COLLAGEN FIBRE BUNDLES IN THE MOLAR ALVEOLAR PROCESS OF THE MOUSE MANDIBLE.

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SUMMARY.

The purpose of the investigation was to establish a three-dimensional concept of the anatomy and patterns of arrangement of the principal collagen fibre bundles in the alveolar process of the molar region of the mouse mandible. The mouse was examined because it was one of the models used by previous investigators in similar studies, and because the mouse mandible provided an isolated group of molars with simple root forms for study purposes.

Comparisons have been made between the findings of this study and the investigations conducted by Cohn (1966, 1972) and by Quigley (1970). These authors concluded that the principal collagen fibre bundles of the mouse and some other animals, coursed through the interdental alveolar bone to extend between adjacent teeth. These fibre bundles were called transalveolar collagen fibre bundles (Cohn 1966, 1972).

In the present investigation, specimens were sectioned at eight microns in the horizontal, the mesiodistal and the buccolingual planes. Histologically prepared sections were examined with the light microscope after staining with the silver method of Gordon and Sweet (Appendix p.6.7). This technique of demonstrating collagen fibre bundles was selected after evaluation of a variety of collagen staining methods.

This investigation showed that in the molar region of the mouse mandible, many of the principal collagen fibre bundles passed completely through the

interdental alveolus, and were continuous with principal fibres of the opposite side of the alveolus.

The patterns of arrangement were best demonstrated in sections cut in the mesiodistal plane through the mid-sagittal region of the teeth. The presence of trans-alveolar fibres was confirmed in horizontal sections with a frequency which was consistent with the patterns of arrangement seen in the mesiodistal plane of section.

Examination of sections cut in the buccolingual plane revealed that some principal collagen fibre bundles passed through the buccal and lingual plates of alveolar bone, to be inserted into the mucoperiosteum.

A localised variation in the morphological pattern of the principal collagen fibre bundles was observed in the interdental regions. This finding may have significance in being related to the distal migration of molar teeth in the mouse.

The results of previous investigations of the morphology and patterns of arrangement of periodontal collagen fibre bundles in animals and man have been reviewed and compared with the findings of this study. As a consequence, a modified concept of the anatomy and pattern of arrangement of the principal collagen fibre bundles in the mouse periodontium has emerged from the present study.

SIGNED STATEMENT.

This project report is submitted as a partial requirement for the Degree of Master of Dental Surgery in the University of Adelaide.

The report contains no material which has been accepted for the award of any other 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 where due reference is made in the text of the report.

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INTRODUCTION.

There is some lack of agreement regarding the function of the periodontal collagen fibre bundles in the support and physiological movements of the teeth of animals and of humans. Some of this disagreement has occurred because conclusions have been based upon empirical investigations of the teeth and their supporting tissues.

For example, Dreyer (1965) concluded that the forces which produced migration of the molar teeth of rats were resultants of functional forces. He further contended that the magnitude and direction of these resultant forces were determined by the angulation of the roots of the teeth to their occlusal surfaces. Moss and Picton (1967, 1970, 1974) however produced experimental mesial and distal drift of the molar teeth of monkeys and their conclusions were in contradiction to those of Dreyer.

This dependance on empiricism in the study of the function of the periodontium of animals is partly due to inadequate knowledge of the normal anatomy and arrangement of the collagen fibre bundles of the periodontium.

Therefore, there is a need to ascertain

(a) the depth and direction of penetration of the alveolar bone by the principal collagen fibre bundles,

(b) the possible extension of these bundles to adjacent teeth through the alveolar bone, and

(c) variations in anatomy and patterns of collagen fibre bundle arrangement which may be associated with the physiological movements of function and migration of teeth.

During research work in the Department of Dental Health of The University of Adelaide, certain patterns of arrangement and anatomical variations of periodontal collagen fibre bundles have been observed. These observations were considered to be significant, and formed the basis of the investigation carried out by the present author.

The observations were also of interest when compared with the findings of Cohn (1966, 1972) and Quigley (1970) who concluded that the principal collagen fibre bundles of mice and of marmosets passed completely through the interdental alveolus, directly connecting the roots of adjacent teeth.

AIMS OF THE INVESTIGATION.

To establish at the histological level, a three-dimensional concept of the anatomy and patterns of arrangement of the collagen fibre bundles in the molar alveolar process of the mouse mandible. To use this knowledge in order to

(i) reassess the findings of previous investigators who have stated that there is direct collagen fibre bundle connection through the alveolar bone between adjacent members of groups of teeth in mice and

(ii) achieve a better understanding of the role of collagen fibre bundles in the movements and the support of the molar teeth of mice.

CHAPTER 1.

REVIEW OF LITERATURE.

A. TRANSALVEOLAR COLLAGEN FIBRE BUNDLES.

It has been established that some principal collagen fibre bundles are anchored through the full thickness of the cementum (Gianelly and Goldman 1971, Glickman 1972). Until recently however, there has been little progress in the determination of the depth to which collagen fibres of the ligament penetrate the alveolar bone. Recent investigations carried out by Cohn (1966, 1972) and Quigley (1970) have introduced a new concept of the anatomy of the collagen fibre bundles in the periodontium.

In 1970, Quigley studied the molar teeth of white mice and hamsters by sectioning the three teeth in the axial mesiodistal plane. He used a method developed by Quigley and Zwarych (1963) who claimed that prior to fixation, a 1 per cent aqueous solution of the enzyme collagenase selectively digested the collagen. Quigley contended that he was able to remove previously calcified collagen, so that only collagen which had not been calcified during life remained to be identified by staining. He illustrated his findings by comparing adjacent experimental and control sections. Quigley claimed that the control showed slight signs of a fibre pattern in the interdental alveolar bone whereas the experimental sections demonstrated a very clear pattern of collagenous fibre bundles within the alveolar bone.

Quigley (1970) also claimed that it was the perforating (Sharpey's) fibres which were not digested by the enzyme, and that they passed completely through the alveolar septum as they extended from tooth to tooth. He described the fibre bundles as being spiral in nature, and conjectured that this spiral or wavy morphology provided a margin of safety against rupture at the alveolar margin when maximum pull was exerted on the fibres of the periodontal ligament.

Cohn (1972) studied the periodontium of the molar teeth of adult mice and marmosets. He stained sections with Van Gieson's stain for collagen and with the colloidal iron method of Bohacek and Gupta (1968).

Cohn concluded that termination of Sharpey's fibres did not occur immediately after penetration of the alveolar surface, but continued without interruption through the entire thickness of the surrounding alveolus. He also stated that in the interdental region, the fibres were continuous with corresponding bundles of the adjacent tooth and that on the mucosal surfaces they blended with overlying collagenous fibres. Interdentally, the fibres formed a series of arches placed at intervals one above the other, with the highest curvature of each arch located within the interdental bone. Cohn preferred the term "transalveolar" to describe these fibres. He related these findings to an earlier investigation (Cohn, 1966) in which lack of function was used to produce disuse atrophy in the molar periodontium of mice. In areas where interdental bone was resorbed, transseptal fibres seemed to appear. Cohn claimed that these were not new fibres, but trans-alveolar fibres which had remained after resorption of the crestal bone.

More recently Cohn (1975) examined dental specimens from preserved adult cadavers and from adult humans immediately after death. After histological processing and immersion in a commercial fabric softener, the blocks were sectioned at 10 microns. He found that many of the cementoalveolar fibres traversed the entire thickness of the alveolus where they became transalveolar fibres. The transalveolar fibres did not penetrate or traverse Haversian systems but ended at the periphery of each system or deviated around them. Cohn claimed that the fibres joined the roots of adjacent teeth, and the root surfaces of the interradicular region. He further contended that in buccolingual sections fibres were seen to pass through the alveolar process to become attached to the periosteum of the alveolar process.

Cohn considered that his findings lent more detail to the explanation of tooth support and showed a previously unrecognised interdependence of adjacent teeth. He also concluded that the orientation and distribution of transalveolar fibres represented a functional adaptation to occlusal forces.

The foregoing is recent work which is most relevant to this study. The following section relates to other less recent concepts.

B. THE COLLAGENOUS TISSUES OF THE PERIODONTIUM. CONCEPTS OF STRUCTURE AND FUNCTION DEVELOPED FROM INVESTIGATIONS OF MAN AND ANIMALS.

Collagen fibre bundles of the periodontal ligament.

In 1848 Tomes discussed the surfaces of the dental alveoli which he described as being "everywhere closely invested with periosteum", which on the free surface was "clothed" with mucous membrane continuous with that of the mouth. He stated that in an histological section including alveolus and tooth, there would be seen the periosteum of the free surface, the periosteum of the inner surface of the alveolus, and the periosteum of the tooth. He described the latter as the dental periosteum or peridental membrane. It seems that he regarded the intervening soft tissue between tooth and bone, as being composed of two membranes although he made no mention of their fusion.

In describing the periodontal ligament, Black (1886) referred to it as the alveolar dental membrane and used the descriptive terms "principal fibres" and "indifferent tissue". He implied that this membrane was more than a periosteum.

The terms alveolodental periosteum and periodontal membrane were used synonymously by Tomes and Tomes (1896) who asserted that the soft tissues investing the root and lining the socket were one and the same membrane. It was also their opinion that there was a marked histological difference between fibre bundles lying next to the alveolar bone and those adjacent to the cementum. Furthermore, they stated that there was no discontinuity as the bundles passed from alveolus to cementum. Tomes and Tomes referred to the embedding of the fibres in the calcified alveolar tissues as being very like the fibres embedded in other bone. They therefore considered that the alveolar fibres should be called Sharpey's fibres which were first described by Sharpey (1856) (cited by Quigley, 1970) as connective tissue fibre bundles which penetrated the circumferential lamellae on the surface of bone.

Noyes (1897) further defined the tissue of the alveolodental periosteum by attributing to it the functions of location, nutrition, sensation, support and provision of formative elements for the alveolus and cementum.

Waugh (1904) stated that the principal collagen fibres of the periodontal ligament were essentially parallel with one another except where they deviated to accommodate vessels and nerves. He further contended that the principal fibres passed directly from the alveolus to cementum. Waugh also believed that in young people, a large part of the alveolar septum remained uncalcified prior to maturity, and the fibres passed directly across the septum to the neighbouring teeth. With increasing age, the fibres were thought to become surrounded by bone and incorporated in the structure of the septum as it developed occlusally.

Recent concepts of the anatomy of the periodontium were reviewed by Gianelly and Goldman (1971). They referred to the cementum, periodontal ligament and alveolar bone as constituting the attachment apparatus. The largest collagen fibres of the ligament were called principal fibres and these were arranged in four relatively distinct bundle groups :

1. an alveolar crest group which extended from the coronal area of the tooth to the alveolar crest,
2. a horizontal group which travelled perpendicularly from the tooth to the alveolar bone,
3. the oblique group in which the fibres extended from the tooth to a more crestal location on the alveolar bone, and
4. an apical group which radiated from the tooth to the bone.

The most prevalent group was the oblique group which constituted almost two-thirds of the fibres. The structure of the individual groups was continually modified to meet changing functional requirements.

In addition to the four main groups of principal fibre bundles in man, Glickman (1972) described other well-defined bundles. These bundles intermingled at right angles or splayed around and between regularly arranged fibre bundles. He also described less regularly arranged collagen fibre bundles in the interstitial connective tissue between the principal fibre groups.

Glickman (1972) stated that the principal fibres of the periodontal ligament were embedded for a considerable distance into the alveolar bone, where they were called Sharpey's fibres. Some Sharpey's fibres were described as being completely calcified, but most contained an uncalcified central core with a calcified outer layer. Glickman found a direct relationship between the length of Sharpey's fibres and the depth of bundle bone. Bundle bone is the name given to the bone adjacent to the periodontal ligament because of its content of Sharpey's fibres (or bundles).

Melcher and Eastoe (1969) referred to the principal fibres of the periodontal ligament as "those collagen fibre bundles which sometimes appeared to extend between cementum and alveolar bone and were embedded at both ends in hard tissue". The embedded parts were termed Sharpey's fibres. In addition to the groups of principal fibres already discussed, Melcher and Eastoe described the radial arrangement of fibre bundles around the crests of the interradicular bony septa of multi-rooted teeth.

Gianelly and Goldman (1971) stated that the density and direction of the collagen fibre bundles of a functioning tooth were modified by the intensity and direction of the forces of occlusion. They noted that the ligament was wider in a functioning tooth than a non-functioning tooth. In addition, the ligament of a non-functioning tooth underwent regressive changes and exhibited a poorly organised and poorly developed structure consisting of loose connective tissue almost devoid of fibre bundles.

In addition to the various groups of fibres, a specific arrangement or plexus of collagen fibres was thought to exist within the periodontal ligament. Sicher (1923, 1942) studied the erupting molars of guinea pigs and the incisors of rats. He described an "intermediate plexus" as a mechanism which allowed the rearrangement of the periodontal fibres. He further suggested that this mechanism might explain why there were no demonstrable ruptures of the ligament fibres following the application of force of a magnitude which could have been expected to cause such fractures. According to Sicher, the bundles did tear. However, he believed that the effect was in the intermediate zone where an unsplicing of the bundles occurred which could escape microscopic observation.

The existence of an intermediate plexus has been questioned by other investigators (Eccles 1959, Trott 1962, Zwarych and Quigley 1965) who believed that the "plexus" was an histological artefact. They claimed that some of the fibre bundles followed a tortuous path, particularly in the centre of the ligament. Therefore, they considered that there was likely to be more severance of these bundles, producing the appearance of an intermediate plexus. The plexus appeared to be primarily associated with tooth eruption.

Sicher (1966) described an intermediate plexus in human teeth during the first and fastest stage of eruption before the teeth occluded. Kraw and Enlow (1967) concluded that the important concept was the need for an intermediate plexus and not the exact location of this adjustment or linkage zone. They considered that a linkage zone could exist in a number of discreet areas or that it could be diffusely located. These authors concluded that it might be a simplistic approach to consider only a middle zone as "the" linkage region. However, Gianelly and Goldman (1971) believed that an adjustment zone existed, but they preferred to call it the ligament plexus.

Shackleford (1971a, 1971b) used the scanning electron microscope to study the periodontiums of the rat, dog, armadillo and man. He observed that individual collagen fibre bundles of 130 to 150 nanometres in diameter coursed in all directions throughout the area between bone and cementum, without particular orientation. He also showed that the fibres anastomosed with the principal collagen fibre bundles to form a continuous fibrous matrix. Shackleford defined these fibres as indifferent fibres, and the matrix which they formed as the indifferent fibre plexus. The density of the fibre plexus was found to be reduced immediately adjacent to alveolar bone and cementum where the principal fibres inserted into the mineralised matrix. Shackleford found that, in general, the alveolodental orientation of the intermediate fibres increased in direct proportion to their size. This tendency continued until diameters of the highly orientated principal fibres were reached. Therefore, the only truly indifferent fibres were the smallest ones, which made up a large proportion of the periodontal mass.

Shackleford (1971a, 1971b) believed that the indifferent fibre plexus played an important part in the formation of a fibrous continuum. This continuum permitted the distribution of a given force over a wider area than would be possible, if only the principal fibres were involved. It was Shackleford's opinion that the entire periodontium would be involved in resistance to a force of any direction or magnitude.

Frank, Lindemann and Vedrine (1958) sectioned small blocks of human alveolar bone at a thickness of 40 nanometres :s and studied decalcified and undecalcified sections with the electron microscope. They described two methods in which the collagen fibres of the periodontal ligament entered the alveolar bone. The first method was in the form of Sharpey's fibres which were usually unmineralized. In the second method the collagen fibres entered the bone in a haphazard manner and contributed to the collagen matrix of the bone where they were impregnated with bone salts. A less mineralized zone of the alveolar bone adjacent to the periodontal membrane and containing Sharpey's fibres, was called the preosseous area.

Eccles (1959) studied the developing principal fibres of the molar teeth of the albino rat. The ages of the specimens used were from 10 to 37 days, which included the period from the beginning to the completion of root formation of the first molar. This selection of specimens enabled Eccles to examine the stages of collagen fibre development in different regions. His investigations supported the theory that function played an important part in stimulating development and orientation of the fibre bundles of the periodontal membrane. He did not find a well-defined permanent intermediate plexus in the fully erupted rat molar. However, he did demonstrate the existence of a plexus during the period of active eruption when adaptation took place between the fibre bundles attached to the tooth and those attached to bone.

Supraalveolar collagen fibre bundles.

Additional tooth stabilisation was considered by Gianelly and Goldman (1971) to be derived from the supraalveolar connective tissue fibres which were not regarded as components of the periodontal ligament proper. These fibres were classified by Gianelly and Goldman (1971) as follows :

1. dentogingival fibres which passed coronally from the supraalveolar cementum into the gingival tissue,
2. dentoperiosteal fibres which emerged from the cementum in the same area as the dentogingival fibres,
3. horizontal fibres which passed transversly

from the cementum towards the gingival epithelium,

4. the circular fibre system which was part of the free gingiva and circled the tooth in a ring-like fashion, and
5. transseptal fibres which extended over the supraalveolar septum to the adjacent tooth.

Two additional fibre groups in the gingival tissues have been described by Gianelly and Goldman (1971). One group extended from the labial to the lingual papilla and followed the line of the col. The other group was vertically directed towards the mucogingival junction.

Glenwright (1970) carried out an histological investigation of the gingival collagen fibres in the Rhesus monkey. The gum overlying unerupted teeth was found to be rich in fibres lying in the mesiodistal direction. These fibres appeared to persist throughout eruption into the mature state and Glenwright described them as "longitudinal gingival fibres". He considered these fibres to be distinct from the circular fibres and functionally important in the maintenance of arch stability.

CHAPTER 2.

MATERIALS AND METHODS.

A. ANIMALS USED AND THE PREPARATION OF SPECIMENS:

Sixty male albino mice were used in this investigation. Forty of these were twelve weeks old, and twenty were six weeks old. The animals were obtained from the Waite Agricultural Research Institute. Their weight varied from 21.5 to 25.6 grammes and the average weight was 23.2 grammes.

The animals were killed by ether inhalation. The mandibles were immediately removed, hemisectioned and immersed in fixative. After fixation, excess soft tissue was removed prior to decalcification.

An initial group of specimens used for bucolingual and transverse sectioning was unsatisfactory because soft tissue on the buccal and lingual sides of the specimens had been removed. With later specimens, care was taken not to remove or damage this soft tissue.

The identity of the six week old and the twelve week old specimens was maintained throughout the processing, blocking, sectioning and staining procedures. It was found that the collagen fibre bundle patterns were better defined in the specimens from the twelve week old animals. Therefore, the findings illustrated and discussed were all derived from sections of the specimens from twelve week old animals.

B. HISTOLOGICAL PROCEDURES AND STAINING TECHNIQUES:

The specimens were fixed in ten per cent buffered neutral formalin (Appendix p.6.1) for forty-eight hours. After fixation the specimens were decalcified by the formic acid-sodium citrate method (Appendix p.6.1), the completion of which was determined by radiographic examination. Following decalcification, the specimens were neutralised and then washed in gently running water for twenty-four hours.

The use of magnifying goggles reduced the manipulation difficulties experienced during the mandibular dissection and specimen blocking stages.

A single-edged razor blade was used for gross sectioning of the specimens to simplify their orientation in the moulds prior to the blocking procedure. This was done following neutralisation, because to do so after impregnation was difficult and also involved the remelting of the solidified paraffin in the specimen prior to blocking.

Those specimens which were to be sectioned in the axial mesiodistal plane of the molar teeth, were prepared in the following way. The specimens were severed with a single-edged razor blade in the axial mesiodistal plane at the lingual surface of the molar teeth. This provided a flat surface which facilitated the orientation of the specimens in the metal moulds prior to blocking.

The specimens to be sectioned in the axial buccolingual plane were severed in the axial buccolingual plane at the mesial surfaces of the first molar teeth. After impregnation the specimens were visually orientated in the metal moulds such that sections cut from the blocks would be in the required plane.

The specimens to be sectioned in the horizontal plane were positioned in the moulds so that the first sections would be from the coronal region. This placement was simplified by the prior removal, at the level of the molar occlusal plane, of the incisor tooth and the ramus.

The specimens were prepared for histological sectioning by the double embedding method of Peterfi (Appendix p.6.2), followed by blocking in paraffin, using the "Tissue Tek II Tissue Embedding Centre".

The specimens were sectioned at eight micrometres and every fifth slide was stained with the silver method of Gordon and Sweet (Appendix p.6.7). The light microscope was then used to select the stained sections which best demonstrated the collagen fibre bundles. Sections adjacent to those selected were then also stained with the silver method of Gordon and Sweet (Appendix p.6.7). This was considered to be the most effective method of locating sections which would be useful in this study.

The silver impregnation method of Gordon and Sweet (Appendix p.6.7) was used in this investigation. Prior to this selection, the following histological staining techniques (Appendix p.6.4 to p.6.13) were evaluated.

1. The colloidal iron method of Bohacek and Gupta (Appendix p.6.4). This technique usually demonstrated the collagen fibre

bundle patterns but did not provide sufficient contrast for photographic recording.

2. The one step trichrome method of Gomori (Appendix p.6.5). The staining of cells and blood vessels dominated and reduced the clarity of the collagen fibre bundle patterns.
3. The trichrome method of Masson (Appendix p.6.9). Results were similar to those obtained with the method of Gomori.
4. The silver impregnation method of Naoumenko and Feigin (Appendix p.6.11). The definition and consistency of staining was inferior to that of the method of Gordon and Sweet.
5. The Van Gieson stain for collagen (Appendix p.6.13) was found to demonstrate the collagen fibre bundles well, but was not used because sections stained with Van Gieson were more suitable for colour photography, which was not used in this investigation.
6. Of the six histological staining techniques evaluated, the one which best demonstrated the collagen fibre bundles was the silver impregnation method of Gordon and Sweet. It was the only technique which showed the component fibres of some of the fibre bundles, and the excellent black and white definition was ideal for black and white photography. There was also no change with age in the quality of the stained sections.

Silver impregnation techniques involve the risk of sections lifting from the slides owing to the presence of free ammonia. The frequency of this problem was reduced by floating the sections onto glass slides and immediately placing them in an oven at 62 degrees centigrade for ninety minutes. It was also important not to proceed with the stages of preparation of the silver solution (Appendix p.6.7) until there was no trace of free ammonia, which was determined by smell.

C. PHOTOMICROGRAPHIC AND PHOTOGRAPHIC EQUIPMENT:

Sections which demonstrated the patterns of arrangement of the periodontal fibre bundles, and the morphology of individual fibre bundles have been identified and recorded photomicrographically. The instrument used for this phase of the investigation was the Zeiss Axiomat.

Photographic copying, developing, printing and enlargement were carried out in the photography department of the Dental School at the University of Adelaide. The following equipment was used:

The Leitz Reprovit II camera system for photographing line drawings on acetate sheets.

The Durst Laborator 54 Universal Camera and the Leitz Focomat 1c for photographic printing and enlargement.

The FC Auto Glazing Machine for the glazing of photographic prints was manufactured by F. Charten and Co. Ltd.

CHAPTER 3.

FINDINGS.

The findings of the investigation will be described in four sections, the first three of which will relate respectively to patterns of arrangement of collagen fibre bundles seen in the mesiodistal, the buccolingual and the horizontal planes. The fourth section will describe a localised variation in the morphology of the principal collagen fibre bundles observed in the mesiodistal and horizontal planes of section.

A. PATTERNS OF ARRANGEMENT OF COLLAGEN FIBRE BUNDLES VIEWED IN THE MESIODISTAL PLANE.

In this plane of section, it was found that some collagen fibre bundles were continuous from the cementum of one tooth, through the interdental alveolus to the cementum of the adjacent tooth.

These fibre bundles, described by Cohn (1970) as transalveolar fibres, appeared to be more numerous in the coronal half of the interdental alveolus. The number of transalveolar fibre bundles which could be demonstrated diminished progressively towards the apical region.

The principal, supraalveolar and transalveolar, collagen fibre bundles in the interdental region are illustrated in Figs. 1 to 6, p.3.3 to p.3.8. These photomicrographs show typical findings of the arrangement, morphology and distribution of these fibre bundles. Transalveolar collagen fibre bundles are shown at greater magnification in Figs. 4 and 5, p.3.6 and p.3.7.

The principal collagen fibre bundles of the periodontal ligament did not change direction occlusally or apically after they entered the interdental alveolus to become transalveolar fibre bundles. Therefore, variations in the orientation of the fibre bundles within the alveolus were closely related to the angles of incidence of the principal collagen fibre bundles to the alveolar wall. The patterns of arrangement of these contiguous fibre bundles were seen to vary. The variation was apparent in sections from the same animal, but was more evident in sections from different animals. This variation is demonstrated by the photomicrographs in Fig.3 p.3.5. (Text continues on p.3.9).

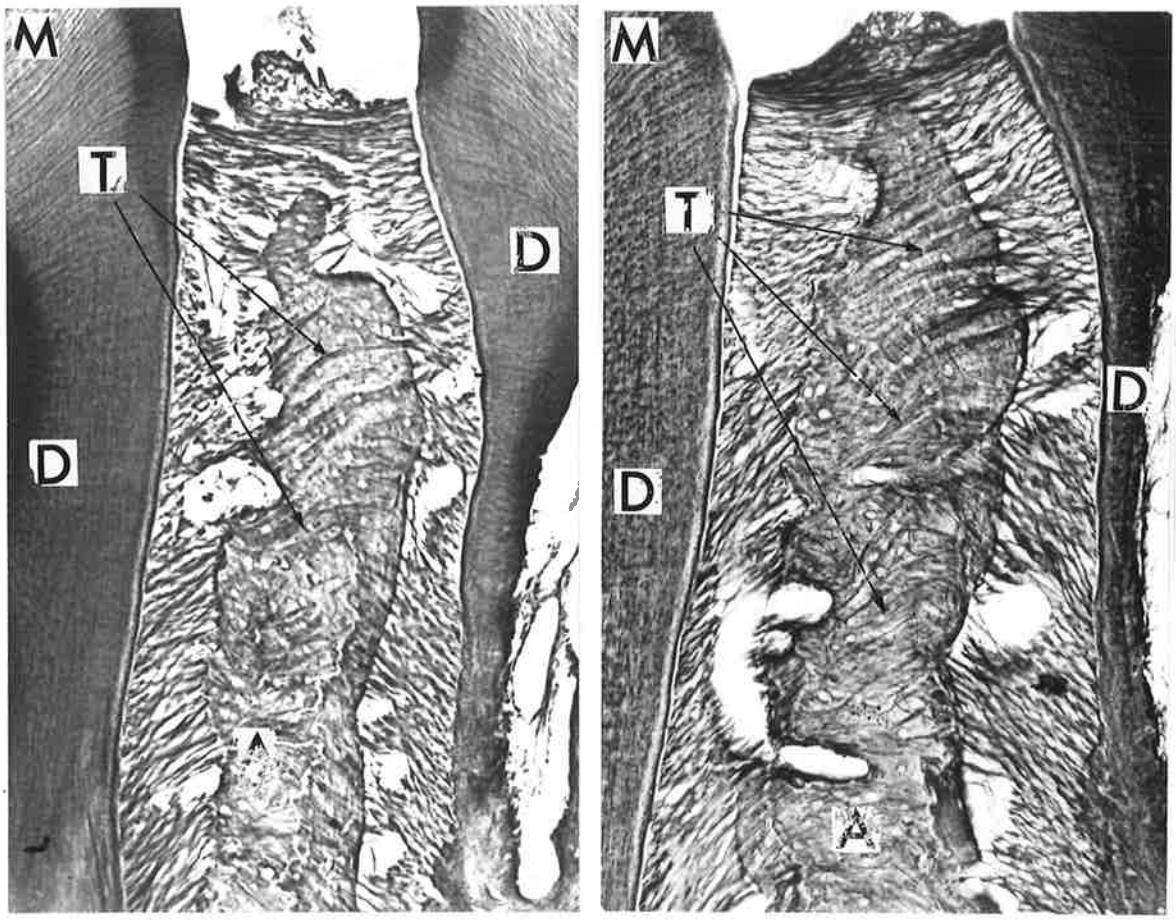

FIG. 1.

Photomicrographs from mesiodistal sections, demonstrating the presence of transalveolar collagen fibre bundles in the interdental alveolar bone of the first and second molars of the mouse mandible. A, interdental alveolar bone; D, dentine; M, mesial; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X 100.

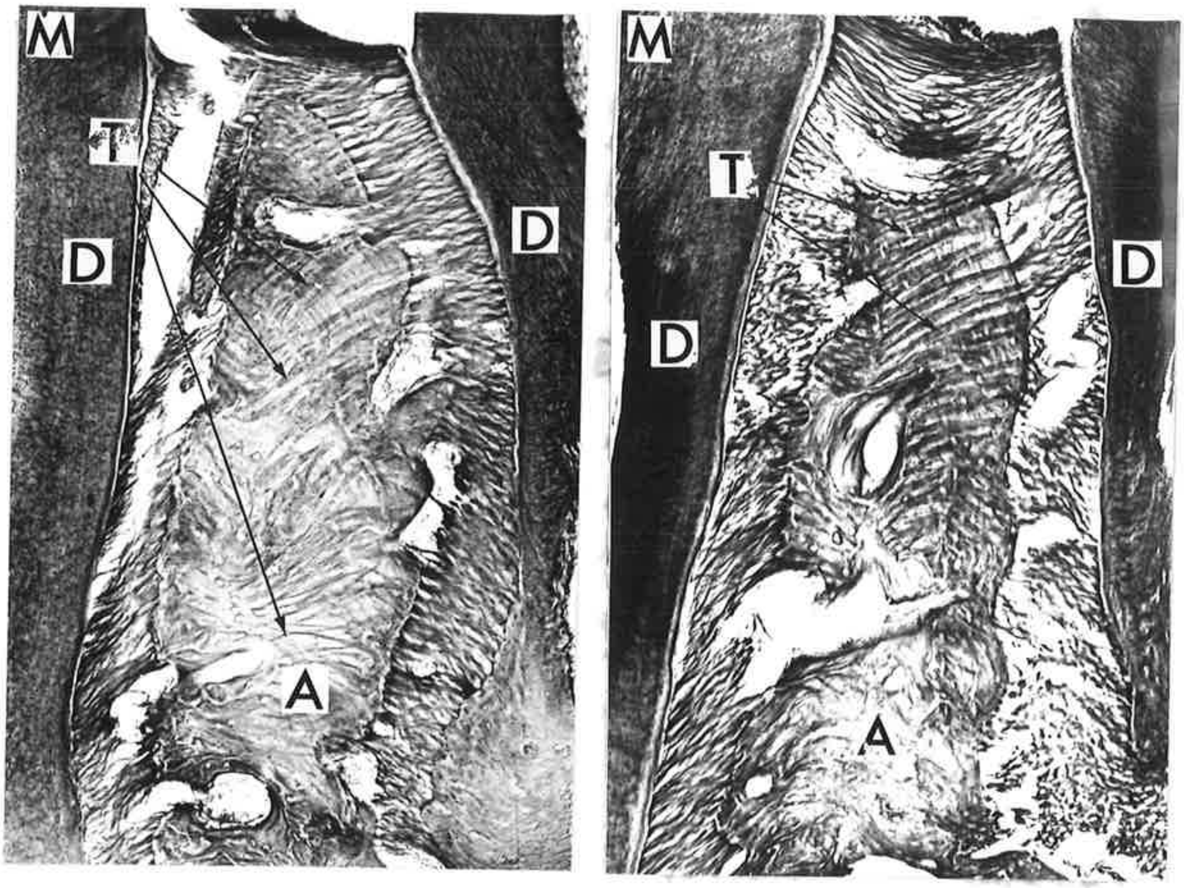

FIG. 2.

Photomicrographs from mesiodistal sections demonstrating the presence of transalveolar collagen fibre bundles in the interdental alveolar bone of the first and second mandibular molars of the mouse mandible.
A, interdental alveolar bone; D, dentine; M, mesial; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X 150.

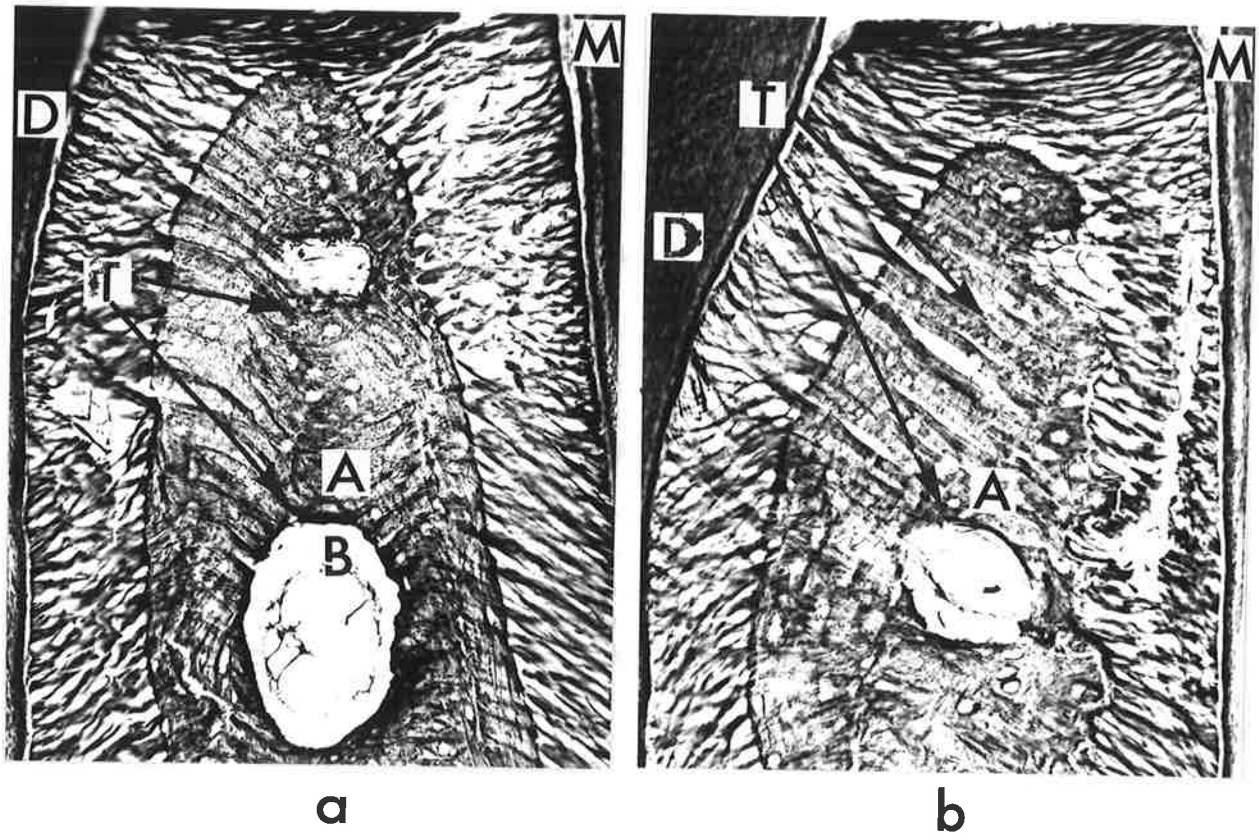

FIG. 3.

Photomicrographs from mesiodistal sections from different animals, showing interdental regions of first and second mandibular molars of the mouse. Comparison of the transalveolar fibre bundles of a. and b. demonstrates the variation in their patterns of arrangement within the interdental alveolus. A, alveolar bone; D, dentine; M, mesial; T, transalveolar collagen fibre bundles. Gordon and Sweet silver impregnation. X 150. Photographic enlargement X 50%.

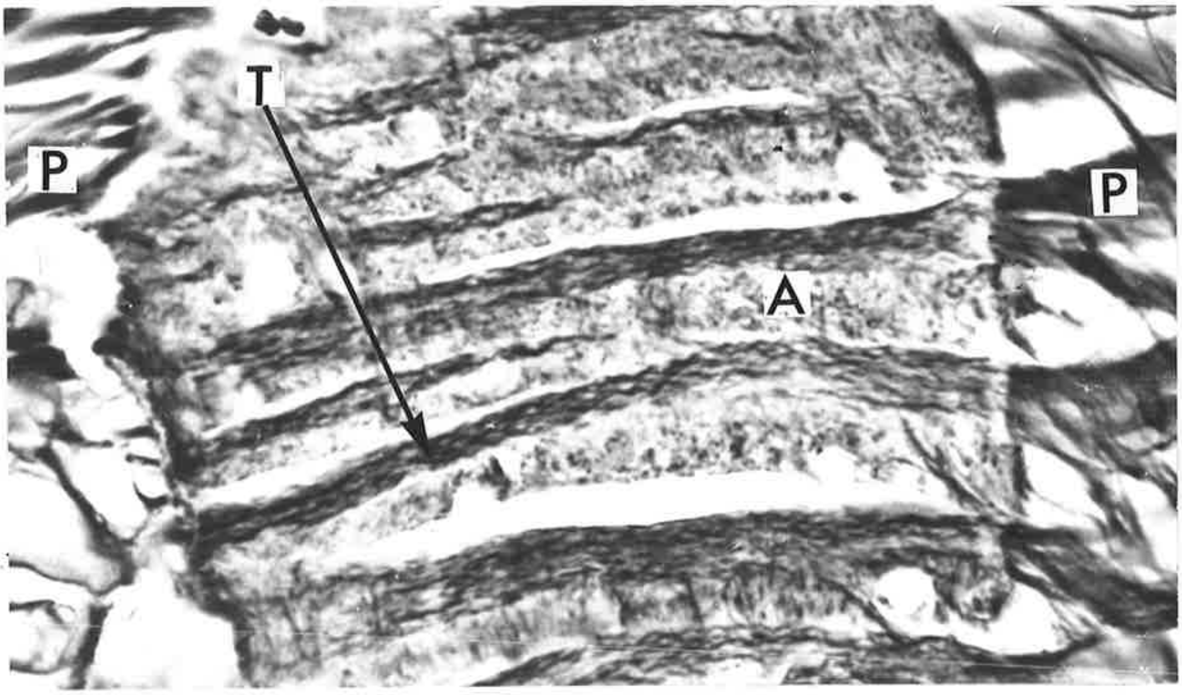

FIG. 4.

Photomicrograph from a mesiodistal section, showing transalveolar collagen fibre bundles in the coronal half of the interdental alveolar bone of first and second mandibular molars. A, alveolar bone; P, principal collagen fibres of the periodontal ligament; T, transalveolar collagen fibre bundle.
Gordon and Sweet silver impregnation. X 400.

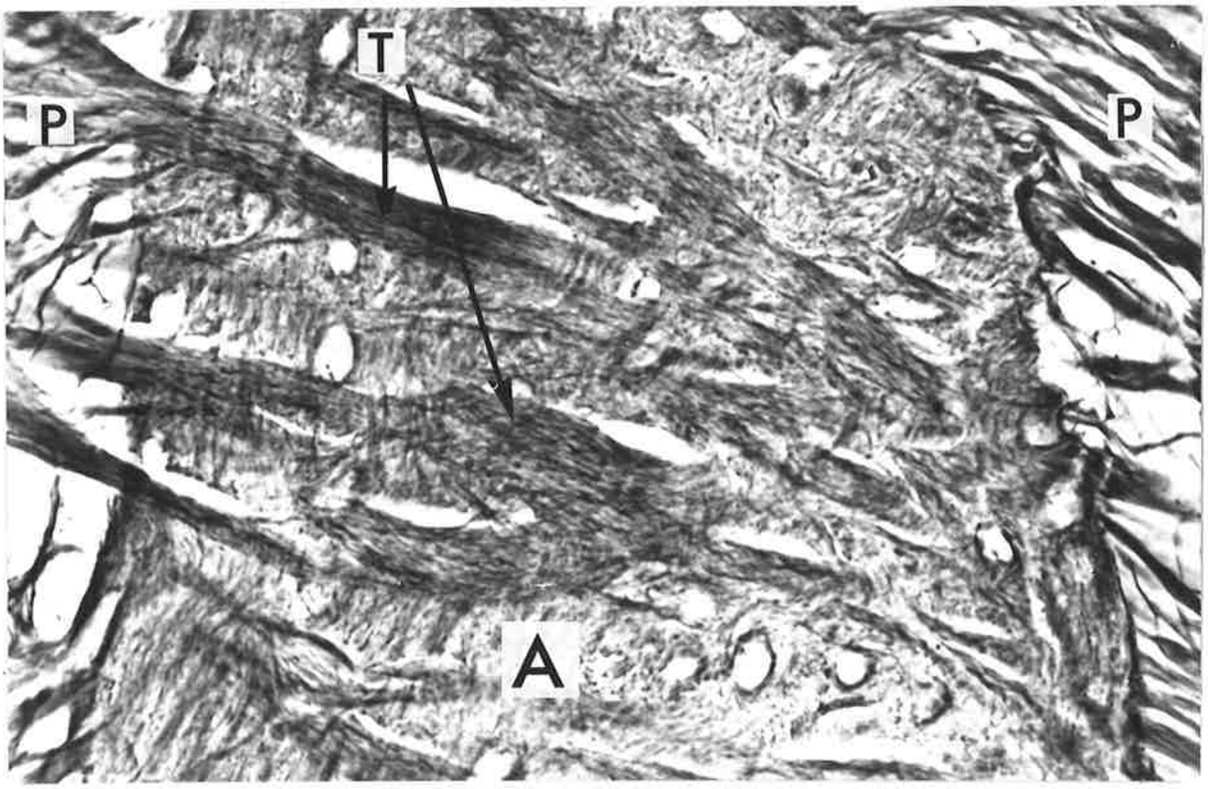

FIG. 5.

Photomicrograph from a mesiodistal section showing collagen fibre bundles in the middle third of the interdental alveolar bone of first and second mandibular molars. A, alveolar bone; P, principal fibres of the periodontal ligament; T, transalveolar collagen fibre bundles. Gordon and Sweet silver impregnation. X 400.

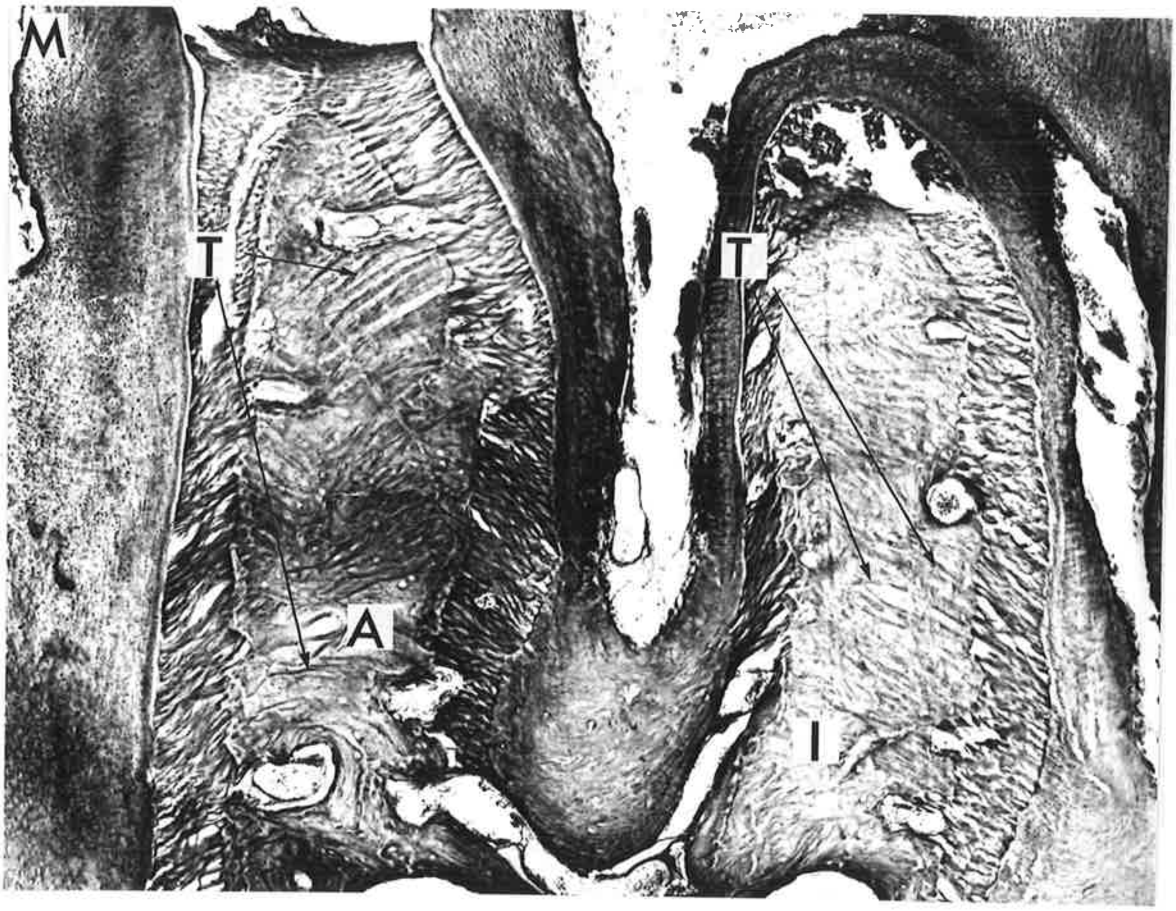

FIG. 6.

Photomicrograph from a mesiodistal section of the interdental region of the first and second molars and the interradicular region of the second molar. A, interdental alveolar bone; I, interradicular alveolar bone; M, mesial; T, transalveolar collagen fibre bundles. Gordon and Sweet silver impregnation. X 100.

The collagen fibre bundle arrangements seen in the interradicular regions were similar to those which were seen in the interdental region. However, the interradicular transalveolar fibre bundles were less frequently demonstrated and the patterns of arrangement were less distinct (Fig. 6 p.3.8, Fig. 7, p.3.10).

Some mesiodistal sections from each animal showed the presence of transalveolar fibres throughout the length of the interdental septa (Fig. 8 p.3.11). This feature was only demonstrated in sections from the midsagittal region of the teeth. Transalveolar fibres were not demonstrated in the apical region of the interradicular septa.

The principal collagen fibre bundles on the mesial side of the first molars were seen to enter the alveolus, where they appeared to terminate at a depth approximately equivalent to the width of the interdental alveolus, without traversing the full width of alveolar bone. Some fibre bundles passed completely through the alveolus and into the periosteum mesial to the first molar but these were rare and could only be discerned in the gingival third of the alveolus in sections from the midsagittal region.

In all sections examined, there was an overall pattern of arrangement which included the transseptal principal and transalveolar collagen fibre bundles.

The diagram shown on page 3.11 (Fig. 8) was drawn from mesiodistal sections and illustrates the morphology and arrangement of collagen fibre bundles in the interdental and interradicular regions and in the region mesial to the first molar. (Text continues on p.3.12).

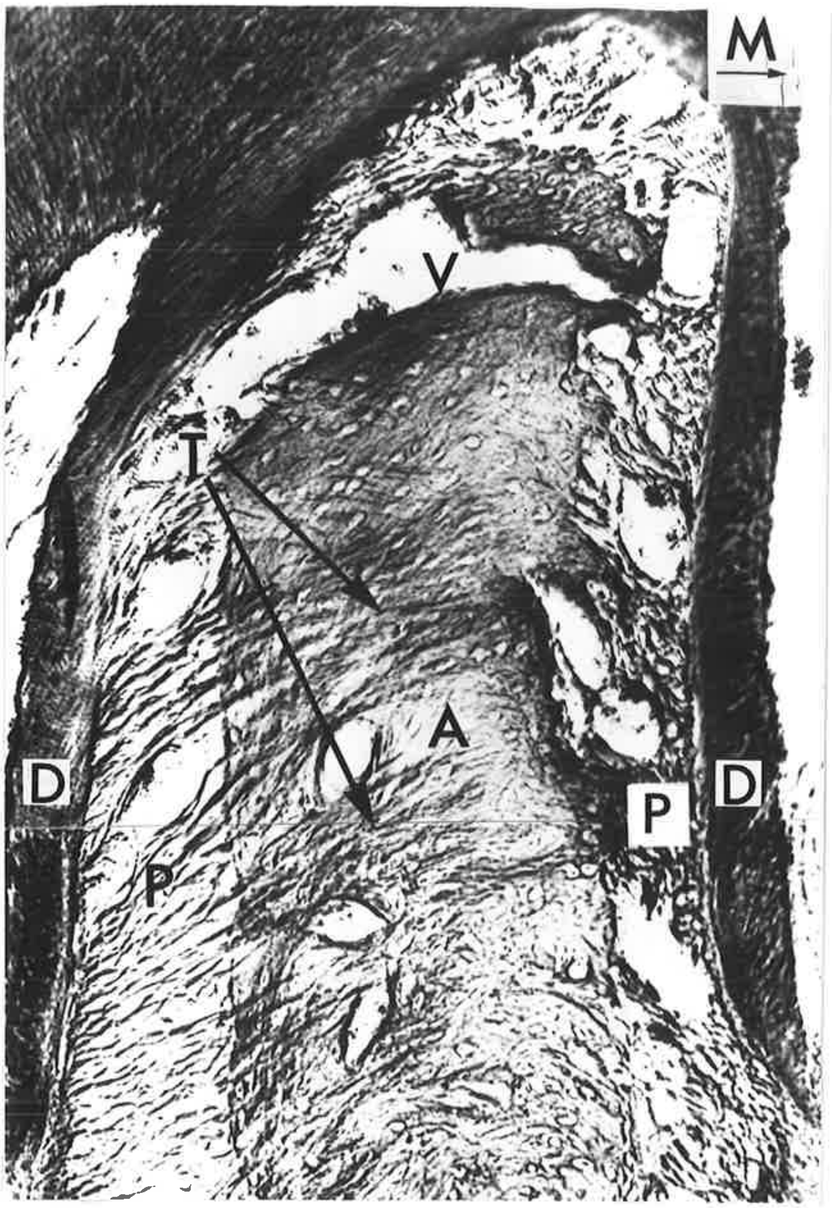

FIG. 7.

Photomicrograph from a mesiodistal section, demonstrating the presence of transalveolar collagen fibre bundles in the interradicular region of the mandibular second molar. A, alveolar bone; D, dentine; M, mesial; P, periodontal ligament; T, transalveolar collagen fibre bundles; V, blood vessel space.
Gordon and Sweet silver impregnation. X. 200.

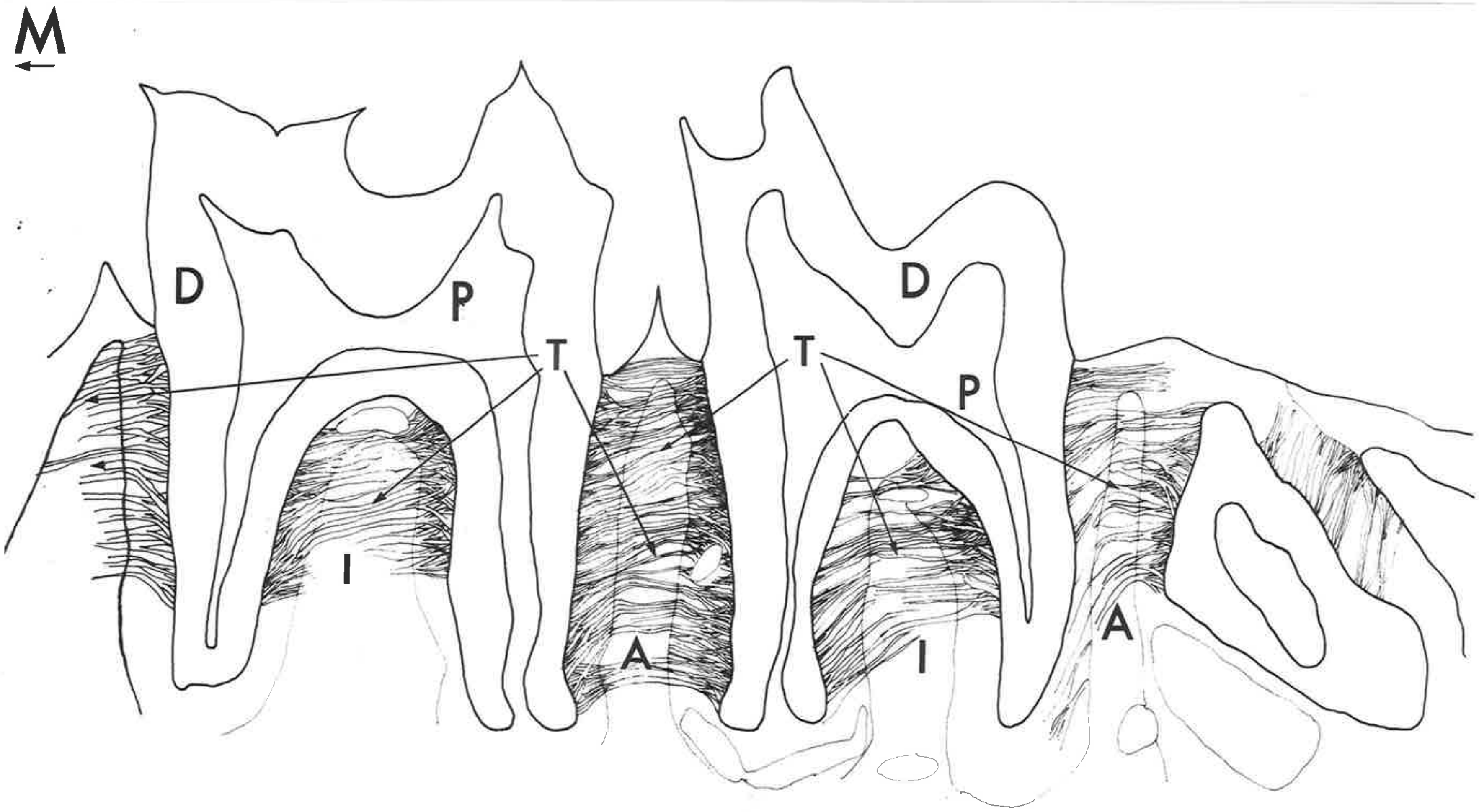

FIG. 8.

Diagram prepared from mesiodistal sections of the molar alveolar process of the mouse mandible, illustrating patterns of arrangement of the collagen fibre bundles. A, interdental alveolus; D, dentine; I, interradicular alveolus; M, mesial; T, trans-alveolar collagen fibre bundles.

B. PATTERNS OF ARRANGEMENT OF COLLAGEN FIBRE BUNDLES VIEWED IN THE BUCCOLINGUAL PLANE.

In this plane of section, it was observed that some principal collagen fibre bundles coursed completely through the buccal or lingual alveolus.

The fibres which passed through the lingual alveolar bone were seen in greater numbers and were better defined in the region of the gingival half of the periodontal ligament. They were fewer and less distinguishable in the region opposite the apical half of the ligament. See Figs. 9, 10, 11, p.3.13 to p.3.15. On the buccal side, demonstration of transalveolar fibres was rare, and they were seen only in the region of the gingival half of the periodontal ligament.

The relation of the transalveolar fibres to the mucoperiosteum could not be clearly demonstrated in any of the sections examined. However, there was some evidence that after passing through the bone, the transalveolar fibre bundles mingled with and became indistinguishable from the connective tissue of the mucoperiosteum.

The fibre bundles passing through the buccal and lingual alveolar bone have been called transalveolar fibre bundles, although they differed from the transalveolar fibre bundles seen in the mesiodistal plane (Cohn 1966), in that after passage through the bone, they became part of the connective tissue of the mucoperiosteum.

Although the transalveolar fibre bundles throughout the lingual alveolus were more clearly and more frequently demonstrated than those in the buccal alveolus,

(Text continues on page 3.16)

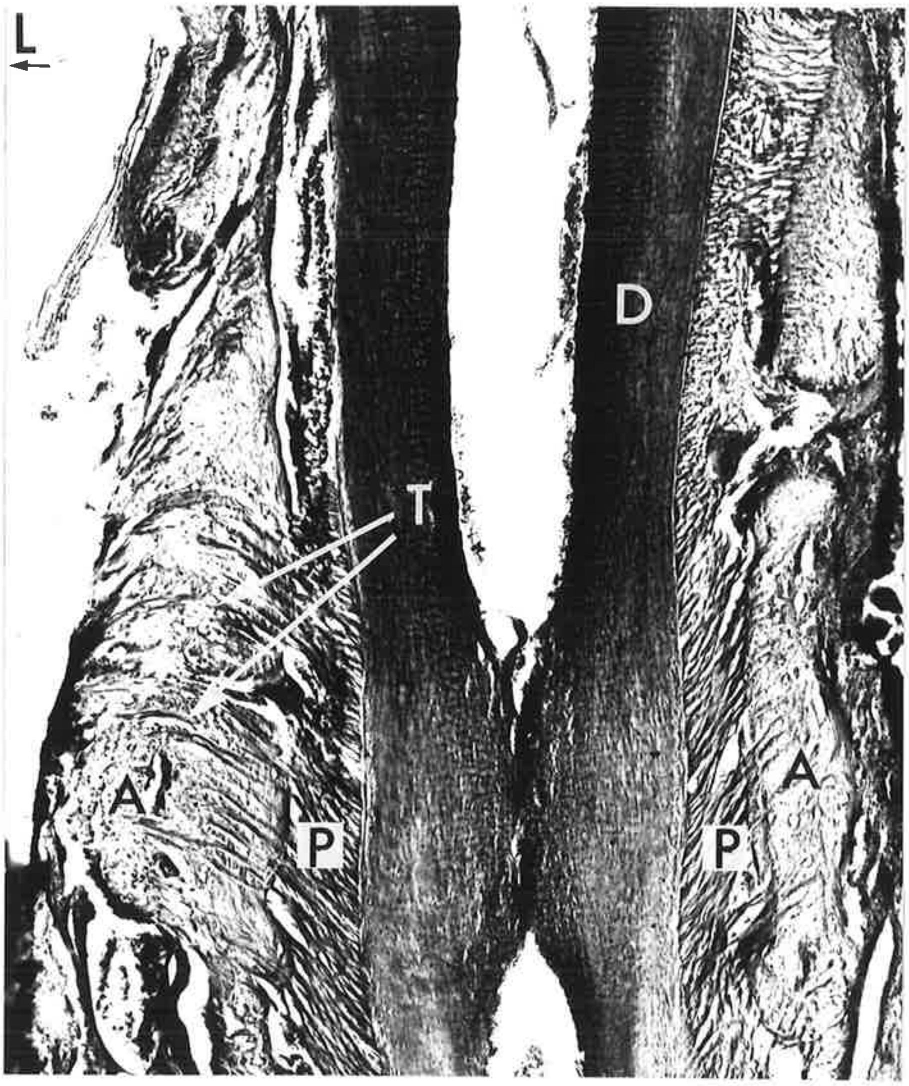

FIG. 9.

Photomicrograph of part of a buccolingual section from the region of the distal root of a mandibular first molar, demonstrating transalveolar collagen fibre bundles. Fibre bundle penetration is greater on the lingual than on the buccal. A, alveolar bone; D, dentine; L, lingual; P, periodontal ligament; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X. 200.

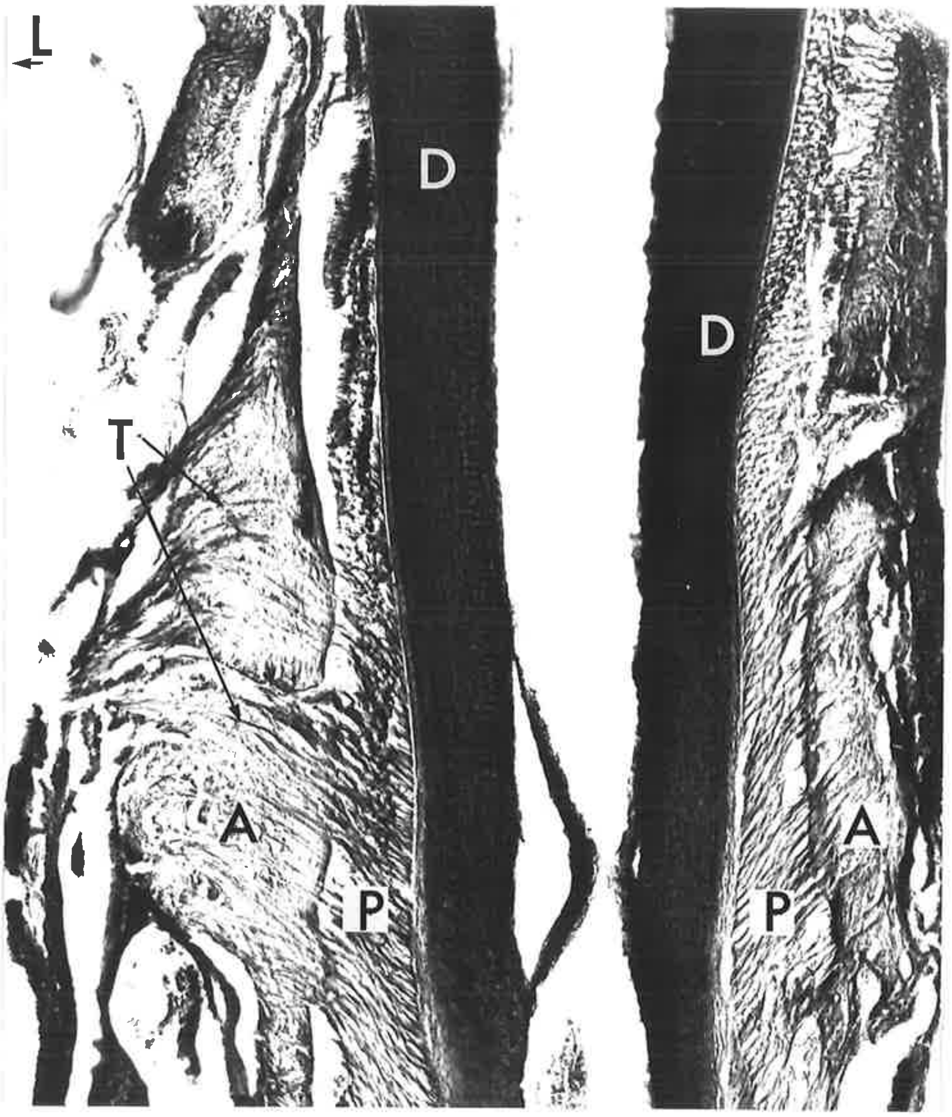

FIG. 10.

Photomicrograph of a buccolingual section from the region of the distal root of a mandibular first molar. A, alveolar bone; D, dentine; L, lingual; P, periodontal ligament; T, transalveolar collagen fibre bundles. Gordon and Sweet silver impregnation. X. 250.

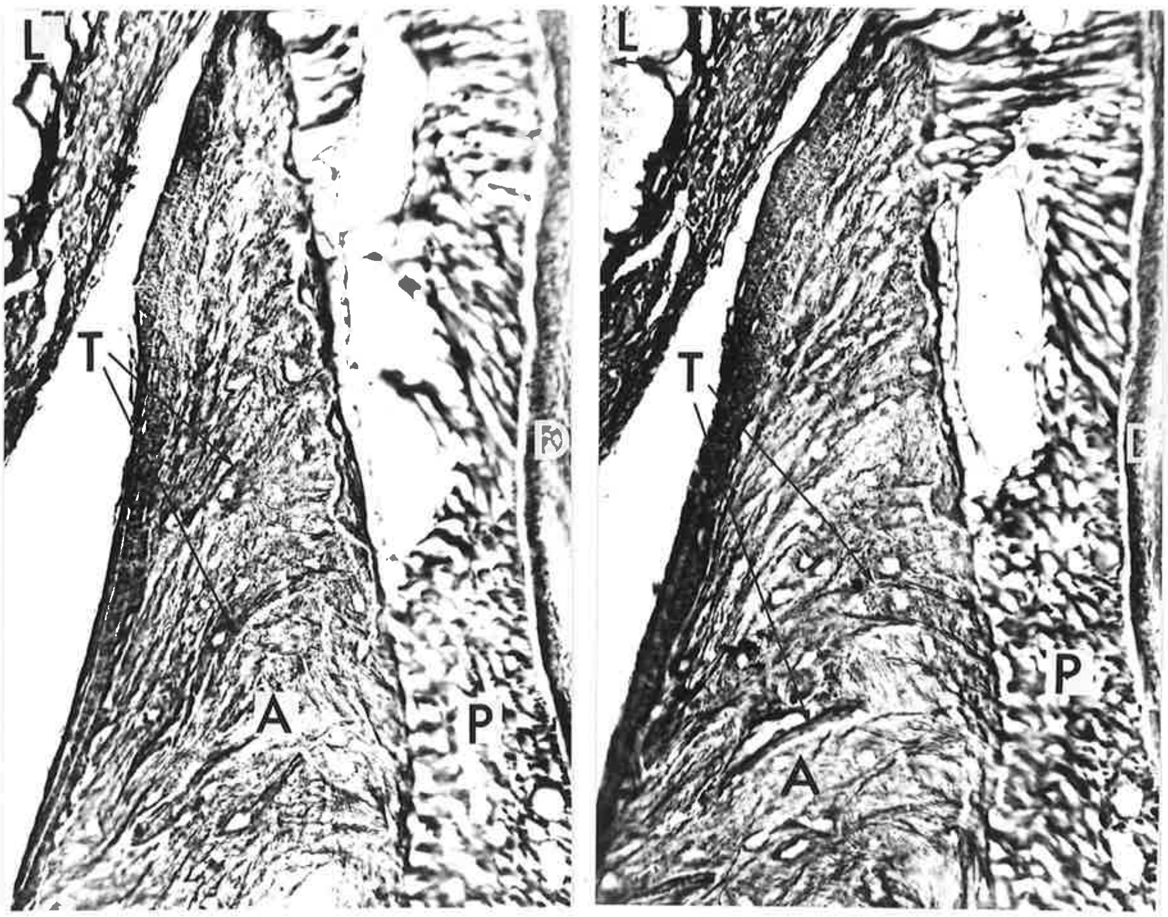

FIG. 11.

Photomicrographs of buccolingual sections illustrating the lingual periodontal ligament and alveolar bone in the region of the distal root of the first molar of the mouse mandible. A, alveolar bone; D, dentine; L, lingual; P, periodontal ligament; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X. 250.
Photographic enlargement X 50%.

the patterns of arrangement of the fibre bundles in the buccal and lingual alveolar bone were similar and the patterns did not vary. A diagram, representative of findings in the buccolingual plane appears on page 3.17 (Fig. 12).

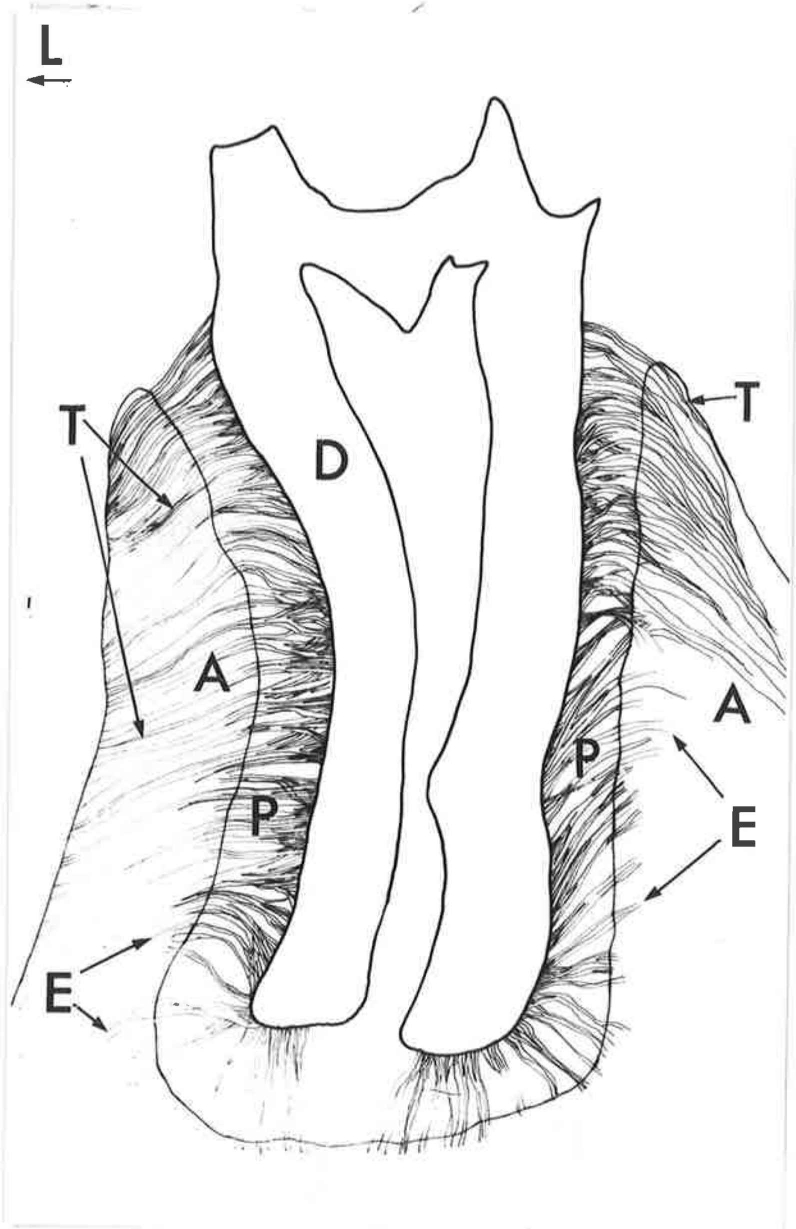

FIG. 12.

Diagram prepared from buccolingual sections, illustrating the collagen fibre bundle arrangement on the buccal and lingual sides of mandibular molar roots. A, alveolar bone; D, dentine; E, extremities beyond which collagen fibre bundles could not be demonstrated; L, lingual; P, periodontal ligament; T, transalveolar collagen fibre bundles.

C. PATTERNS OF ARRANGEMENT OF COLLAGEN FIBRE BUNDLES VIEWED IN THE HORIZONTAL PLANE.

In this plane of section, transalveolar fibre bundles were seen clearly in only a few sections.

The arrangement of fibre bundles seen in horizontal section is illustrated in Figs. 13, 14, 15 and 16 on pages 3.19 to 3.22. All sections in which trans-alveolar bundles could be demonstrated were from the coronal third region of the interdental bone. The pattern is similar to a magnetic field, and was demonstrated in some sections from each specimen.

As in the buccolingual plane, the sections from the horizontal plane demonstrated a consistent pattern of arrangement, with little variation. This was in contrast to the considerable variation of pattern seen in sections from the mesiodistal plane.

In the horizontal sections some fibre bundles were seen to pass completely through the alveolar bone on the lingual side, and also through the alveolar bone mesial to the first molar. However, in this plane of section very few fibre bundles could be seen passing through the alveolar bone on the buccal side. A diagram representative of findings in the horizontal plane of section, appears on page 3.23 (Fig. 17).

(Text continues on p.3.23)

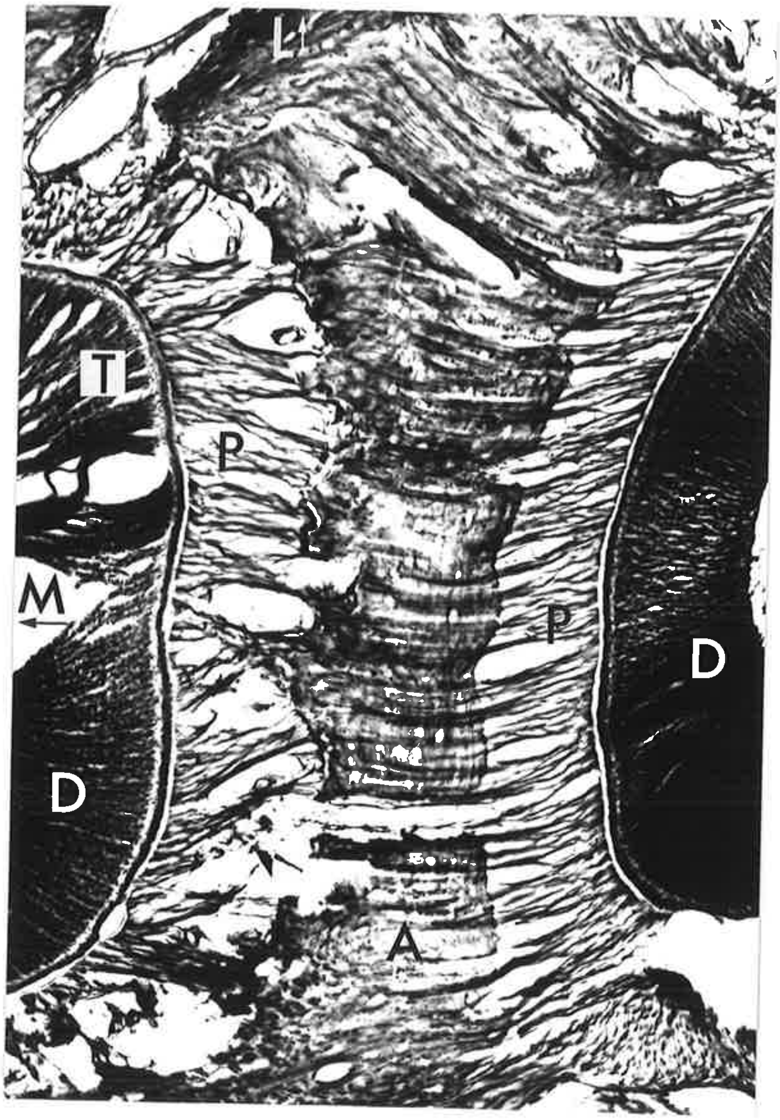

FIG. 13.

Transalveolar collagen fibre bundles are demonstrated in this photomicrograph of a horizontal section from the coronal third of the interdental region of the first and second mandibular molars. A, interdental alveolar bone; D, dentine; L, lingual; M, mesial; P, principal collagen fibre bundles; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X. 100.

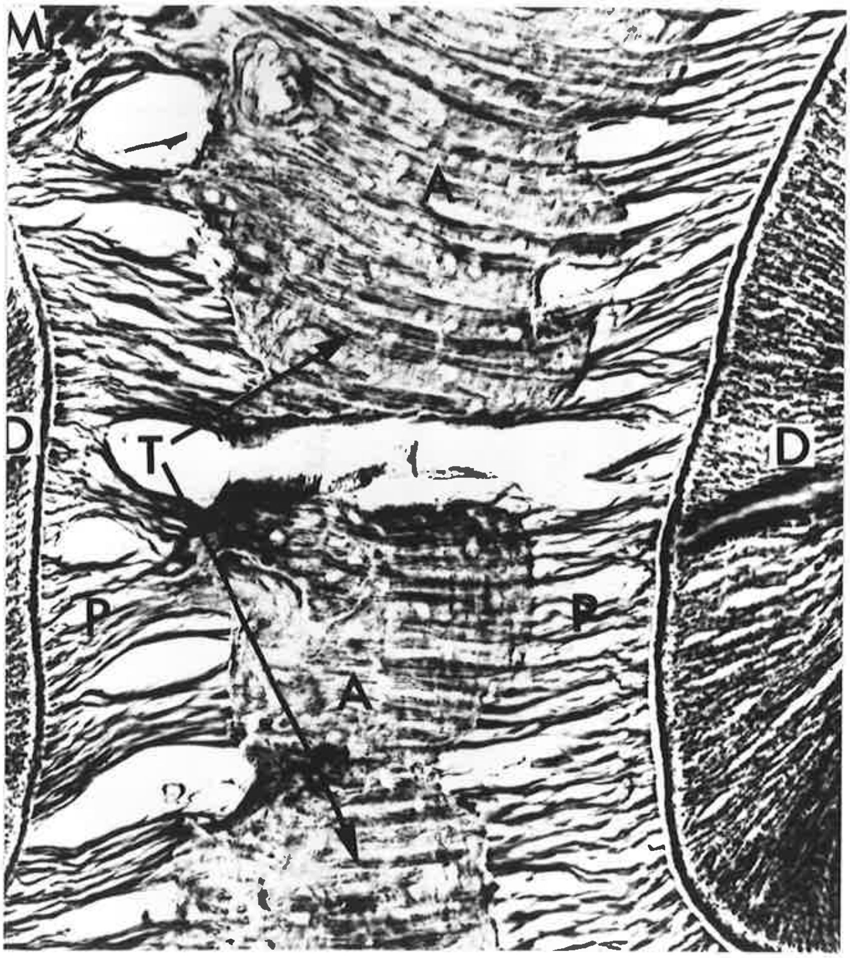

FIG. 14.

Photomicrograph of the interdental area of first and second molars from a horizontal section in the region of the coronal third of the roots. A, interdental alveolar bone; D, dentine; M, mesial; P, periodontal ligament; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X. 400.

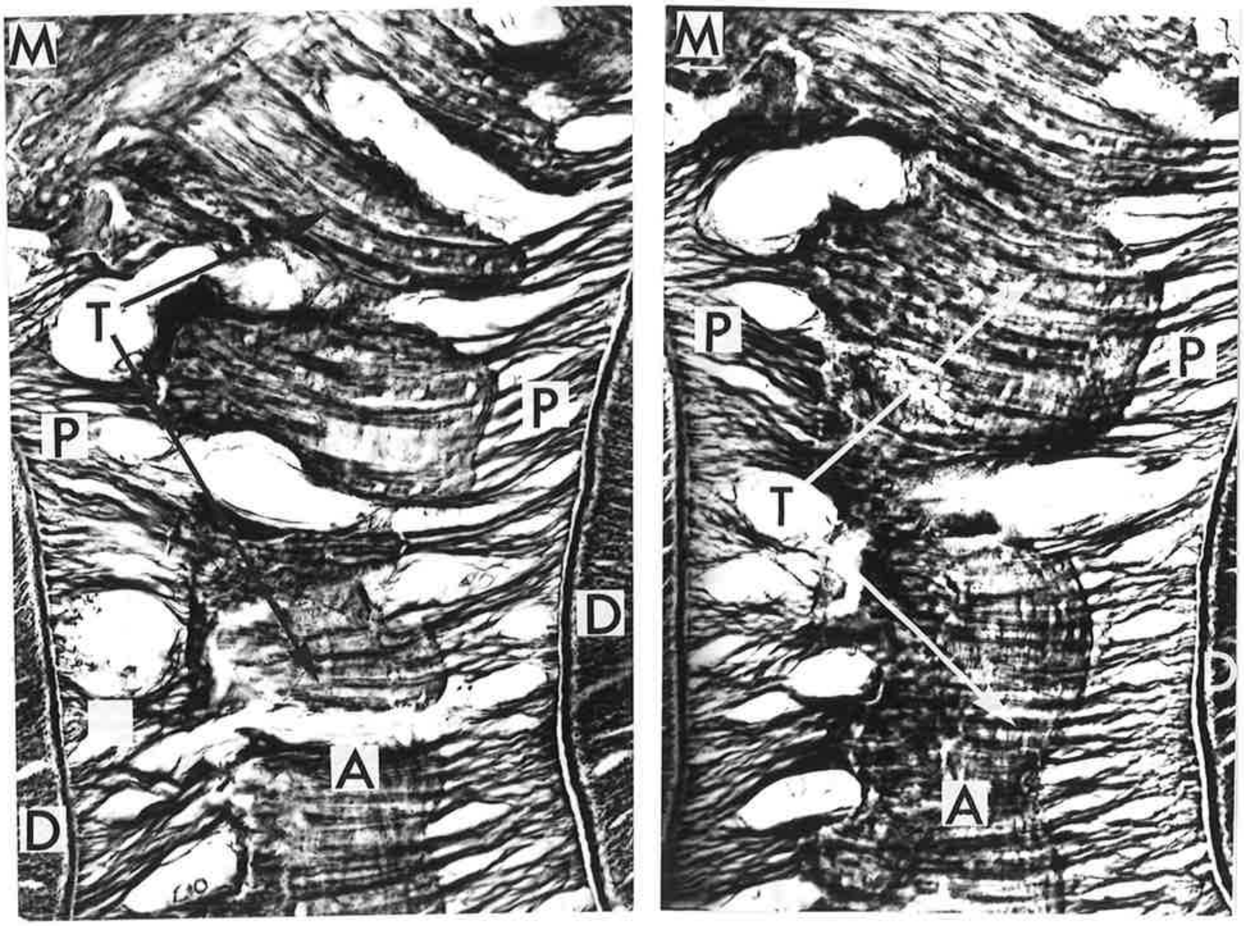

FIG. 15.

Photomicrographs of the interdental area of first and second molars from horizontal sections in the region of the coronal third of the roots. A, interdental alveolar bone; D, dentine; M, mesial; P, periodontal ligament; T, transalveolar collagen fibre bundles. Gordon and Sweet silver impregnation. X. 400.

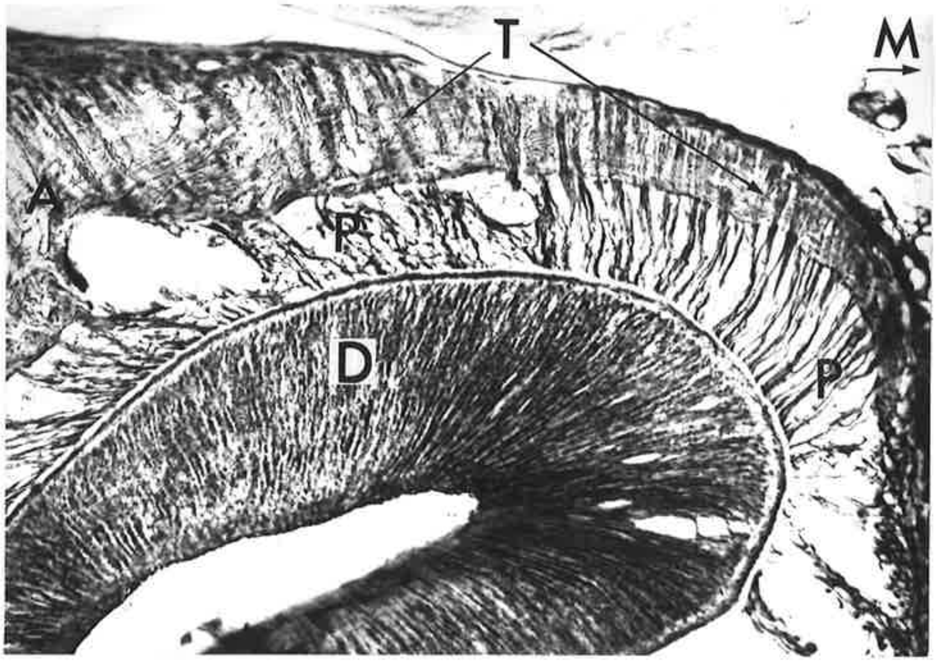

FIG. 16.

The presence of transalveolar collagen fibre bundles is demonstrated in the mesiolingual area of a horizontal section from the region of the coronal third of the roots of the molars of the mouse mandible. A, alveolar bone; D, dentine; M, mesial; P, periodontal ligament; T, transalveolar collagen fibre bundles.
Gordon and Sweet silver impregnation. X. 100.

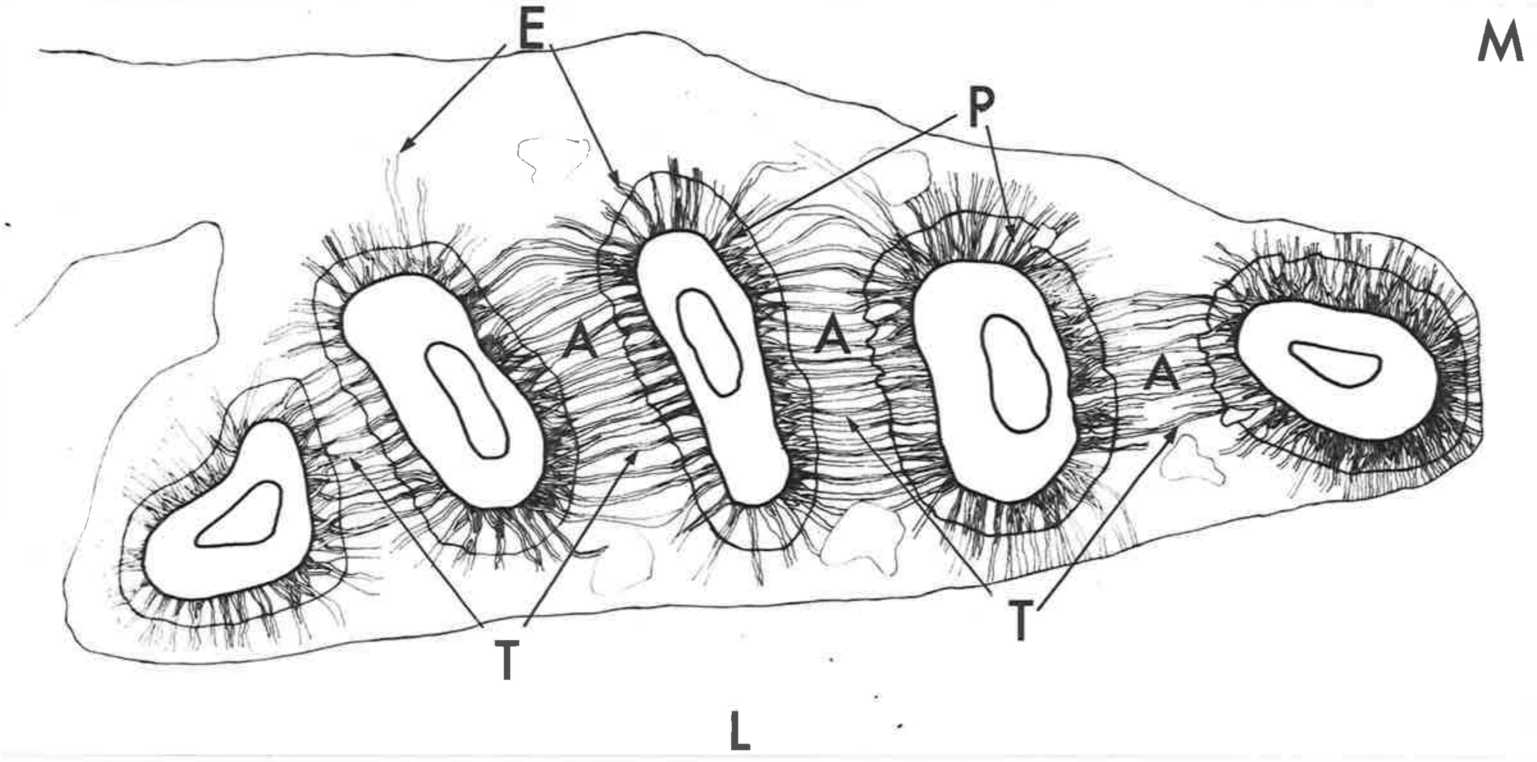

FIG. 17.

A diagram representing collagen fibre bundle patterns seen in horizontal sections from the coronal third regions of the roots of the mandibular molars. A, interdental alveolar bone containing transalveolar collagen fibre bundles; E, extremeties beyond which collagen fibre bundles could not be traced; L, lingual; M, mesial; P, principal collagen fibre bundles; T, transalveolar collagen fibre bundles.

D. LOCALISED VARIATION OF THE MORPHOLOGY OF THE PRINCIPAL COLLAGEN FIBRE BUNDLES.

The investigation also showed a constant difference between the morphology of the principal collagen fibre bundle patterns on the mesial and those on the distal sides of the roots of the molar teeth of the mouse mandible.

On the distal side of the teeth the bundles passed almost directly from the cementum to the alveolus. The bundles were smaller in cross section and more numerous at the cementum surface than at the alveolar surface, but the change in number and width appeared to occur with little change in the direction of the bundles.

On the mesial side of the teeth, there was a much greater change in thickness and number of bundles as they passed from the cementum to the alveolus. The bundles appeared to be drawn together at the alveolar surface to form thick round groups of bundles, which fanned out, flattened and overlapped each other as they passed towards the cementum. The shapes so formed were quite distinguished from the shapes seen in the periodontal ligament of the distal side of the molars.

The resultant shape formed by the arrangement of the collagen fibre bundles is illustrated photomicrographically in Figs. 18 and 19, p.3.25 and 3.26 and diagrammatically in Fig. 20 p.3.28.

The localised variation seen in the interdental region was not conclusively evident in the interradicular region, where the principal fibres on the mesial side of the distal root did not differ sufficiently from those on the distal side of the mesial root. (Text continues on p.3.27)

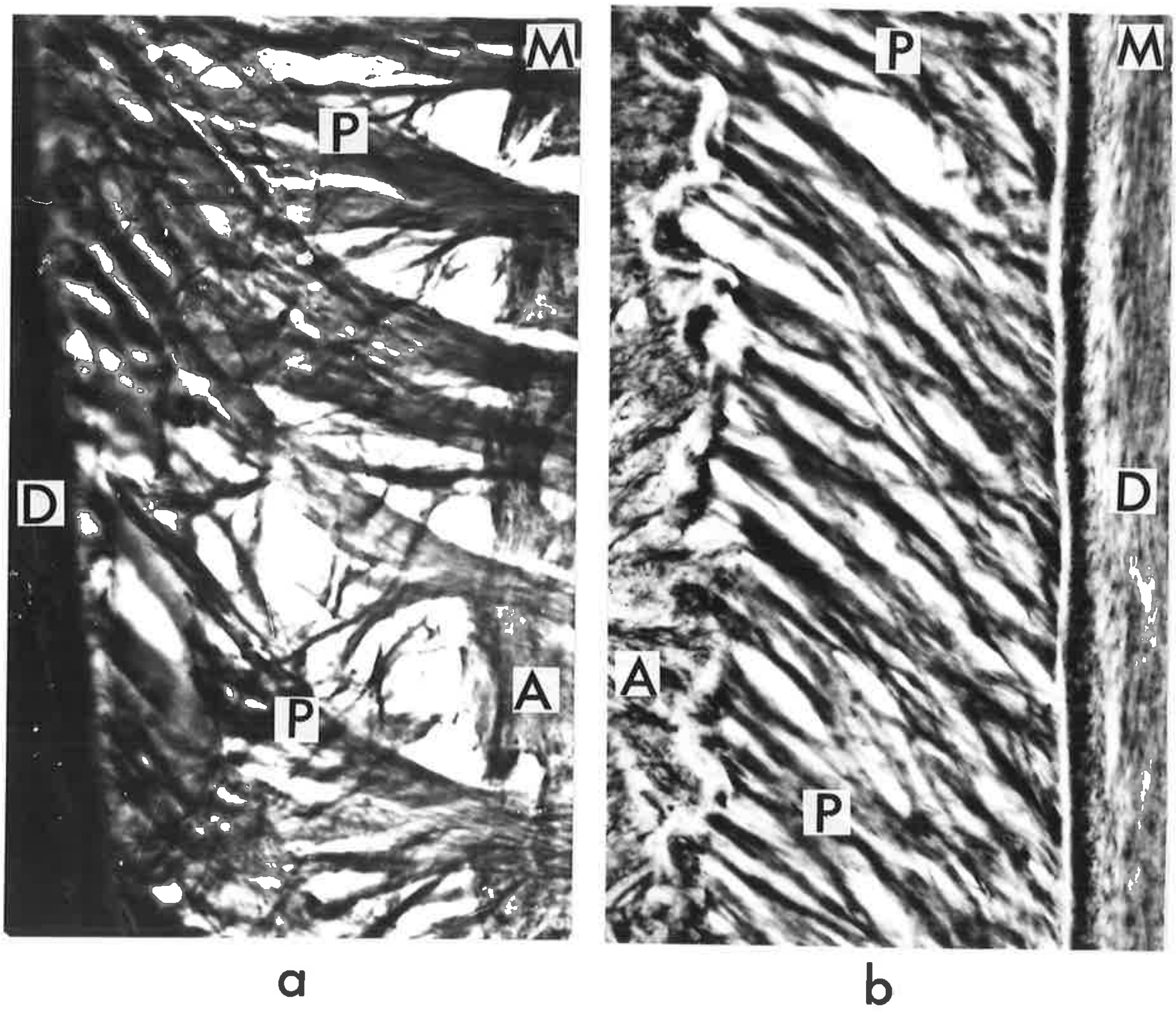

FIG. 18.

Comparison of photomicrographs from mesiodistal sections, demonstrates the difference in the morphology of the principal collagen fibre bundles seen on the mesial (a) and the distal (b) sides of the molar teeth of the mouse mandible. A, interdental alveolar bone; D, dentine; M, mesial; P, principal collagen fibre bundles. Gordon and Sweet silver impregnation. X. 400.

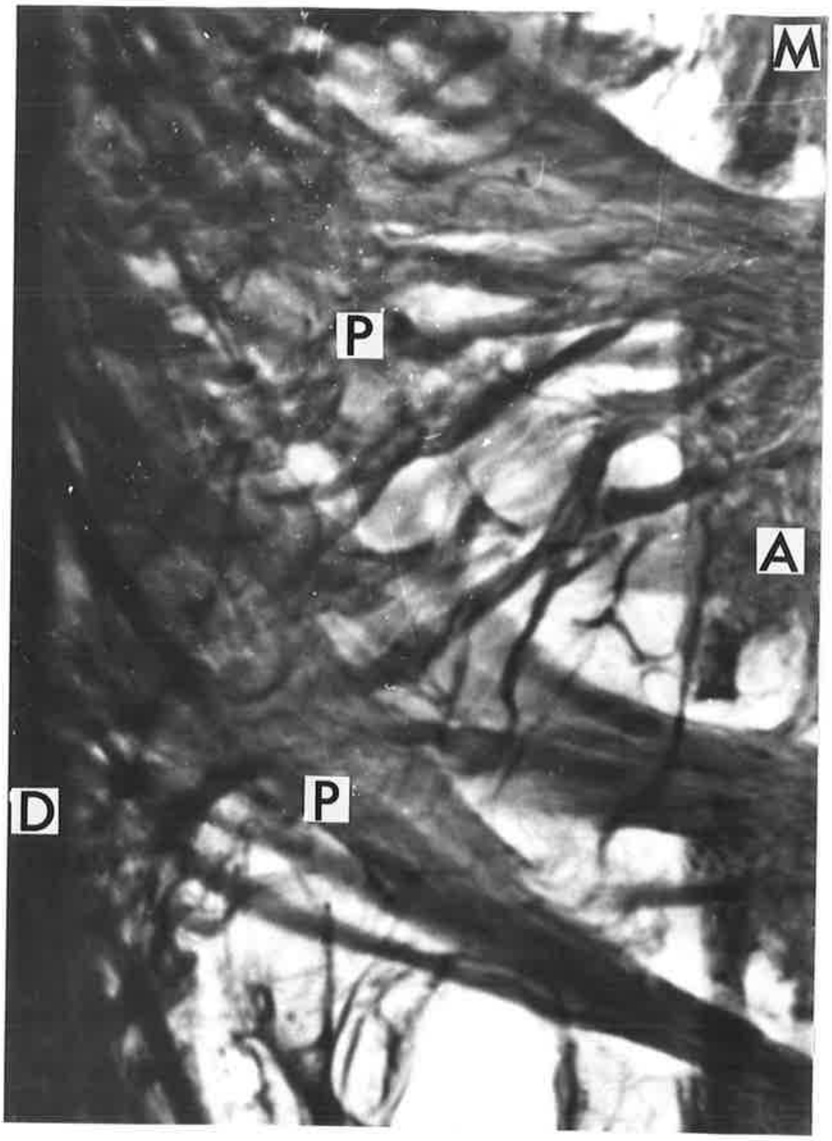

FIG. 19.

Photomicrograph of a mesiodistal section showing principal collagen fibre bundles passing from the mesial side of the second molar to the interdental alveolar bone. The fanning of the collagen fibre bundles as they pass from the alveolar bone to the cementum is apparent. The difference between the thickness of the bundles at the alveolar bone and at the cementum is also demonstrated. A, interdental alveolar bone; D, dentine; M, mesial; P, principal collagen fibre bundles of the periodontal ligament. Gordon and Sweet silver impregnation. X. 400. Photographic enlargement X. 2.

There were no apparent localised variations in the principal fibre bundle morphology in sections cut in the horizontal plane, or in those cut in the buccolingual plane.

In sections from the mesiodistal plane, it was observed that fragmentation and fracture of sections occurred more frequently in the periodontal ligament adjacent to the mesial surface of the interdental bone between the crestal and apical regions.

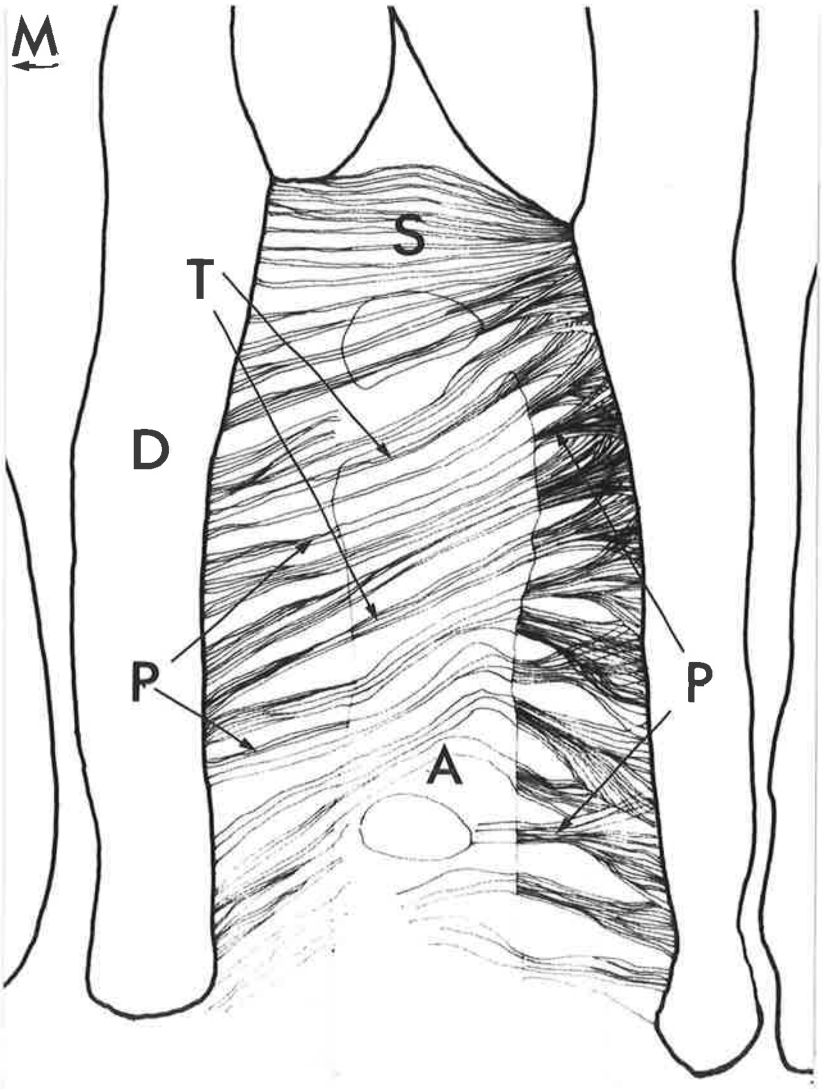

FIG. 20.

Diagram prepared from mesiodistal sections from the mesiodistal plane showing the collagen fibre bundle arrangement in the interdental region of the first and second molars of the mouse mandible. The morphological difference between the principal collagen fibre bundles on opposite sides of the alveolus is apparent. A, interdental alveolar bone; D, dentine of the distal root of the first molar; M, mesial; P, principal collagen fibres; S, transseptal collagen fibres; T, trans-alveolar collagen fibre bundles.

SUMMARY OF FINDINGS.

Collagen fibre bundles were seen to pass without interruption through the interdental, the interradicular, the buccal and the lingual alveolar bone. Fibre bundles passing through the alveolar bone were also observed mesial to the first molar.

It was demonstrated that some collagen fibre bundles passed from the cementum of one tooth to the cementum of the adjacent tooth. A similar connection was also seen between the roots of individual teeth.

A localised difference in principal collagen fibre bundle morphology was seen in the periodontal ligament of the mesial and distal sides of the interdental and interradicular alveolar bone.

CHAPTER 4.

DISCUSSION.

The results of the investigation will be discussed in two sections.

The first section will relate to the light microscope examination of the patterns of arrangement of the collagen fibre bundles of the alveolar process of the mouse mandible.

The second section of the discussion will refer to the morphology of the principal collagen fibre bundles of the periodontal ligaments of the mandibular molars of the mouse, as seen with the light microscope.

A. PATTERNS OF ARRANGEMENT OF COLLAGEN FIBRE BUNDLES.

The term periodontium is a collective noun used to describe the various supporting tissues of the teeth, such as cementum, periodontal ligament, supra-alveolar tissues and alveolar bone. There has been a tendency to regard and study the periodontium on a regional basis. For example, Sicher's (1942) study of the intermediate plexus was confined to the periodontal ligament, Edwards' (1968) work involved mainly the supra-alveolar gingival tissues, and Glenwright (1970) devoted an investigation to the circular and longitudinal gingival fibres in the rhesus monkey. This regional approach to the study of these tissues may have diverted attention from tissue patterns involving the whole of the periodontium.

Quigley (1970) studied the molar teeth of mice and hamsters, and claimed to have shown that all the perforating (Sharpey's) fibres passed completely through the interdental septum. These findings, which were from sections cut in the mesiodistal plane, were only partially confirmed by this investigation, in which few trans-alveolar fibres have been demonstrated in the apical and furcal regions of sections from the mesiodistal plane.

Cohn's investigations (1966, 1972) of mice and marmosets were also confined to the mesiodistal plane, and his conclusions did not differentiate between the two animals. His depicted results showed a regularity and symmetry of pattern not seen in the present study. (Fig. 21 p.4.3). The arc formation reported by Cohn was not consistently apparent in this study, and frequently, the arcial arrangement was present but in an inverted form. Furthermore, the point of entry of the fibres was rarely at the same level on both sides of the alveolar bone. All sections studied demonstrated more variation in the overall patterns of arrangement of the collagen fibre bundles than was indicated in Cohn's description of his findings.

Although there are differences in the detail of the present observations and those of Cohn (1966, 1972) and Quigley (1970) the same phenomenon has been demonstrated in each of these studies. This common finding is that in the mesiodistal plane, there is collagen fibre bundle connection through the alveolar bone, between the roots of adjacent teeth and between the roots of the same tooth.

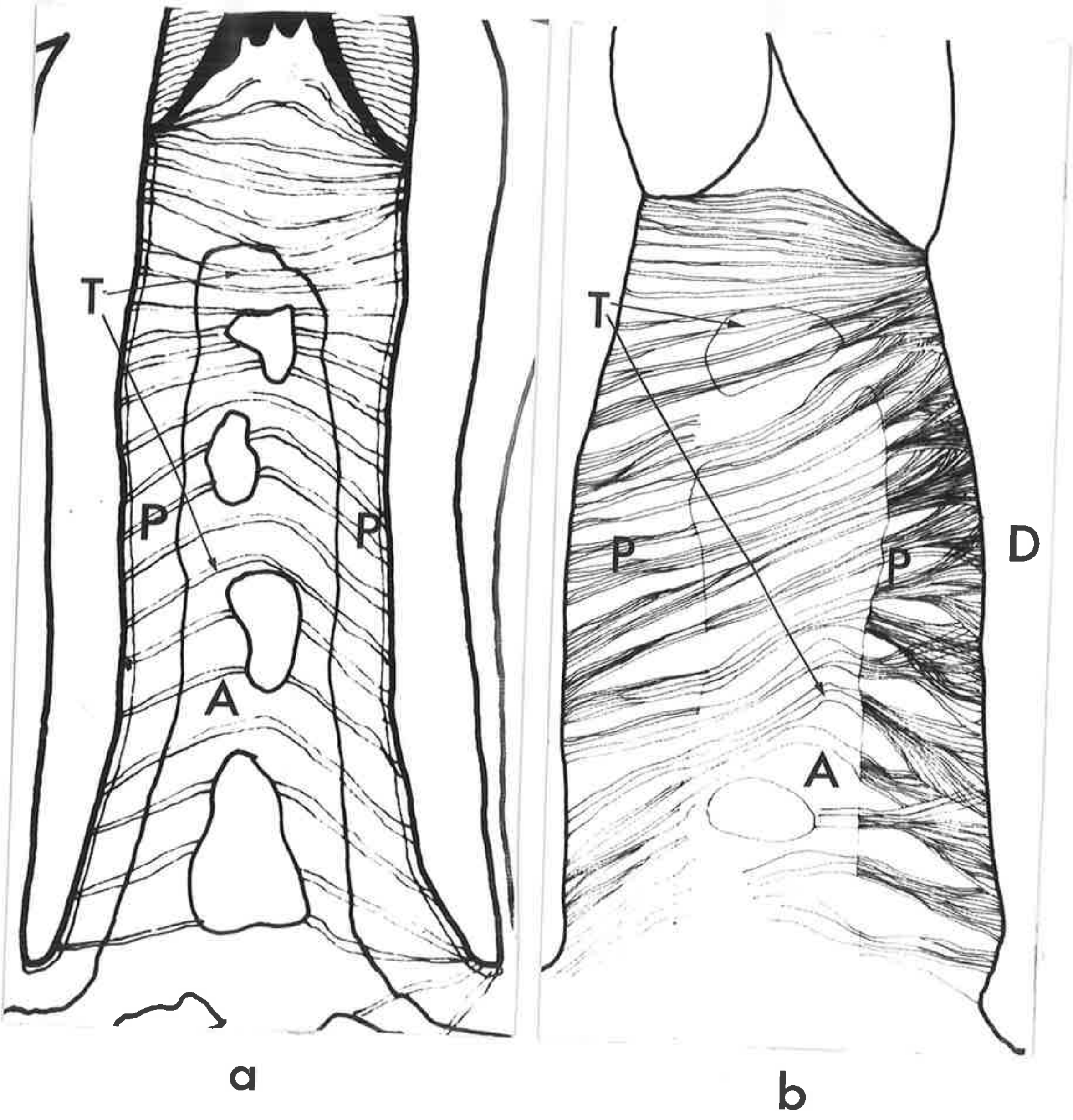

FIG. 21.

Diagrams demonstrating the findings of Cohn (a) and those of this study (b). Both diagrams represent the interdental region of the first and second molars or of second and third molars of the mouse mandible. A, interdental alveolar bone; D, dentine of the mesial root of the second molar; P, principal collagen fibre bundles; T, transalveolar collagen fibre bundles. Localised variation in patterns of collagen fibre bundle arrangement were not depicted by Cohn.

Previous investigations (Cohn 1966, 1972 and Quigley 1970) have used sections from the mesiodistal plane. In the present study, the inclusion of sections from the buccolingual and the horizontal planes has made possible a three dimensional appreciation of the anatomy and patterns of arrangement of the collagen fibre bundles of the mandibular molar alveolar process of the mouse. Direct connection through the bone has been seen between the periodontal ligament and the connective tissue of the periosteum. This has been demonstrated on the buccal and lingual sides of the teeth and also in the alveolus mesial to the first molar. Cohn (1966, 1972) and Quigley (1970) made no reference to findings in the region mesial to the first molar.

The presence of transalveolar fibres in the mouse suggests that the principal collagen fibre bundles of the periodontium may have a greater influence on the anatomical and functional interrelationship of adjacent teeth of mice than was previously thought. The presence of these fibres in the mouse further suggests that there may be inadequacies in some current concepts of the mechanisms of human tooth support, which have been proposed by such authors as Gianelly and Goldman (1971), Glickman (1972), and Melcher and Eastoe (1969).

It is contended by these workers that collagen fibre bundles of the periodontium connect the cementum to the alveolus by insertion into the alveolar wall. They also assert that bundles from the gingival region of the cementum pass over the alveolar crest and directly connect the cementum of adjacent teeth, and that there is a clinically important group of fibre bundles which pass from the cementum of the teeth into the gingival connective tissue. These authors' descriptions of periodontal collagen fibre bundles do not include reference to direct connection of adjacent teeth except by the transseptal collagen fibre bundles.

In the mouse mandible, the presence of transalveolar collagen fibre bundles indicates that functional force applied to a particular tooth would be transmitted more readily than has been thought. Therefore, this force could be expected to have a greater influence on other teeth of the same group. Functional forces would also appear to have a greater effect on the periosteum of the alveolar process, since the passage of transalveolar fibres through the buccal and lingual bone may increase the strength and area of connection between the periodontal ligament and the periosteum.

The anatomy of the teeth of mice is different to that of human teeth, and the action of mastication is confined to anteroposterior and vertical movements. That is, the lateral movements of human mastication do not occur in the mouse. These anatomical and functional differences do not permit direct analogy of the dentitions of the human and of the mouse.

However, the work of Cohn (1975) and Edwards (1976) on human material has shown the presence of some transalveolar collagen fibre bundles in the buccal and lingual bone, and in the crestal region of the interdental alveolar bone. These findings, together with results obtained from animals by Cohn (1966, 1972), Quigley (1970) and the present author, indicate that the role of the principal collagen fibre bundles in human tooth support may be more complex than has been realised.

It also appears that changes in the direction and magnitude of forces acting on the teeth would have an affect throughout the alveolar bone and not only at the surfaces in apposition to the periodontal ligament. This hypothesis is supported by the work of Noble and Martin (1973) who, in experiments to determine the effects of occlusal interference on individual teeth found that changes occurred in the mobility of teeth adjacent to those which were directly involved.

It has been shown in humans (Reitan 1969) that pressure from the root of a tooth causes a slight deflection of the bony wall during normal function. A factor which prevents the permanent bending of bone is that the hydroxyapatite crystals are laid down in a network of collagen fibres which reestablish their normal spatial relationships as soon as the pressure is relieved. The ability of alveolar bone to recover from functional distortion may be increased by the unique presence of transalveolar collagen fibre bundles.

Grimm (1972) and Picton (1965) have shown that bending of crestal bone is detectable with forces of less than 50 grammes. The present author proposes that the distribution of deforming forces in the crestal region is more rapid and more widespread because of the presence of transalveolar collagen fibre bundles.

The findings of bone deformation were also consistent with the hypothesis that strain in bone may be one of the mediating processes which transform an extrinsic force to cellular bone response (Grimm 1972). That is, remodelling changes observed throughout alveolar bone are more readily explained by the implication of transmitted strain in the bone. The transmission of strains may be one of the functions of transalveolar collagen fibre bundles.

The development of methods of minimising the occurrence and degree of relapse of orthodontically moved teeth has been the objective of many investigations. The results of these investigations and their clinical application have been explained by reference to the effects of the supraalveolar collagen fibre bundles and the removal of these effects by certain surgical procedures. (Billberg 1971, Boese 1969, Brain 1969, Edwards 1970, Reitan 1959, Reitan 1967).

Although it has been contended that the action of the supraalveolar collagen fibre bundles is the only intrinsic factor involved, no investigator has demonstrated that surgically induced cessation of this action completely eliminates forces of relapse from within the alveolar process. This discrepancy in cause and effect indicates that there are forces present, the source of which has not been recognised.

If in the human, there are fibres which are analogous to those seen in the mouse, this would help to explain the residual relapse tendency which still exists after surgical interference with the integrity of the supraalveolar collagen fibre bundles.

Sims (1973) described a mesiodistal network of oxytalan fibres in the mandibular molar region of the mouse. He contended that this network provided an uninterrupted oxytalan fibre system spanning the entire molar segment. The orientation, pattern of arrangement and function of the oxytalan fibres and the collagenous fibre bundles are dissimilar. However, both fibre systems unify the teeth and periodontal tissues of the entire molar segment. These observations support the proposal that groups of teeth and their supporting tissues should be regarded as developmental and functional units.

The above view has been expressed by Baume (1956) who made an histological and roentgenographic analysis of the growth and development of the alveolar process of the Rhesus monkey. Baume's investigation was suggested by his observation of patterns in the occurrence and locations of periodontal lesions. Baume hypothesised that these patterns could be ascribed to previous interference with the development of the individual teeth and periodontium with consequent premature degeneration. His findings demonstrated that the teeth and their supporting tissues should be regarded in groups which developed and functioned together as units.

B. PRINCIPAL COLLAGEN FIBRE BUNDLE MORPHOLOGY.

Mesiodistal and horizontal sections of the molar region of the mouse mandible demonstrate a constant morphological difference between the principal collagen fibre bundles on the mesial side, and those on the distal side of the roots of the teeth. It is thought by the present author that this observation may be of functional and physiological significance in relation to the constant phenomenon of distal migration of the molar teeth of the mouse. (Zwarych and Quigley 1965).

Consideration of the findings in the present investigation leads the author to propose that these phenomena may be associated in the following way. The fibre bundles are of greater thickness at the distal alveolar surface than at the mesial alveolar surface. It is therefore suggested that the horizontal vector of occlusal forces may produce a different functional effect on the distal surfaces than on the mesial surfaces of the interdental alveolar bone.

This variation in functional effect may be related to the different patterns of bone deposition and resorption associated with the migration of teeth in the mouse, and seen in other animals including man.

Of the several theories of the mechanism of tooth eruption, the one which receives most credence is the theory of traction from the principal fibres of the periodontal membrane (Ten Cate 1969). This theory has been supported experimentally. For example, in animals with experimentally induced lathyrism in which condition the collagen formation is disorganised, the rate of eruption is reduced. Furthermore, the rate of eruption is unimpeded when the growing base of the tooth has been destroyed.

One of the theoretical bases of this concept of tooth eruption, is that there is within the periodontal ligament, a generation of force in a vertical (eruptive) direction by the process of fibrillogenesis (Thomas 1965 cited by Ten Cate 1969). This theory is also pertinent to this discussion.

Fibroblasts secrete tropocollagen into the so-called extracellular compartment, where polymerization into a fibrous form occurs. It is assumed that when the tropocollagen macromolecules are secreted, they exist in a relatively disordered and mobile state. The macromolecules approach each other, and become aligned in a state of order. It is argued that the decrease in disorder of the tropocollagen macromolecules will produce a force along the axis of the orientating fibrils to prevent the macromolecules from returning to their disordered state.

The system of aggregated macromolecules eventually becomes stabilised by the formation of intramolecular and intermolecular cross-links. During this phase of stabilization, there is a decrease of more than ten per cent of the molecular length of the tropocollagen unit (Olsen 1963). To this contraction can be added a shrinkage factor due to dehydration as lateral aggregation of the adjacent helices takes place (Tomlin and Worthington, 1956).

It is thought that tensions arising from these sources are dissipated by the movement of tooth eruption.

A horizontal vector of the theoretic forces of collagen fibril contraction can be considered in relation to the findings of the present study. It can be postulated that the different arrangement of the collagen fibre bundles on the mesial and on the distal sides of the interdental alveolus could cause the application of different magnitudes of force on each surface of the interdental bone. This force difference may be sufficient to produce different reaction by the bone forming and bone resorbing elements adjacent to these surfaces, which could result in the movement of teeth known as migration or approximal drift.

These findings are of interest as they may relate to the concepts of approximal drift and common direction of tooth movement proposed by Brodie (1934) Dreyer (1965), Picton and Moss (1973, 1974). Brodie stated that the teeth of mammals maintained approximal contact as they migrated towards a common centre, and that the teeth of rodents migrated towards a centre distal to the teeth.

The investigations of Sicher and Weinmann (1944) and of Kronman (1970) supported Brodie's theory. Sicher and Weinmann demonstrated the intermittent distal and slightly buccal movement of molar teeth of the rat and, by labelling alveolar bone increments with alizarin-S, they were able to show that the rate of drift was 60 to 90 micrometres per week. Kronman showed that the teeth of golden hamsters also moved distally.

Dreyer (1965) conducted experiments with rats, in order to determine the response of the jawbones to forces transmitted through the molar teeth during mastication. He concluded that the angulation of roots to the occlusal surfaces of the molar teeth of rats determined the resultant of the functional forces acting on the teeth, and that in the human, this resultant or anterior component of force plays an important part in the mesial movement of teeth, and is responsible for the maintenance of interdental contact. In the present investigation the roots of the mandibular molars of the mouse did not show angulation to which a resultant distal vector of functional forces could be attributed.

The theories of Dreyer were not supported by the work of Moss and Picton (1967, 1970, 1974) who produced experimental mesial drift in adult monkeys. In one of these studies (1967) movement of teeth of the buccal segments was produced by removal of contacts between adjacent teeth. The rate of drift of teeth in normal occlusion was compared with that of contralateral teeth, the opponents of which had been extracted. On both sides of the arch in all animals the amount of drift was the same.

Moss and Picton concluded that any anterior component of occlusal force was not the cause of mesial drift. They also contended that approximal drift of teeth was produced by the action of the supraalveolar collagen fibre bundles. The present author proposes that transalveolar collagen fibre bundles, in the mouse, may supplement the action of the supraalveolar collagen fibre bundles.

In the human periodontium Edwards (1976) observed a morphological variation of the principal collagen fibres which was similar to the variations seen in the mouse by the present author. Edwards demonstrated in the human that greater thickness of principal collagen fibre bundles occurs on the mesial side of the interdental alveolus. The location of this morphological variation in the human is opposite to that observed in the mouse. However, the direction of physiological migration of teeth in the molar region of the human dentition is opposite to the direction of migration of analogous teeth in the mouse.

It was observed by Melcher and Walker (1976) that the periodontal ligament remained attached to teeth extracted from mice, rats and monkeys. This indicated that attachment of the ligament to the tooth is stronger than its attachment to the alveolus. A similarly interesting observation of the present investigation was that rupture and fragmentation of sections occurred more frequently in the periodontal ligament adjacent to the mesial surface of the interdental bone. This apparent difference in tensile strength may be indicative of structural variation on opposite sides of the interdental alveolus, and would support the histomorphological evidence of such a variation.

CONCLUSIONS.

1. In the molar region of the mouse mandible many collagen fibre bundles pass through the interdental, the interradicular and the buccal and lingual plates of alveolar bone. These transalveolar fibre bundles are continuous with the principal collagen fibre bundles of the periodontal ligament, and form a direct connection between the roots of adjacent teeth. Such connection through alveolar bone also exists between the mesial and distal roots of individual teeth and between the roots of the teeth and the mucoperiosteum.

2. This and other recent studies of animals and humans, have shown that functionally and developmentally the teeth should be considered in groups and not as individual units of tooth and periodontium.

3. There are differences in the patterns of arrangement of the principal collagen fibre bundles on the mesial and distal sides of the interdental and interradicular alveolar bone in the molar region of mice. A recent study of the human alveolar process has demonstrated an analogous variation, and it is concluded that this phenomenon may be related to physiological drift of teeth in man and animals. The directions of drift in the mouse and in the human are opposite, and this is thought to be consistent with the observation that the variation seen in the mouse is the reverse of that

seen in man.

4. Any hypotheses relating to the physiological movement of teeth or to the movement of teeth during or after orthodontic treatment, should acknowledge the possible existence and influence of transalveolar collagen fibre bundles.

APPENDIX.

A. PREPARATION OF SPECIMENS:

Fixation -

Ten Per Cent Bufferred Neutral Formalin

37 - 40 per cent formalin	100.0 ml
distilled water	900.0 ml
sodium phosphate monobasic	4.0 gm
sodium phosphate dibasic (anhydrous) .	6.5 gm

Decalcification -

Formic Acid - Sodium Citrate Method

1. Place the specimen in large quantities of formic acid-sodium citrate solution until decalcification is complete. The solutions should be changed daily.

   Solution A.

sodium citrate	50.0 gm
distilled water	250.0 ml

   Solution B.

formic acid 90 per cent	125.0 ml
distilled water	125.0 ml

   Mix solutions A and B in equal portions.

2. Wash in running water from 4 - 8 hours.

Tissue Processing -

After decalcification the specimens were processed at 37°C, using the double embedding method of Peterfi (Culling 1974). Double embedding is the name given to a technique whereby the tissue is first impregnated with celloidin, and subsequently blocked in paraffin. In this way, the advantages of both materials are combined. The process involves the following stages:

(1)	70 per cent alcohol (ethanol)	overnight
(2)	80 per cent alcohol	one hour
(3)	90 per cent alcohol	one hour
(4)	95 per cent alcohol	one hour
(5)	absolute alcohol	one hour
(6)	absolute alcohol	one hour
(7)	absolute alcohol	one hour
(8)	one part absolute alcohol 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

These stages were followed by embedding in paraffin as follows:

(1)	two thirds methyl salicylate one third paraffin wax	one hour
(2)	one half methyl salicylate one half paraffin wax	one hour
(3)	one third methyl salicylate two thirds 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 vacuumed in paraffin wax at 56°C for fifteen minutes prior to blocking in paraffin, using the "Tissue Tek II Tissue Embedding Centre" machine.

B. STAINING TECHNIQUES.

BOHACEK AND GUPTA.

A COLLOIDAL IRON STAINING METHOD.

From the American Journal of Medical Technology (1968)

1. Remove paraffin in Xylol, pass through alcohols down to water.
2. Immerse slides for one minute in 3 per cent acetic water.
3. Without rinsing, immerse slides in equal parts of 5 per cent dialyzed iron.
4. Rinse well in several changes of distilled water.
5. Immerse slides for 10 to 20 minutes in a freshly prepared solution consisting of equal parts of 2 per cent potassium ferrocyanide and 2 per cent hydrochloric acid. (The control is included at this step).
6. Wash thoroughly in distilled water.
7. Counterstain using either 0.1 per cent safranin for two minutes or the Van Gieson method.
8. Dehydrate with alcohols, clear in xylene and mount.

Results

1. With safranin as counterstain: acid mucopolysaccharides, bright blue; nuclei, red; collagen light red; mast cells, bright blue.
2. Using Van Gieson method: acid mucopolysaccharides, bright blue; nuclei, brownish black; collagen, red; mast cells, bright blue.

GOMORI.

A ONE STEP TRICHROME METHOD

From Luna (1968).

Bouin's Solution

Picric acid saturated aqueous solution ..	750.0 ml
37 - 40 per cent formalin	250.0 ml
Glacial acetic acid	50.0 ml

Trichrome Stain

Chromotrope 2 R	0.6 gm
Aniline blue (or light green, SF yellowish)	0.3 gm
Glacial acetic acid	1.0 ml
Phosphotungstic acid	0.8 gm
Distilled water	100.0 ml

Staining Procedure

1. Deparaffinize and hydrate to distilled water.
2. Place in Bouin's solution at 56°C for one hour
3. Wash well in running water or until yellow colour disappears.
4. Stain nuclei with Weigert's iron haematoxylin or Gomori's chromium haematoxylin solution for 10 minutes.
5. Wash in water.
6. Trichrome stain for 15 - 20 minutes.
7. Place in 5 per cent acetic water for 2 minutes. If sections too dark, differentiate in 1 per cent glacial acetic water to which 0.7 gm of phosphotungstic acid has been added. Rinse in distilled water.

8. Dehydrate in 95 per cent alcohol, absolute alcohol, and clear in xylene - 2 changes each.
9. Mount with Histoclad.

Results

Muscle fibres - red

Collagen - green (or blue with aniline)

Nuclei - blue to black.

GORDON AND SWEET.

A METHOD FOR SILVER IMPREGNATION

From Culling (1974).

Special solutions required

1. Silver solution - to 5 ml of 10.2 per cent aqueous silver nitrate add strong ammonia drop by drop until the precipitate which is first formed is just dissolved. Add 5 ml of 3.1 per cent sodium hydroxide. Add strong ammonia drop by drop until the precipitate is just dissolved (the solution should not be completely clear). Make up the solution to 50 ml with fresh distilled water.
2. Acidified potassium permanganate -

0.5 per cent potassium permanganate ..	95 ml
3.0 per cent sulphuric acid	5 ml

Method

1. Dewax, bring to water.
2. Oxidise in acidified pot. permanganate 1 - 5 minutes (4 minutes).
3. Wash in water.
4. Bleach in 1 per cent oxalic acid for two minutes.
5. Wash well in distilled water.
6. Mordant in 4 per cent iron alum for fifteen minutes. (Make silver solution at this stage).
7. Wash quickly - 1 dip in distilled water.
8. Treat with silver solution until section is

transparent (30 seconds to 1 minute).

9. Quick rinse in distilled H_2O.
10. Reduce in 10 per cent formalin for 1 - 2 minutes.
11. Wash in tap water then distilled water.
12. Tone in 0.2 per cent gold chloride for 5 minutes.
13. Rinse in distilled water.
14. Fix in 5 per cent hypo for 5 minutes.
15. Wash in water 1 - 2 minutes.
16. Counterstain with 0.25 per cent neutral red.
17. Dehydrate clear and mount.

Results

Collagen fibres black.

MASSON.

A TRICHROME STAINING METHOD

From Luna (1968).

Solutions

Bouin's Solution.

Weigert's Iron Haematoxylin Solution.

Biebrich Scarlet-Acid Fuchsin Solution -

Biebrich scarlet, aqueous 1 per cent	90.0 ml
Acid fuchsin, aqueous 1 per cent	10.0 ml
Glacial acetic acid	1.0 ml

Phosphomolybdic-Phosphotungstic Acid Solution -

Phosphomolybdic acid	5.0 gm
Phosphotungstic acid	5.0 gm
Distilled water	200.0 ml

Aniline Blue Solution -

Aniline blue	2.5 gm
Glacial acetic acid	2.0 ml
Distilled water	100.0 ml

2 per cent Light Green Solution.

1 per cent Glacial Acetic Acid Solution -

Glacial acetic acid	1.0 ml
Distilled water	100.0 ml

Staining Procedure

1. Deparaffinise and hydrate to distilled water.
2. Mordant in preheated Bouin's solution for one hour at 56°C or overnight at room temperature if formalin fixed.
3. Cool and wash in running water until yellow

colour disappears.

4. Rinse in distilled water.
5. Weigert's iron haematoxylin solution for ten minutes. Wash in running water for ten minutes.
6. Rinse in distilled water.
7. Biebrich scarlet-acid fuchsin solution for two minutes. Save solution.
8. Rinse in distilled water.
9. Phosphomolybdic-phosphotungstic acid solution for ten to fifteen minutes before aniline blue solution. (Aqueous phosphotungstic acid 5 per cent for fifteen minutes before light green counterstain). Discard solution.
10. Aniline blue solution for five minutes or light green solution for one minute. Save solution.
11. Rinse in distilled water.
12. Glacial acetic acid solution for three to five minutes. Discard solution.
13. Dehydrate in 95 per cent alcohol, absolute alcohol, and clear in xylene, two changes each.
14. Mount with Permount.

<u>Colour Results</u>

Nuclei - Black
Cytoplasm, Keratin, Muscle Fibres and Intercellular Fibres - Red
Collagen - Blue

NAOUMANKO AND FEIGIN.

A SILVER SOLUTION FOR STAINING RETICULIN

From Stain Technology (1974).

1. Sections deparaffinised and hydrated to distilled water.
2. Immerse in solution containing 45 ml 0.25 per cent potassium permanganate and 0.5 ml of 0.67 per cent acetic acid in distilled H_2O for two minutes.
3. Rinse in distilled H_2O for fifteen seconds.
4. Immerse in 1 per cent oxalic acid for two minutes.
5. Rinse - two changes of distilled H_2O: one minute each.
6. Immerse for 6 minutes or longer in a solution made by adding in order:

ammonium nitrate (8 per cent)	-	7.0 cc.
distilled H_2O	-	35.0 cc.
NaOH (4 per cent)	-	8.0 cc.
$AgNO_3$	-	3.8 cc.

7. Rinse briefly in 70 per cent ethyl alcohol (one slow dip).
8. Immerse successively in two changes of .2 per cent formalin, briefly and with constant motion in the first, longer in the second - for a total of two minutes.
9. Rinse with distilled water - one minute.
10. Immerse $AgCl_2$ (1 per cent) for one minute.
11. Rinse with distilled H_2O for one minute.

12. Immerse in 5 per cent sodium thiosulphate (hypo) for one minute.
13. Rinse in distilled H_2O for one minute.
14. Dehydrate and mount.

Results -

Collagen fibres black.

VAN GIESON.

A METHOD FOR STAINING COLLAGEN

From Luna (1968).

Solutions - Weigert's iron haematoxylin

Solution A:

haematoxylin crystals	1.09 m
alcohol 95 per cent	100.0 ml

Solution B:

ferric chloride, 29 per cent aqueous	4.0 ml
distilled water	95.0 ml
hydrochloric acid, concentrated	1.0 ml

Working Solution:

equal parts of Solution A and Solution B.

Van Gieson's solution:

acid fuchsin 1 per cent aqueous	2.5 ml
picric acid, saturated aqueous	97.5 ml

Staining procedure:

(1) deparaffinize and hydrate to distilled water

(2) Weigert's haematoxylin solution for ten minutes

(3) wash in distilled water

(4) Van Gieson's solution for one to three minutes

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

(6) mount with Permount or Histoclad.

Results

Collagen stains red.

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