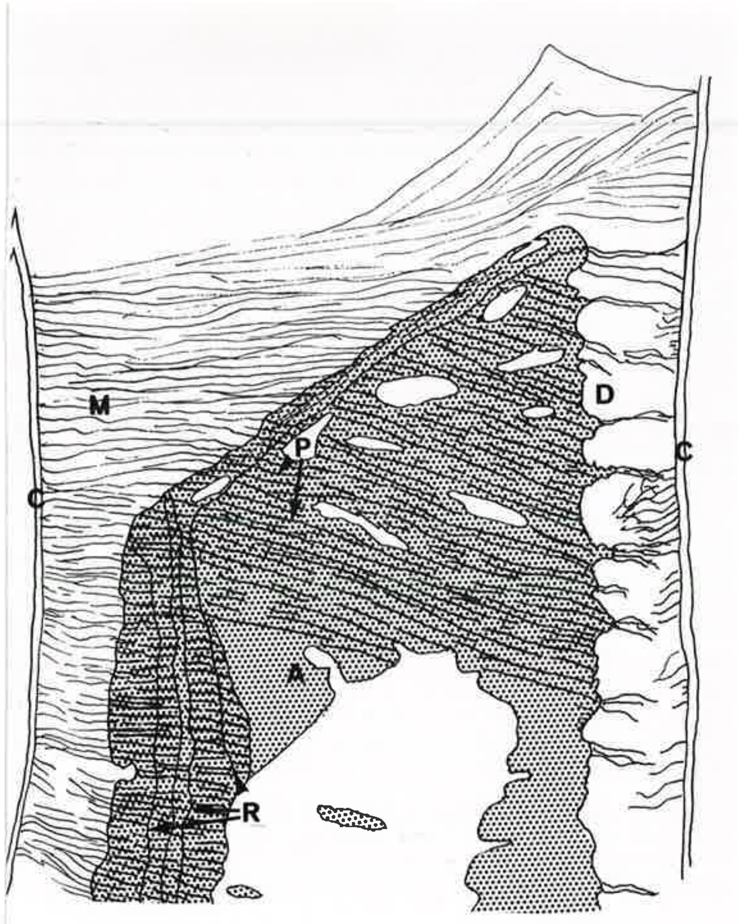


Fig.31. Diagrammatic representation of three consecutive serial sections. Crestal perforating fibres terminate at the reversal line. Below the crest, the perforating fibres (P) traverse the reversal line (R) and continue as the principal fibres of the periodontal ligament. A, alveolar bone; C, cementum; D, distal; M, mesial.
Area 13; 11 year old female; mesiodistal section.

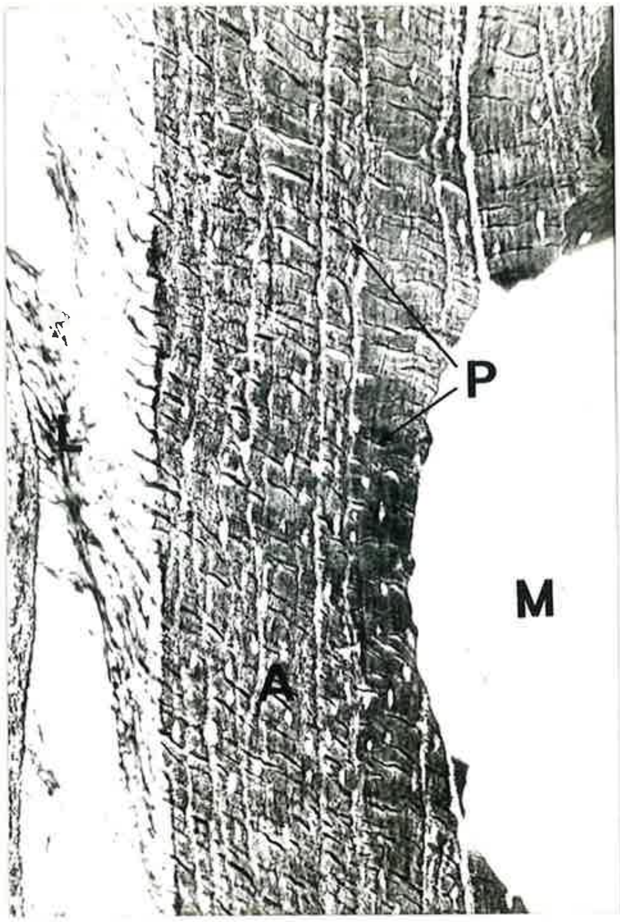


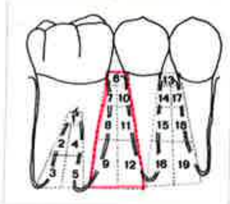
Fig.32. Perforating fibre bundles (P) penetrate the alveolar bone (A) to terminate at the marrow space (M).
Area 18; 11 year old female; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

PREMOLAR-MOLAR REGION.

Mesiodistal sections of the interdental area between the mandibular right second premolar and first molar (19 year old male).

The interdental alveolar bone was of the lamellated form similar to that observed in the 19 to 21 year old premolar region.

Area 6, 7, 8, 9, 10, 11, 12.



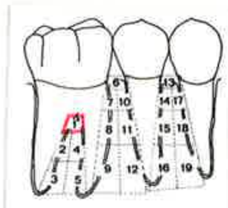
The perforating fibre bundle pattern was similar to that observed in the premolar region. In Area 6 perforating fibre bundles were traced on serial sections and were found to traverse the alveolar bone crest from the mesial to the distal surface and to then continue as the fibre bundles of the periodontal ligament. In the more apical areas, the perforating fibre bundles entered the bone at an upward inclination which was greater in Areas 10 and 11 than in Areas 7 and 8. The degree of fibre bundle penetration was also greater on the mesial than on the distal surface of the interseptal crest.

MOLAR REGION.

Mesiodistal sections of the interradicular area of the mandibular right first and second molars (two 19 year old males).

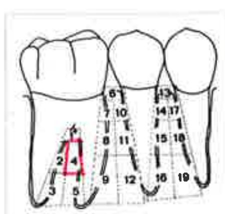
The interradicular alveolar bone was composed of an outer layer of lamellated bone separated by cancellous trabeculae (fig.33 p.3.47) similar to that observed in the interdental region of the 19 to 21 year old premolar specimens (fig.5 p.3.9).

Area 1.



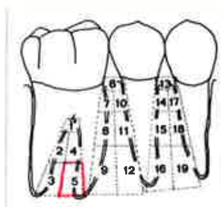
Perforating fibre bundles were seen to penetrate the alveolar crest as a slight upward inclination and to varying depths. A few perforating fibre bundles traced on serial sections divided into fibres after entering the bone and traversed the bone in an arc upwards to anastomose with similar fibres from the adjacent surface (fig.34 p.3.48).

Area 4.



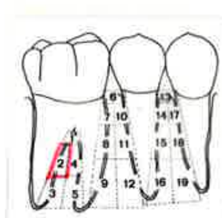
Perforating fibre bundles were observed penetrating the bone and traversing the reversal lines to terminate at the innermost reversal line. The inclination of the fibres was greater than that observed in Area 1. The fibres terminated as finger-like projections but were not divergent, unlike those noted in the interdental areas. The reversal lines were more irregular compared with the corresponding mesial region (Area 18) of the interdental bone. Intrinsic bone matrix fibres were mainly orientated parallel to the long axis of the interradicular bone.

Area 5.



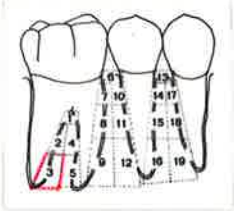
Perforating fibre bundles were observed penetrating the alveolar bone at an occlusal inclination to terminate at the innermost reversal line. The bone in this region was more lamellated than that observed in Areas 4 and 1. The perforating fibre bundles divided into loosely woven fibres which terminated as finger-like projections similar to those observed in Area 19. The orientation of the intrinsic bone matrix fibres was generally parallel to the long axis of the interradicular bone.

Area 2.



The perforating fibre bundles penetrated the alveolar bone to a minimal depth and terminated at the innermost reversal line in a similar manner to that observed in Area 15 of the 19 to 21 year old premolar specimens. However, the fibres did not terminate as divergent finger-like projections. The occlusal inclination of the fibres was less than that noted on the adjacent mesial surface (Area 4). Howship's lacunae indicated areas of alveolar bone resorption in this area. Intrinsic bone matrix fibres were randomly orientated through the bone.

Area 3.



The pattern of perforating fibre bundle penetration was similar to that observed in Area 2. The penetration was minimal and the fibres terminated at the first reversal line. Howship's lacunae were observed in this area indicating that bone resorption had occurred. The inclination of the fibres towards the occlusal plane was less than that noted in Area 2. The general orientation of the intrinsic bone matrix fibres was parallel to the long axis of the interradicular bone.

/3.49

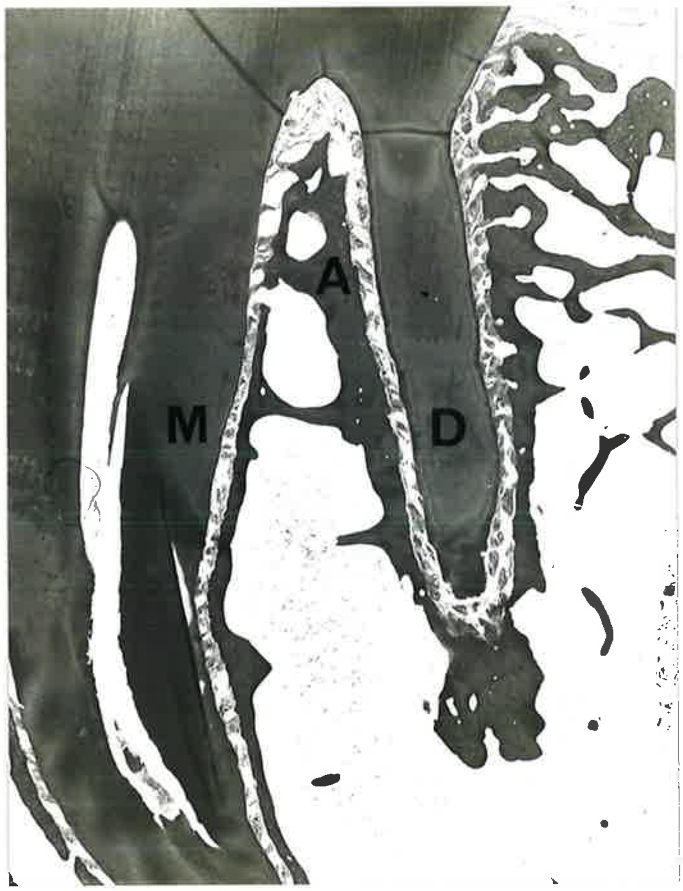


Fig.33. Cancellous interradicular bone (A) of the mandibular right first molar.
D, dentine; M, mesial.
Areas 1-5; 19 year old male (a); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 8.

Fig.34. Perforating fibres (P) enter the alveolar bone (A) from the mesial (M) and distal and from serial sections anastomose within the bone.
L, periodontal ligament; R, reversal line.
Area 1; 19 year old male (b); mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 500.

Horizontal sections of the mandibular right first and second molars (19 year old male).

Interdental Area.

In the crestal third, perforating fibre bundles were observed to enter from the mesial and distal aspects and anastomose within the dense lamellated bone (fig.35 p.3.50). The perforating fibre bundle pattern in the middle and apical thirds of the interdental bone was similar to that observed in the corresponding areas of the 19 to 21 year old premolar region.

Buccal Plate Area.

Perforating fibre bundles penetrated the dense lamellated bone overlying the buccal surface of both the molars in the crestal area. The fibre bundles traversed the bone (fig.36 p.3.51) and emerged to anastomose with the collagen fibre bundles of the overlying periosteum.

In the middle and apical thirds of the buccal alveolar bone, the perforating fibre bundles penetrated the bone and terminated at the innermost reversal line in a similar manner to that observed in the 19 to 21 year old premolar area.

Lingual Plate Area.

Complete penetration of the lingual lamellated bone by the perforating fibre bundles was not observed. The fibre bundle pattern was similar to that observed on the lingual aspect of the 19 to 21 year old premolar segments.

/3.52

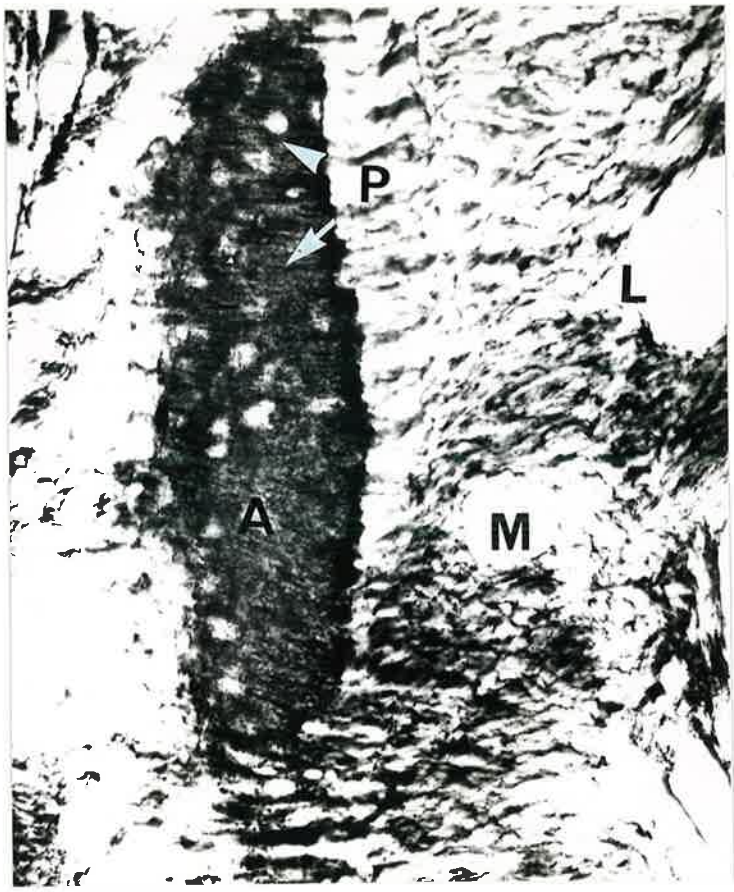


Fig.35. Perforating fibre bundles (P) enter the alveolar bone (A) in the crestal third from the mesial (M) and distal to anastomose within the bone.
L, periodontal ligament.
Area 1; 19 year old male; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 200.

Fig.36. Perforating fibre bundles (P) traverse the buccal alveolar bone (A) overlying the mandibular right first molar from the periodontal ligament (L) to the periosteum (PR). Crestal third; 19 year old male; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 250.

Buccolingual section of the mandibular right first molar (19 year old male).

The buccal and lingual alveolar bone overlying the molar was lamellated in form in the crestal third and more Haversian in nature towards the apical third.

Buccal alveolar bone.

Perforating fibre bundles were observed traversing the lamellated bone in the crestal third (fig.37 p.3.53). The perforating fibre bundles entered the lamellated buccal bone in an horizontal path and then curved apically to emerge and anastomose with the collagen fibres of the overlying periosteum.

In the middle and apical third areas, the perforating fibre bundles penetrated the dense lamellated bone and terminated at the innermost reversal line (fig.38 p.3.54) adjacent to the Haversian systems.

Lingual alveolar bone.

The complete penetration of the crestal third on the lingual aspect by the perforating fibre bundles was not observed. The perforating fibre bundles entered the alveolar bone from the periodontal ligament side and curved apically to terminate at the innermost reversal line (fig.39. p.3.55).

In the middle and apical third areas the perforating fibre bundles entered the bone at a slightly apical angulation and terminated at the innermost reversal line. The depth of penetration of the perforating fibre bundles was greater on the lingual aspect than on the buccal aspect.

/3.56

Fig.37. Perforating fibre bundles (P) enter the alveolar bone (A) from the periodontal ligament (L) and traverse the bone (A) overlying the mandibular right first molar. The fibres curve apically and emerge to join with the fibres of the overlying periosteum (PR). Crestal third; 19 year old male; bucco-lingual section.
Gordon and Sweet silver and Van Gieson.
X 200.

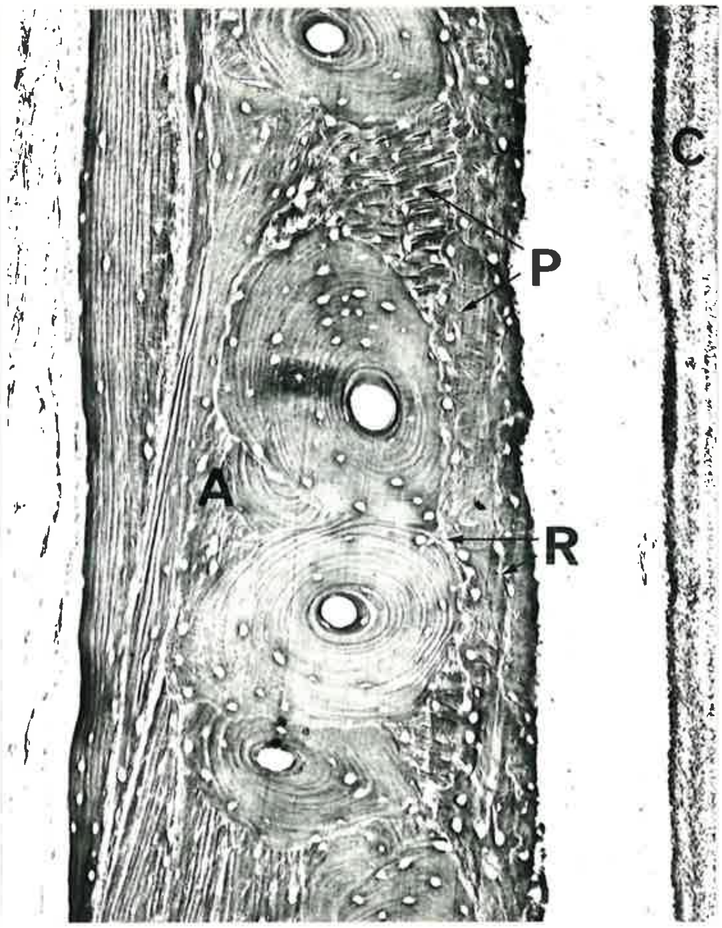


Fig.38. Perforating fibre bundles (P) penetrate the buccal bone (A) overlying the mandibular right first molar to terminate at the innermost reversal line (R).
C, cementum.
Middle third area; 19 year old male; bucco-lingual section.
Gordon and Sweet silver and Van Gieson.
X 100.

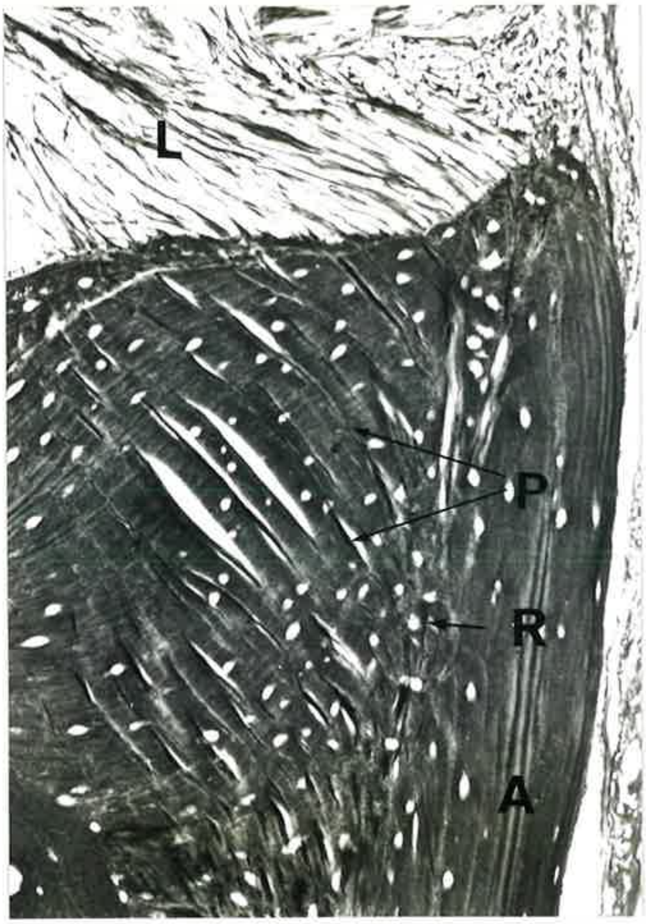
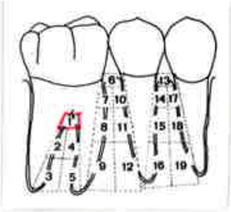


Fig.39. Perforating fibre bundles (P) penetrate the lingual lamellated bone (A) overlying the mandibular right first molar, curve apically, and terminate at the innermost reversal line (R).
L, periodontal ligament.
Crestal third area; 19 year old male; bucco-lingual section.
Gordon and Sweet silver and Van Gieson.
X 100.

Mesiodistal sections of the interradicular area of the mandibular right first molar (46 year old male).

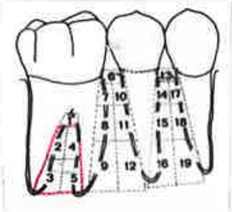
The interradicular bone was more cancellous in form (fig.40 p.3.58) and the perforating fibre bundles did not stain as intensely (fig.41 p.3.59) as those observed in the 19 to 21 year old males.

Area 1.



Perforating fibre bundles were not observed completely traversing the alveolar bone crest (fig.42 p.3.60). The perforating fibre bundles entered the bone on the mesial aspect and terminated at the innermost reversal line. There was minimal fibre bundle penetration on the distal surface which was characterized by Howship's lacunae indicating that bone resorption had occurred in this area.

Area 2, 3, 4 and 5.



The general arrangement of the perforating fibre bundles in these areas of the interradicular bone was similar to that observed in the 19 to 21 year old specimens although the arrangement of the periodontal ligament fibre bundles was more irregular and the number of bundles was less. In Area 4 of the interradicular bone, a continuation of the perforating fibre bundles through the marrow space was noted (fig.43 p.3.61). The perforating bundles entered the bundle bone and emerged at the marrow space which they traversed and then inserted into the adjacent cancellous bone.

/3.62

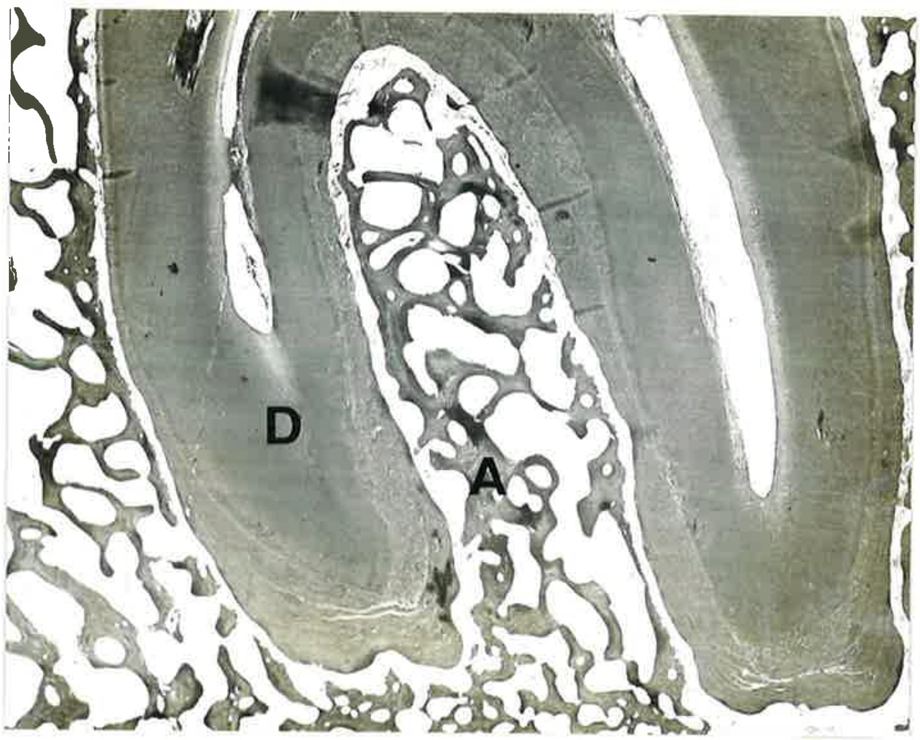


Fig.40. Trabecular alveolar bone (A) in the inter-radicular area of the mandibular right first molar.
D, dentine.
Areas 1-5; 46 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 8.

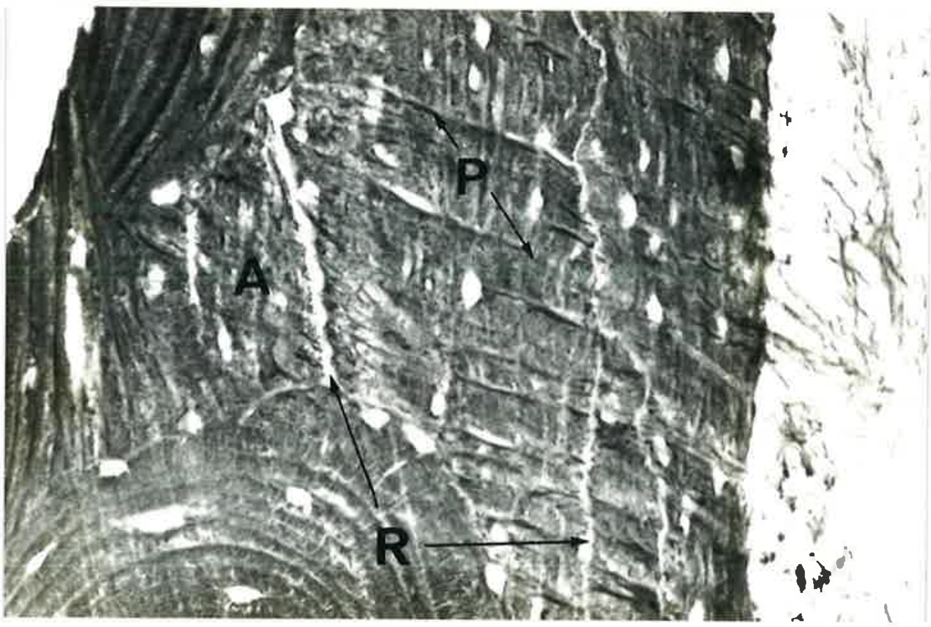


Fig.41. Lightly stained perforating fibre bundles (P) penetrate the alveolar bone (A) to terminate at the innermost reversal line (R).
Area 4; 46 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 200.

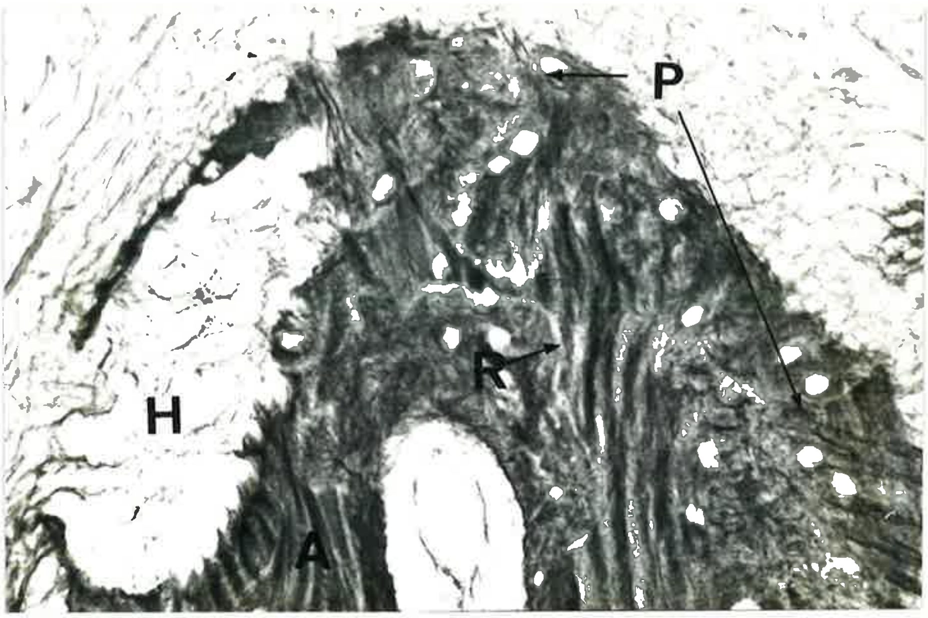


Fig.42. Perforating fibre bundles (P) penetrate the alveolar bone (A) and terminate at the innermost reversal line (R). Large area of resorption (H) on the distal surface.
L, periodontal ligament.
Area 1; 46 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 200.

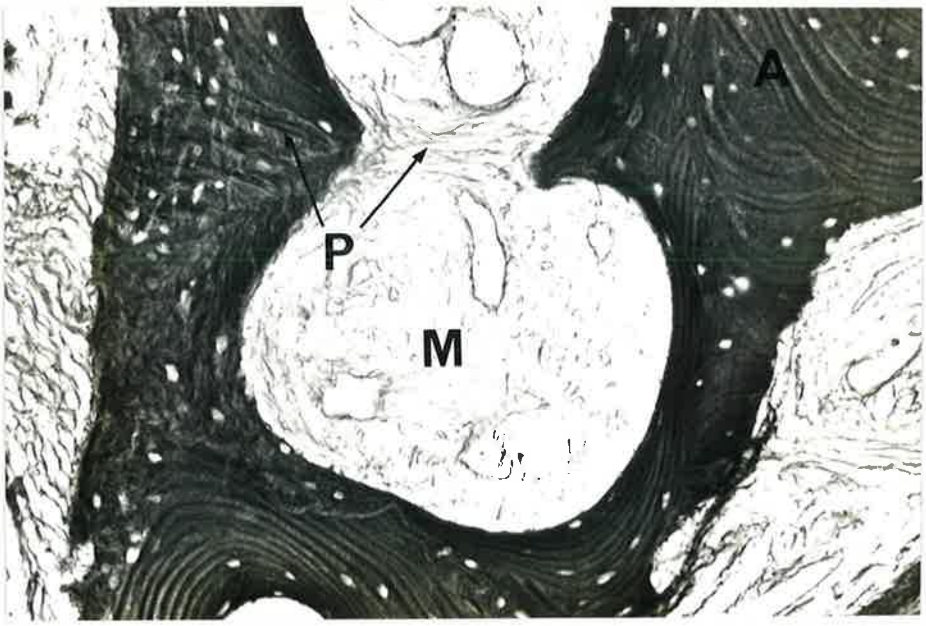


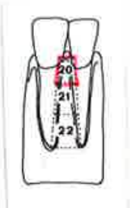
Fig.43. Perforating fibre bundles (P) traverse the lamellated alveolar bone (A) and then cross the marrow space (M) to insert into the bone on the adjacent side.
Area 4; 46 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 100.

INCISOR REGION.

Mesiodistal sections of the interdental area between the mandibular right central and lateral incisors (19 year old male).

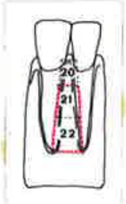
The interdental septum consisted of lamellated bone and was narrower mesiodistally than that observed in the 19 to 21 year old premolar areas (fig.5 p.3.9). There were fewer marrow spaces and these were observed mainly in Area 21 and 22 of the interdental septum (fig. 44 p.3.64).

Area 20.



Perforating fibre bundles were observed passing through the entire thickness of the interdental septum (fig.45 p.3.65). The fibre bundles entered the bone from the mesial and distal and angled upwards towards the occlusal plane and appeared to anastomose at a reversal line. There was no apparent pattern with respect to the position at which the fibre bundles anastomosed.

Area 21 and 22.



Complete penetration by the perforating fibre bundles was not observed in these areas of the interdental bone. The fibre bundles penetrated from the mesial and distal surfaces and terminated at the innermost reversal line on the mesial and distal, respectively. There was a greater fibre bundle penetration on the mesial aspect of the interdental bone. A greater occlusal inclination of the perforating fibre bundles was noted on the mesial aspect comparable to Area 17, 18 and 19 of the 19 to 21 year old premolar specimens.

/3.66

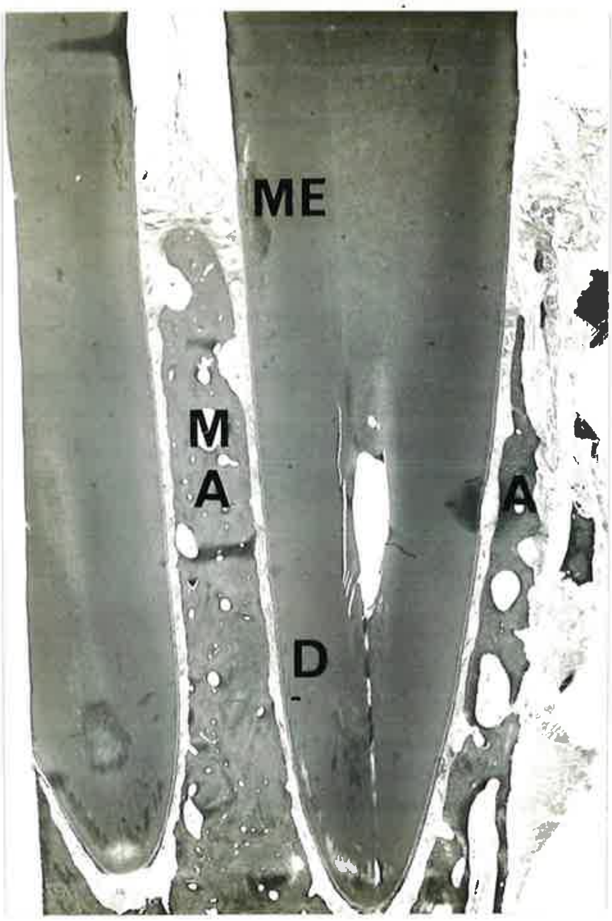


Fig.44. Narrow interdental alveolar bone (A) with minimal marrow spaces (M) between the mandibular right central and lateral incisor.
D, dentine; ME, mesial.
Areas 20-22; 19 year old male; mesio-distal section.
Gordon and Sweet silver and Van Gieson.
X 8.

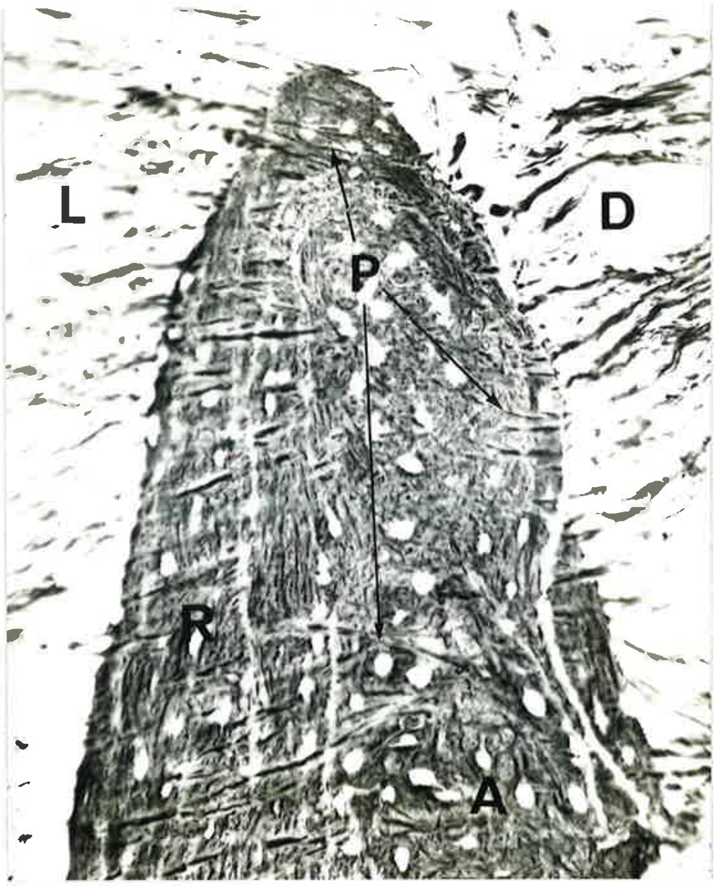
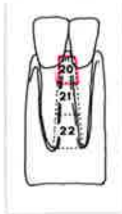


Fig.45. Perforating fibre bundles (P) enter the lamellated bone (A) from the mesial and distal (D) and anastomose within the bone.
L, periodontal ligament; R, reversal line.
Area 20; 19 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 225.

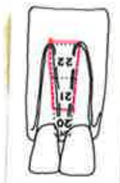
Horizontal sections of the interdental area between the mandibular left central and lateral incisors (19 year old male).

Area 20.



Perforating fibre bundles were observed penetrating the interdental septum on the mesial and distal and also the buccal and lingual aspects to varying depths (fig.46 p.3.68). In the crestal third, the fibre bundles penetrated the deepest on the mesial surface and the least on the buccal and lingual surfaces. On the lingual aspect the fibre bundles divided into fibres after entering the mesial surface of the bone and terminated at the innermost reversal line as divergent fingerlike projections (fig. 46 p.3.68). The distal surface was characterized by deeply penetrating fibre bundles towards the labial aspect and areas of resorption indicated by Howship's lacunae towards the lingual aspect. A few fibre bundles were observed which curved outwards towards the labial aspect of the interdental septum. They anastomosed at the innermost reversal line on both the mesial and distal aspects with the perforating fibre bundles entering from each side (fig. 47 p.3.69).

Areas 21 and 22.



The interdental bone was more cancellous with numerous reversal lines on both the mesial and distal aspects. The perforating fibre bundles in these areas penetrated the alveolar bone and terminated at the inner-most reversal line, on both the mesial and distal aspects. The penetration was greater on the mesial aspect than on the distal surface similar to the pattern observed in Area 14 and 17 respectively of the 19 to 21 year old premolar specimens.

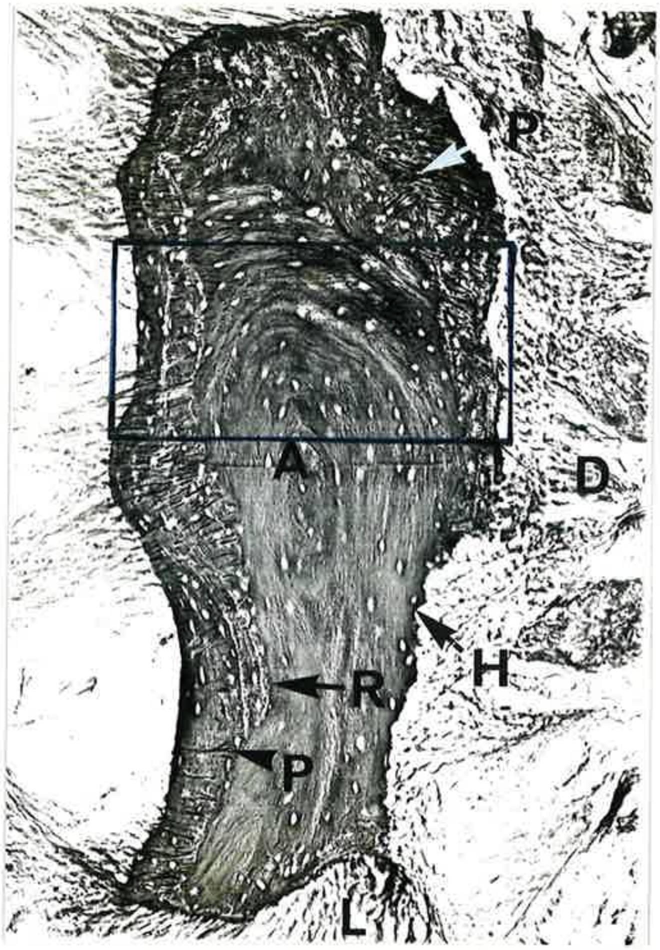


Fig. 46. Perforating fibre bundles (P) penetrate the alveolar bone to varying extents on the mesial, distal (D), labial and lingual (L) surfaces. On the linguo-mesial aspect the perforating fibres terminated at the innermost reversal line (R) as divergent fingerlike projections. H, Howship's lacunae.
Area 20; 19 year old male; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 80.

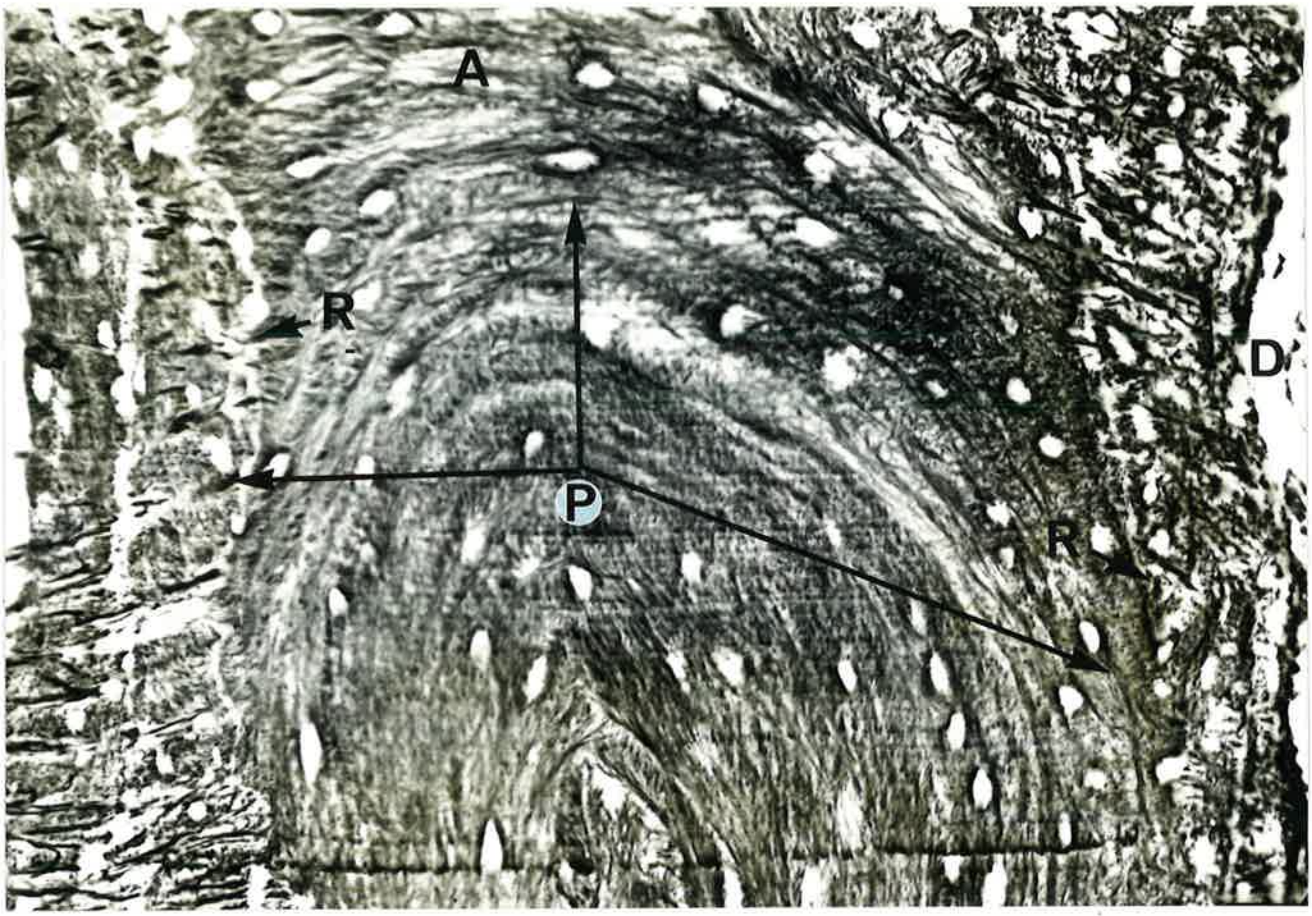


Fig. 47. Higher magnification of Fig. 46 (inset). Perforating fibre bundles (P) on the labial aspect of the alveolar bone (A) curve labially and anastomose with the fibre bundles entering from the mesial and distal (D) surfaces at the innermost reversal lines (R). X 400.

Horizontal sections of the buccal and lingual alveolar bone overlying the mandibular left central and lateral incisors (19 year old male).

Complete penetration of the entire thickness of the labial cortical plate was noted in the more coronal regions overlying the central and lateral incisors (fig. 48 p.3.71). The bundles of the perforating fibres emerged on the labial surface of the bone and anastomosed with the collagen bundles of the overlying periosteum. Perforation of the lingual cortical plate by the fibre bundles was not observed.

In the more apical areas the perforating fibres penetrated to the innermost reversal lines and then terminated as divergent finger-like projections in a similar pattern to that described in the corresponding 19 to 21 year old premolar region.

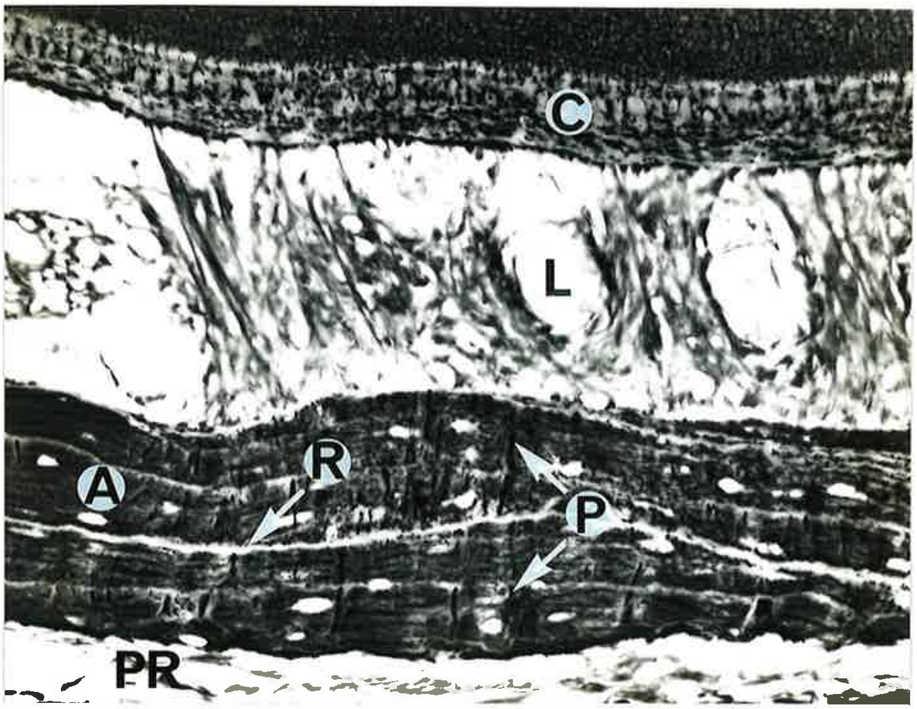


Fig.48. Perforating fibre bundles (P) traverse the alveolar bone (A) overlying the mandibular left lateral incisor to emerge and anastomose with the collagen fibres of the periosteum (PR).
C, cementum; L, periodontal ligament; R, reversal lines.
Crestal third area; 19 year old male; horizontal section.
Gordon and Sweet silver and Van Gieson.
X 250.

GENERAL SUMMARY OF THE MORPHOLOGY OF THE PERFORATING FIBRE BUNDLES.

A difference in the morphology of the principal fibre bundles of the periodontal ligament on the mesial aspect of the alveolar bone was observed in comparison with the adjacent bundles on the distal surface. The insertion of these bundles on the mesial aspect was generally seen as a convergence of a number of principal bundles into narrow dense bundles which then increased in thickness to insert into the interdental and inter-radicular bone. After insertion the perforating fibre bundles tended to narrow prior to dividing into fibres (fig.49 p.3.74).

On the distal surface the number of principal fibre bundles which joined together was less and as a result the perforating fibre bundles were less fan shaped. The thickness of the resultant larger bundles was less than that observed on the mesial aspect prior to insertion into the bone (fig.49 p.3.74).

Although not as obvious, there appeared to be a similar difference in the morphology of the insertion of the principal fibre bundles into the buccal and lingual aspects of the alveolar bone in the 19 to 21 year old premolar specimens. The general morphology of the bundle insertion on the buccal aspect of the mandibular right premolars was similar to that observed on the mesial surface of the interdental bone and the insertion pattern on the lingual similar to that on the distal surface.

Where the perforating fibre bundles terminated at a reversal line the generally close parallel arrangement of the fibres forming the bundles divided into divergent finger-like projections (fig.50 p.3.75).

The difference in the insertion morphology of the perforating fibre bundles and their finger-like termination was also observed in the 68 year old male premolar specimen and also the 46 year old male molar segment.

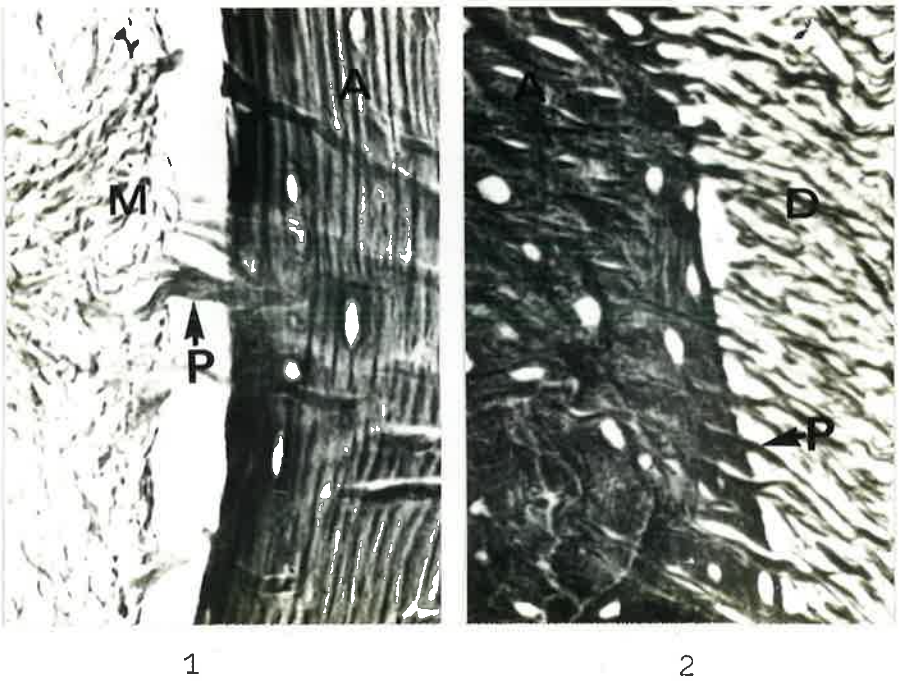


Fig.49. Greater thickness of the perforating fibre bundles (P) at their point of insertion into the alveolar bone (A) on the mesial aspect 1 (M) than on the distal surface 2 (D).
Areas 17 and 14 respectively; 19 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 400.

Fig.50. Divergent fingerlike projections of the perforating fibre bundles (P) where they terminate within the alveolar bone (A) at the innermost reversal line (R).
Area 17; 19 year old male; mesiodistal section.
Gordon and Sweet silver and Van Gieson.
X 225.

CHAPTER 4.

DISCUSSION.

Perforating (transalveolar) fibre bundles have been demonstrated in the molar periodontium of the mouse (Quigley, 1970; Cohn, 1970, 1972a), the marmoset (Cohn, 1972b) and Macaca mulatta (Cohn, 1973). The basic histological arrangement of the teeth and periodontium of these experimental animals is generally considered by these authors to be similar to that of man. The present investigation was commenced in December 1974 to examine the pattern and arrangement of perforating (Sharpey) fibre bundles in the posterior interdental and interradicular alveolar bone of the human mandible. Subsequently, Cohn (1975) has demonstrated these fibre bundles in the interdental alveolar bone and the labial and lingual alveolar wall of the maxillary central and lateral incisors, and also the canines. He reports that the fibre bundles are observed wherever lamellated bone without Haversian systems was present. Although he states that he had studied the alveolar bone of the maxillary and mandibular premolar and molar teeth, and had observed transalveolar (perforating) fibres in the interdental and interradicular crestal regions, he did not provide the necessary photographic evidence of fibres in these areas. He also states in his findings of the interradicular region, that, "because of technical difficulties, evidence for the periodontal continuity of these arches was lacking. It was therefore assumed that the ends of each arch were attached at approximately the same level on adjacent teeth". Unfortunately, Cohn does not state what these technical difficulties were, nor the basis for his assumption. The fact that this assumption was allowed in such an eminent journal is also surprising.

The findings of the present investigation provide new and more detailed information on the arrangement of the human "perforating" (Sharpey) fibre bundles not published in Cohn's (1975) study of the human "transalveolar" (Sharpey) fibre bundles. The Concise Oxford dictionary definition of "perforate" is "to penetrate through or into", which adequately describes the collagen fibre pattern in the alveolar bone determined in the present study. Contrary to the findings of Cohn in the human the present study clearly demonstrates that it is, in fact, only the fibres in the crestal third which completely traverse the alveolar bone and not in the more apical regions. Consequently, Cohn's use of the term "transalveolar" should be restricted to a description of those crestal third fibres which completely traverse or cross the crestal bone.

The present investigation demonstrates perforating fibre bundles traversing the interdental, interradicular, labial and buccal alveolar bone of the incisors, premolars, and molar crestal regions. These observations were consistently made in the fourteen specimens comprising the 11 to 21 year age group. However, in the two male specimens aged 46 and 68 years, complete traversal of the crestal alveolar bone by the fibre bundles could not be confirmed. Both these specimens demonstrate bone destruction estimated to be the original crestal third and evidence of chronic periodontal disease in sections stained with haematoxylin and eosin. The observation of numerous reversal lines in the alveolar bone also indicates that considerable bone remodelling has occurred. These two factors could have explained why the incomplete traversal of the remaining crestal alveolar bone by the perforating fibre in these specimens, was more typical of the fibre pattern in the middle regions of the alveolar wall.

The presence of perforating fibre bundles traversing the interdental and interradicular crests of alveolar bone can be explained by Sicher's 1959 concept. In this concept, Sicher states that an intermediate plexus in the transseptal (supra crestal) fibres is obvious during the eruption of human teeth. These fibres and their intermediate plexus then become incorporated within the crestal bone as anastomosing perforating fibres during the bony development of the interdental septa of young humans. Waugh (1904) describes this process of transseptal fibre incorporation while the tooth continues to erupt and the alveolar crest develops towards the oral mucosa. However, this concept of resulting perforating fibre anastomosis is purely speculative as no evidence of an intermediate plexus was observed in the present study. The perforating fibres observed in the labial and buccal bone supporting the mandibular incisors, premolars and first molar may also be incorporated into the forming lamellated bone in this area, as implied by Baume (1956) in his description of the tooth and its related bone as a developmental entity.

The greater degree of perforating fibre bundle penetration in the crestal third of the interdental and interradicular bone compared with the more apical areas can be explained by the number and pattern of reversal lines in these crestal regions which indicate that considerable bone deposition has occurred. As new bone is deposited on both sides of the crest around the existing perforating fibres, the length of these fibres enclosed in bone increases, as reported by Kraw and Enlow (1967).

Greater perforating fibre penetration and evidence of bone deposition in the crestal, middle and apical thirds of the mesial aspect of the interdental and interradicular bone was observed in all specimens compared with the distal side. These findings confirm those of Stein and Weinmann (1925) who postulated that the greater bone deposition observed on the mesial surface of the interdental bone was evidence of mesial drift. Evidence of bone resorption in the present study was only observed on the distal aspect of the interdental and interradicular bone. In the present study it was not known whether these resorption patterns reflected physiological drift, or may have occurred as a result of dental trauma incurred at the time of the motor vehicle accident since some of the victims survived for periods of up to 48 hours after the accident.

Oppenheim (1911) has shown that resorption can continue for a period of at least four days after force application. Accordingly, the trauma due to head injuries may have initiated some resorption in the period of time before death. In two of the six 19 year old premolar segments from the present study, resorption was only observed in the apical third. This finding suggests that physiological tooth movement may not be a simple bodily movement but occurs in part as a tipping movement. A tipping movement would involve resorption on the distal apical surface of the interdental alveolar bone and greater bone deposition on the mesial crestal surface similar to that observed in the present study.

The occlusal inclination of the perforating fibre bundles in the crestal and middle third of the interdental bone differed on mesial and distal surfaces in the 19 to 21 year old premolar mesiodistal sections. There was an increased fibre angulation on the mesial surface of the interdental bone. It is speculated that one reason to account for this fibre angulation might be the need to resist an increased occlusal force on the distal aspect of the tooth crown. A difference in the mesiodistal angulation was also confirmed in the examination of sections cut in the horizontal plane. Such a vector of force could possibly result from the individual's curve of Spee and the occlusal interdigitation. The contrasting angulation of the perforating fibres in the 68 year old premolar specimen may also have been related to these same factors. However, confirmation of the occlusal relationships was not possible as the individuals in this study had suffered maxillo-facial injuries which altered their original occlusal relationships but did not influence the perforating fibre bundle relationships in each segment of teeth examined.

The reduced number of perforating fibre bundles observed in the apical areas of the 19 to 21 year old premolar interdental bone suggested that functional force vectors occurring in these areas were not as great as in the crestal region. Cohn (1975) reported, in somewhat vague terms, that the vertical interface between the lamellae of the alveolar bone where the perforating fibre bundles overlapped, was constant in a particular alveolar septum but varied from the middle to each side of the alveolar wall in different specimens. He suggested that the mesiodistal location of the site of overlap within the alveolar bone represented a functional adaptation of these fibres to occlusal and perhaps muscular forces. In the present study, the anastomosis of the perforating fibres appeared to be unrelated to a particular interface and varied in position from the mesial to the distal aspect in the same crest.

Cohn (1975), stated that he had observed trans-alveolar fibres in the more apical portions of the inter-dental septum between the incisors, and the incisors and canines of both jaws. The present author did not observe perforating fibres completely traversing the interdental septum in the apical region. The fibres penetrated to the innermost reversal lines on both the mesial and distal aspect but were separated by a central region of trabecular bone and Haversian systems (fig.51 p.4.13). Although it was only possible to obtain one specimen in the mandibular incisor region for the present investigation, the findings in this specimen were nevertheless contrary to those of Cohn who did not list the number of specimens he examined, nor the age or sex of the subjects.

In 1975, Cohn described the greater degree of interweaving of the transalveolar fibres observed in the alveolar process of man compared with the mouse. In the present human findings, interweaving of some of the fibres was observed, but it was not a constant feature.

Cohn stated that transalveolar fibre bundles traversed only the crestal, middle and apical thirds of the vestibular and lingual alveolar walls of anterior teeth. In contrast, the present investigation demonstrated these fibres in both the anterior and posterior regions of the mandible. However, complete perforating fibre bundle traversal was only observed in the crestal third areas of the vestibular aspect.

In contrast to Cohn, the present author did not routinely observe the perforating (transalveolar) fibres penetrating the labial and buccal alveolar wall in the form of angled arches pointing occlusally as described by Cohn. However, perforating fibres in the form of angled arches were observed in the mandibular right first premolar region of one 21 year old specimen. The other fibre pattern which he described was noted in the incisor and the premolar region of the remaining 19 to 21 year old specimens. That is, they passed in a straight line approximately parallel to each other through the wall. The difference in the incisor region may again be related to the study of only one incisor segment. However, this does not account for the similar pattern seen in the four human premolar segments which were also examined histologically. The reason for this difference could not be explained, since Cohn did not state the age nor the region of the specimens where the two patterns were observed.

The sections in the present study were stained with Gordon and Sweet's silver counter-stained with Van Gieson in contrast to the modified Mallory stain used by Cohn (1975). The difference in staining technique would not, in the present author's opinion, account for the marked differences in the findings, since the perforating (transalveolar) fibres in both studies were identified by their morphological appearance.

In the present investigation, the existence of some intrinsic bone matrix fibres aligned perpendicular to the perforating (Sharpey) fibres in the crestal areas confirmed the findings of Weinmann and Sicher (1955). Weinmann and Sicher however, did not discuss the completely random orientation of the bone matrix fibres on the distal aspect of both the interdental and interradicular crests. Furthermore, they did not mention a change from a perpendicular orientation to the perforating fibres to one parallel to the long axis of the interdental and interradicular bone in the more apical areas as noted in the present study.

Cohn (1972a) described the transalveolar (perforating) fibre bundles of the mouse as traversing the interdental bone in an arc which curved occlusally towards the crest and then downwards to join with the fibres of the periodontal ligament of the adjacent tooth (fig.52 p.4.14). Dunstan (1975) has shown that the transalveolar (perforating) fibre bundles of the mouse have a noticeably different arrangement. He described the path of these bundles as being generally in a straight line, entering the interdental crest on the mesial aspect with an inclination towards the occlusal plane, and emerging on the distal surface at a more crestal level. Furthermore, Dunstan observed a difference in the arrangement of the fibre bundles on the mesial and distal surfaces of the interdental bone. On the distal aspect the fibres emerged from the bone and spread out in a fan-like arrangement (fig.52 p.4.14). Cohn (1972a, 1975) did not refer to any differences in the level of entrance and exit of the perforating fibre bundles nor any change in their morphology in either the mouse or man.

A comparable finding of a change in the general morphology of the emerging perforating fibres from mesial to distal was observed by the present author in the human material. The perforating fibre bundles formed a broad base as they emerged from the mesial surface of the interdental bone and then fanned outwards towards the cementum (fig.49 p.3.74). On the distal aspect, the fibre bundles retained their individuality and emerged from the bone surface without the broad base observed on the mesial surface. Also these fibre bundles did not tend to fan outwards to the same degree as they traversed the periodontal space. The present morphological findings are the mirror image of the findings by Dunstan (1975) in the mouse, where the fibres on the distal aspect of the interdental bone have a more definite fanshaped appearance. Mice molars have been shown to exhibit distal migration (Moss and Picton 1974). As human teeth tend to migrate mesially (Stein and Weinman 1925) the pattern of the perforating fibres emerging from the interdental and interradicular bone of the mouse and man have significant similarities. These patterns may be related to the different directions of approximal drift in man and mouse.

J. G. Edwards (1971) described a surgical technique for the prevention of relapse in extraction cases for teenage orthodontic patients. He found that by surgically excising the excess gingival tissue present after the teeth were approximated, opening of the extraction space was eliminated or diminished in all the cases treated. The present findings of perforating fibre continuity through the interdental and interradicular crests suggest that the simple surgical removal of the interdental gingivae between the approximated teeth may be insufficient. Based on the present author's findings, movement of one tooth in the mandibular area could effect the proximal teeth by the transmission of tension, in particular, and probably pressure. Thus, in the case of an approximated mandibular canine and second premolar the tension of the perforating crestal fibres on the distal of the mesially moved premolar and the mesial of the distally moved canine could cause the space to open.

The findings by the present author of perforating fibres completely traversing the labial and buccal alveolar bone in the crestal areas supporting the mandibular incisors, premolars, and molars has a direct relationship to other investigations of orthodontic rotational tooth relapse. In 1970, J. G. Edwards described a surgical procedure for the prevention of rotational tooth relapse. He derotated mandibular premolar teeth in teenage orthodontic patients using fixed appliances after first tattooing the attached gingivae surrounding these experimental teeth. Edwards stated that the tattoo marks deviated from their original position towards the direction of rotation of the teeth. The amount of movement of the tattoos corresponded to the degree of tooth rotation. After two months of mechanical retention, he surgically cut all the fibrous attachments surrounding the derotated teeth to a depth of 3mm below the alveolar crest. He observed that most of the tattoos returned to their original position within 20 to 40 hours after surgery. Edwards stated that the gingiva covering one of the orthodontically derotated teeth remained distorted and a deeper incision was required.

The present author suggests that not only are the supra-alveolar gingival fibres a causative factor in gingival distortion (J. G. Edwards, 1970) but that the distortion of the mucosa could also be caused by the tension of the periodontal ligament fibres being transmitted via the perforating fibres, observed traversing the buccal bone, through to the overlying periosteum of the buccal mucosa (fig. 53, p. 4.15).

The possible involvement of these perforating crestal fibres as a causative factor in rotational tooth relapse and the shallow depth of the present gingival slice-cut procedures may explain the rotational relapses described by Pinson and Strahn (1974) and Walsh (1975).

In contrast to the findings by Grant and Bernick (1972) who stated that the perforating (Sharpey) fibres of aged human alveolar bone did not stain with silver nitrate, the present investigation demonstrated these fibres, although they were less intensely stained than in the younger specimens. This difference may be due to the particular staining technique and counterstain used in the present study. The observation of noticeably thicker principal fibre bundles made by Grant and Bernick was not confirmed in the present study.

Further information would be provided to clarify the differences found in the present study and that reported by Cohn (1975) by a histological study of a greater number of young and older aged specimens. This study should include the horizontal and buccolingual planes to determine the effects of age on the morphology of the perforating fibres passing through the buccal and possibly the lingual cortical plates.

COHN EDWARDS

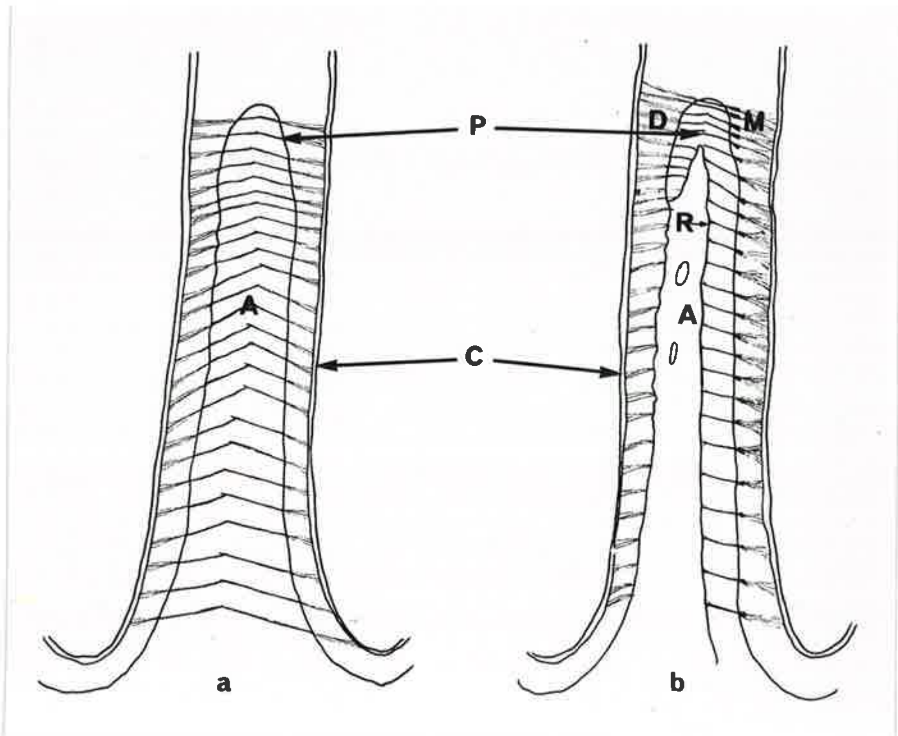
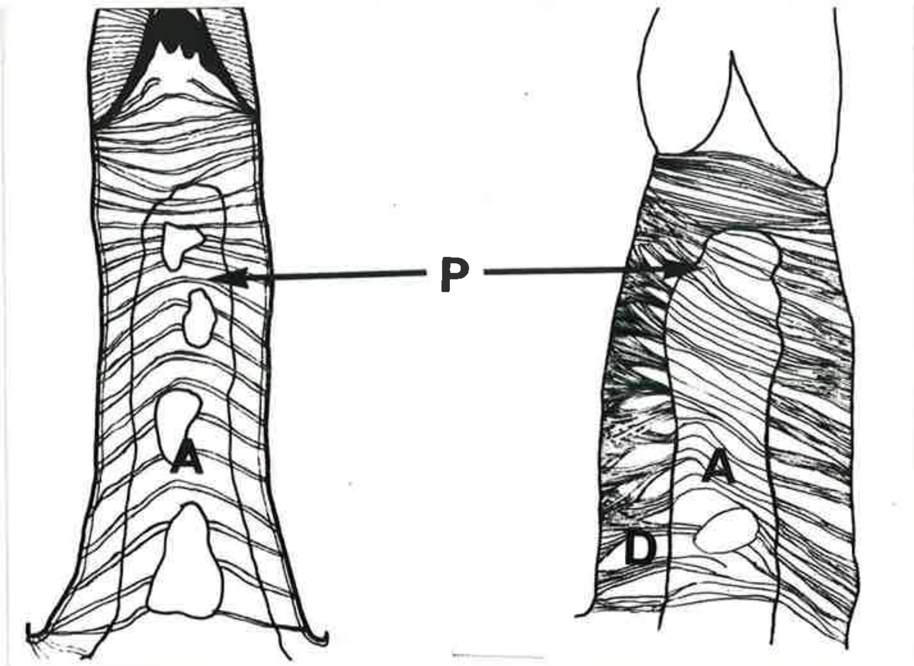


Fig. 51 Diagrammatic representation of the perforating fibre bundle pattern (P) in the alveolar process (A) of the human mandible as described by Cohn (1975), a; and the present findings, b. M, mesial; D, distal; C, cementum. Mandibular incisor area, mesiodistal section.

COHN DUNSTAN



(a) (b)

Fig. 52 Diagrammatic representation of the perforating fibre pattern of the mouse as described by Cohn 1972, (a); and Dunstan 1975, (b). A, alveolar bone; D, distal; P, perforating fibres.

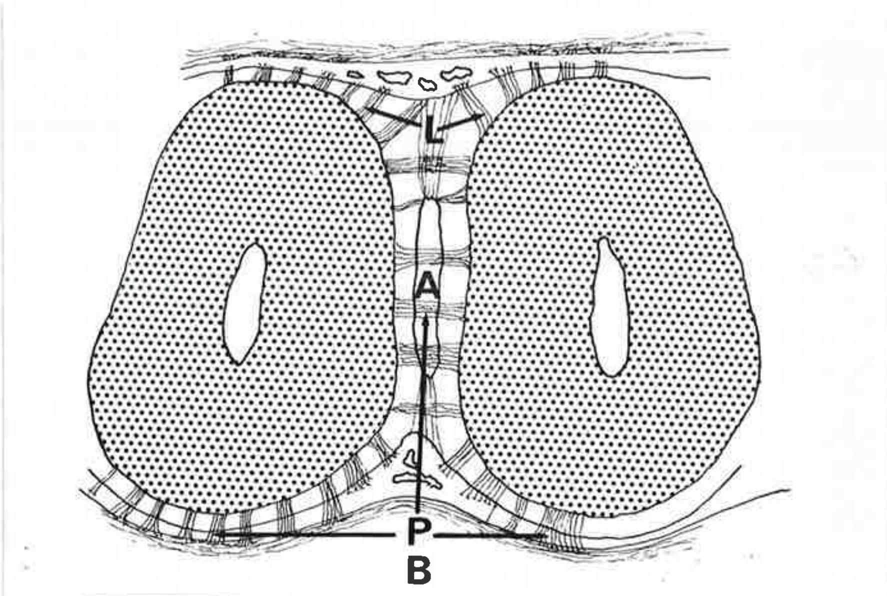


Fig. 53 Findings of the present investigation summarised as a diagrammatic representation of the perforating fibre bundle pattern (P) in the alveolar bone (A) of the mandibular premolar area.
L, periodontal ligament; B, buccal.
Crestal third, 19-21 year old specimens, horizontal plane.

CHAPTER 5.

CONCLUSIONS.

1) Gordon and Sweet's silver stain counterstained with Van Gieson proved to be the most suitable method for the demonstration and black and white photographic recording of perforating fibre bundles in the human mandible.

2) Perforating fibre bundles have been demonstrated passing through the crestal third of the interdental, interradicular, buccal and labial bone in the mandibular molar, premolar, and incisor region of 19-21 males and females. These fibre bundles were also demonstrated in the crestal third of the interdental bone between the mandibular right first and second premolars obtained from an 11 year old female. However, no interradicular bone was available in this specimen.

3) A theory has been advanced to account for the presence of the perforating fibre bundles and their arrangement within the crestal regions of bone.

4) A difference in the degree of perforating fibre bundle penetration and angulation has been observed between the mesial and distal aspects of the inter-dental and interradicular bone. A morphological difference in the principal collagen fibre bundles at their site of insertion into the alveolar bone on the mesial, distal, buccal (labial), and lingual surfaces has been described and discussed.

5) Specific differences have been demonstrated and

discussed between the findings of Cohn (1975) and those of the present investigation.

6) Age changes in the perforating fibre bundles of the human mandible and their pattern have been described.

7) The role of perforating fibre bundles which completely traverse the interdental, interradicular, buccal, and labial alveolar bone in the human mandible and their implications in the management of orthodontic relapse has been considered.

CHAPTER 6.

APPENDIX I.

PREPARATION OF SPECIMENS.

I. 1 The buffered neutral formalin solution was made to the following formula:

37 - 40 per cent formalin	100.0 cm^3
distilled water	900.0 cm^3
sodium dihydrogen phosphate	4.0 g
disodium hydrogen orthophosphate	6.5 g

I. 2 The tissues were processed at 37 C using the Double Embedding Technique. After neutralization in 5 per cent sodium sulphate, the decalcified sections were treated according to the following stages:

(1) 70 per cent ethanol .... overnight

(2) 80 per cent ethanol .... one hour

(3) 90 per cent ethanol .... one hour

(4) 95 per cent ethanol .... one hour

(5) absolute ethanol .... one hour

(6) absolute ethanol .... one hour

(7) absolute ethanol .... one hour

(8) one-part absolute ethanol .. one hour
one-part methyl salicylate . one hour

(9) methyl salicylate plus
0.5 per cent celloidin .... two days

(10) methyl salicylate plus
1 per cent celloidin .... two days

I. 3 The Paraffin embedding method:

(1) 2/3 methyl salicylate -
1/3 paraffin wax ...... one hour

(2) 1/2 methyl salicylate -
1/2 paraffin wax ...... one hour

(3) 1/3 methyl salicylate -
2/3 paraffin wax ...... one hour

(4) paraffin wax first change .. two hours

(5) paraffin wax second change . two hours

(6) paraffin wax third change .. overnight.

The specimens were then vacuumed in clean paraffin wax at 56 C for fifteen minutes prior to blocking.

APPENDIX II.

PREPARATION OF GLASS SLIDES.

II. 1 Albuminized slides.

Clean glass slides were washed in detergent, then in tap water and finally rinsed in distilled water. The slides were then dipped into a solution made from a mixture of one egg white and 400.0 cm^3 of distilled water. The albuminized slides were then placed in a draining rack and dried in an oven at 37 C.

II. 2 Gelatinized slides.

Clean glass slides were soaked in a dichromate cleaning solution, washed in running tap water for 6 hours and then thoroughly rinsed in distilled water. The slides were allowed to drain for 2-3 seconds and then dipped into the subbing solution which was prepared as follows: 5.0 g of U.S.P. gelatin were dissolved in 100.0 cm^3 of warm distilled water; then 0.5 g of chrome alum (chromium potassium sulphate) was added. After the solution had cooled, it was filtered through Whatman 1 filter paper. After the slides had been dipped in this subbing solution, they were allowed to dry (vertically) in a dust free atmosphere. It is important that the slides are not allowed to dry between the cleaning and coating procedures, as the subbing solution will not properly rewet the dried slides.

APPENDIX III.

STAINING TECHNIQUES.

Routine Mayer's Haematoxylin and Eosin Stain

(a) Preparation of Mayer's Haematoxylin:

haematoxylin crystals	....	1.0 g
distilled water	....	1000.0 cm^3
sodium iodate	....	0.2 g
ammonium or potassium alum	....	50.0 g
citric acid	....	1.0 g
chloral hydrate	....	50.0 g

Dissolve the alum in water, without heat; add the haematoxylin in this solution. Then add the sodium iodate, citric acid and the chloral hydrate, shake until all components are in complete solution. The final colour of the stain is reddish-violet.

(b) Preparation of 1 per cent Stock Alcoholic Eosin:

eosin Y, water soluble	....	1.0 g
distilled water	....	20.0 cm^3
dissolve and add alcohol, 95 per cent		80.0 cm^3

(c) Working Eosin Solution:

eosin stock solution	....	one part
alcohol, 80 per cent	....	three parts

Just before use add 0.5 cm^3 of glacial acetic acid to each 100.0 cm^3 of stain and stir.

(d) Method:

(1) Deparaffinise and hydrate the sections to water.

(2) Immerse in Mayer's haematoxylin for 15 minutes.

(3) Wash in running tap water for 20 minutes.

(4) Counterstain with eosin from 15 seconds to 2 minutes depending on the age of the eosin, and the depth of the counterstain desired. For even staining results, the slides were dipped several times before allowing them to set in the eosin for the desired time.

(5) Dehydrate in 95 per cent and absolute alcohols, with 2 changes of 2 minutes each or until excess eosin is removed.

(6) Clear in xylol, 2 changes of 2 minutes each.

(7) Mount.

(e) Staining Reactions:

Amyloid	-	pink to red
Nuclei	-	blue
Background	-	unstained
Elastica	-	pink to red.

Heidenhain's Aniline Blue Method

Preparation of solutions:

(a) 1 per cent Azocarmine B solution

azocarmine B	1.0 g
distilled water	100.0 cm^3

Bring to boil, filter at 56 C, cool and add 1.0 cm^3 glacial acetic acid. The solution must be kept in a refrigerator and filtered before use.

(b) 1 per cent Aniline-Alcohol Solution

aniline 1 per cent	1.0 cm^3
alcohol 95 per cent	100.0 cm^3

(c) 5 per cent Phosphotungstic Acid Solution

phosphotungstic acid	5.0 g
distilled water	100.0 cm^3

(d) Aniline Blue Solution

aniline blue, water soluble	0.5 g
orange G	2.0 g
distilled water	300.0 cm^3
glacial acetic acid	8.0 cm^3

Method:

(1) deparaffinize and hydrate to distilled water

(2) place in pre-heated azocarmine B solution at 56 C for 15 minutes

(3) rinse in distilled water

(4) differentiate in aniline alcohol solution until cytoplasm and connective tissue are pale pink and the nuclei stand out sharply. Control differentiation by rinsing in 1 per cent acetic acid in 95 per cent alcohol

(5) mordant in phosphotungstic acid for 15 minutes

(6) rinse in distilled water

(7) aniline blue solution for 15 minutes

(8) rinse in distilled water

(9) dehydrate in 95 per cent alcohol, absolute alcohol and clear in xylene, two changes, two minutes each

(10) mount.

Staining Reactions

chromatin, osteocytes, neuroglia	-	red
collagen, reticulum	-	blue
muscle	-	red to yellow
osteoid material in decalcified sections	-	blue

Mallory's Phosphotungstic Acid Haematoxylin (P.T.A.H.)

Preparation of solution:

haematoxylin	1.0 g
phosphotungstic acid	20.0 g
distilled water	100.0 cm^3

Dissolve the haematoxylin and phosphotungstic acid separately in distilled water, using gentle heat. When cool, combine the solutions, and make up to one litre.

Ripening

This is accomplished immediately by adding 0.177 g of potassium permanganate, or by exposing to light and warmth for 5 - 6 weeks.

Method

Paraffin embedded material not fixed in Zenker should be immersed in 3 per cent potassium dichromate for 12 - 24 hours and then well washed in running water.

(1) After post chroming bring the sections to water.

(2) Transfer to Lugol's iodine for 15 minutes.

(3) Decolourize in 95 per cent alcohol for 1 hour.

(4) Wash in distilled water.

(5) Place in 0.25 per cent aqueous potassium permanganate for 5 minutes.

(6) Wash in water for 2 minutes.

(7) Rinse in distilled water.

(8) Place in 5 per cent oxalic acid for 10 minutes.

(9) Rinse in distilled water.

(10) Wash in water for 5 minutes and rinse in distilled water.

(11) Stain in P.T.A.H. for 12-24 hours.

(12) Dehydrate through 95 per cent and absolute alcohol. This must be done rapidly as alcohol removes the red staining.

(13) Clear in xylol and mount in D.P.X.

Staining reactions

nuclei, fibrin, neuroglia, fibroglia	- blue
collagen, reticulin and ground substance of bone	- yellow to brick red

Modified Mallory Stain (Humason 1967)

Preparation of solutions

Mallory I:

acid fuchsin, C.I. 42685	1.0 g
distilled water	100.0 cm^3

Phosphomolybdic acid:

phosphomolybdic acid	1.0 g
distilled water	100.0 cm^3

Mallory II:

aniline blue, WS, C.I. 42780	0.5 g
orange G, C.I. 16230	2.0 g

distilled water 100.0 cm^3

Method

(1) Deparaffinize and hydrate slides to water; remove $HgCl_2$. If $HgCl_2$ is absent from fixative, mordant in saturated aqueous $HgCl_2$, plus 5% glacial acetic acid: 10 minutes. Wash, treat with Lugol's and sodium thiosulfate, wash and rinse in distilled water.

(2) Stain in Mallory I: 15 seconds.

(3) Rinse in distilled water: 10 or more seconds, to differentiate reds.

(4) Treat with phosphomolybdic acid: 1-5 minutes.

(5) Rinse briefly in distilled water.

(6) Stain in Mallory II: 2 minutes.

(7) Rinse in distilled water.

(8) Differentiate aniline blue in 90% ethyl alcohol.

(9) Dehydrate in absolute alcohol, clear and mount.

Staining reaction:

Nuclei - red

Collagen - dark blue

Mucus, connective tissue and hyaline substance - blue

Bone matrix - red.

The Gordon and Sweet Silver Impregnation Method for Reticulin Fibres.

Preparation of solutions:

(a) Silver Solution:

To 5.0 cm^3 of 10.2 per cent aqueous silver nitrate add strong ammonia (.880) drop by drop until the precipitate, which is first formed, is just dissolved. Add 5.0 cm^3 of 3.1 per cent sodium hydroxide. Add strong ammonia (0.880) drop by drop until the resulting precipitate is just dissolved (the solution should not be completely clear). Make up the solution to 50.0 cm^3 with fresh glass distilled water

(b) Acidified Potassium Permanganate

0.5 per cent potassium permanganate	95.0 cm^3
3.0 per cent sulphuric acid	5.0 cm^3

Method:

(1) bring the sections to water

(2) oxidize in acidified potassium permanganate for 1 - 5 minutes

(3) wash in water

(4) bleach in 1 per cent oxalic acid for 3 - 5 minutes

(5) rinse in glass distilled water

(6) wash in tap water and then in 2 - 3 changes of glass distilled water

(7) mordant in 2.5 per cent iron alum for 10 minutes

(8) wash in 2 - 3 changes of glass distilled water

(9) treat with silver solution until section is transparent (30 seconds)

(10) wash well in several changes of glass distilled water

(11) reduce in 10 per cent aqueous formalin

(12) wash in tap water and then in glass distilled water

(13) tone in 0.2 per cent gold chloride for 10 - 15 minutes

(14) rinse in glass distilled water

(15) fix in 5 per cent sodium thiosulphate for 5 minutes

(16) wash in water for 1 - 2 minutes.

These stained sections were then counter-stained for 1½ minutes in Van Gieson, made up as follows:

saturated aqueous solution of picric acid (approximately 1 per cent)	100.0 cm^3
1 per cent acid fuchsin	10.0 cm^3

(17) rinse in water rapidly

(18) dehydrate, clear and mount.

Staining Reaction

reticulin fibres only	-	black
collagen fibres	-	brown to mauve.

CHAPTER 7.

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