VASCULAR MORPHOLOGY OF RAT MOLAR PERIODONTIUM

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ERRATA

p viii	line 16	kindlova should be Kindlova
p 3.3	line 23	altough should be although
p 3.6	line 7	Lift should be Luft
p 3.6	line 26	bipsied should be biopsied
p 3.20	line 19	prevalant should be prevalent
p 3.53	line 9	ude should be due
p 3.76	line 21	steriological should be stereological
p 4.4	line 5	legnth should be length
p 4.11	line 20	5" x 6" should be 12.5cm x 15cm
p 5.2	line 16	sampe should be sample

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SUMMARY

The periodontal ligament is a unique joint that is able to withstand enormous forces without damage. Many investigations have been conducted to determine the normal morphology of this gomphosis as a prelude to experimentation designed to clarify the mechanisms that enable the ligament to function in the presence of severe mechanical insult. The fibrillar and cellular components of the ligament have received most attention. The vascular component and the ground substance have been investigated,but a thorough review of the literature reveals that there are many aspects of the periodontal microcirculation that remain without any information or are, at best, unconfirmed. This project was designed to reassess the information available on the vascular architecture of the rat molar periodontium. In addition, a rudimentary classification of the blood vessels present was attempted. A corrosion casting technique was selected because it enabled complete three-dimensional imaging of the vascular network to be obtained. This technique had last been used to investigate rat molar periodontium in 1962 (kindlova and Matena) and at that time a number of technical difficulties allowed only an incomplete description. These difficulties were resolved by adopting the vascular casting technique proposed by Murakami (1971, 1975) with modifications by Gannon (1981) and examining the casts in the scanning electron microscope (SEM).

Male Sprague-Dawley rats approximately 9 weeks old were cannulated via the common carotid arteries. Blood was washed out by cutting the external jugular veins for fluid egress and perfusing a saline solution through the arteries. This solution contained papavarine (10^{-7}gm/ml) heparin (10 IU/ml) and PVP40 (to make a blood colloid pressure of 25mm Hg), and was warmed to 37^{o}C. At the completion of blood washout,prepolymerized methyl methacrylate was introduced. When polymerization was complete, both hard and soft tissues

were corroded using a sequence of KOH (20%), HCl (10%), enzymes and ultrasonic cleaning. The specimens so obtained were rendered conductive using osmium tetroxide vapour and gold coating and viewed in the scanning electron microscope. Stereo-pair photomicrographs were obtained and morphological descriptions were made from the three-dimensional images produced with an appropriate viewer.

The findings highlighted the vascular architecture of the different regions of the rat molar periodontium. The ligament proper contained capillaries and postcapillary venules arranged in occluso-apically oriented tracts. Arterial elements were rare in the ligament. The vessels anastomosed freely with both the gingival network and the plexus in the alveolar bone. Around the perimeter of the socket,the vessels ran uninterrupted from the apex to the coronal extremity. Over the interradicular septum,the vessels arose from rami of the alveolar network and coursed only 200-400 micrometres in an occluso-apical direction,before re-entering the bone. A concentration of larger venous elements was found at the crest of the interradicular septum.

The network at the gingival crevice consisted of a flat capillary plexus extending from the cemento-enamel junction up to the crest of the free gingival margin. From within the middle third of this plexus arose a number of twisted vascular loops,consisting predominantly of postcapillary venules. In the interproximal col,the vascular loops became quite complex structures resembling the arrangement of vessels in the kidney glomeruli or the intestinal villi. The alveolar bone medulla contained a plexus of vessels that primarily served to supply and drain the ligament vessels. Very few microcirculatory vessels devoted to the service of the bone marrow itself were noted.

It was proposed that the occluso-apical orientation of ligament vessels was the most suited to the maintenance of the patency of the blood vessel lumen during intrusion of a tooth into its socket. The role of the vascular

loops in the production of crevicular fluid was discussed, but the arrangement suggests that the vessels may play a role in countercurrent exchange which, in other organs, is suited to maintenance of homeostasis or the actual absorption of substances into the blood stream. Some of the theories explaining the role of the vascular architecture in the dissipation of functional forces were supported by the observations in this project. However, other theories were not confirmed.

The morphological descriptions provided in this project establish a sound basis for further research into the mechanisms of dissipation of functional forces. It is suggested that the vascular casting technique can be utilised to obtain additional information in this area.

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I dedicate this project to my children, Thomas and Lucinda, who have been my constant source of inspiration. To dear Helen, I also extend my thanks for her support.

STATEMENT

This 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 this report contains no material previously published or written by another person except when due reference is made in the text of the report.

W.T. WEEKES

SECTION 1

INTRODUCTION

The vasculature of the periodontal ligament has been the subject of a number of research investigations over the years but has received less attention than the cellular and fibrillar elements of the ligament. Many of the projects have been able to provide a superficial insight into the vascular architecture of the ligament, but few publications have attempted to categorize the vessel types. Material from humans has been reported infrequently and reliance has had to be made on animal models, particularly the rat and the monkey.

Not only is the blood supply important for nutritional purposes, it is also implicated in other roles. The exact mechanism that enables a tooth to withstand the severe functional forces applied to it still remains somewhat of an enigma. A number of reports have shown that the blood vessels make a significant contribution to the dissipation of potentially damaging forces (Wills, Picton and Davies 1976, Walker, Ng and Burke 1978, Ng, Walker, Zingg and Burke 1981). This functional requirement of the vascular bed has no counterpart elsewhere in the body - most stress bearing structures or joints between two hard tissue components consist of avascular articular surfaces. Bone resorption and deposition require increased nutrition over resting tissues and it has been shown that blood vessels are intimately related to both resorptive and depository surfaces (Bernick 1962). In addition to these functional moieties, Sims (1983) has described an arrangement within the ligament where the blood vessels bear a close association with oxytalan fibres and nerves. The exact function of this arrangement has not been determined, but it may be related to control of the vascular system via proprioceptive mechanisms. Packman, Shoher and Stein (1977) provided evidence of a vascular autoregulatory phenomenon in the ligament and this may be associated with the structures found by Sims. The gingival crevice has been shown to produce an

exudate, particularly in the presence of inflammation, that is said to arise from the blood vessels. This exudate can provide protection to the gingival tissues by removing noxious agents from the crevice (Cimasoni 1974).

It is obvious that the vasculature of the periodontal ligament serves a variety of functional needs. Precise information on blood vessel architecture and a categorization of the vessel types present in the ligament is necessary before an accurate understanding of the functions of the ligament can be achieved. To date many aspects are still unknown or at best remain unconfirmed.

Very early research work provided a description of arteries only (Hayashi 1932). Venous drainage was described by Cohen (1959),but usually the vessels in the ligament microvascular bed were described as no more than capillaries and "blood vessels". More concrete categorization was provided by Kindlova and Matena (1962),who described the course of arteries and veins. However, it was not possible to confirm their descriptions from the illustrations and the schematic diagram that they presented did not appear to be a realistic representation in some areas. India ink perfusions of laboratory animals, particularly the rat, constituted the majority of the research work on periodontal vasculature. The descriptions of vascular architecture provided were brief and rather vague and blood vessel categorization was rarely attempted by researchers using this technique.

It was felt necessary, therefore, to conduct an investigation that was able to provide information on vascular architecture as well as allow a detailed classification of vessel types to be made. Workers in other fields using a vascular casting SEM technique (Murakami 1971, Nowell and Lohse 1974, Hodde, Miodonski, Bakker and Veltman 1977, Hodde and Nowell 1980, Gannon 1981, Hodde 1981) seemed to be able to provide for other organs the sort of information that was lacking in previous research into the periodontal ligament. This technique was adopted in this project to study the vascular system of the rat molar periodontium. The rat was chosen because of its

ease in handling and because the molars in the maxilla and mandible are claimed to resemble human molars both morphologically and functionally (Schour and Massler 1971),even though they possess a greater number of roots.

SECTION 2

PROJECT AIMS

It was proposed that this project be an investigation into the vasculature of the rat molar periodontium. Recently developed techniques using methyl methacrylate vascular casts viewed in the SEM have enabled both precise descriptions of vascular architecture and reasonably accurate categorizations of vessel types to be made. These techniques have not previously been applied to the rat molar periodontium and it was aimed to utilize them in this project so that deficiencies in our knowledge of vascular supply to the periodontium could be upgraded.

Specific aims were:

(1) to re-evaluate the findings of earlier investigations, particularly those presented by Kindlova and Matena (1962)

(2) to provide a description of the vascular architecture previously poorly defined, in particular

  (a) the occurrence of any regional variation in the periodontal ligament

  (b) the arrangement of the interproximal col region

  (c) anastomoses between gingival and ligament networks

  (d) the arrangement of vessels in the alveolar bone medulla in both the interdental and interradicular regions

  (e) the presence of "glomi" within the ligament or gingival crevice regions

  (f) anastomoses between periodontal ligaments of adjacent teeth, either over or through the interdental septum

(3) to categorize the vessels present in the various components of the periodontium

(4) to discuss the significance of the findings in relation to the unique function of the periodontium and the periodontal ligament in particular

It was anticipated that this project would establish a sound morphological model of rat molar periodontal vasculature. This knowledge would then provide a proper foundation on which to base research into the functional mechanics of the periodontium, especially the ability of the ligament to withstand high loads without damage - a feature of particular interest to orthodontists.

SECTION 3

REVIEW OF THE LITERATURE

The normal vasculature of the periodontium has been studied in man and a variety of animals, with the majority of work being conducted on animal models. Animals used have been the monkey, rats, mice, hamsters, cats and dogs. A number of different techniques have been employed to elucidate the vascular architecture. The two most popular methods have been either histological examination or perfusion with a substance that fills the lumen of the blood vessels so that they can be made visible. It is proposed in this literature review to examine first the findings of the histological and ultrastructural studies and then to follow with a resume of the results of the different perfusion techniques. Once the techniques have been described and the findings reported, a comparison will be made between the architecture of different animal models.

HISTOLOGIC AND ULTRASTRUCTURAL INVESTIGATIONS

The first comprehensive examination of periodontal vascularization using a histologic examination was carried out by Carranza, Itoiz, Cabrini and Dotto in 1966. They used Wistar rats, C3H Mice, hamsters, guinea pigs, cats and dogs for their investigation and observed blood vessels after using the Wachstein and Meisel technique for demonstration of adenosine triphosphatase activity. They reported that the gingival blood supply in rodent molars arose mainly from mucosal vessels and was, to a certain extent, independent of the blood supply to periodontal ligament and alveolar bone. Vascular loops were in close association with the crevicular epithelium (epithelial cuff) forming a dense circumferential plexus. The gingivae, both marginal and attached, were very vascular,with frequent anastomoses between epithelial and subepithelial blood vessels (figure 1).

In the periodontal ligament,larger blood vessels ran parallel to the

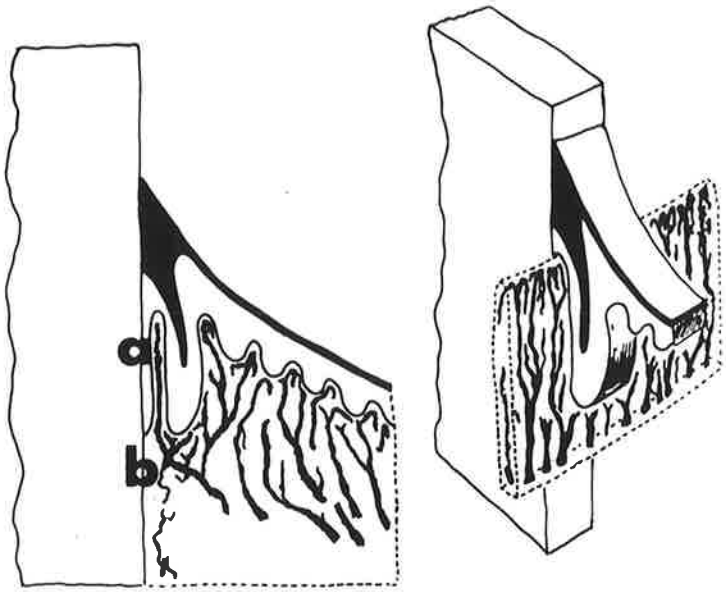


Fig. 1. Schematic representation of blood supply to rodent gingiva. **a:** vascular loops against the epithelial cuff. **b:** connections between gingival and periodontal blood vessels.

Figure 1 Schematic representation of blood supply to rodent gingiva (from Carranza, Itoiz, Cabrini and Dotto 1966).

long axis of the root,giving out irregular branches that intertwined forming a plexus around the root. Collateral supply to the ligament came via the alveolar bone, particularly in the middle and apical thirds. These vessels entered the ligament at right angles to it and immediately bent to take a course parallel to the long axis of the tooth. The presence of horizontal communicating branches between these principal vessels was not reported. The blood vessels were closer to the bone than to the cementum. In rat and mouse molars,there were connections between the periodontium and the pulp, mainly in the apical third and bifurcation areas. The periodontal vasculature of the guinea pigs showed significant variation due to the fact that the molar is a continuously erupting tooth. Since this pattern of continual eruption does not resemble the situation present in the human dentition, and other animals, the particular features of this system will not be discussed.

Although Carranza et al. described the general arrangement of blood vessels in the various animals, they did not attempt to identify the type of vessels they were describing. Thus, no mention of arterial, capillary or venous supply was made and so the architecture of the microvascular bed of the ligament was not defined.

A study using vital microscopy in conjunction with histology was conducted by Hock and Nuki (1971). They observed the morphology of normal and inflamed gingiva of ferrets, opossums, cats, dogs and rhesus monkeys. Their results seemed to indicate that all animals had comparable vascular patterns altbugh only specimens from the dog were illustrated.

In non-inflamed gingiva, the blood vessels lay in a well-organised network. The afferent supply terminated in straight vessels running directly beneath the marginal epithelium, parallel to the gingival margin. Efferent blood appeared to drain into vessels in attached gingiva. The width of the erythrocyte stream in the majority of vessels varied from 10 to 19 micrometres.

Main afferent and efferent vessels lay at 90 degrees to the gingival margin and were 20 to 45 micrometres in width. Numerous capillary vessels, 5 to 9 micrometres in width, connected afferent to afferent, afferent to efferent and efferent to efferent vessels. Reversal of flow was occasionally seen in certain vessels 10 to 19 micrometres wide, connecting afferent to afferent or efferent to efferent vessels.

Freeman and Ten Cate (1971) in their study of the development of the periodontium in mice, confirmed the observation that blood vessels occur closer to the alveolar bone than the root surface. Again, no classification of the vessel type was made.

Avery and his co-workers in 1975 were able to categorize "capillary" and "precapillary" vessels in the mouse periodontium. The capillaries were classified as type A-1-alpha (after Bennett, Luft and Hampton 1959), since no intracellular fenestrations were observed. The endothelial nucleus protruded into the lumen together with thin finger-like projections of the endothelial cell. The inter-cellular junctions of the capillaries were tortuous with occasional tight junctions. The vessels were completely surrounded by a distinct basement membrane and incompletely surrounded by pericytes. The precapillary vessels had an incomplete muscular coating and thicker endothelial cell walls, with a distinct basement membrane separating these two layers. Although Avery et al. did not discuss vascular architecture of the periodontal ligament, they did give an insight into the types of vessels present which is of major importance to the adequate identification of periodontal vasculature.

Sims in 1975 described the characteristic anastomosing vascular system in the mouse periodontium, adding further support for the architecture described by Carranza, Itoiz, Cabrini and Dotto (1966). Again, however, no attempt was made to detail the types of blood vessels present.

In 1976 Sims examined human material and confirmed that the bicuspid periodontal vasculature had a predominantly occluso-apical orientation. The

vessels were interrelated by a series of lateral communicating branches which resulted in complex anastomosing vascular system that included elliptical formations. It was suggested that this type of vascular intercommunication was ideally arranged to permit the rapid topographic differentiation of vascular flow according to the physiologic requirements of masticatory function. In 1977 Sims studied oxytalan meshwork associations in the periodontium of the mouse mandible and his observations of the vascular architecture of the mouse periodontal ligament were as follows:

(1) The principal vessels in the periodontal ligament had an occluso-apical alignment and were interconnected by means of lateral anastomosing branches.
Where the communicating branches were curved, the vascular system formed elliptical arrangements.

(2) The periodontal ligaments of individual molars were connected directly to each other via groups of vessels contained in canals through the interseptal bone.

(3) The above canals interconnected the teeth in a manner similar to the rat (Garfunkel and Sciaky 1971).

The ultrastructure of small blood vessels in the gingiva were described by Gavin and Trotter (1968). All the vessels that they found in the marginal gingiva of dogs and cats were capillaries, although some vessels classified as capillaries could have been venules. Ninety per cent of these vessels had a continuous endothelial basal lamina and the authors commented that, in the gingiva at least, the absence of capillary fenestration confounds the theory that the gingival crevicular fluid is a result of increased vascular permeability (Cimasoni 1974).

A similar region was investigated by Kindlova and Plackova in 1973 using both histological and ultrastructural examinations of the marginal gingiva of Wistar rats. They used a similar technique to Carranza et al. (1966) for the demonstration of adenosine tri-phosphatase activity in the

walls of vessels beneath the junctional and crevicular epithelium. Their findings supported those of Gavin and Trotter (1968) because typical features of fenestration of endothelial cells were not seen in capillaries supplying the junctional epithelium. However, intracellular pores and fenestrations were characteristic of the capillaries of the crevicular epithelium. Both papers provided similar vessel classifications using the criteria of Bennett, Lift and Hampton (1959) and Simon (1965).

Kindlova and Plackova described the presence of rich capillary loops below the junctional epithelium but not below the crevicular epithelium. They went on to say that there was evidence that the capillary loops under the junctional epithelium were characteristic of a newly formed capillary network. It was also suggested that these loops were not arterio-venous anastomoses.

The rabbit gingival vascular bed was studied by Mohamed, Waterhouse and Friederici in 1973 using transmission electron microscopy. They found capillaries, postcapillary venules, muscular venules and terminal arterioles in the free gingival microvasculature of rabbit incisors and molars. Approximately 30% of the capillaries were fenestrated and 97% of these fenestrated capillaries were of the venous limb type. These workers provided further information on the vessel types present in the gingiva,although the rabbit is an experimental animal infrequently used in periodontal vascular research.

The detail available from the examination of material in the TEM by Gavin and Trotter (1968), Mohamed et al. (1973) and Kindlova and Plackova (1973) was far superior to that obtained from the histologic techniques previously used to study the periodontium. Unfortunately very few follow-up studies using the TEM have been undertaken. The most recent reports are by Gilchrist (1978) and Barker (1980). Gilchrist used human material bipsied from the site of orthodontic extractions and was able to suggest a classification of the periodontal vasculature based on ultrastructural morphology. He found four vessel types which he classified as follows:

Type I: simplest type yet most numerous

Type IIa: obvious yet incomplete periendothelial cellular investment

Type IIb: complete periendothelial cellular investment

Type III: complete multilayered periendothelial cellular investment

Gilchrist found that these vessels corresponded to Rhodin's (1968) Classifications as follows:

Type I: venous capillary

Type IIa: postcapillary venule

Type IIb: collecting venule

Type III: collecting venule

As far as the architectural arrangement was concerned, Gilchrist reported as follows:

> The Type I vessels were found in the middle and dental annular thirds. They were found in the areas of dense collagen and also in the looser tissue between the principal periodontal fibre bundles. They appeared not to course towards or away from the root surface but rather apico-gingivally or circumferentially.
>
> Type II vessels were almost exclusively confined to the middle annular third of the ligament space. A greater tendency to be located in areas of looser connective tissue between the principal fibre bundles was noted. All vessels, but particularly the Type II vessels, tended to be collected in clumps,between which was much relatively avascular tissue.
>
> Type III vessels were confined to the middle annular third exclusively and tended to be axially oriented.

In conclusion Gilchrist described certain trends in the vascular architecture. Firstly, vessels of any of the types described were more common in the crestal third than the middle third. Secondly, the vessels were collected in concentrations and, thirdly, no perforating vessels were observed, but this latter observation may have been due to the method and site of specimen sampling.

Barker (1980) used almost identical samples and techniques to provide similar results,but also to discover new information about the human periodontal blood supply. He found only two types of periodontal vessel and he described them as small and large vessels of the periodontal ligament. The small vessels, which he classified as pericytic venules, were distributed in the cemental one-half of the ligament. These vessels lay in loose connective tissue between the principal fibre bundles. Some of these small vessels passed through foramina in the aveolar wall of the tooth socket to enter the trabecular spaces where they came into close association with the walls of "alveolar large vessels" (Barker's classification) to become "vasa vasorum". The large ligamentous vessels were not definitively classified but there was substantial evidence to suggest that they were not veins but lymphatics. These large vessels communicated with similar vessels in the alveolar bone via foramina and had their greatest diameter in the ligament just at the entrance to a foramen and became smaller in diameter in the ligament the further away from a foramen they were situated. The smaller diameter extensions of these vessels were closely associated with the small pericytic venules and appeared therefore to have a drainage function.

Apart from the identification of what might be lymphatics,the most striking feature of the results of both Gilchrist and Barker was the lack of any vessels on the arterial side of the circulation. This may not necessarily be applicable to all surfaces of the ligament, however, because the site of biopsy of their material was a thin sliver of buccal bone including only the middle and coronal two-thirds of the alveolar crest. It could be possible that the site of entry of any arterial supply to the ligament was away from this region and therefore the presence of only venous elements would be expected at those sites beyond that where the arterial supply divided to become capillary and eventually venular elements.

It can be seen that histologic techniques provide only an incomplete picture of the vasculature of the periodontium. The TEM studies of Gavin and Trotter (1968), Kindlova and Plackova (1973), Mohamed Waterhouse and Friederici (1973), Gilchrist (1978) and Barker (1980) have been able to provide a classification of specific vessels in the periodontium and it seems that this method of examination will prove to be the most satisfactory way of determining the fine structure of the periodontal ligament. The main problem is, and will always be, that it is extremely difficult to obtain samples of human material that are large enough and that are fresh enough to provide detail on the normal physiologic morphology. In many instances the best way of preventing tissue degeneration is to perfuse fixative into the living tissue. This technique is obviously out of the question in the human model but can be readily applied to animals. The great lack of literature regarding the TEM examination of the periodontal ligament vasculature indicates a rich field for further investigation.

PERFUSION TECHNIQUES

There are many techniques whereby the blood vessels are perfused with a medium which, by suitable processing, renders the filled lumen visible. The most common perfusion techniques use India ink, undiluted or with gelatin, Teichmann's paste coloured with cinnibar, barium sulphate, latex or methyl methacrylate. Other less common perfusions consist of a mercury mass, potassium dichromate followed by diluted lead acetate to precipitate lead chromate and other vascular casting media such as Microfil and Tensol No. 7, a perspex precursor. The latex and methyl methacrylate perfusions lend themselves to direct examination once the surrounding tissues are corroded away. The other techniques require, initially, decalcification followed by either radiography in the case of radiopaque materials like barium sulphate, or by clearing of the tissue to render the filled vessels visible. The cleared specimens have to be sectioned to allow adequate

visualization of the particular area of interest and this sectioning renders the elucidation of a three-dimensional object more difficult. Most investigations reported have examined either enlargements of radiographs or sections under the light microscope. The methyl methacrylate resin has lately been used for observation in the scanning electron microscope with a great improvement in magnification and depth of field. This resin appears to be the most promising medium for describing the vascular architecture of any anatomical structure including the periodontal ligament. A more detailed analysis of the advantages and disadvantages of these techniques will appear in the discussion at the end of the review of the literature.

The first definitive work on the periodontal blood supply was provided by Hayashi in 1932. His technique for studying human jaws was to perfuse with carmine gelatin followed by microscopic investigation after serial sectioning. The diagrams drawn by Hayashi are duplicated later in this report (figures 13 a, b) Hayashi described interalveolar branches of the dental arteries which arose at the base of each alveolus and ran coronally on both the labial and lingual aspects. Each dental artery entered the periodontal ligament and gave off further side branches which ascended ray-like to surround the tooth root.

The interalveolar branches gave off side branches which perforated the socket wall to enter the periodontal ligament and course coronally. These branches anastomosed with one another and the periodontal branches that arose directly from the dental artery to form longitudinal periodontal arteries. In the maxilla however, the interalveolar branches gave off a longitudinally arranged series of branches to supply the lingual buttress of spongy bone before the vessels entered the ligament. In cross-section the longitudinal periodontal arteries gave off branches at right angles which united to form a vascular circle around the tooth.

In general, the alveolar bone and periodontal membrane of the upper jaw had a richer blood supply than the lower jaw and the alveolar bone of both jaws was more vascular on the lingual aspect. In both jaws the front teeth possessed more periodontal vessels on the labial than the lingual side, but the reverse was true of the premolar and molar teeth. This work by Hayashi stood virtually alone for twenty years and it was not until the 1950's that investigations into periodontal vasculature were re-established.

Keller and D.W. Cohen in 1955 used an India ink perfusion in dogs and found that the gingival tissues were supplied by ascending vessels on the periosteal side of the alveolar process. This was confirmed 2 years later by Schuback and Goldman (1957) who also investigated dogs but used a mercury-gelatin medium. The specimens were radiographed and the resulting films were examined under the light microscope. They found a similar arrangement of periosteal vessels on both buccal and lingual sides, which anastomosed on the crest of the ridge to form a gingival plexus.

In 1958 Bevilacqua used India ink to describe the blood vessels in the periodontium of rats. He found ascending branches from the apical region, branches from the alveolar arteries, and descending branches from the gingiva,finding their way to the "pericementum" and ending there. These vessels were arranged in a basketlike network around the roots and followed a tortuous course.

L. Cohen conducted a study of the mandible of the cat in 1959 and supplemented his examination by also investigating monkey, dog and human mandibles. He used a variety of techniques including India ink and gelatin, carmine gelatine and radiopaque material. His findings, however, were rather sparse. The majority of blood vessels arising from the inferior dental arteries passed upwards to the alveolar border whereas the lower border of the mandible was supplied mainly by periosteal vessels. The

vessels to the ascending rami of the mandible arose from the muscles of mastication which were inserted therein.

L. Cohen in another report in 1959 expanded his findings on venous drainage in the human mandible by studying six cadavers within forty-eight hours after death. The age and sex of the cadavers was not disclosed. A modified Sahla needle was used to trephinate the cortical bone so that a radiopaque substance could be deposited in the cancellous bone and the mandibles were radiographed immediately after the injection. Cohen noted that the venous egress from the mandible was upwards to the pterygoid plexus via the inferior dental veins and also downwards to the facial and jugular veins. The periosteal veins drained into the facial vein and were probably responsible for the main venous drainage of the mandible. Cohen did not explain how the material flowed out of veins only and not arteries as well. In neither paper did he mention his criteria for classifying vessels into arteries or veins.

Also in 1959,Kindlova and Matena published an investigation into the periodontium of the rat mandibular incisor using latex corrosion casts. Their work was the most comprehensive to date and was the forerunner of some detailed papers on the periodontium of rat molars. This first paper established the technique and also defined the criteria for identifying the vessels cast in latex. These criteria were as follows:

"the arterioles appear on the cast as straight and round with branches at right angles, which are wavy below the site of branching and which branch out into a spatial rete above the site of branching.
the veins are slightly flattened and form a spatial rete with comparatively large meshes."

These authors also used histologic sections of the same area to confirm the results in the latex cast and one suspects that the classifi-

cations of the vessels may have depended more upon the histology of the vessel wall rather than from the unsophisticated criteria quoted above. Since their article dealt solely with the continuously erupting incisor, which is of little relevance in the present investigation, only brief mention will be made of their findings. The incisor periodontium was supplied by branches of either the mandibular or mental artery and the vessels in the periodontal crevice formed two continuous layers, one of which was arterial and close to the tooth, and the other was venous and coursed closer to the alveolar bone. The venous net had blood flowing into it from not only the incisor but also the area of the molars and the alveolar bone.

L. Cohen published an article in 1960 which further investigated the mandible of cats using Carmine gelatin. He reported that the blood supply to the periodontal membrane was derived from the apical region of the tooth, the alveolar bone and from the gingival tissue. A complete description of the architectural arrangement of the vessel in the ligament was not provided. The arterial architecture within the bone marrow was also described. The vessels showed little or no decrease in calibre with branches the same size as themselves and they pursued a straight course, changing direction by sudden angulations. This characteristic was also found in the periodontal membrane as well and complements Kindlova and Matena's report of arterial elements in the periodontium of the rat incisor.

In addition Cohen noticed that some of the vessels lay close to the socket wall in grooves and he concluded that the vessels were therefore protected from the effects of pressure. Cohen also commented on an anastomosis between the periodontal vessels and those of the gingiva around the necks of the teeth,which is contrary to the mainly independent gingival vasculature found by Carranza et al. (1966).

The vascular supply to developing teeth in rats was investigated by

Bernick in 1960 using an India ink-gelatin perfusion. He also confirmed the occluso-apical orientation of blood vessels coming from the apical area and being joined by perforating vessels arising from the interseptal bone. No mention was made of any vessels coursing horizontally within the ligament. The vessels were limited to the bony half of the periodontal ligament. Bernick was able to confirm the observation of Cohen (1960) where gingival vessels arose from the periodontium,although Bernick found these vessels were located directly underneath the basal surface of the epithelial attachment. At the transseptal region a rich anastomosis was formed by vessels from the periodontal vessels of the adjacent teeth and crestal branches of the interseptal vessels. From this area twigs arose to terminate in direct apposition to the basal surface of the gingival epithelium.

A few years later,Bernick (1962) published details of changes to the vasculature of rat molars with age. He reported that the basic pattern he had previously described was unchanged although he noted that at the apex of the developing root, vascular twigs coursed towards the forming acellular cementum. As the animals aged, vascular twigs crossed the periodontal ligament to terminate adjacent to the border of the cellular cementum, a component that increased with age, until in animals over one year of age there was a definite hypercementosis. This was in contrast to the lack of vessels to the acellular cementum superiorly.

The other main age change was a progressive thickening of the alveolar socket wall with a corresponding reduction in the number of vessels penetrating from the alveolar bone marrow. Eventually there was a complete absence of perforating vessels in rats greater than one year of age. Bernick commented that, in general, blood vessels were absent in the cemental half of the periodontal ligament,except in areas of formation of cellular cementum and also in areas of root resorption which

were observed in older rats. As with previous workers, Bernick could not provide any information on the types of vessels encountered and did not describe any venous drainage.

In the same year Kindlova and Matena (1962) published their description of the architecture of the rat mandibular molar periodontal vasculature,after studying latex casts in conjunction with histologic sections. These workers confirmed the general pattern of vessels running in the ligament and were able to refine the description of the vessels at the gingival cuff region (figures 2, 3). They also pointed out that the occlusally running arteries in the ligament were interconnected by a fine capillary network. At the alveolar crest, the arteries linked to form a horizontal arterial circulus from which branched a continuous row of freely coiling capillary loops. This row encircled the whole tooth and was similar to the arrangement described briefly by Bernick (1960). These workers also observed that the blood supply to the gingiva was basically independent of that to the periodontium, a finding which was supported later by Carranza et al. (1966), but which was contrary to previous investigations (Cohen 1960, Bernick 1960).

Kindlova and Matena were the first to discuss the venous drainage of the periodontium and found that the veins had their origin at the alveolar crest and that they collected blood not only from the capillary loops of the horizontal circulus but also from some veins from the gingival plexus. These veins then drained apically and either continued down the periodontal ligament or entered the alveolar bone. As they coursed apically the number of intercommunicating branches grew so that there was a rich basket-like plexus encircling the root apex.

The work of Kindlova and Matena provided the most complete picture of the vascular architecture but did not give any basis for a morphological classification of the vascular elements. The criteria mentioned in their

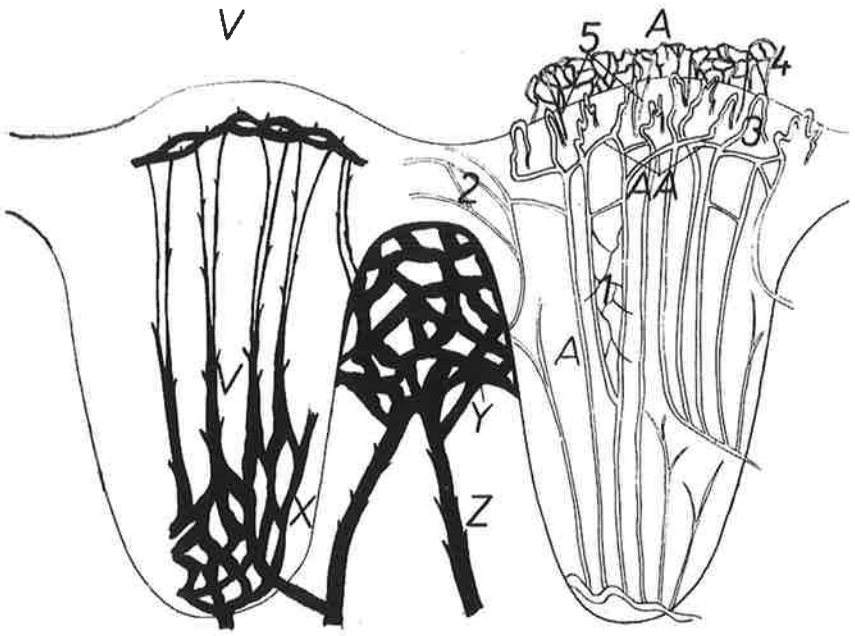


Figure 2. Detail of arterial and venous arrangements of the rat molar periodontium (from Kindlova and Matena 1962).

V = veins
A = arteries
AA = horizontal arterial circulus
X = venous rete below apex
Y = interradicular venous rete
Z = venous drainage from septum
1 = capillary network of ligament
2 = capillaries above septum
3 = coiled capillary loops
4 = capillary network of gingiva
5 = communication between gingival and periodontal capillaries

Figure 3. Detail of vascular architecture below the gingival crevice of the rat molar (from Kindlova and Matena 1962).

3 = coiled capillary loops
5 = communication between gingival and periodontal capillaries

1959 article were not repeated and even if one assumes that they were used for classification, one still is rather wary of the conclusions drawn because no discussion of size, cross-sectional shape or angle of branching occurred when the classification was made. As a consequence, one has to accept the authors' word that the structures they described were really present. It is not possible, from the illustrations provided, to substantiate the classifications made and there is therefore a need for some confirmatory work in this area. Kindlova and Matena used the light microscope which has a very poor depth of field. The advent of the scanning electron microscope (SEM) has enabled more useful information to be gleaned from vascular corrosion casts,particularly with the development of new casting materials, such as methyl methacrylate, which have a number of advantages over the latex casts. The latex casts are known to have poor dimensional stability because they tend to droop under their own weight and consequently any description of architectural arrangement must be qualified because of this problem. However, the examination of latex casts under the light microscope represented the state of the art in 1962 and has provided an extremely useful basis for future development and discussion.

Boyer and Neptune (1962) produced a paper on the blood supply to the teeth and adjacent tissues of the rat, the rabbit, the opossum and the hamster. They perfused each animal with potassium dichromate followed by a diluted lead acetate,which produced a precipitate of lead chromate that was visible when the tissues were cleared. In the rat mandibular molar the blood supply of the periodontium originated from the inferior alveolar artery. The vessels continued superiorly in the ligament until they extended above the crest of the alveolus at the level of the necks of the molar teeth. No horizontal ramifications between the periodontal vessels were reported. Boyer and Neptune agreed with Kindlova and Matena (1962) by describing the apparently independent blood supply of the gingiva. In the maxillary molar

the vessels supplying the buccal mucosa and gingiva perforated the alveolar bone to supply the peridontium at the apex and through the socket wall at all levels. The communication was not direct but coursed from gingival vessels to medullary bone vessels to periodontal vessels. The vascular arrangement of the rabbit mandible and maxilla was similar to that of the rat. Adjacent periodontal plexuses in the maxilla were connected by a direct communication through the interdental alveolar bone. There might also be a communication between the periodontal vessels and the greater palatine vessels. Boyer and Neptune described the vasculature of the opossum,without giving any great detail to the arrangement of the periodontium. The inferior alveolar artery supplied the mandibular teeth and the periodontal plexus extended above the crest of the alveolar bone as in the rat. In the maxilla the periodontal plexus communicated with the blood vessels and sinuses in the medullary bone. The hamster had a general blood supply very similar to the rat. In the hamster and the rat, the periodontal vessels of the molar were often supplied by periodontal vessels of the incisor. This work, as in others, did not describe the type of vessels nor did it discuss venous drainage and thus gave only an incomplete picture of vascular architecture.

A study of the vascular architecture of the mandible using human cadavers was published by Castelli in 1963. He detailed the blood supply to the various portions of the mandible and his findings significant to this review, were that the veins of the alveolar bone,and especially, from the periodontal ligament, joined one another and also the veins of the interalveolar septi. The course of these veins did not parallel that of the arteries. These findings are in accord with Kindlova and Matena's description of venous drainage in rats. Castelli perfused with China-ink to determine the overall vascular patterns,but also used Teichmann's paste with cinnibar which had a particle size of 8 to 12 micrometres,which prevented the paste from entering vessels with a smaller diameter than the particles. Thus Castelli was able to identify the arterial side of the vascular supply.

Unfortunately he made no comment on the arterial arrangement within the periodontal ligament,except to say that the alveolar-dental branches of the inferior alveolar artery supplied the teeth and adjacent tissue including the dental pulp, alveolar bone, interalveolar septi and periodontal membranes. These vessels passing through the alveolar walls anastomosed with the capillary network in the gingiva. The size of the alveolar-dental branches was 280 micrometres in diameter.

Heulke and Castelli conducted an investigation into the blood supply of the rat mandible in 1965. They used a variety of perfusion materials to obtain their results. Arteries to the incisor periodontium were small tortuous vessels which divided into abundant capillaries after reaching the membrane, thus suggesting that arterial elements were rare in the periodontium of the incisor. Their only comment on the supply to the periodontium of the molar was that it was derived from branches of the superior branch of the inferior alveolar artery. The venous drainage of the molars was through channels which passed through bone to the gingival veins. Concentrations of venous channels were seen in the interradicular septi and around the apices of the molar teeth and this observation supported Kindlova and Matena's (1962) report of a basket-like apical venous plexus around the molars.

Cernavskis and Hunter (1965) used microangiography to look at the vasculature of the rat mandible. They asserted that the main supply to the incisor was from an unnamed branch of the external maxillary (facial) artery and not the inferior alveolar artery. They also found that the periodontium of the molars was supplied by branches of the vessels from the superior surface of the incisor. These branches broke up into rich capillary networks that also supplied the pulp and surrounding bone of the molars. This aspect of periodontal blood supply to the molars agreed with the findings of Boyer and Neptune (1962) in the rat and the hamster. Cernavskis and Hunter, however, did not supply any classification of the

blood vessels nor did they discuss venous drainage.

The monkey was investigated with latex corrosion casts by Kindlova in 1965. The main blood supply to the teeth of the mandible was via the inferior dental artery. However, in the monkey, some of the blood supply to the lower incisor teeth came from branches of the lingual artery which perforated the alveolar bone. Kindlova found that the main periodontal vessels were located adjacent to the bony wall of the socket. These main vessels gave off branches towards the tooth,which formed a flat capillary network of irregular mesh.

The arrangement of the vascular plexus was different in the marginal part of the periodontium (figure 4). At the coronal extremity of the ligament (C) there was a narrow band of a more dense capillary network. From this, single capillaries were given off which ran a coiled course upwards and returned to the network from which they originated. Above this arrangement was a further network of tenuously looped capillaries with coiled arterial parts (E) of greater calibre than those more apically. They encircled a thick venous limb and then drained into it. This latter structure was adjacent to the epithelial attachment of the gingiva. These coiled loops were more prevalant on posterior teeth and occurred most frequently interdentally. At the crest of the alveolar ridge there was anastomosis between the vessels of the periodontal ligament and those of the gingival connective tissue. From this anastomosis arose a few rows of straight slender loops which supplied the crest of the unattached gingiva and some anastomosed with the capillary loops supplying the epithelium of the gingiva facing the oral cavity.

The basis of Kindlova's classification was again not made clear and she did not fully discuss the venous side of the vascular tree. She also admitted that a variation in structure in the interradicular area could not be fully described because of inadequate filling of the venous system

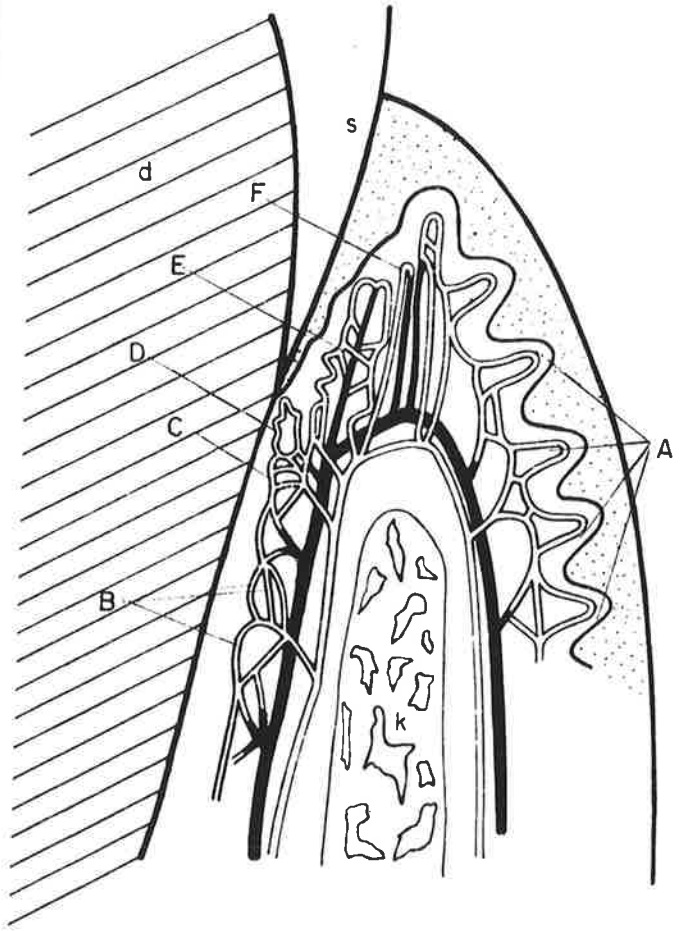


Figure 4. Detail of the vascular arrangement of the periodontal ligament and gingivae of Macacus Rhesus (from Kindlova 1965).

A = Subepithelial capillary network of gingiva
B = capillary network of periodontal crevice
C = band of denser capillary network in the periodontal crevice
D = coiled capillaries resembling glomeruli
E = capillary loops with the amply coiled arterial part
F = simple capillary loops
s = enamel
d = dentine
k = bone

by back perfusion. This suggests that her findings were based on incomplete vascular casts of either the arterial side or the venous side,but not both together. It is not possible to define the junction between the particular elements of the vascular tree because they gradually merge into each other. It is also not possible to precisely stop the latex perfusion at any point in the vasculature and the admission by Kindlova of inadequate venous filling makes one wonder about the accuracy of her classifications.

The monkey was again the subject of experiments in 1965 and on this occasion Castelli and Dempster used the India ink perfusion technique to publish a study on the response of the periodontal vasculature to experimental pressure applied to the tooth. Their discussions on the vascular arrangements of the periodontium were primarily concerned with the central incisors and premolars,which they said appeared to resemble the arrangement of man. They described afferent arterioles to the periodontium which were less than 100 micrometres in diameter and entered the periodontal membrane in the apical two thirds of the root after passing through cribiform openings in the alveolar wall. Once these vessels entered the membrane they immediately formed into capillary branches with a polyhedric plexiform pattern orientated parallel to the long axis of the root. The diameter of the capillaries was 9 to 10 micrometres. There were also thicker more irregular vessels described as venules which anastomosed with each other to form an elongate plexiform pattern in the mid socket region and a coarser venous plexus in the apical and cervical thirds of the periodontium. The venules tended to form a layer close to the socket wall while the capillaries were closer to the cementum. The veins drained either towards the apex of the tooth or through the alveolar wall into the network of the bone marrow. In the apical region they formed,with the pulpal veins, a dense plexus or venous cap in the marrow spaces surrounding the periapical region of the tooth. There is an outlet for the veins of the gingival crevice area via an anastomosis with the periodontal veins.

The dento-gingival junction was the subject of an investigation by Egelberg (1966a). He perfused mongrel dogs with an India ink-gelatin mixture and removed a portion of the marginal gingiva from the buccal and lingual surfaces. From these specimens the surface layer of the gingival crevice was removed and examined. The effect of chronic marginal gingivitis on the vasculature was also studied. Egelberg found that just under the crevicular epithelium the capillary plexus was different from that under the oral epithelium of the gingiva. The main variation was the absence of vascular loops under the crevicular epithelium and thus there was only a flat vascular plexus extending from the gingival margin right down to the base of the crevice. This is in marked contrast to the findings of Kindlova in the monkey (1965), and Kindlova and Matena in the rat (1962), where it was found that there was a plexus of coiled capillaries underneath the crevicular epithelium.

Egelberg measured the diameter of the vessels as varying between 7 and 40 micrometres,where the predominant number of vessels was larger than 7 micrometres. He suggested that vessels of the terminal circulation of a diameter larger than 7 to 8 micrometres (with the exception of arterioles) should not be considered true capillaries,but rather should be classed as postcapillary venules, small venules and venules. Egelberg did not determine how one could differentiate between arteries and venules.

In chronically inflamed gingiva the pattern of the capillary plexus was altered. There was an increased number of blood vessels and these were arranged in a bed rich in loop formations. On histologic sections taken concurrently, it was observed that the loop formations were associated with epithelial projections into the connective tissue in areas of inflammation.

Kindlova (1967) studied the effect of periodontitis on the vascular supply to rat molars. Her description of the normal arrangement in her control animals differed from previous reports using similar techniques

(Kindlova and Matena 1962). The earlier report described separate periodontal and gingival networks,whereas the later report indicates anastomoses between the two networks. Interconnections between the periodontal vessels were described as fine in 1962,but thick branches were reported in 1967. These discrepancies were not discussed by Kindlova.

The normal vascular anatomy of the rat gingival crevice was different from that of the dog as described by Egelberg (1966a) due to the presence of capillary loops in the rat,which were only present during periodontitis in the dog. The changes occurring in mild inflammation in the rat consisted of the formation of new capillaries that formed a dense capillary plexus under the epithelial attachment. These capillaries were dilated and had varicosities. Greater amounts of inflammation caused the recession of the epithelial attachment down the root of the tooth. As this occurred, some of the keratinized oral epithelium grew into the gingival crevice and it brought with it the characteristic vascular pattern found under the normal oral epithelium. The major changes to the blood vessel pattern were limited in area and only occurred directly under the attachment epithelium. Basically Kindlova enlarged on the findings of Egelberg and also highlighted the different reactivity observed under the different types of epithelium.

Saunders in 1967 studied the vascular supply of monkeys and the human foetus using microangiographic studies of a radiopaque injection medium. The main finding of interest was the continuity of periodontal and gingival vessels in the monkey which compares with the findings of Kindlova (1965).

Also in 1967, Cutright and Bhaskar perfused monkeys with silicone rubber and examined decalcified sections. They found the characteristic arrangement of vessels in the periodontium,with vessels passing occlusally derived from the intra-alveolar and apical arteries. The middle part of the periodontal plexus was supplied by only the intra-alveolar arteries and the cervical portion was derived from the intra-alveolar arteries as

well as the arteries from the vascular plexus of the gingiva. These workers found that the periodontium of the anterior mandibular teeth was supplied, on their labial aspect, by vessels penetrating the labial alveolar bone,having arisen from the labial soft tissues. They found no periodontal vessels supplied by the lingual artery and this was in direct contradiction to Kindlova's earlier investigations in the monkey. They also found no evidence of coiled capillaries in the periodontal ligament which were first described by Wedl (1881, cited in Kindlova and Matena 1962) and to which Kindlova draws similarities when describing the vessels she saw under the attachment of the crevicular epithelium of monkeys.

In a later article, Cutright and Bhaskar (1969) investigated the developing teeth of monkeys and found that the periodontal vasculature was derived from the vascular plexus of the dental sac. This plexus gave off branches to the dental papilla and the authors found that this communication continued as the tooth matured,so that there was an anastomosis between the pulp and the periodontal ligament via the apical foramen and also via accessory canals formed by entrapment of the communicating vessels in the tooth germ stage as the root elongated. This apical pulpal-periodontal anastomosis had not been discussed previously in the monkey although it had been reported in the rat (Garfunkel and Sciaky 1971). It helps to explain a pathway where infectious diseases can extend from one area to another and in particular the relationship between pulpal inflammation and periodontal disease.

Folke and Stallard in 1967 used a novel technique to provide a comprehensive view of the periodontal microcirculation. They injected plastic microspheres with a diameter of 15 ($\pm$5) micrometres into the blood stream of squirrel monkeys. They took histologic sections and looked for regions where the microspheres were trapped in the blood vessels. The blood supply to the oral epithelium was from branches of the periosteal

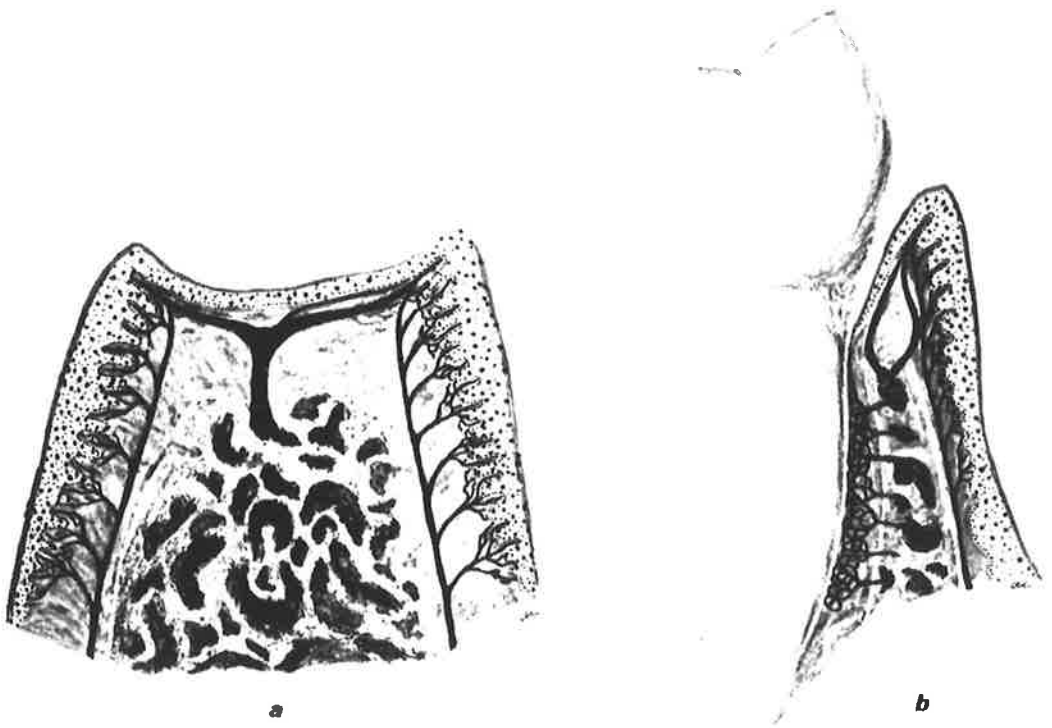


Figure 5. Detail of the arterial supply to the gingival region of the squirrel monkey (from Folke and Stallard 1967).

blood vessels passing to the connective tissue - epithelial interface at right angles to the epithelial surface. Underneath the epithelial attachment of the gingival crevice, however, the vascular plexus ran parallel to the epithelial surface and originated from infra-bony arteries leaving the alveolar process at the crestal area (figure 5). This finding agreed with Egelberg (1966a),but did not conform with Kindlova's report (1965) which described the vessels under the crevicular epithelium as being part of the periodontal plexus. In the 'col' region, the epithelial attachment was supplied by vessels from the crestal bone as well as from the periodontal ligament.

The blood supply to the periodontal ligament was from vessels passing from the alveolar bone through cribriform openings at every level. This agreed with findings by earlier workers investigating both the monkey (Castelli and Dempster 1965, Kindlova 1965, Cutright and Bhaskar 1967) and the rat (Kindlova and Matena 1962, Boyer and Neptune 1962, Carranza et al. 1966). However, Folke and Stallard could not verify the existence of vessels running parallel to the long axis of the tooth. They postulated that,if these vessels did exist,they must be either arterial or venular in nature and of a diameter large enough to allow the microspheres to pass through. They suggested that the micro-vasculature of the periodontal ligament differed from the gingiva either by way of having a greater number of preferential channels or by the vessels having an overall increase in diameter.

Folke and Stallard did not discuss the possibility that the lack of microspheres in the ligament could be due to other factors such as:

(1) the presence of a vascular bed of small vessel size sited upstream of the ligament,which prevented the passage of microspheres:

(2) the vessels of the ligament all being too small to permit entry of the microspheres

(3) vascular spasm which might occur in response to the perfusion and shut down the periodontal vasculature.

However, if Folke and Stallard's theories were correct,then it could be concluded that the periodontal ligament of the monkey does not contain vessels of capillary size. The majority of the literature does not support such a concept.

Folke and Stallard also noted that the position of the vascular plexus in the periodontium was closer to the alveolar bone than the tooth root, but no comment was made on whether or not this arrangement occurred along the whole length of the ligament from the apex up to the crest of the alveolar ridge. Consequently it was assumed by the present author that there was no variation detected in this pattern with height from the alveolar crest. Folke and Stallard were able to establish the general extent of the arterial distribution and the approximate junction between the arterioles and true capillaries,because the size of their microspheres meant that they would be trapped in or at the entrance to the terminal capillary bed. It is interesting that the presence of a terminal plexus of capillaries within the periodontal ligament was not confirmed in their report.

The question of derivation of vessels under the crevicular epithelium was investigated by Kindlova in 1968 using latex corrosion casts of developing rat molars. She found that a vascular plexus which supplied the enamel organ formed around the crown of the developing tooth. As the tooth erupted, the plexus over the crown disappeared until, at the end of eruption, the enamel epithelium was reduced to a narrow band at the neck of the tooth. At this time it was observed that there was a period of inflammation which subsided on completion of eruption leaving the coiled capillary loops previously described (Kindlova and Matena 1962). It was thus suggested by Kindlova that these capillary loops were not derived

from either the gingival vascular plexus or the periodontal vasculature but that they developed independently as vessels of the enamel organ and erupted with the tooth.

In 1970 an abstract appeared of a paper produced in Russia by Soloviev. He looked at the vascular network of the periodontium of dogs and confirmed that most vessels penetrated the periodontal membrane from the interalveolar septa. He also noted that the network was better developed in the mesial and distal regions of the periodontium but he did not comment on why this was so. The arteries were obvious and anastomosed freely. He also encountered vascular glomeruli consisting of epithelial cells and networks of capillaries. The method of this investigation was not described in the abstract.

Dogs and monkeys were the subjects of experiments conducted by Khouw and Goldhaber (1970) who perfused these animals with a colloidal suspension of carbon. The specimens were sectioned and cleared and observed in the light microscope. They discussed the findings for dogs and monkeys together and did not describe any differences between the two species. The vessels of the normal periodontal ligament formed an unevenly distributed network surrounding the tooth, located approximately in the middle third between the bone and the cementum. Per unit area, this was the most densely vascularized part of the ligament. The connective tissue next to the bone was richer in vessels than the connective tissue lining the cementum. The compact layer of bone of the socket wall had many perforations through which vessels from the underlying medullary bone entered the periodontal ligament. Coronally, the vessels of the periodontal ligament were continuous with those of the oral mucosa and underlying connective tissue.

In 1970 Kindlova produced a more comprehensive paper on the development of the marginal periodontium in the rat molar than she did in 1968. In this paper she made some observations that seemed to contradict the

findings made by herself and her co-worker in 1962. The basic pattern of development that she described remained unaltered from her 1968 publication. However, the circular vessel around the cervical portion of the vascular network over the enamel organ was described as a vein. This vessel persisted as the tooth erupted and Kindlova found that the periodontal membrane spread from this vessel in an apical direction. In the 1962 article, a horizontal arterial circulus was described from which capillary loops arose to extend under the crevicular epithelium. This horizontal arterial formation must be the same one that is now described as a circular vein. This contradiction was not discussed and it seems that the criteria used by Kindlova and Matena (1962) for identifying arteries and veins may not be accurate. From this report it seems that the coiled capillary loops are part of the gingival plexus rather than the periodontal plexus as drescribed by Kindlova and Matena (1962).

A further study using an India ink perfusion was reported by Garfunkel and Sciaky in 1971. They looked at the vascularization of rat periodontal tissue of all teeth and found that,in the periodontal ligament of the molars,the principal vessels ran parallel to the long axis of the tooth and were joined at right angles by finer interconnecting branches. The interdental papilla was supplied by blood vessels from the gingival and periodontal networks,in agreement with Cohen (1960) and Saunders (1967), but contrary to Kindlova (1962). There were also links in this region from vessels on the buccal and lingual sides. In addition they noticed that the central annular region of the ligament had a paucity of blood vessels. Other findings were links between the periodontal vessels, the vessels of medullary bone and the supraperiosteal vessels of the alveolar mucosa,which supported the findings of Bernick in 1960. A connection between vessels in adjacent periodontal ligaments was found as well.

Garfunkel and Sciaky described a number of blood vessels passing from

the crestal supraperiosteal network,extending either into the bone marrow or the capillary network surrounding the roots, where there was a basket-like network surrounding the periodontal region of the molar. In the mandibular molar, two parallel but interconnected networks were found in the periodontal space, one close to the root and the other closer to the alveolar bone. This is in disagreement with the majority of findings in this region and in this animal, but later work has confirmed the presence of a dual network in the canine periodontal ligament (Kishi and Takahashi 1977).

Kindlova and Trnkova (1972) used histologic examination together with latex corrosion casts to investigate the vascular arrangement beneath the sulcular and junctional epithelium in dog gingiva. They found that the flat capillary network, described by Egelberg (1966a) as a feature of healthy gingival tissues, appeared quite often (32% of their sample). They also found, however, a number of other different blood vessel arrangements indicating a high degree of variability in the arrangement of crevicular capillaries. The most common vascular pattern was a network of coiled capillary loops beneath the entire crevicular epithelium that was observed in 39% of their sample. The authors detailed three other less common arrangements. The first was a flat network similar to that described by Egelberg (1966a) but with obvious capillary loops at the gingival margin. The second had a majority of capillaries with a vertical arrangement in the apical segment and the third consisted of the entire region containing oblique capillaries with few anastomoses. Kindlova and Trnkova mentioned a similar variability of vascular pattern under the oral part of the gingival epithelium,but they did not provide a description. They commented that this variability might be a characteristic feature of the dog periodontium but they added that the presence of an inflammatory infiltrate into the region of examination might indicate that the variability was due to different irritations that the tissues had previously undergone.

Soderholm and Egelberg (1973) reported on the morphological changes in gingival blood vessels in developing gingivitis in dogs. In dogs with clinically healthy gingiva the network of vessels lay adjacent to the epithelium of the gingival crevice. Few loop formations were found and, when present,they were mainly adjacent to the gingival margin. In chronically inflamed dog gingiva increased numbers of vessels have been found with greater diameters and abundant loops near the gingival margin and apically along the dento-gingival junction. During the development of gingivitis the authors found two characteristic changes:

(1) Widening of the vessels,which occurred in the venular segments usually within a week from the commencement of the experiment.

(2) Changes in the course of the vessels,which were first observed in the vessels connecting the afferent and efferent aspects of the terminal vascular systems. With increasing time a greater proportion of the efferent side showed changes in architecture to form new vascular loops. These changes in vascular course occurred later than the widening of the venular elements.

Soderholm and Egelberg made a similar comment to Kindlova and Trnkova (1972) by suggesting that previous inflammatory episodes could influence the observed vascular patterns.

The organisation of dog and cat gingival vasculature was described by Nuki and Hock (1974) after examination by vital microscopy, histology and perfusion with silicone latex compound. They found no discernible difference between the arrangement and structure of vessels within the free gingiva of maxillary and mandibular canines and molars in cats and dogs.

A regular arrangement of vessels occurred in clinically non-inflamed gingiva around newly erupted teeth. Vessels underlying the crevicular epithelium were continuous with vessels of the periodontal ligament,while vessels underlying buccal epithelium were continuous with vessels of the

oral mucosa. Vessels in crestal gingiva represented the capillary connections between crevicular and buccal networks. However, these individual components could not be distinguished 3 weeks after eruption due to numerous anastomosing vessels between the subepithelial vascular networks throughout the free gingiva.

Arteries from the alveolar mucosa supplying the central and proximal buccal gingiva branched into arterioles, 44 to 25 micrometres wide at the level of the crestal bone, while arteries from the periodontal ligament branched into arterioles subjacent to the epithelial attachment. These arterioles tended to run parallel with the gingival margin. They branched into smaller arterioles and precapillary arterioles, 18 to 15 micrometres wide, within the apical region (500 micrometres immediately coronal to the epithelial attachment) and mid region of the gingiva. These terminal arterioles then ramified into capillary networks underlying crevicular, crestal and buccal gingival epithelium.

Venular capillaries draining the capillary systems ran into post-capillary venules, 8 to 30 micrometres wide, lying within 250 to 500 micrometres of the margin midway between the buccal and crevicular aspects of the free gingiva. Postcapillary venules and venous capillaries anastomosed frequently with each other in planes parallel to both buccal and oral gingival surfaces and eventually formed small venules in the apical free gingiva, often in close proximity to the circumferential arterioles. The venules continued on into the vascular beds of the periodontal ligament and oral mucosa.

Venules in alveolar mucosa anastomosed to form small veins running along the vestibular sulcus. There were frequent anastomoses in the venous system around all aspects of a tooth and between the venous systems of adjacent teeth.

Nuki and Hock (1974) identified a basic "capillary unit" that was repeated throughout the vascular network of non-inflamed gingiva (figure 6a). This unit consisted of a minimum of two terminal arterial capillaries, four primary venular capillaries and a varying number of connecting vessels. Two of the primary venular capillaries branched into postcapillary venules within 300 micrometres of the gingival margin, while the other two venular capillaries continued apically to anastomose with other similar vessels. The majority of units extended 400 to 1000 micrometres mesio-distally and lay within 300 to 500 micrometres of the gingival margin. Connecting vessels of each unit connected with those of adjacent vessels. Vessels in one unit interlaced with those of other units (figure 6b).

The effects of inflammation on the vascular morphology of the gingival tissues were also studied by Nuki and Hock (1974). Measurements made on vessels in vital gingiva showed that the width of many vessels increased by 5 to 10 micrometres, while vessel length often increased by 400 to 1000 micrometres. Elongated vessels became twisted spiralled or looped (figure 7). In specimens exhibiting overt inflammation, the capillary unit broke down to form one or two loop structures with a minimum of one arterial capillary and two venous vessels. With the disappearance of many of the connecting vessels, the spatial relationship of the marginal capillaries changed so that the subepithelial anastomosing capillaries lay more closely opposed to either buccal or crevicular epithelium.

These vessel loops replaced the entire network of capillaries, precapillary arterioles and postcapillary venules of crestal gingiva and much of the superficial networks underlying crevicular and buccal gingival surfaces.

Hock (1975) examined the gingival vasculature around the erupting deciduous teeth of dogs and cats using the same techniques as Nuki and Hock (1974). She found that a regular network of vessels became established

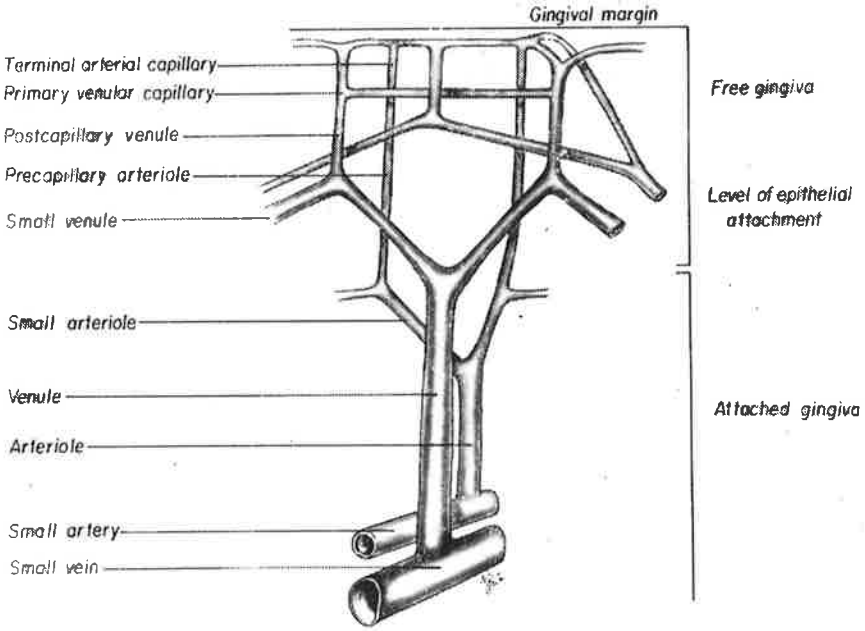


Figure 6a. Diagram of organisation of gingival microvasculature derived from Microfil perfused specimens and serial tissue sections (from Nuki and Hock 1974).

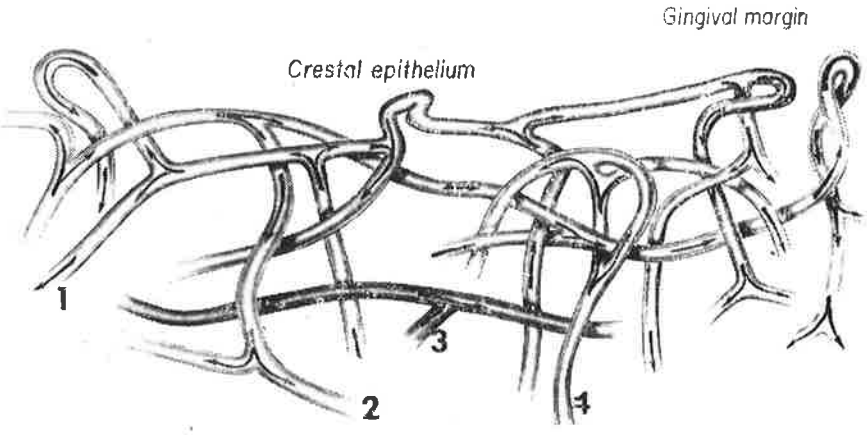


Figure 6b. Diagram from tracing of videotape record to show relationship of four capillary units in vascular network (from Nuki and Hock 1974).

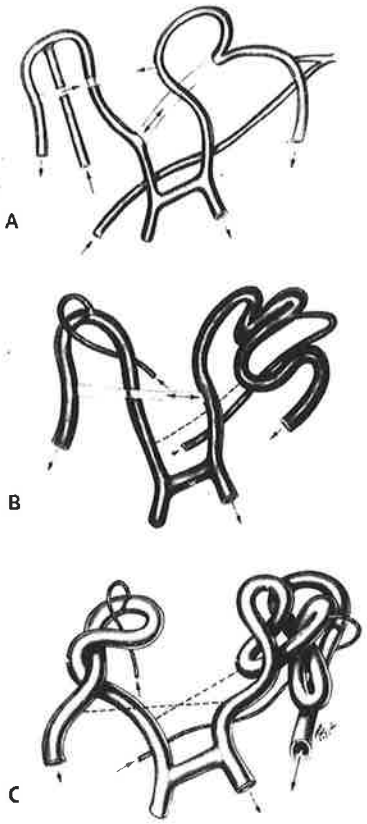


Figure 7. Diagrams illustrating the change from a regular network to loops. A. Regular network. B. Intermediate stage. C. Loops. Note also changes in vessel dimensions (from Hock and Nuki 1974).

soon after the beginning of tooth eruption. This network persisted until the onset of histologically detectable chronic inflammation. In a few specimens where chronic inflammation became established, characteristic vascular changes occurred during which vessel loops replaced the network pattern. As vessel loops became established, vessel width increased by up to 5 micrometres, vessel length by up to 2000 micrometres and intervascular distance by 25 to 50 micrometres. The longer vessels were looped and twisted or spiralled.

In 1976 another paper using a vascular perfusion technique was published. Ichikawa, Watanabe and Yamamura reported on the vascular architecture of oral tissues of dogs using methyl methacrylate corrosion casts and the scanning electron microscope. This was the first report of oral vasculature using this technique. Unfortunately the results described were rather sparse with no new information. The authors described big arteries and veins running vertically with numerous capillaries branching from them forming networks in the "so-called" intermediate layer of the periodontal plexus. These networks formed a reticular ring around the void space in which the tooth existed before its dissolution. No discussion was made of the gingiva or connections of the periodontal ligament vessels to other structures. In addition, the illustrations showed casts of an incompletely perfused vascular plexus.

In 1977 Kishi and Takahashi also published a scanning electron microscopic study of the vascular architecture of the periodontal ligament of mongrel dogs. This article proved to be most informative,with excellent pictures of complete methyl methacrylate vascular casts. However, no illustration of the ligament vasculature in its entirety from the apex to the gingival extremity was provided.

As an adjunct to their experiment,the authors were able to corrode

away the soft tissues with a proteinase and leave bone intact. This technique illustrated the relationship of the vessels to the bone and the bony fenestrations that had not been possible to determine in other corrosion cast investigations. These workers were able to identify the sources of the blood vessels of the periodontal ligament. The maxillary teeth were supplied by branches of the infraorbital artery and the mandibular teeth were supplied by the inferior alveolar artery. In general the ligament was supplied from four directions:

(1) from small arteriolar twigs arising from vessels supplying the pulp

(2) from small arteriolar twigs from the vascular plexus of the alveolar bone marrow entering the ligament through openings in the alveolar wall and distributing to most parts of the ligament

(3) from periosteal arterioles supplying the crevicular epithelium joining the vascular network of the ligament at its most coronal portion (this type was very rare).

(4) from periosteal arterioles penetrating the tip of the alveolar crest to supply the ligament direct.

These results were in basic agreement with previous workers in other models (Carranza et al. 1966, Kindlova and Matena 1962, Bernick 1960, Boyer and Neptune 1962, Castelli and Dempster 1965, Cernavskis and Hunter 1967, Folke and Stallard 1967). It was also noticed that the vessels supplying the pulp coursed in a well defined 'S' fashion before entering the apical foramen.

Kishi and Takahashi went on to provide a detailed description of the architecture of the ligament microvasculature. In the apical quarter of the socket there was a two layered arrangement,with the superior layer (i.e. closer to the tooth) running parallel to the long axis of the tooth forming a "fence-like" network. Below this was an inferior layer (i.e. closer to the socket wall);with vessels running at right angles to the

superior layer. The superior layer consisted of units of two capillaries, arranged such that one was closer to the tooth root and one closer to the alveolar bone. These capillaries were joined to each other with horizontal branches to form a "rope-ladder" or fence-like network. These units occurred about 120 to 160 micrometres apart and within them were numerous arterio-venous, arterio-arterio and venous-venous anastomoses. The inferior vascular layer consisted of the arteries coming out from the openings of the alveolar wall and the venules of the ligament.

The middle half of the ligament had the dual layered vascular arrangement of the apical quarter,but towards the coronal end of the alveolus bundles of thick vessels were observed to traverse circularly. These circular vessels became better defined the further coronally they were positioned.

In the most coronal portion of the ligament the two layers were still present,but the superior layer gave off many hairpin shaped vascular loops. The inferior layer had circularly or longitudinally oriented bundles of thick vessels,each of which was made up of a thick venule and one or two arterioles and ran obliquely upwards along the alveolar wall. These bundles were closely related to the superior layer with repeated branching and anastomoses of the arterioles and capillaries. These vascular bundles also emitted branches at regular intervals,which coursed parallel to the long axis of the tooth and finally joined the superior layer.

Immediately above the crest of the alveolar ridge the superior and inferior layers suddenly fused together to form a vascular plexus quite different from those of the other regions. Some of the vessels in this plexus originated from the vessels in the subepithelial plexus of the internal margin of the gingiva,but this source occurred infrequently. The major supply to this plexus was from periosteal arteries. From the

network formed from the principal vessels, numerous hairpin-shaped loops arose and projected upwards to surround the tooth root.

The general arrangement just described has some similarities with Kindlova and Matena's (1962) description of the microvasculature of the rat periodontal ligament,particularly in relation to the horizontal vascular circulus and the capillary loops in the region of the gingival attachment.

Birn (1966) in his discussion of blood supply to human periodontal ligament,assumed that all perforations of the alveolar wall contained blood vessels. Kishi and Takahashi showed that most perforations did in fact contain a blood vessel and the larger openings contained several arterioles and a lesser number of thick venules. However, some of the smaller openings were observed to contain no vessels at all and this factor must be considered when drawing assumptions from Birn's work.

Kishi and Takahashi, however, did not mention any criteria that were used to classify the vessels that they described. This is unfortunate since the complete vascular casts that were obtained would have lent themselves admirably to an overall morphological analysis with a concomitant vessel classification.

De Almeida and Bohm (1979) studied the gingival vasculature of Wistar rats injected with colloidal carbon. Thick cleared sections were examined with a stereoscopic microscope. They found that the gingiva was richly vascularized and the vessels were continuous with those of the mucosa. Beneath the external epithelium was a network of capillaries with a diameter of 8 micrometres. At the point where the attached gingiva began, there was a longitudinal belt of vessels, from which small ramifications sprang up, forming loops. The loops, with a diameter of 10 to 25 micrometres, were separated from one another and were not continuous with the more

superficial capillary network.

One of the most recent articles available is by Boc and Peterson (1981) who investigated revascularization in dogs after a posterior mandibular alveolar osteotomy. They used silicone rubber in addition to radiopaque perfusion media and found that the periodontal ligament vasculature, as well as being supplied by the inferior alveolar artery, had many branches derived from medullary bone circulation and also from the vessels that extended from the buccal and lingual attached gingiva down into the medullary space, along the periodontal ligament. These vessels appeared to come up and over the cortical plates of bone and extend down well into the anastomosing network of medullary vasculature. The medullary bone was richly vascular, with clear anastomosing channels to the periodontal ligament, cortical bone and neurovascular bundle. After the alveolar osteotomy,the blood supply to the periodontal ligament was decreased,as its supply from the inferior alveolar artery was cut. However, its vitality was retained and its vascularity re-established via the lingual periosteal vessels that extended over the cortical bone crest, down along the periodontal ligament and into the medullary bone. This work has been the first to describe an anastomosis of periosteal and medullary vessels via the periodontal ligament and this aspect warrants further investigation. No mention was made in this article of the vascular supply to the gingival crevice area.

Hellum and Ostrup (1981a,b) also investigated the blood supply to the body of the mandible of the dog by using a Microfil perfusion together with a radiopaque perfusion in a different artery to define the vascular territories of the particular vessels. They found that the inferior alveolar artery supplied the entire mandibular body and subperiosteal vessels, the attached gingiva and a portion of the buccal mucosa. The lingual mucosa of the body of the mandible below the gingiva, including

the muscle attachments, was supplied by the sublingual artery, a branch of the facial artery. Branches of the inferior alveolar artery to the interradicular alveolar bone were seen to anastomose within the periodontal ligament and gingival tissues and to terminate as end arteries in the marginal gingiva. Several anastomoses were found between the medullary and the periosteal territories, mainly in the alveolar process, where they frequently appeared as a periodontal - periosteal communication. An anastomosis at the symphyseal region was also evident.

The authors of this article found that the differentiation between arterial and venous perfusion was difficult and their discussion did not mention venous drainage. This may have been due to the fact that the Microfil and Colorpaque media did not penetrate the entire vascular tree. It could also mean that the sectioning of the mandible prior to clearing and examination destroyed the three-dimensionality of the vessel networks, making it impossible to follow the course of the vessels from the arterial to the venous side.

Hellum and Ostrup (1981b) also investigated the effect that blocking the inferior alveolar artery had on opening up the collateral blood supply and found that after 30 days the blood supply to the mandibular body was almost completely returned to normal via the facial artery and its sublingual branch. No mention was made of whether this mandibular blood supply was reconstituted for example, by ingrowth of new vessels or by anastomoses between the microvascular beds of the different arteries.

SYNOPSIS OF THE PERIODONTAL VASCULATURE

The majority of publications detailing the vascular architecture of the periodontium have appeared between 1960 and 1970, and up to date the information that has been obtained gives only a partial insight into the blood supply to the periodontal ligament and surrounding structures. The

reasons for this lack of completeness seem to be that the perfusion techniques which were used in the majority of the research papers have not been able to either completely fill the vasculature or render the filled vasculature easily visualized in its entirety in three dimensions. Most investigations have used the light microscope and very few studies have been undertaken with either the transmission or the scanning electron microscope. There is a very rich area for future research into the periodontal ligament vasculature because as yet no definitive ultrastructural classifications exist.

As far as the vascular architecture of the periodontal ligament is concerned, there has been basic agreement on a number of features. Most attention has been turned to the rat and monkey with lesser information being available on man, mice, cats, dogs and hamsters. This discussion will deal with the information available to present a picture of the vascular architecture of each animal model. Areas of little or no information or disputed areas will be highlighted.

The Vascularization of the Periodontium of the Rat

The rat has been used extensively in vascular research and there is a great deal of information available about the blood supply to the teeth, periodontium and jaws. The rat molar has been claimed to resemble the structure and morphology of human teeth (Schour and Massler 1971) and so will be discussed in detail. The continually erupting incisor, although of great interest in the examination of tooth formation and eruption, has no direct relevance in this study,which is concerned primarily with determining an anatomical norm for the adult situation in the rat molar. Consequently, only brief consideration will be afforded the incisor.

It was generally accepted that the blood supply to the mandibular teeth and their supporting structures came,in the main, from branches of

the inferior dental artery (Kindlova and Matena 1962, Boyer and Neptune 1962, Huelke and Castelli 1965, Carranza et al. 1966). There was also evidence in the mandible that supply to the molars came indirectly from vessels that arose from the periodontal ligament of the incisor. (Boyer and Neptune 1962, Cernavskis and Hunter 1965, Garfunkel and Sciaky 1971).

The periodontium of the rat maxilla was supplied by the greater palatine vessels, vessels deep to the muco-buccal fold and the superior alveolar vessels. The palatine and cheek vessels provided a liberal blood supply to the gingiva and soft tissues and branches from these vessels perforated the alveolar bone. The superior alveolar vessels supplied the periosteum and then penetrated it to reach the alveolar bone and subsequently supplied the pulp and periodontal ligament (Boyer and Neptune 1962). The gingiva was supplied mainly by periosteal vessels arising as branches of the mucosal vessels (Carranza et al. 1966),which were presumably branches of the facial (external maxillary) artery (Cernavskis and Hunter 1965).

The periodontal ligament of the mandibular molars obtained its blood supply directly from branches of the inferior dental artery entering the ligament at the region of the apex of the tooth and also indirectly from branches penetrating the alveolar bone at all levels and anastomosing with the ascending branches from the apex (figure 8). The main branches in the ligament had an occluso-apical orientation (Bevilacqua 1958, Bernick 1962, Boyer and Neptune 1962, Kindlova and Matena 1962, Carranza et al. 1966, Garfunkel and Sciaky 1971). Finer branches connected these principal vessels (Kindlova and Matena 1962, Garfunkel and Sciaky 1971). The arrangement of vessels in the periodontal ligament of maxillary molars was essentially the same as the mandibular molars (Garfunkel and Sciaky 1971). Some authors indicated that the vessels originating apically maintained their integrity right up to the alveolar crest (Boyer and Neptune 1962.

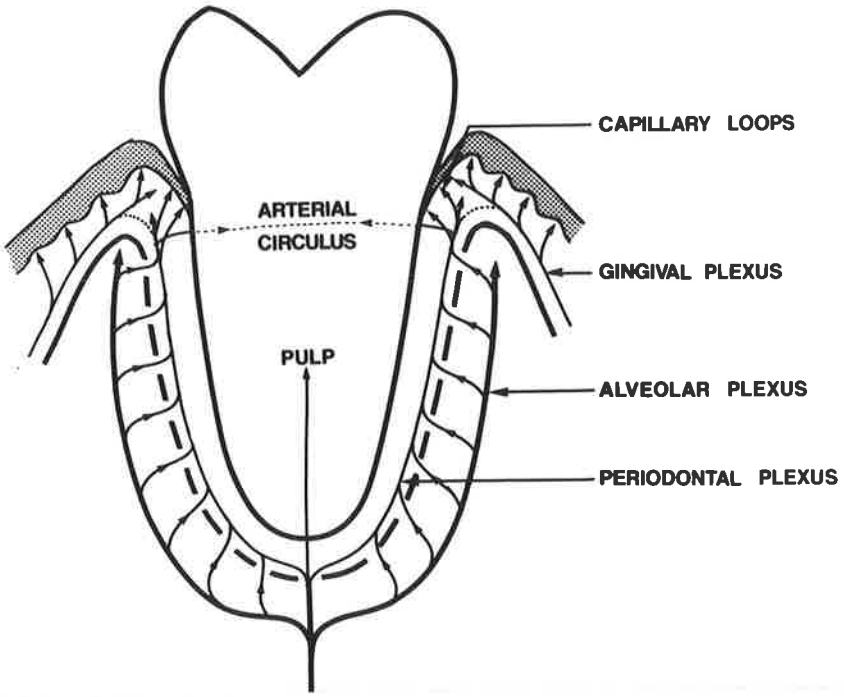


Figure 8. Schematic representation of the arterial supply to the rat molar, drawn in bucco-lingual cross-section, representing a synthesis of the arterial architecture most commonly reported.

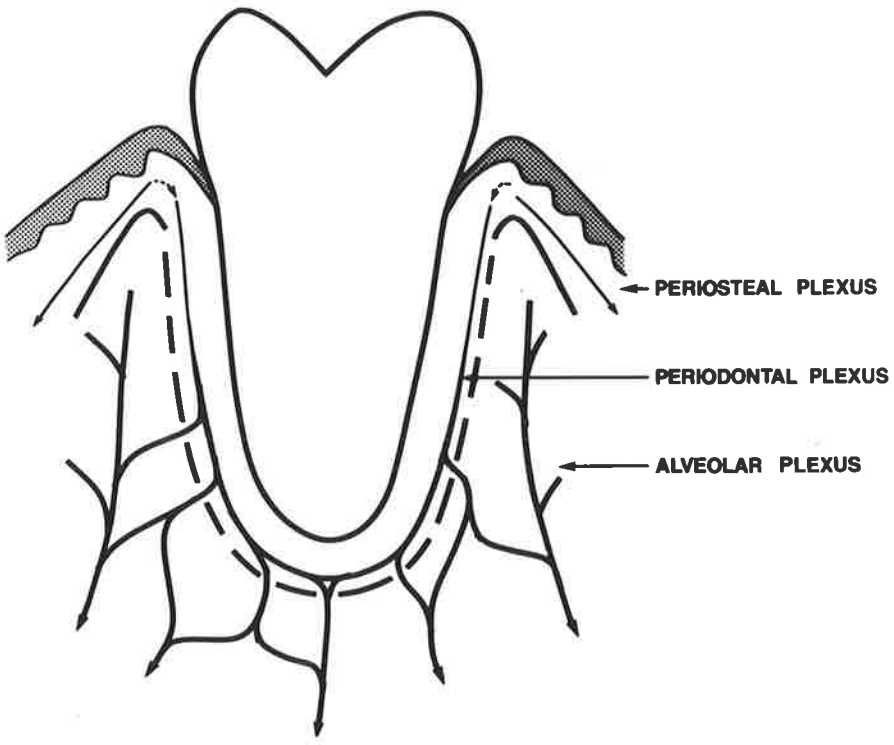


Figure 9. Schematic representation of the venous drainage away from the rat molar drawn in bucco-lingual cross-section and representing a synthesis of the most commonly reported venous architecture.

Carranza et al. 1966).

The position of the blood vessels within the ligament was reported to be closer to the alveolar bone than to the tooth (Carranza et al. 1966, Bernick 1962),but there was some disagreement. Garfunkel and Sciaky (1971) reported two parallel networks in the periodontal ligament of the mandibular molar, one close to the root and the other external to it. These particular authors did not elaborate on their findings and it is not known whether the outermost network was closer to the alveolar bone than the tooth, nor is it known whether the innermost network was associated with any cementum formation or resorption. Bernick (1962) reported that, although the network was, in the main, closer to the alveolar bone, there were branches that approached the tooth in areas of formation of cellular cementum and also in areas of root resorption.

The arrangement of vessels in the gingival crevice has not been comprehensively discussed. Kindlova and Matena (1962) did not seem to acknowledge that a distinct crevicular plexus could exist. They reported a plexus under the gingival plexus facing the oral cavity which had infrequent connections with the periodontal ligament. The gingival extremity of the periodontal ligament had a horizontal arterial circulus formed by the ligament vessels from which branched coiled capillary loops. These loops were considered to be part of the ligament plexus. No other author has confirmed the presence of the horizontal circulus. Carranza et al. (1966) reported a dense plexus of vascular loops beneath the epithelial cuff. The marginal region of the gingival tissues ended in finger-like terminal loops (Garfunkel and Sciaky 1971) and loops in the gingival crevice were also found by De Almeida and Bohm (1979).

The types of vessels in the periodontal ligament have been described by Kindlova and Matena (1962) as both arterial and venous. Other authors

have classified the vasculature no further than just "blood vessels" or "capillaries". Kindlova and Matena described arterioles that coursed occluso-apically as being joined by a fine capillary network. The horizontal arterial circulus they described also gave off capillaries and these emptied into veins that drained towards the apex of the tooth. These veins were joined by some veins from the gingiva and branched often to form a venous plexus. Some branches passed through the alveolar wall and the remainder continued to branch as they went apically,until the tooth apex was surrounded with a "basket-like" venous plexus (figure 9). Once the veins from this plexus entered the medullary bone they anastomosed with other vessels of the bone marrow and soon drained away by the shortest route. Huelke and Castelli (1965) confirmed the concentration of venous channels in the interradicular septa and about the apices of the molar teeth. However, they described the venous drainage of the molar teeth and their supporting structures as being through channels which passed through bone to the gingival veins. Unfortunately, these authors did not elaborate on this description.

Blood vessels passing from one periodontal ligament through the alveolar bone and connecting with blood vessels of the adjacent ligament have been described by Garfunkel and Sciaky (1971) and Carranza et al.(1966).

Although the periodontal vascular architecture of the rat has been the most widely reported of all animal models, there are still large deficiencies in the information available. A definitive categorization of the vessels within the periodontal ligament has not yet been conducted. Kindlova and Matena's (1962) descriptions were based on vague criteria and have not ever been confirmed,despite the fact that 20 years have elapsed since their investigations were conducted. No author has been able to provide a complete three-dimensional model of vascular architecture of the ligament from apex to gingival crevice,even though corrosion casts have lent themselves to

this type of presentation. Illustrations available to date only show selected isolated areas and do not provide an overall view of the entire periodontal vasculature.

The Vascularization of the Periodontium of the Monkey

There were many similarities between the distribution of blood vessels in the periodontium of the rat and the monkey. There were also, however, some dissimilarities,but these were not so great as to disallow a correlation between the two animal models.

The main blood supply to the periodontium depended on the position of the tooth. In the maxilla the superior alveolar artery was the major afferent artery with branches from the palatine vessels also entering the periodontal ligament via the palatal mucous membrane (Kindlova 1965). The palatine vessels supplied mainly the palatal aspect of the central and lateral incisors,whereas the labial aspect of this tooth, including the labial gingiva and alveolar bone, was supplied by branches of the facial artery (Castelli and Dempster 1965).

The blood supply to the mandible was derived from the inferior alveolar artery. The lingual artery, however, supplied branches to the anterior mandibular teeth (Kindlova 1965, Castelli and Dempster 1965). The labial periodontal ligaments of the anterior teeth were described by Cutright and Bhaskar (1967) to be supplied by vessels that traversed the labial soft tissues, entered the labial alveolar bone and penetrated through this bone to reach the periodontal ligament.

These last-mentioned workers were not able to confirm the presence of lingual blood supply to the mandibular anterior teeth.

The blood vessels entered the periodontal ligament mainly through cribriform openings in the alveolar plate,which were most numerous in the

middle and apical two thirds (Castelli and Dempster 1965, Khouw and Goldhaber 1970). The vessels that passed through the cribriform plate were arterioles and were less than 100 micrometres in diameter. Once they entered the ligament space they immediately broke into a polyhedric plexiform pattern oriented parallel to the long axis of the tooth. These capillaries formed a layer close to the cementum. Venules were present in the ligament and anastomosed with one another to form a mesh closer to the alveolar wall than the capillary layer (Castelli and Dempster 1965).

Kindlova's (1965) description of the arrangement of vessels was similar to that just mentioned, but did not identify the exact position of the capillaries in relation to the surrounding structures (figure 10). Kindlova described the flat capillary network as occurring closer to the tooth than the main periodontal vessels, but did not mention if they were close to the cementum or were away from it. Khouw and Goldhaber (1970) reported an unevenly distributed network of blood vessels located in approximately the middle third between the bone and the cementum. However, they elaborated by saying that the middle third had the greatest vascularity per unit area, but that the connective tissue next to the bone was richer in vessels than the connective tissue lining cementum. This aspect of the positioning of the plexus of blood vessels in the periodontal ligament remains unresolved.

Kindlova (1965) did not describe arteries in the periodontal ligament in her text, but her illustrations showed a vessel, which was labelled as an artery in her figure 8, coursing the whole length of the ligament and from which the capillary network branched. One assumes in Kindlova's publication that these arterial elements ran uninterrupted from the apical regions to the region of the alveolar crest, but this aspect is unclear. Cutright and Bhaskar (1967) described the source of vasculature

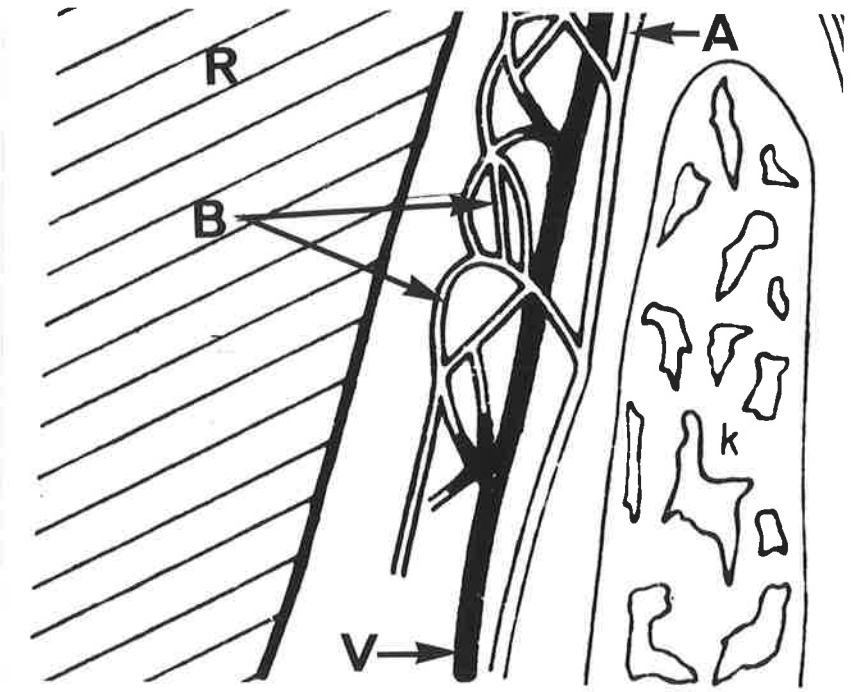


Figure 10. Schematic representation of the vascular network of the periodontal ligament of the monkey (from Kindlova 1965).

A = Artery
B = Capillary plexus
R = Tooth root
V = Vein
k = Alveolar bone

in the apical region as the intra-alveolar arteries,whereas the cervical portion obtained its supply not only from the intra-alveolar arteries but also from the vascular plexus of the gingiva. It is assumed from this description that no vessel ran intact from the apical region of the ligament up to the coronal portion and that each portion of the ligament vasculature received its blood supply locally and then anastomosed with adjacent regions.

The venous drainage consisted of venules arranged in an elongate plexiform pattern,which often met to form a common stellate enlargement. There were two directions of venous drainage. Firstly, towards the apex of the tooth and secondly, through the cribriform plate of the alveolar wall toward networks in the bone marrow. The latter veins joined with those from the apex to form a venous cap in the bone marrow (Castelli and Dempster 1965). Kindlova (1965) drew a diagram of the veins draining the periodontal ligament. These veins received blood from the capillary network and ran straight down in an occluso-apical direction. She did not elaborate on these vessels in the text of her paper nor did she describe the basis for her classification.

The gingival tissues were reported to have a number of different features according to their situation. For instance, the attached gingiva facing the oral cavity was said to be different from the unattached gingiva of the crevice facing the tooth and this was described as being slightly different from the tissues in the 'col' area (Kaplan, Pameijer and Ruben 1977). The vascular supply to these different areas has also been described to differ depending on the area of observation (Folke and Stallard 1967, Kindlova 1965). The vascular supply to the oral mucosa and attached gingiva had its main arterial supply from the periosteal vessels,from which branched smaller vessels forming capillary loops which ran perpendicular to the surface of the oral cavity (Folke and Stallard 1967).

The vasculature below the crevicular epithelium has had conflicting descriptions. Folke and Stallard (1967) reported that the epithelial attachment area had its arterial supply derived primarily from infra-bony vessels leaving the alveolar process at the crestal area pursuing a course close to, and parallel to, the epithelium. In the 'col' region the vessels were similarly derived but, after running perpendicular to the 'col' epithelium from the alveolar bone, bent sharply to the surface to run parallel to the basement membrane.

Kindlova (1965) showed a much more intricate arrangement of vessels in the crevicular region. At the coronal extremity of the periodontal ligament, just under the junctional epithelium, the flat periodontal capillary network was condensed into a narrow band. From these vessels single capillaries were given off and, having run a coiled course, returned to the network. Above these, subjacent to the crevicular epithelium were found tenuously looped capillaries with clearly coiled arterial parts of a greater calibre than those found more apically. These coiled arterial parts encircled a thick venous limb and then drained into it. This arrangement was found on all aspects of the teeth but was more numerous in the 'col' region. Kindlova (1965) also reported an anastomosis between the main vessels supplying the periodontal ligament and the gingival connective tissue. In the region of this anastomosis arose two or three rows of straight slender loops with arterial and venous limbs of equal length,which extended into the crest of the free gingiva where a further anastomosis with the capillary loops supplying the epithelium facing the oral cavity occurred. Saunders (1967) confirmed this gingival-periodontal anastomosis.

The literature available on the periodontal vasculature of the monkey is sparse and most experimentors used different techniques. As a consequence, the information that has been provided is often conflicting and therefore

not directly comparative. Until more research is carried out in the monkey,there are a number of areas which remain with incomplete information. Still unconfirmed is the exact architectural morphology particularly in the region of the crevicular epithelium. Also the classification of the particular vessel types present into arteries, arterioles, capillaries, venules and veins has not been undertaken with any accuracy or sound basis. The monkey is an animal model which has the closest resemblance to man and, as a consequence,it is surprising that more work has not been instigated. This may be ude to the high cost of keeping monkeys and the increased inconvenience of handling, but it is apparent that more studies on this animal model are needed before a complete understanding of periodontal vasculature is obtained.

The Vascularization of the Periodontium of Man

There has not been a great deal of literature on the vascular morphology of human periodontium and that which was available provided only incomplete information. All reported work was carried out on cadavers, mainly of elderly people, and also on infants. Information was available on the distribution of blood vessels within the jaws (figure 11) but there was a distinct lack of reporting on the architecture of the periodontal ligament and the gingival crevice region specifically.

Castelli (1963) reported that the mandible received its arterial supply from the inferior alveolar artery,which provided vessels in the cortical bone of the mandibular body and alveolar-dental branches to teeth and adjacent tissue. In the incisive area, anastomosis occurred between the inferior alveolar artery and vessels from the sublingual region in the area of attachment of the geniohyoid, genioglossus and anterior digastric muscles. The alveolar dental branches were branches of the inferior alveolar artery and consisted of eight to twelve main channels,averaging

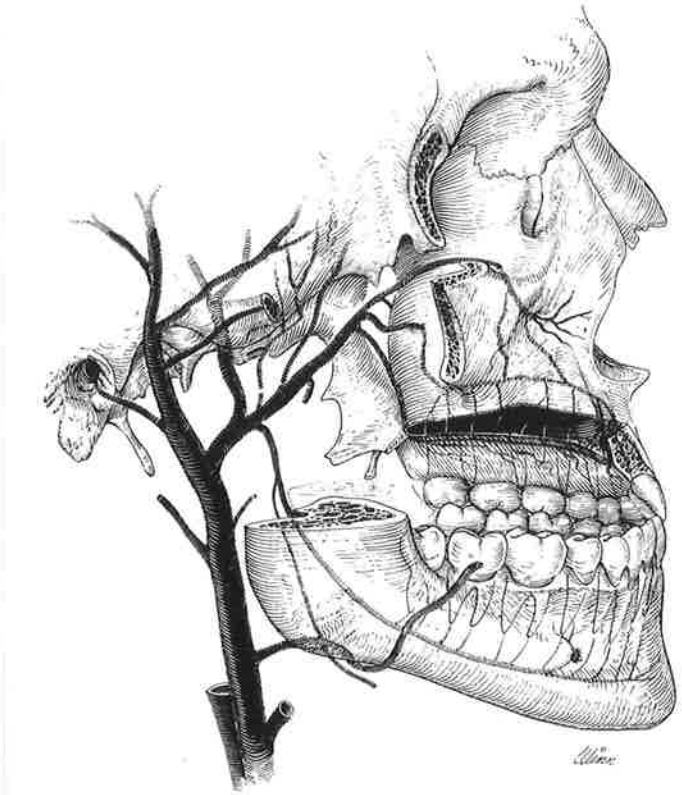


Figure 11. Schematic composite illustration of the blood supply to the maxilla and the mandible of the human (from Bell, Proffit and White 1980).

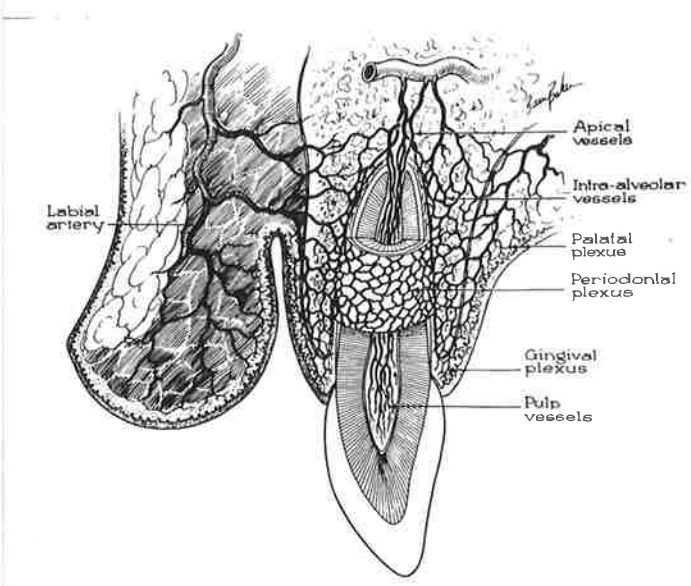


Figure 12. Schematic composite illustration of the blood supply to the anterior maxillary region. (from Bell, Proffit and White 1980).

280 micrometres in diameter with a varied number of finer branches. These arteries surrounded each alveolus,and having passed upwards through the alveolar walls,anastomosed with the capillary network of the gingiva. These arteries also supplied the pulp, alveolar bone, interalveolar septi, and the periodontal ligament.

It is unclear how these vessels entered the periodontal ligament,but an investigation by Birn (1966) assumed that each perforation in the tooth socket wall contained blood vessels which entered the periodontal ligament. Birn noted perforations at all levels but most frequently in the gingival third, less in the apical third and least in the middle third, except in the multi-rooted teeth where the supply is equal in apical and middle thirds. Birn also found that the blood supply (really the area of perforations per unit surface area) increased gradually tooth by tooth, toward the posterior teeth, being least to the incisor and greatest to the second molar. In all teeth the ligament was mainly supplied by small blood vessels,but the number of large vessels increased from the incisors towards the molars with most vessels, small or large, found in the gingival and apical thirds of the alveolus. Variations were also reported by Birn between the various surfaces of the tooth. For instance, the mesial and distal surfaces had a very slightly better supply than the buccal and lingual surfaces and,in the lower jaw,the blood supply was slightly less to the distal than the mesial of the ligament. The lower jaw also received a slightly smaller distribution of blood vessels than the upper.

Studies on the arrangement of vessels within the human periodontal ligament are sparse. A diagram of the arterial arrangement of the blood supply to the periodontium of the upper incisors of humans was published by Bell, Proffit and White in 1980 (figure 12). However, this diagram was presumably based on findings from monkey experiments, and although descriptive, cannot be regarded as an accurate representation of actual

human periodontal vascular morphology.

The most comprehensive description was published by Hayashi in 1932. According to Hayashi the periodontium was supplied either directly by dental arteries or indirectly by interalveolar branches of the dental arteries (figure 13a,b).The former arteries entered the ligament at the apical region and branched to supply the pulp and the periodontium. The latter arteries gave off perforating branches which passed through the socket wall to enter the ligament and course coronally. These perforating branches anastomosed with one another, as well as with the periodontal branches arising directly from the dental artery, to form longitudinal periodontal arteries.

In cross-section these longitudinal arteries gave off small vessels at right angles,which supplied the periodontal membrane and cementum and also united to form a vascular circle around the tooth.

Investigations into the types of vessels occuring in the ligament were limited. Gilchrist (1978) found only venous capillaries, postcapillary venules and collecting venules. Barker (1980), discovered what he described as pericytic venules. These were either partly or completely surrounded by pericytes and were thus all categorized as venous capillaries or postcapillary venules (Rhodin 1968). Gilchrist noticed that the most frequent vessel type was the venous capillary and these and the pericytic venules described by Barker were found to be closer to tooth than alveolar bone. The vessels described by Gilchrist were predominantly axially oriented,but the venous capillaries were also seen to course circumferentially. Barker found communications between medullary and periodontal vessels via foramina in the alveolar wall,but Gilchrist did not.

Few articles were found to describe the venous drainage in the human jaws and none discussed in detail the venous drainage of the periodontal ligament. Castelli (1963) described veins in the periodontal ligament

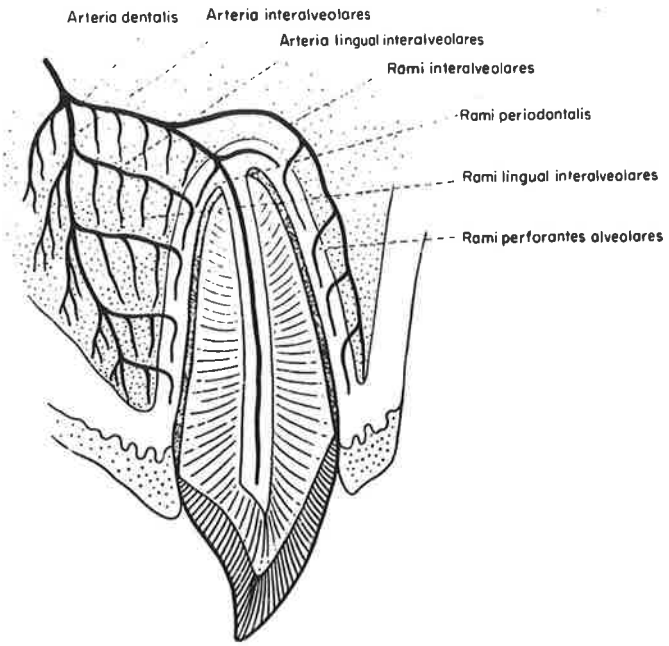


Figure 13a. Diagram of the distribution of the periodontal arteries about single rooted teeth of the upper jaw (from Hayashi 1932).

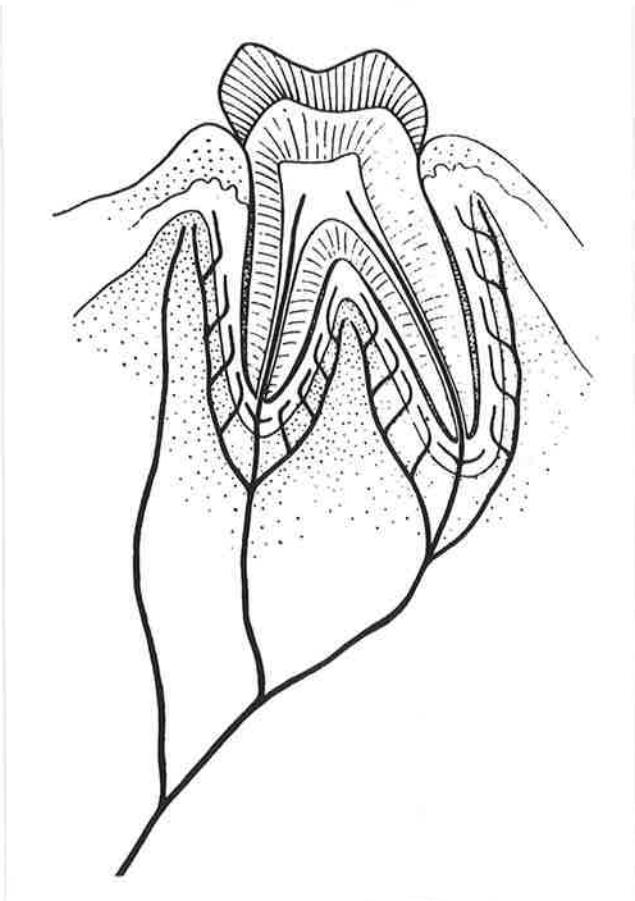


Figure 13b. Diagram of the distribution pattern of the periodontal arteries about a molar tooth (from Hayashi 1932).

that joined one another and also joined the veins in the interalveolar septi. Within the septi were large venous vessels together with many small capillary vessels,forming venous nets which were linked with the venous network surrounding the apex of each alveolus. From L. Cohen's (1959) descriptions it appeared that the veins of the mandible converged and passed into the facial veins (both anterior and posterior) and, via these, drainage occurred into the jugular vein. There were also veins called inferior dental veins that accompanied the arteries of the same name. These veins drained upward to the pterygoid plexus. Cohen (1959) felt that the veins of the periostium had a large surface area and drained into the facial veins and were responsible for the main venous drainage of the mandible.

Bouyssou, Bader, Lodter and Duffaut (1970) described arterio-venous glomi in the periodontal ligament,particularly in the coronal half. These glomi consisted of a markedly collapsed arterial part which had thick walls and also voluminous venous parts with thin dilated walls. These authors noted that these structures disappeared around functionless teeth.

The blood supply to the human gingiva has not been studied extensively. Accepted textbooks on periodontal anatomy report only scantily and mainly describe animal vascular architecture in this region, predominantly of monkey and dog (Carranza 1979, Goldman and D.W. Cohen 1980).

Forsslund (1959) and Saunders and Rockert (1967) agreed that interdental branches of the superior and inferior dental arteries perforated the alveolar crest to end in the capillary network of the gingiva. These small arteries anastomosed with arteries supplying the vestibular and oral mucosa. The gingival tissues were also supplied by the greater palatine artery and superior labial branches of the facial and infraorbital arteries in the maxilla in the regions of distribution of these arteries. In the mandible

the collateral supply to the gingiva was afforded by the sublingual branch of the lingual artery and by branches from the buccal, inferior labial and mental arteries in the corresponding distribution area (Saunders and Rockert 1967).

According to Forsslund (1959) the gingival capillary network was formed by the division of the perforating branches into finer vessels to form a plexus. Capillary loops originated from this plexus subepithelially. The gingival vascular net anastomosed with vessels in the floor of the mouth and in the lips and with the vessels in the periodontal membrane. The capillary loops existed in the connective tissue where it protruded into the epithelium in finger-like papillae. These papillae disappeared towards the inner surface of the gingival sulcus and were highest in the external aspect of the epithelium. The capillary loops originated from an arteriole and emptied into a venule. Forsslund (1959) also discovered an anastomosing pattern of capillaries as well as the loop pattern. This anastomosis pattern occurred in healthy gingiva but was more frequent in inflamed gingival tissue. The number of capillary loops was observed to be 46 per square millimetre in healthy tissue and 21 per square millimetre in gingivitis. Kamijo, Suzuki, Takahashi, Wakatsuki, Maeda and Takeishi (1964) found a slightly greater concentration of capillary loops per unit area but confirmed the reduction in number when the gingival tissues were inflamed.

Glavind and Loe (1966) found similar capillary densities in the normal gingiva of pregnant and non-pregnant women and also found that the densities were comparable when these two groups of women displayed mildly inflamed gingiva. These workers did not say whether there was a change in the density when comparing the diseased to the non-diseased state. The mean number of capillaries per 0.5 square millimetres in the free and attached gingiva was 59 and 61 respectively.

The information regarding the venous outflow from the gingiva is scarce. Saunders and Rockert (1967) expounded that some of the veins terminated in tributaries of the anterior and deep facial, buccal, maxillary, palatine and lingual veins.

Much more information needs to be elicited regarding the vasculature of the human periodontium. The paucity of findings is obviously due to the difficulty in obtaining suitable material in fresh condition for experimental work. Most authors relied on cadavers, or used vital microscopy. The latter being limited to obtaining information on only the most superifical structures.

The Vascularization of the Periodontium of Dogs and Cats

The blood supply to dogs was described by Perint in 1949. The maxilla and maxillary teeth were supplied by branches of the internal maxillary artery. The superior posterior alveolar artery gave branches to the antral mucosa and the molar roots as well as the premolar teeth and the superior anterior alveolar artery supplied the nasal mucosa and 'frontal' teeth. The superior labial artery supplied the gingiva and the labial frenum, and finally the palatine artery also contributed to the supply. In the mandible the blood supply was via the inferior alveolar artery, the inferior labial artery, (which anastomosed with the inferior alveolar artery), and lastly periosteal arteries which formed a double blood supply to the mandible,but were independent of the inferior alveolar artery.

Soloviev (1970) found that the periodontal ligament of the dog was supplied by arteries that penetrated from the interalveolar septa. These arteries gave off numerous capillaries that intertwined around the larger vessels to form a vascular network in the 'intermediate layer' of the periodontal network (Soloviev 1970, Ichikawa et al. 1976). Veins were present and both arteries and veins ran vertically (Ichikawa et al. 1976,

Carranza et al. 1966). Carranza and his colleagues found that the blood vessels were closer to bone than to cementum and that they had a 'plane-like' vascular plexus at the region of the epithelial cuff. The comprehensive work of Kishi and Takahashi (1977) supported this arrangement and showed a dual plexus that consisted of a rope ladder-like arrangement of vertical capillaries overlying a coarser plexus that was oriented more horizontally. This plexus was situated closer to the alveolar bone and also anastomosed with perforating vessels.

Towards the crest of the alveolar plate the presence of relatively thick circularly oriented bundles was reported in the layer closer to the bone (Kishi and Takahashi 1977),and this was similar to the situation described by Kindlova and Matena (1962) in the rat. The layer closer to the tooth gave off many hairpin shaped capillary loops towards the tooth,which was again in agreement with findings in the rat (Kindlova and Matena 1962). Above the alveolar crest both layers fused to provide a fine vascular plexus under the crevicular epithelium. Boc and Peterson (1981) found that the periodontal ligament was supplied by vessels from the attached gingiva as well as vessels perforating from the medullary bone.

The gingival tissues of the dog were supplied by the blood vessels of the periosteum,which ascended from the lower border of the mandible and anastomosed at the crest of the alveolar ridge. They also provided capillary loops to the connective tissue papillae of the epithelium. (Keller and Cohen 1955, Goldman 1956, Schuback and Goldman 1957, Nuki and Hock 1974, Kishi and Takahashi 1977). Arteries from the periodontal ligament also supplied the gingival tissues and branched into a microvascular plexus at the level of the epithelial attachment (Nuki and Hock 1974).

Egelberg (1966a) reported that the dentogingival junction had a

distinct layer of blood vessels close to the crevicular epithelium. No capillary loops, such as those that occurred under the oral epithelium, were present and the diameters of the vessels ranged from 7 to 40 micrometres. This flat subcrevicular epithelial network was similar to that found by Carranza et al. (1966) and Kishi and Takahashi (1977) at the epithelial cuff.

In addition, Nuki and Hock (1974) described a basic capillary unit that was repeated regularly throughout the gingival tissues. This regular network was a normal occurrence that appeared as soon as the tooth erupted and persisted until such time as the gingiva became inflamed.

Kindlova and Trnkova (1972), however, found this regular type of arrangement in only a third of their sample and reported a high degree of variability in the architecture of the vessels in the crevice. The vessels underyling the crevicular epithelium were continuous with vessels of the periodontal ligament via venules draining the capillary plexus (Nuki and Hock 1974).

The simple flat network of blood vessels in this region was quite distinct from the arrangement found by Kindlova and Matena (1962) in the rat, and Kindlova (1965) in the monkey.

In the cat mandible, L. Cohen (1959, 1960) reported that the inferior dental artery was the principal nutrient artery and that an anastomosis existed between this artery and the periosteal blood vessels. The vascularity of branches of the inferior dental artery was greater towards the alveolar bone than the inferior border of the mandible. The periodontal ligament was supplied by vessels from the apical region of the tooth, from the alveolar bone and from the gingival tissue. Cohen also suggested that arteries were present in the ligament, but did not elaborate on what other types of vessels might have been present. The

marrow of the cat mandible was supplied by a capillary network but no sinusoids were observed. The mandible was drained by a single venous channel.

Apart from the studies of Kishi and Takahashi (1977),insufficient work has been conducted on the overall vascular architecture of the cat and dog. No confirmatory work is available and indeed specific details are lacking. The need for further research, particularly in relation to a basis for classification of the blood vessels present, is again emphasized.

The Vascularization of the Periodontium of Mice

Only Carranza et al. (1966) had carried out experiments looking specifically at the periodontal vascularization of mice. These authors also included investigations on rats, dogs, cats, hamsters and guinea pigs in their paper and gave a generalized description to cover all these animal models. Only a few specific references to the mouse were provided and it was assumed,therefore, that the mouse had a vascular arrangement similar to that for the rat, where the principal vessels ran parallel to the long axis of the root and gave out branches that intertwined forming a periodontal plexus. This plexus was closer to bone than cementum, presumably along the whole length of the ligament, but this point needs clarification. Connections were seen between periodontal and pulpal vessels and occurred particularly in the apical region. This arrangement was confirmed by Sims (1977) in his histological investigations of oxytalan fibres in mouse periodontium where he found the vessels had an occluso-apical orientation and were interconnected by lateral anastomosing branches. He also found that adjacent molars were connected directly via groups of vessels contained in canals passing through the interseptal bone.

Of all the animal models, the vascular morphology of the periodontium of the mouse has been the least investigated. This is most likely due to the animal's small size, making it difficult to carry out the perfusions that are most useful in the study of vascular architecture.

THE ROLE OF THE PERIODONTAL VASCULATURE

The role played by the microcirculation in the performance of an organ or structure is either:

(1) primarily nutritional to the tissue or

(2) possessing a regulatory, homeostatic or operant function

(paraphrased from Knisely (1940) by Sobin and Tremer (1977)).

Sobin and Tremer (1977) stated that "it is axiomatic that form and function of the circulatory system are interdependent, and that the anatomy clearly reflects the physiology of the part". An examination of the circulatory system of different organs such as kidney, liver and lung shows striking differences among them and these differences are related to the different functional roles played by these organs in their entirety. Carrying this concept further, there appears to be no reason to suggest that the microcirculation of the periodontium is not intimately related to the function of the ligament. Since the role of the periodontal ligament in permitting the tooth to withstand functional loading without damage is quite unique in the body, one can infer that the circulatory anatomy might also be quite unique. In function the periodontium is able to withstand extremely high forces (Mansour and Reynick 1975) with no damage to itself or surrounding structures. Also the oral environment is extremely hostile from a microbiological viewpoint and, although dental disease is endemic in the human, the oral structures are able to withstand challenge from this environment surprisingly well. In addition, the architectural arrangement of the periodontal microcirculation has been implicated in the ability of the periodontium to carry out its multiple and varied function.

The large venous plexus found in the periodontium of rat and monkey (Kindlova and Matena 1962, Castelli and Dempster 1965) has been described as a reservoir of blood which, when combined with a similar venous collection in the bone marrow, can act as a compensatory mechanism for transitory changes in pressure within the ligament during function. Thus, if a tooth is tipped, the increase in pressure in the region of compression is relieved by an outflow of blood to a region of relatively lower pressure, into the region of tension or into the sinusoids present in the bone marrow, for instance (Castelli and Dempster 1965). Bien (1966) postulated that the apical venous rete provided an efficient diffusion exchange mechanism and also a mechanism for maintaining steep pressure gradients across very thin membranes. These properties are required for maintaining respiratory needs, waste disposal and temperature regulation, etc. but are also necessary to prevent gas cavitation occurring during high compressive forces.

Bien further postulated that if this venous rete failed to function then tiny bubbles of carbon dioxide would be liberated at the apex causing a local drop in pH which would in turn cause decalcification of the root manifest by root resorption. This theory could explain how root resorption can occur without necrosis of the apical tissue following the exertion of excessive forces by orthodontic or prosthetic appliances, or by the presence of a malocclusion.

It has also been postulated that the movement of the tooth in its socket has the effect of applying intermittent pressure onto the "basket-like" venous plexus at the apex of the tooth which acts to empty the veins and thus stimulate the blood circulation (Kindlova and Matena 1962). In addition it has been mentioned that the course of some vessels partly embedded in the alveolar bone helps protect them from collapse in the face of an increase in pressure during function (L. Cohen 1960). This,

however, cannot be possible if one assumes that the periodontal ligament resembles a closed system filled with incompressible fluid. This is because of the fact that the application of a force on such a system will create an equal force on all other parts of that closed system. This hydraulic action has maximum effect if the closed system is homogenous. The periodontal ligament is far from homogenous and it is not a closed system, since it has vessels entering and leaving it, and so this hydraulic effect must be lessened somewhat. However, within the relatively confined interstitial tissues of the ligament,a localized hydraulic action might cause obliteration of the blood vessel lumen,whether the vessels lie in bony grooves in the socket wall or not.

The spread of infection has also been attributed to be enhanced by the venous architecture (L. Cohen 1959, Castelli and Dempster 1965), as the direction of the infective process follows closely the path of the veins and lymphatics. By the same token, however, the vascular system has been acknowledged as playing a role in the defence mechanisms against infection, particularly in the gingival crevice region. Egelberg (1966a) postulated that the arrangement of the apparently venular plexus subjacent to the crevicular epithelium was conducive to the exudation of tissue fluid from the gingival crevice by virtue of the greater permeability of these venous elements. He also mentioned, and has support from other workers (Kindlova and Plackova 1973), that the vessels underneath the crevicular epithelium, particularly the junctional epithelium, are easily injured by extraneous noxae. Thus one cannot be sure if the particular morphology of this area developed in response to environmental irritation,or was related to the morphological characteristics of the junctional epithelium itself (Kindlova and Plackova 1973).

The morphology of the vascular plexus beneath the crevicular epithelium has been further implicated in the preservation of the integrity

of the "epithelial cuff". Waerhaug (1960) suggested that the tight seal of the unattached gingiva around the cervical region of the tooth was maintained by the tonicity of the tissue provided by the subgingival vascular plexus. Forsslund (1959) found that the architecture of the gingiva facing the oral cavity was remarkably stable and allowed the maximum amount of circulation through the gingival tissues at all times. He proposed that this constant circulation may form part of the tissues' local resistance to infection and also resistance to the constant thermal and mechanical stresses to which the gingiva is frequently subjected. Bien (1966) proposed that the dense gingival plexus was an area where replenishment of the "squeeze film" could occur. The application of a force on a tooth could be dampened because the interstitial fluid is forced out of the space between the tooth and the socket as the tooth is intruded. When the force is relieved, the interstitial fluids (or squeeze film) are replenished not only by a reflux of interstitial fluids but also by fluid diffused from the blood vessels of the dense gingival and apical networks. The arrangements of vessels in the gingival and apical regions has been considered by Bien to be support for his concept that these networks operate as a defence against the high pressure generated in the periodontium by the forces of mastication. Also, as mentioned earlier, the architectural arrangement of these regions provides an efficient diffusion exchange mechanism which enables normal physiological processes to occur across the blood vessel wall, despite the fact that blood flow may have been occluded by the application of a force onto the tooth.

Bien was not able to cite any proof for his hypotheses. Wills, Picton and Davies (1976) however, were able to determine that the resistance to displacement of a tooth after the application of an intrusive force was provided, in part, by the fluid systems of the periodontium. They ascertained that the fluid systems contributed 30% of the resistance to displacement. The authors made mention of the fact that, although the

blood vessels only occupy a small portion of the periodontal ligament (0.5 to 1 per cent by their estimates),any reduction in the volume of the vascular bed has a large effect on the intrusion. In the early stages of intrusion, the blood may be looked upon as having an energy-dissipating function providing a major viscous component to the displacement and recovery of the tooth.

The role of the vasculature in bone and cementum resorption has been discussed by Gianelly (1969) and Bernick (1962). Gianelly found that orthodontic forces that occluded the lumen of the blood vessels were associated with undermining resorption of the alveolar bone. In areas where the orthodontic force was less and the vessels maintained their patency, the predominent resorption was of the frontal type. Gianelly postulated that the vascular network acted as a hydraulic system that transmitted a force, on stimulation, which created a pressure increase in the attachment apparatus. According to Bassett's theories,such a pressure could distort the crystal structure of the adjacent bone creating piezoelectric currents (Bassett 1965) which might then result in osteoclastic activity in the immediate area.

Contrasting this latter viewpoint, Gianelly suggested that where the blood vessels were occluded,the nutritional supply to that area was lost and so also was the hydraulic transmission of force. However, these hydraulic forces might be established in adjacent areas where vascular occlusion had not occurred,and in these circumstances would be favourable for undermining resorption to begin. Bernick (1962) noticed that the blood vessels in rats formed a network in the periodontal ligament that was closer to bone than to tooth,except in areas where there was new formation of cellular cementum,or where there was resorption of the root surfaces. Bernick suggested that the occurrence of vascular twigs from the periodontal network to these areas added one more proof that blood vessels are necessary

in the process of bone and tooth repair as well as growth.

The periodontal vasculature is thus implicated in a wide range of different functions where complex and poorly understood mechanisms are involved. Much research is being performed to shed light on these areas, but before any of these investigations can be completely valid they must be based on a thorough understanding of the normal anatomy and morphology. Thus, further insight into the role of the periodontal vasculature awaits a comprehensive description of the normal. Once the description of normal anatomy has been achieved in detail,then it will be possible to undertake further studies such as:

(1) examine the aging changes in the vasculature

(2) use animals as functional testing models to detect the changes in vasculature incident to the application of a tooth moving force.

VASCULAR PERFUSION TECHNIQUES

The overwhelming majority of investigations into periodontal ligament vascularization have been conducted using various perfusion techniques that render the blood vessels visible. Only a relatively few studies have been performed on a histologic or ultrastructural basis. The information available about the types of vessels present in the ligament is very scanty indeed. The major gap in the research into periodontal ligament vasculature can be filled by TEM examination of animal models that closely resemble man. However, the three-dimensional description of a vascular bed using ultrastructural evidence can only be established easily using a statistical basis for random sampling of serial sections (Weibel 1981). This does not provide direct visualization of the architectural arrangement and is also open to the errors present in any statistical sample. The most satisfactory method of obtaining information about vascular architecture therefore is to fill the total vascular tree

with a material that can enable examination of the entire microcirculation still in its three-dimensional form and not necessitate interpretation from two dimensional data. A number of techniques have been developed to visualize the microcirculation of the periodontium. An overview of each technique will be presented here and pertinent features including advantages and disadvantages of each technique will be discussed below.

India ink in gelatin

The most common perfusion technique used has been the perfusion of a gelatin mixture coloured with either carbon particules in the form of India ink or a dye such as cinnabar. This medium is injected intra-arterially subsequent to blood washout with a physiological saline solution. Once the gelatin has set, the specimen is fixed, decalcified and sectioned into relatively thick pieces from 100 to 250 micrometres wide (Bernick 1962, Cernavskis and Hunter 1965) and then cleared. The sections are viewed under a stereo-microscope or light microscope. The main advantage of this technique is that the sections can be stained for concomitant histologic examination and as a consequence the relationship between blood vessels and other structures can be determined.

The disadvantages of the technique revolve around two main factors. Firstly, the injection of a viscous particulate medium into the vascular system cannot be guaranteed to produce complete filling of all blood vessels, because particles, even though they may be as small as 1 micrometre in diameter, can aggregate and occlude the smaller vessels (Hellem and Ostrup 1981a). As well, it must be difficult to get the viscous material to flow into the finest vessels. The second factor is the sectioning of the specimen which must be implemented to help overcome the very poor depth of field of the light microscope. This step destroys the three-dimensionality of the vascular tree and makes any discussion on spatial relationships extremely difficult and subject to errors of interpretation. Although this technique

has been used extensively and has helped provide a general outline of the vascular system of the periodontium,it is limited because it has not been able to provide a classification of all the vessel types in the vascular tree,particularly in relation to the venous side. Nor has it been able to provide a complete three-dimensional architectural description of the blood supply to the periodontium.

Other types of perfusion medium have been used,but the specimens have been treated similarly with fixing, decalcification and clearing followed by sectioning. The other media mentioned in the literature are:

(1) Mercury-gelatin mass (Goldman 1956)

(2) Teichmann's paste with cinnibar (Castelli and Dempster 1965)

(3) Microfil, a silicone rubber compound (Cutright and Bhaskar 1967, 1969).

Microangiography

Another commonly used technique is the injection of a radiopaque medium which subsequently renders the vasculature visible after the specimen is placed on fine grain photographic film and irradiated (Cohen 1959, Saunders 1967, Koivumaa and Lassila 1971). The specimen must be fixed and decalcified prior to irradiating. This technique again has the disadvantage of particulate matter being injected into the blood vessels and the associated blockage and lack of filling that can occur. Furthermore, the radiographic procedure renders a three-dimensional specimen only in two dimensions. This deficiency can be partly overcome by using stereo-pairs,but even so the resolution obtained cannot give sufficient detail of the capillary networks to be of any real value in studies of micro-vasculature.

Microspheres

A perfusion technique using microspheres of a size ranging around

15 micrometres in diameter was reported by Folke and Stallard (1967). They used histologically stained serial sections and traced them on glass plates to build up a three-dimensional picture. The hypothesis was that the microspheres became trapped in blood vessels where the diameter was sufficiently small to prevent further passage. If the microspheres were of a diameter of 15 (±5) micrometres, then they would penetrate blood vessels down to this diameter and no further. This position in the vascular tree was thought by Folke and Stallard to correspond to the junction between the arteriolar and capillary networks. However, the 30% variation in size of the microsphere means that the range of vessel diameters in which the spheres will be trapped is from 10 to 20 micrometres. Within this size range, the type of vessel encountered can vary from a small arteriole to a capillary to a postcapillary venule. In addition, the aggregation of microspheres within the vessel lumen could drastically influence the completeness of the perfusion. As a consequence, the classification of a vessel based on the entrapment of a microsphere cannot be at all accurate.

Vital Microscopy

Vital microscopy is a method that has been used to examine the vascularity of readily accessible structures and has the advantage of being able to observe in vivo the normal undisturbed anatomy and physiology. Forsslund (1959) examined the gingival tissues of humans as did Kamijo, Suzuki, Takahashi, Wakatsuki, Maeda and Takeishi (1964) and Glavind and Loe (1967), but only the most superficial blood vessels of the buccal gingiva were described. Gangler and Merte (1979, 1983) developed a technique whereby both the periodontal ligament vessels themselves and those of gingival crevice could be viewed with and without the application of a

force to the tooth. Some invasion of the alveolar bone and tooth structure was necessary but this did not have any apparent effect on the area under observation. Gangler and Merte have to date only described the continuously erupting incisor of the rat which unfortunately, has only limited relevance to this project.

Injection replica or vascular casting technique

The examination of hollow structures by filling them with liquids which solidify has been occurring for centuries (Hodde and Nowell 1980). Many methods have been developed that consist of clearing the filled or perfused tissues followed by sectioning of the specimen in order to render the blood vessels visible. The main disadvantage of this method is the loss of the three-dimensionality that occurs after sectioning of the specimen. To overcome this problem the use of corrosion casts has become widespread. This technique has enabled observation of an internal replica of the vascular tree in its entirety without the surrounding tissues blocking direct vision. Initially these corrosion casts were studied under the light microscope and the short depth of field of this instrument limited the usefulness of the three-dimensional casts. The advent of the SEM, however, has enabled more information to be obtained from vascular casts because of the great depth of field that these microscopes are able to generate.

The first use of the SEM to study corrosion casts of blood vessels was in 1970 by Nowell, Pangborn and Tyler using latex casts. Murakami in 1971 was the first to study acrylic resin corrosion casts in the SEM. There are a number of casting materials that are available and the selection of a particular material depends not only on the organ or tissue being investigated but also on whether the material meets the criteria for an ideal injection media. These criteria are listed in the Appendix.

The two most common injection media used in corrosion cast SEM

microscopy are rubber compounds (latex) and polymer resins (methyl methacrylate), since they most satisfactorily fit the criteria (Gannon 1978). The advantages of the rubber compounds are:

(1) they can produce complete replication at the microvascular level,

(2) because vulcanization occurs with a change in pH, there is minimal increase in viscosity during preparation of the animal and filling of the vascular system,

(3) the opacity of some latex media allows orientation of the specimen during dissection under light microscopy,

(4) the elasticity of the vulcanized medium allows virtually distortion-free gross dissection.

The disadvantages are:

(1) casts of diluted latex require freeze drying or critical point drying to preserve spatial interrelationship,

(2) they do not consistently replicate luminal surface microstructures,

(3) the elasticity which enhances gross dissection inhibits accurate micro-dissection of single vessels,

(4) the casts tend to droop under their own weight following corrosion,

(5) the degree and direction of shrinkage during hardening and specimen preparation is uncertain.

(Adapted from Gannon 1978 and Hodde and Nowell 1980).

The advantages of methyl methacrylate are:

(1) the resins can be prepolymerized to a known viscosity (Gannon 1981),

(2) the casts can support their own weight and are easily air dried,

(3) complete replication of the internal lumen of a blood vessel including microstructural detail is readily achieved,

(4) the pliable casts developed by Murakami (1975) are readily dissected without gross distortion to the remaining portions of the cast.

The disadvantages are:

(1) the increase in viscosity during the injection period as the resin is polymerizing from the time it is mixed,

(2) the components of the resin are injurious to human health and so they must be handled with care,

(3) shrinkage during polymerization.

The development of the corrosion casting or injection replica technique has probably produced more useful information than any other method with regard to vascular architecture of other organs. The major advantage of the technique is that an impression of the internal lumen of the blood vessels can be made and all surrounding tissues can be removed to enable a complete three dimensional network to be observed. The disadvantages include the loss of spatial relationships to other structures, for instance bone and cementum, which are of interest in this project. These relationships are important when discussing the role a blood supply plays in providing nutrition, in dissipating forces and in aiding immunological defence mechanisms. Another disadvantage of the technique is the loss of surrounding soft tissue that would be useful for ultrastructural or histological classification. Thus, to classify vessels, one has to rely on the internal luminal surface detail as well as the vascular architecture. This is not as satisfactory as classification from ultrastructural morphology. The problem of the accuracy of replication in the casting medium is ever present, as is polymerization or hardening dimensional changes and the difficulties in obtaining complete filling of the vessels that occur in any perfusion.

The latex corrosion cast was used by Kindlova and Matena (1959, 1962) and Kindlova (1965) to detail the vascularity of periodontal ligaments in the rat and monkey. These workers observed the casts under the light microscope and were confronted with the problem of coping with the very

poor depth of field using this instrument. The use of these latex materials in the SEM has been reported (Nowell et al. 1970) but it was found that methyl methacrylate corrosion casts were more satisfactory. The use of this technique to view periodontal vasculature in dogs has been reported (Kishi and Takahashi 1977) and appears to have provided the most detailed information that is available to date. The SEM corrosion cast technique using methyl methacrylate seems to be the most able to fill in the gaps of information now present. The technique has been reported to provide complete replication of the internal lumen of the blood vessels to give an easily examined three-dimensional structure from which the answers to many questions pertaining to periodontal vascular architecture can be gleaned.

CLASSIFICATION OF BLOOD VESSELS

Blood vessels can be categorized on an ultrastructural basis after examination in the TEM. This method provides information about vessel diameter, about the types of cells that are present in the vessel wall and about the vessel's relationship to structures such as bone, cementum surface, collagen fibres and the other components of the periodontal ligament. However, the information is only available in a two dimensional form and the three dimensional spatial relationships must be determined by a time-consuming steriological technique. As a consequence, TEM examination does not conveniently give an indication of the vessel's relationship to the other components of the vascular tree. Classification of blood vessels based on their position within a vascular tree (i.e. vascular architecture) can be made relatively easily using SEM examination of vascular casts. A search of the literature reveals several useful references which indicate methods of classification based on either ultrastructure or vessel architecture and distribution which can be applied to the examination of vascular casts,such that an accurate categorization

of the vessels can be made.

Endothelial Cell Morphology

Rhodin in 1967 and 1968 published two comprehensive papers on the classification of vascular elements of subdermal microcirculation of the rabbit skin. He concentrated particularly on the ultrastructural differentiation of arteries through capillaries to veins. In his work he determined criteria which can be used to identify any particular blood vessel. Since this project will not specifically be using ultrastructural examination, the criteria that Rhodin used for identification can not all be applicable. However, some of his criteria relating to internal diameter and endothelial cell morphology are of immediate application in the corrosion cast technique and are detailed in table 1.

The luminal surface morphology of arteries, capillaries and veins is most important in this project as the internal surface detail is readily imprinted onto the vascular casting.

Further evidence of differentiation between vessel types,based on endothelial cell morphology,was provided by Gnepp and Green in 1979,who published a paper comparing the luminal surface detail of lymphatics, arteries and veins of dogs. Specimens of the aorta showed that the endothelial cell nuclei were orientated in the direction of flow and protruded further into the lumen than did the endothelial cell nuclei of lymphatics. This was also true of the endothelial cells of the vena cava. While the nuclei of arteries, veins and lymphatics were approximately the same size, their shapes and distribution of chromatin were markedly different. The arterial endothelial nuclei were elongated and most commonly exhibited a fine chromatin pattern with randomly orientated irregular grooves.

Wolff (1977) emphasized that there were no distinct boundaries

VESSEL	INTERNAL DIAMETER (MICROMETRES)	ENDOTHELIAL CELL MORPHOLOGY
Arteriole	100 - 50	- Cells are flat and 50 micrometres long - nucleus is 2 micrometers high - usually overlapping of upstream cell on downstream cell
Terminal arteriole	Less than 50	- similar to above
Precapillary sphincters	7-15 (these taper off to an arterial capillary within 50 micrometres)	- shorter cells than for terminal arteriole - nucleus is shorter, thicker and more lobated - the entire endothelial cell protrudes - toward the lumen and reduces the diameter of the lumen
Venous capillary	Up to 8	- endothelial cell nucleus was flat
Postcapillary venule	8 - 30	- endothelial cells are flat & considerably larger than cells of the venous capillaries - slight overlapping of the adjoining endothelial cell occurs
Collecting venule	30 - 50	- similar features to postcapillary venule
Small collecting veins	100 - 300	- similar to above

Table 1: Criteria for categorizing blood vessels according to internal diameter and endothelial cell morphology (from Rhodin 1967, 1968).

between small arteries, arterioles and terminal arterioles. He pointed out that,along this sequence,the endothelial cells became more flattened, changing from a lancet-like shape with their long axis parallel to that of the vessel to a more disk-like shape, while the number of inter-endothelial contacts per vascular cross-section decreased.

The venous endothelial nuclei were described by Gnepp and Green (1979) as more angular in outline and less elongated than those of the arteries and lymphatics. They also showed a fine chromatin pattern and grooves orientated predominantly in the direction of blood flow.

Hodde and Nowell (1980) gave examples of vascular casts showing imprints of the endothelial cell nucleus as well as the cell borders. They also said that the imprint patterns were different and characteristic for arteries and veins and could be used as criteria to identify these vessels as such in the casts.

Hodde (1981) stated that arterial endothelial cells had an oblong shape and ovoidal nuclei (with microvillous protrusions), oriented in the long-axis of the vessel. The venous endothelial cells had a random shape with circular nuclei (without microvillous protrusions). Hodde used the differences in endothelial cell morphology as replicated in the vascular cast as criteria for distinguishing arteries from veins.

Langille and Adamson (1981) described a technique whereby brief perfusion of a silver nitrate solution caused a build up of silver precipitate at the cell boundaries,which left an impression of these boundaries in the vascular cast. This technique shows how classification,based on cell border morphology amongst other things,can be readily achieved.

Vascular Architecture

Wiedeman (1962 and 1963) published an investigation into the lengths

and diameters of blood vessels from distributing artery to collecting vein in the living circulation of the bat wing. She was able to provide information, based on the spatial relationships of the particular vascular elements, that would enable identification of a vessel to be made and she suggested some different types of vascular arrangement that can exist.

Wiedeman defined the distal limits of the particular types of vessels. A major artery was said to end at its first bifurcation,while the major vein was considered to begin at the point where two large veins form a junction. Small veins were defined as vessels which empty into the major vein and their origin was generally found to be parallel to an arterial arcade. Venules were defined as those vessels which empty into small veins. Venules received blood from postcapillary vessels which in turn originated from the capillary network. A capillary was defined as a distributing vessel which arose as a side branch of an arteriole and ended at a point where an inflowing tributary joined it forming a postcapillary venule.

The average lengths of the various types of blood vessels found by Wiedeman are tabulated below:

VESSEL	AVERAGE LENGTH (MILLIMETRES)
Artery	17.0
Small Artery	3.5
Arteriole	0.95
Capillary	0.23
Postcapillary venule	0.21
Venule	1.0
Small Vein	3.4
Vein	16.6

Table 2: Average length of blood vessels (from Wiedeman 1962)

The diameter of a major vein compared to a major artery was found to be greater again by one half. The major vein had twice as many inflowing tributaries as the major artery had branches. The cross-sectional area of the vein was more than twice that of the artery. The diameter of a small vein was twice that of a small artery and small veins had one half as many vessels again flowing into it, than the artery had branches. The total cross sectional area of the small veins was seven times as large as the arteries. The average diameter of a venule was three times that of an arteriole,with each having the same number of tributaries or branches. The total cross-sectional area of the venules was 25 times that of the arterioles. Postcapillary venules had a diameter twice as large as the capillaries and were three times more numerous. Each capillary gave rise to three postcapillary venules, and,although there were equal numbers of postcapillary venules emptying venules as there were arterial capillaries originating from arterioles, there were almost twice as many venules to receive these small vessels as there were arterioles to supply the capillaries.

Wiedeman also tabulated measured diameters of the capillaries of various mammals and tissue beds (e.g. dog small intestine and heart, rabbit muscle, human conjunctiva etc.) and found that they ranged from 2 micrometres to 12 micrometres,with an average of around 4 to 5 micrometres. She also calculated that the venous system accounts for 80% of the total vascular volume.

Guyton (1971) also discussed cross-sectional area of blood vessels and reported the total cross-sectional areas of each vessel type in humans. This has been reproduced in table 3.

In addition, Guyton described the quantities of blood in the different parts of the circulation (figure 14). The most interesting feature was that, despite the fact that the cross-sectional area of the capillaries

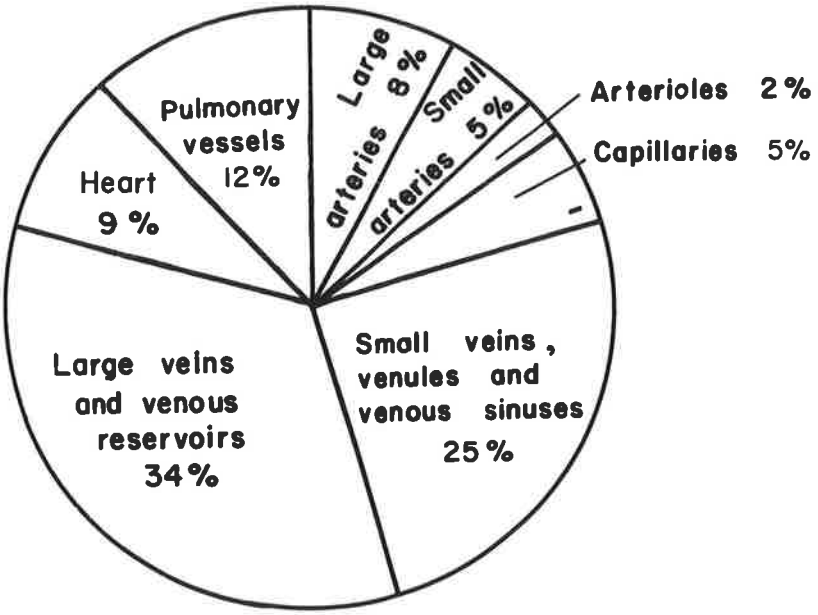


Figure 14. Percentage of total blood volume in each part of the circulatory system (from Guyton 1971).

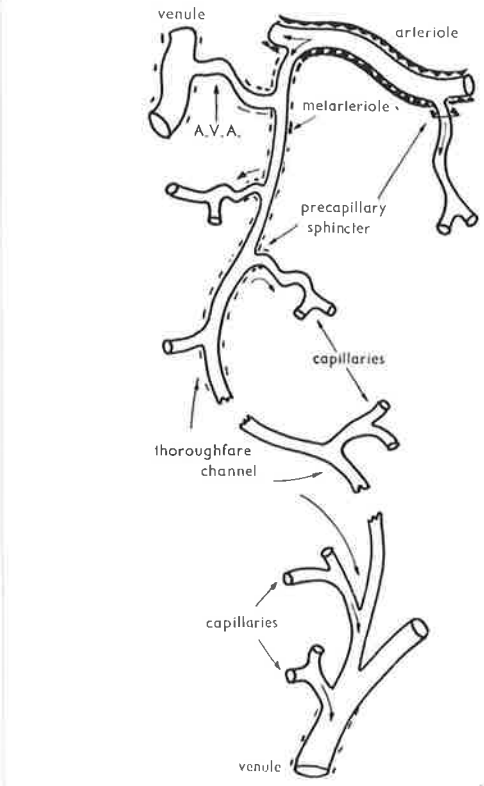


Figure 15. Diagram of a functional unit of a capillary bed, together with a metarteriolar-venular anastomosis (A-V-A) and a precapillary branching off directly from an arteriole (from Forsslund 1959, modified from Chambers and Zweifach 1944).

was 2500 square centimetres,these capillaries only contained about 5% of the total blood volume. This obviously indicates that these vessels are either relatively very short or that perhaps only a small number of capillaries are patent at any one time. It is these types of ratios (i.e. length, diameter etc.) that provide a valuable aid in vessel classification when examining the vascular casts.

VESSEL	CROSS-SECTION AREA (SQUARE CENTIMETRES)
Aorta	2.5
Small arteries	20.0
Arterioles	40.0
Capillaries	2500.0
Venules	250.0
Small Veins	80.0
Vanae Cavae	8.0

Table 3: Cross-sectional area of blood vessels (from Guyton 1971).

Forsslund (1959) discussed the morphology of a capillary bed from an arteriole to a venule,placing particular emphasis on the arrangement that occurred in the skin and the gingiva (figure 15). He stated that, morphologically,the capillaries can be divided into four types:

(1) arteriolo-venular bridges

(2) true or non-muscular capillaries

(3) arterio-venous anastomoses

(4) sinusoids

In addition,Forsslund mentioned that some writers classified capillaries into two additional types:

(5) precapillaries (arterial capillaries)

(6) venous capillaries (prevenules)

Forsslund used the precapillary category to include that part of the capillary that included the so-called precapillary sphincter.

The first three of the above categories were found in skin and the particular features of each are described below:

(1) Arteriolo-venular bridges.

These vessels provided the most direct path between the arterial and venous sides. The vessel walls contained thin smooth muscle cells that were widely separated. Between the muscle cells,the vessels were able to function as true capillaries. The muscle cells strengthened the vessel wall and prevented the vessel from passive collapse with variation in blood pressure. These bridges varied considerably in length and often increased in diameter as they extended distally. The proximal muscular part of the bridge was called a metarteriole. In the distal region the bridge functioned as a true capillary,but since it was an extension of an arteriolo-venular bridge,it was termed an arteriolo-venular (or a-v) capillary. The diameter of this a-v capillary varied from 12 to 50 micrometres,but was predominantly around 15 micrometres. The arteriolo-venular bridges did not show a direct course between arterial and venous sides,but showed a variety of branching patterns:

(a) A direct path

(b) A fountain-shaped pattern with a central trunk that divided into two or three branches

(c) A horseshoe pattern

In the ear of the rat almost all minute vessels extending to the epithelial surface were arteriolo-venular bridges. This might reflect the fact that blood supply to the ear has to serve only metabolic and nutritional requirements alone and has no specific

functional requirement.

(2) True capillaries

The wall of these vessels consisted solely of endothelial cells, numbering from two to five around the circumference. True capillaries occurred as lateral branches of arteriolo-venular bridges and were often collapsed. As the capillary branched from a metarteriole, that part of the capillary nearest the muscular vessel formed a muscular sphincter (or precapillary sphincter). The capillaries emptied into a-v capillaries, prevenules or venules. Each arteriolo-venular bridge with its lateral capillaries formed a morphologic unit. Sometimes there was anastomoses between such units via true capillaries and this formed the vascular pattern of the capillary bed. A capillary could arise directly from an arteriole and have its capillary sphincter proximal to this origin. The capillary subsequently emptied into a venule.

(3) Arterio-venous anastomoses

These were small shunts between arteries and veins. The majority of arterio-venous anastomoses were found in those parts of the body most exposed to thermal and mechanical stimuli. As a consequence, it might be reasonably proposed that this type of anastomosis would be predominant in the periodontal ligament.

These types of architectural arrangements can be an important guide to classification of the vascular patterns found in the corrosion casts of blood vessels. The commentary by Forsslund (1959) emphasized the fact that not all capillaries were open at any one time and their patency depended on the metabolic and functional requirements of the particular area where they existed. This phenomenon highlights the problem of ensuring all vessels are filled when performing vascular perfusions and must be born in mind when examining casts.

In summary then it is possible to provide a reasonably accurate classification of the internal replica of the periodontal vasculature using the following features as a basis:

(1) Diameter of the vessel

(2) Shape of the vessel

(3) Patterns of branching and anastomoses

(4) Cellular impressions on the cast surface of

  (a) endothelial cell nuclei

  (b) endothelial cell borders

  (c) precapillary sphincters

These features are summarized in Table 4.

ASPECTS OF THE PERIODONTAL CIRCULATION REQUIRING CLARIFICATION

The vascular architecture of the rat has been investigated more than other animal models and yet gaps still exist in the information collected. The most important questions that need to be resolved in all animals are

(1) what categories of blood vessels are present in the periodontium?

(2) what exactly is the architecture of blood vessels at the gingival margin?

(3) what is the spatial relationship between blood vessels, tooth surface, cementum and alveolar bone?

(4) are separate arterial and venous networks,as described by Kindlova and Matena,present?

(5) what is the morphology of the vascular junctions?

(6) what is the arrangement of lymphatics?

In regard to the first question,only a few authors have attempted to classify blood vessels. However, those who have gave only very brief criteria for their classification or none at all. Kindlova and Matena,in their 1962 publication,freely discussed arteries and veins as did Kindlova in 1965,but

VESSEL CATEGORY	ENDOTHELIAL CELL IMPRINTS	SHAPE OF LUMEN	PATTERN OF BRANCHING	INTERNAL DIAMETER RANGE (MICROMETRES)
Arterioles	Cells have an oblong shape with spindle shaped or ovoidal nuclei imprints oriented along direction of flow. The nuclei imprints show microvillous protrusions.	Straight but wavy below the site of branching. Round cross-section.	Relatively few branches which have the main trunk almost at right angles.	50 - 100
Terminal Arterioles	As for arterioles	As for arterioles	As for arterioles	8 - 50
Capillaries	No characteristic imprints have been described in the literature, although the endothelial cells number from two to five around the circumference.		Arise as side branches of an arteriolo-venular bridge or a terminal arteriole and end at a point where joined by an inflowing tributary to form a postcapillary venule. May not all be patent at any one time.	4 - 7
Postcapillary Venules	Cells have a random shape with circular or oval nuclei imprints which have no microvillous protrusions.	Slightly flattened cross-section	Receive blood from capillary networks. Diameter is twice that of capillaries and they are three times more numerous Branches join at more acute angle than arterial branches. Could comprise the distal part of an arteriolo-venular bridge.	8 - 30
Collecting Venules	As for postcapillary venules		Receive blood from post-capillary venules and empty into small veins. Average diameter is three times that of arterioles but each has the same number of branches.	30 - 50
Small Collecting Veins	As for postcapillary venules			50 - 300

Table 4: Synopsis of criteria used for classification of replicated blood vessels. (Adapted from Kindlova and Matena 1959, Forsslund 1959, Wiedeman 1962, 1963, Rhodin 1967, 1968, Hodde 1981).

these authors did not discuss the criteria for their categorization.

Few authors discussed vessel diameter,which would be extremely useful in identification. Some authors who did mention diameter (Castelli 1963, Castelli and Dempster 1965, Folke and Stallard 1967, Kishi and Takahashi 1977) did so for the principal vessels only and did not use that information as a means of general classification. Nuki and Hock (1974), however, did base their classification of blood vessels of the gingiva on the diameter of the erythrocyte stream. Although patterns of anastomosis were discussed by these authors,the actual detail of branching and aborization was generally not described and this again would have been a further adjunct to classification as proposed by Weideman (1962). However, Nuki and Hock (1974) described a repeating morphologic unit in dog gingival tissues,but to date no authors have been able to provide similar descriptions for the other regions of the periodontium.

Strangely, the tenor of the majority of discussions was to describe the blood supply to an area and to ignore the venous drainage away from it. This seems to indicate that vessels that might be described as veins were not observed,due to the fact that the particular techniques used did not render the veins visible. In investigations that back-perfused via the venous system,the indication here is that the perfusion medium could not adequately delineate between the efferent and afferent parts of the vascular bed.

As a general criticism of previous investigations,one has to ask whether the techniques developed so far have been capable of filling the vascular tree completely and,as a consequence,is it timely to develop different techniques in an attempt to gain more complete information. The first area for further research thus appears to be to concentrate on obtaining visibility of the vascular tree in its entirety. Once a completely visible vascular tree has been obtained,one can conduct a basic classification of the vascular elements present. A more accurate and precise classification will

require concomitant TEM examination. To date no such basic investigation has been conducted.

The remaining questions regarding vascular architecture of the gingival margin and periodontal relationships will also be resolved by more complete information provided by improved techniques.

SECTION 4

METHODOLOGY

SAMPLE

Sprague-Dawley rats were selected as the experimental animal because they were readily obtainable, easy to handle and house, and were overall cost effective. The molar teeth of the rat have been said to resemble human molars in shape, proportion and function (Scour and Massler 1971). The molars do, however, possess a greater number of roots than their counterparts in the human dentition. Nevertheless, the advantages of using this animal outweigh the disadvantages,to the extent that the rat becomes one of the most suitable models for studying the vascular anatomy of the molar periodontium. The animals were bred at the Central Animal House of the Waite Agricultural Research Institute in Adelaide, South Australia. Male rats were obtained at 9 weeks of age and kept in the Animal House of the Dental Department, Royal Adelaide Hospital under controlled humidity, temperature and lighting. They were fed a normal laboratory diet with water ad libitum. Perfusions were conducted on animals aged 9-12 weeks,which was the equivalent age to a young adult human. Forty-eight rats were perfused and they ranged in weight from 200 to 425 grams,with most animals being approximately 275 grams.

ANAESTHESIA

Initially the animals were weighed and then placed in a dessicating jar containing ether. They remained in the jar until they could no longer hold up their heads. They were then removed and given an intra-peritoneal injection of sodium phenobarbitone (Nembutal 60mg/ml, Abbott Laboratories Pty. Ltd. Sydney, Australia),diluted 1 to 2 with physiological saline to give a dosage of 20mg/ml. Approximately 0.4 ml. were given for every 100 gms. of body weight. Anaesthesia was rapid and profound.

SURGICAL TECHNIQUE

Anaesthesia was tested by squeezing the animal's foot and touching the cornea of the eye. Surgery began if no reactions were elicited.

Superficial dissection of the ventral surface of the neck exposed both the right and left external jugular veins. They were freed of the overlying fascia and connective tissue envelope and a braided black silk suture was placed underneath each vein. Deeper dissection of the neck, cutting through the sternomastoid and sternohyoid muscles revealed the common carotid arteries of both sides and the overlying internal jugular veins. Black silk sutures were passed beneath each artery. The suture under the right common carotid was then tied as far proximally as possible and the vessel was cannulated distal to the tie with plastic tubing (size SP10, Dural Plastics Pty. Ltd.) The cannula was ligated into place securely with the black silk suture. The left common carotid artery was then ligated and cannulated. The external jugular veins were tied and cut distally to the tie to allow for the egress of blood. The animal was then ready for blood washout followed by vascular casting with methyl methacrylate resin.

PERFUSION TECHNIQUE

The equipment used for blood washout and subsequent resin casting was modified from that published by Gannon (1978) (figure 16). It consisted of a one litre aspirating bottle with the aspirating nipple at the bottom. Around the neck of the bottle was a clamp which securely held a rubber stopper. The clamp had an extension arm to hold a sphygmomanometer gauge. Two short pieces of glass tubing passed through the stopper and a long piece of rubber tubing was connected to one length of glass tubing and a Kartell 'Y' junction connected to the other. Attached to the end of the long piece of rubber tubing was a three-way stopcock,which was inserted

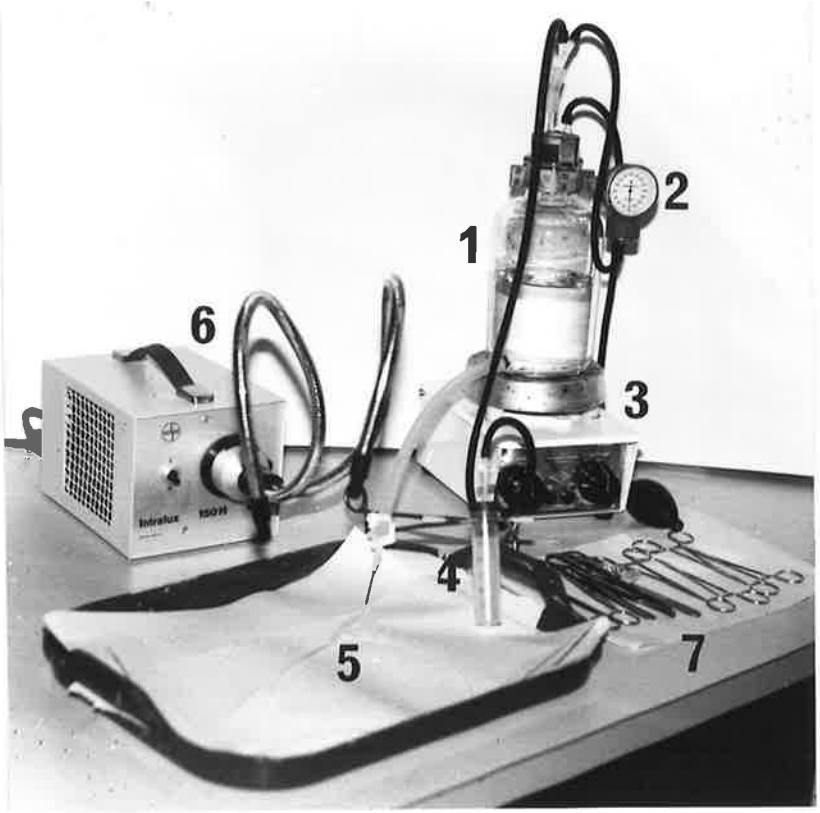


Figure 16. Perfusion Equipment

1 = One-litre aspirating bottle
2 = Sphygmomanometer gauge
3 = Heated magnetic stirrer
4 = 20.0 ml. syringe
5 = Cannula tubing
6 = Light source
7 = Surgical instruments

into the hub of an eighteen gauge needle. The needle was pushed through a stopper large enough to fit into a 20.0 ml. disposable plastic syringe. This syringe held the casting resin,which was mixed once washout was complete. A sphygmomanometer pump and sphygmomanometer gauge were connected to the remaining arms of the 'Y' junction by a short legnth of rubber tubing. This enabled the contents of the aspirating bottle to be pressurised to any desired pressure up to 300 mm Hg. The aspirating nipple had a large diameter silicone rubber tube pushed over it,to which was connected another three-way tap. This tap was connected,via the hub of an 18 gauge needle,to a 'T' junction piece of tubing. To each end of the 'T' junction was a series of tubing of diminishing diameter, each piece inserted into the end of the size slightly larger than itself until at the end was tubing of size SP10. These pieces of tubing were just large enough to be comfortably inserted into the carotid arteries and remained in place for the entire procedure. The 18 gauge needle hub accepted either the tap from the tubing off the aspirating nipple for the saline washout or the tip of the disposable plastic syringe for the resin perfusion.

Perfusion began as soon as each cannula had been inserted into one of the carotid arteries. The washout solution in the aspirating bottle was pressurized to about 240 mm Hg and the three way stopcock to the cannula tubing was opened. The stopcock to the 20.0 ml. syringe remained closed. Once washout was complete (as evidenced by the lack of blood escaping from the external jugular veins),the tubing was clamped to prevent both the loss of the perfusate from the animal and the introduction of air bubbles. The three-way stopcock from the aspirating nipple was closed and tubing removed from the 18 gauge needle hub. Just prior to terminating the washout, 40.0 ml. of the resin was mixed and placed into two 20.0 ml. disposable syringes. One of these was then inserted into the 18 gauge needle hub. The rubber stopper attached to the tubing from the top of the aspirating bottle was

put into the top of the syringe and clamped into place. The pressure in the aspirating bottle was increased to 300 mm. Hg and the stopcock to the disposable syringe opened. After the first 20.0 ml. of resin had been perfused the remaining 20.0 ml. was introduced by changing the syringe. Once all the casting medium had been perfused both external jugular veins were clamped and the pressure reduced to 20 mm. Hg. When the resin in the disposable syringe had polymerized, the equipment was disconnected after clamping the cannula into the carotids and the animal was placed into warm tap water (approx. $50^{o}C$).

PERFUSION MEDIA

Washout Medium

For each rat, 300.0 ml. of blood washout medium was prepared. Double distilled water which was millipore filtered through a 0.22 micrometre filter (Millipore Corporation, Bedford, Mass.) using a vacuum filtration unit (Gellman, Ann Arbor, Mich.) was used throughout. The following ingredients were added:

(1) Sodium Chloride (Ajax Chemicals, Sydney, Aust.):

9.0 gm. for every 1.0 litre of water

(2) Heparin (Heparin Sodium injection B.P. Mucous, Glaxo Australia Pty. Ltd.):

1.0 ml. of 1000 IU/ml heparin for every 100.0 ml. of water, to make a concentration of 10 IU/ml.

(3) Papavarine HCl (120 mg./10.0 ml., David Bull Laboratories Pty. Ltd.):

0.1 ml. of papavarine for every 1.0 litre of water.

(4) Polyvinylpyrrolidone, M.W. 40,000 (PVP40, Sigma Pty. Ltd.):

58.74 gm. for every 1.0 litre of water which provides a blood colloid pressure of 25 mm. Hg.

The solution was mixed in the 1.0 litre aspirating bottle on a heated magnetic stirrer (Cat. No. 212, SEM Pty. Ltd.) until a temperature of 45-50^{o}C was reached.

Casting Medium

The casting medium was prepared according to the formula developed by Murakami (1975) and refined by Gannon (1980). Initially 150.0 ml. of methylacrylate monomer (Polysciences, Inc., Washington, PA., Cat. No. 0834) were added to 1.5 gm. of 2:4 dichloro-benzoyl peroxide paste (Polysciences Inc., Warrington, PA., Cat. No. 0441). Six 25.0 ml. screw-capped scintillation vials (Wheaton Pty. Ltd.) were each filled with 20.0 ml. of this monomer, plus initiator, and placed in line in front of an erythremal fluorescent tube (FL8E, Oliphant Pty. Ltd.). Two vials containing 20.0 ml. of water were placed at each end of the row of six vials. The lamp was turned on and left for 27.5 minutes. At the end of this time the vials were allowed to cool to room temperature and their viscosity was measured using a modified Ostwald viscometer. This prepolymerization produced monomer with a viscosity of approximately 3 centistokes,which was stored until required at -4^{o}C. The shelf life of the prepolymerized monomer was 3 to 4 months.

To prepare the resin for casting, 12.0 ml. of hydroxypropyl methacrylate (Polysciences Inc. Warrington, PA., Cat. No. 0730) were mixed with 0.6 ml. of N-n dimethylaniline accelerator (Polysciences Inc. Warrington, PA., Cat. No. 0231). 28.0 ml. of the prepolymerized monomer were added to 0.4 gm. of Benzoyl Peroxide (water wet 78% active, Polysciences Inc. Warrington, PA., Cat. No. 3968). Once the Benzoyl Peroxide was dissolved in the monomer, the hydroxypropyl methacrylate containing the accelerator was added to it and this mixutre was placed into two 20.0 ml. disposable syringes and perfused as quickly as possible. Polymerization proceeded as soon as the resin was mixed and was completed in approximately ten to twenty minutes.

TISSUE CORROSION

Once the resin had polymerized, the equipment was disconnected and the animal was placed into a plastic container filled with very warm tap water,to which was added a heaped teaspoon of Bio-Ad (Colgate-Palmolive Pty. Ltd., Sydney, Aust.). The Bio-Ad helped hasten disintegration of the soft tissues. Leaving the animal for 24-48 hours in this solution rather than placing it immediately in caustic colutions also helped to provide more rapid corrosion. After soaking for this period of time,the mandibles were dissected free and, with the head, were then placed into a 20% solution of potassium hydroxide (Analytical and Research Chemical Co., Bowden, S. Aust.) in the same plastic container. After 4-7 days the potassium hydroxide was removed by overflow rinsing with tap water. Rinsing with cold water for 1-2 hours was followed by warm water for half an hour. Once all the debris was removed the water was carefully tipped from the container so that the partly corroded head and mandibles were not disturbed. The water was then replaced with approximately 10% hydrochloric acid (BDH chemicals (Aust.) Pty. Ltd.) for 4 hours. The acid was removed by further overflow rinsing for 1 to 2 hours.

Following acid corrosion some of the specimens were subjected to enzyme digestion in order to remove any further soft tissue debris. Three different enzymes were used,but none had any apparent advantage over the others. The enzymes used and recipes are as follows:

(1) Trypsin

50.0 mg. in 50.0 ml. of 0.1M phosphate buffer pH6. The temperature of the solution was maintained at $37^{o}C$ and the specimen was subjected to the enzyme action for 30 minutes. See appendix for formula of phosphate buffer.

(2) Pepsin

0.4 gm of 1:60,000 Pepsin in 100.0 ml of 0.1N HCl. The temperature of the solution was maintained at 37°C and the specimen subjected to digestion for 20 hours.

See appendix for formula of 0.1N HCl.

(3) Collagenase

50.0 mg. of collagenase in 100.0 ml. of TRIS buffer pH 7.4. Specimen subjected to digestion for 18 hours in solution maintained at 37°C.

See appendix for details on TRIS buffer.

After rinsing, the specimen was placed in a beaker containing distilled water and a few drops of detergent (Extran concentrate 100, BDH chemicals (Aust.) Pty. Ltd.). The beaker was then placed into an ultrasonic cleaner for 5 to 10 minutes. Examination of the specimen at this time under a stereomicroscope (Olympus Model VM2) revealed an apparently clean cast. However, subsequent SEM examination often revealed what appeared to be tissue remnants.

It was considered by this author that the tissue digestion was sufficient to remove all soft and hard tissue. This indicates that the apparent remanants were in fact either areas of extravasation or areas where the resin had permeated the blood vessel wall and entered the interstitial tissue spaces (Casley Smith and Vincent 1978).

Finally the specimens were carefully washed by putting them through a series of 3 beakers containing double distilled water prior to storage in a jar of the same water.

SECTIONING THE CAST

The specimens were sectioned in order to observe the vessels deep within

the socket and the medullary bone. To do this,the casts were first frozen in distilled water. The casts were readily visualised in the ice-block and easily oriented. The actual sectioning was carried out using a new razor blade (Blu-Strike, U.S.A.) by carefully sawing through the block of ice and the specimen within it. If the razor blade was used to vigorously slice through the ice-block it was found that the ice fractured and damaged the cast. Gentle back and forth cutting when the ice-block was melting, not straight from the freezer, obviated this problem and enabled good clean cuts through the specimens,leaving them otherwise undamaged.

The casts were sectioned in one of two planes,either sagittally or coronally. Four casts of the molar region were obtained for each animal representing the four quadrants. In each case,the casts on the left side were sectioned in one plane and the right side in another. The diagram below (figure 17) indicates the position of each plane of section for casts of the mandible.

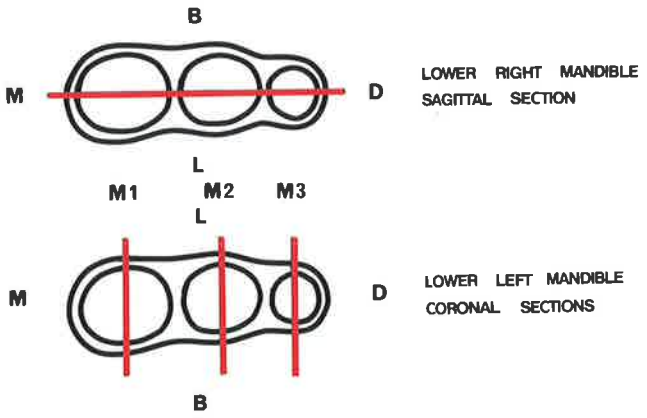


Figure 17. Schematic representation of position and direction of planes used for sectioning the vascular casts.

Sectioning in the sagittal plane enabled the three molars to be viewed in mesio-distal cross-section,whereas sectioning in the coronal plane allowed a bucco-lingual cross-section to be visualised. Three cuts had to be made in the coronal plane to expose the root sockets sufficiently.

DRYING THE CAST

Once sectioned,the specimens were replaced in double distilled water. They were dried by first placing a small quantity of detergent into their container. The specimens were then carefully placed on a piece of gauze and allowed to dry in the air. The detergent acted to reduce surface tension and allow the casts to dry without distortion.

RENDERING THE CAST CONDUCTIVE

The specimens were mounted on aluminium stubs using silver dag (Dag 915, Silver in M1BK, Achison Colloids Company, Plymouth). Conductivity preparation was according to the technique of Murakami, Unerhira, Kawakami and Kobutsu (1973). The stubs holding the specimens were placed into a dessicating jar containing 1.0 gm of osmium tetroxide crystals (Johnson Mathey Chemicals Ltd., Hartfordshire England) for 24-48 hours. They were then allowed to stand for 1 hour in the fume cupboard to allow excess osmium tetroxide vapour to sublimate,prior to placement into a tightly closed jar containing a small amount of hydrazine hydrate (Ajax Chemicals, Sydney, Australia). The final step was to sputter coat with gold using a C.S. Pty. Ltd. Minicoater.

The specimens were coated for a total of 7-9 minutes using 15 ma current at 150-200 micrometres pressure. Successive coats were applied in 30 second bursts and the specimens were allowed to cool for 20 seconds between coats. Each specimen was re-oriented within the chamber after every 2 minutes of coating. This ensured the most complete coating possible.

EXAMINING THE CAST

The mounted specimens were examined in an ETEC Autoscan scanning electron microscope at 20KV accelerating voltage and a working distance of 24 to 26 centimetres. The final condenser aperture was 200 micrometres. Areas of interest were selected for photography. After each area was photographed the specimen was tilted through 6^{o}. The area just photographed was recentred on the screen and refocused with the Z control. This produced a stereo-pair to enable 3-dimensional viewing of the specimen.

PHOTOGRAPHY

Photographs were taken on the photographic equipment attached to the SEM, using Ilford FP4 120 mm. black and white film. Specimen coding, magnification factor and scale, accelerating voltage and working distance were automatically recorded on the film. Magnifications recorded were for the size of the image on the negative.

DEVELOPING AND PRINTING

The film was developed using Ilford ID2 Developer and Ilford Hypam Rapid Fixer, following manufacturer's instructions in a Patterson developing tank. The negatives were printed onto Ilfospeed grade 2 glossy paper using a Durst Laborator 54 enlarger. Enlargement factors used were such that the whole negative image was printed onto paper cut into 5" x 6" rectangles. Occasionally negatives were enlarged to a greater magnification onto the same size paper. All magnifications quoted in this report are for the printed image, not the negative. The print was developed in Ilfospeed paper developer and fixed in Ilford Hypam Rapid Fixer according to the manufacturer's directions. The prints were dried in an air dryer (Model RCD-33, FC Manufacturing Co. Ltd., Osaka, Japan) and stored in paper envelopes.

EXAMINATION OF PHOTOMICROGRAPHS

All photomicrographs were examined in pairs using a Stereo Aids viewer (Rd. No. 70.485). This enabled all images to be viewed in three dimensions. More information was available using this method of viewing than by examining 2-dimensional images of higher magnification.

SECTION 5

FINDINGS

The findings have been divided broadly into two portions. The first deals with the classification of blood vessels and examples are given to show how the criteria outlined in table 4 can be applied to provide a definitive categorization of the vessels found in the periodontium.

The second and major portion concentrates on vascular architecture. The description of the vasculature will be related to each particular anatomically distinct region of the periodontium which has been divided into the following components:

(1) alveolar bone

(2) periodontal ligament

(3) gingival crevice

(4) interproximal col

(5) masticatory and vestibular mucosa

Present findings have shown that,as these periodontal components differed anatomically and functionally,so also did the vascular architecture of each region.

It must be pointed out that any comment about a vessel's relationship to other structures, such as dentine, enamel or epithelium, is really only a deduction based on existing knowledge of histologic examination of the same region. This is because all of the rat tissues are removed during the preparation of the cast for examination. However, the overall morphology of the vascular cast retains almost exactly the morphology of the vascularized tissues prior to corrosion and so the description of vascular relationships to other structures is less difficult than it might otherwise be (figure 20).

All photomicrographs studied during this report were stereo-pairs. Without a three-dimensional examination of the vascular casts,much of the

detail was lost. A low power view when studied in three-dimensions using an appropriate viewer reveals more information than a two-fold increase in magnification (Wergin and Pawley 1980),due to the perspective that can be obtained with this type of image. Because of this fact, the present author feels that unless the vascular casts are viewed with three-dimensional imaging, it is impossible to obtain all the information available,no matter what the magnification.

VESSEL CLASSIFICATION

An example of an arteriole is depicted in figure 21. It had an internal diameter of 55 micrometres and demonstrated elongated spindle-shaped endothelial cell nuclei imprints oriented along the long axis of the vessel. The vessel was round in cross-section and had comparatively few branches.

Figure 22 shows a vessel that can be classified as a terminal arteriole. The endothelial cell nuclei imprints were deeper in this example,but still displayed the sampe spindle shape, oriented along the long axis of the vessel, as seen in figure 21.

Two capillaries branching from a terminal arteriole, illustrated in figure 23, have an average diameter of 4 micrometres. The constriction in diameter at the site of branching could represent a precapillary sphincter or an arterial cushion. Endothelial cell nuclei imprints were very clearly defined on many of the replicated capillaries. More often than not,the lumen of the capillary was formed by only one or two endothelial cells and the ratio of the size of the nucleus to the vessel diameter meant that the nucleus covered a broad flat area wrapped around the replica. This resulted in the imprint of the endothelial cell nuclei being indistinct in small diameter vessels. The capillaries in the periodontium did not show any anastomosis into a typical capillary bed. Instead they seemed to play a

purely distributive role supplying the ligament. They could almost have been very small terminal arterioles.

A postcapillary venule can be seen accompanying the terminal arteriole in figure 24. The endothelial cell nuclei imprints of the venule were rounder, tending oval, did not show any specific orientation and had a flat floor when compared to the arteriolar nuclei imprints. These latter imprints showed microvillous protrusions which were not present in the venular imprints. The vessel diameter was 20 micrometres.

The endothelial cell nuclei imprints of a collecting venule (figure 25) were similar to the postcapillary venule. The diameter of this particular vessel was 35 micrometres and most examples of this type of vessel had a flattened cross-section and many tributaries which joined at an acute angle.

The regions of the periodontium under examination in the present report received their original supply from, and provided ultimate drainage into, vessels which were branches of the carotid arteries and jugular veins and were larger than those listed in table 4. The only feature that distinguished these vessels from their smaller ramifications was an increased diameter. In all other parameters they were identical to their immediate branches. Since these vessels occurred at sites removed from those investigated, it was not felt necessary to include examples in this report.

THE ALVEOLAR BONE

The arterial supply to the alveolar bone and indeed most of the periodontium was found to come from arteries (with a diameter of 100 micrometres or more) which were most likely branches of the inferior dental artery in the mandible and posterior superior dental artery in the maxilla. Branches of these arteries supplied the pulp and the interradicular septum. The periodontal ligament was supplied mainly by indirect branches emanating from within the bone medulla and only a small proportion of its vascularity

came from the apex via direct branches of the main artery. In the mandible the interradicular septum was supplied by six to eight terminal arterioles with a diameter of 20 micrometres,which followed fairly closely the course of larger venous vessels (figure 26). These vessels gave off only a very few branches while traversing the deeper parts of the bone medulla. As they neared the periodontal ligament, however, they branched more frequently (about every 150 to 200 micrometres) to supply the ligament over the entire interradicular septum. The branches were usually perpendicular to the main trunk. These arterioles entered the ligament at all levels and did not break into a capillary bed during their course through the bone. In fact,they appeared to supply the periodontal ligament only and did not provide any detectable branches to the medulla.

The venous drainage from the interradicular septum was directed away from the periodontal ligament. It appeared that the venous arrangement predominantly served to drain the ligament and a small number of sinusoidal vessels deep within the septum,rather than the bone medulla itself,which did not contain a microvascular plexus. Venous vessels from the ligament, approximately 15 to 20 micrometres in diameter, penetrated the alveolar bone and soon joined larger vessels of approximately 30 micrometres in diameter. These vessels coursed toward the middle of the septum and joined other vessels of similar size. The ultimate venous drainage away from the septum was via four or five central vessels with a diameter of 60 to 80 micrometres, with the occasional vessel being as large as 100 micrometres. The smallest venous elements were classified as postcapillary venules,and these joined collecting venules which drained into small collecting veins.

The venous drainage from the crest of the interradicular septum was mostly via collecting venules (35 to 45 micrometres in diameter),which ran very close to the periodontal ligament. On each side of the septum, however, the majority of the drainage was through postcapillary venules

with a diameter of 20 to 25 micrometres, which coursed for approximately 300 to 400 micrometres before coalescing to form collecting venules. Thus it seemed that a greater volume of venous blood needed to be carried away from the crest of the interradicular septum than from the sides.

In the middle of the interradicular septum there was a plexus of sinusoid vessels with a diameter of 30 to 50 micrometres and a distinctly different architectural arrangement (figure 26). Intertwined amongst the venous vessels was a fine open plexus,which was the only small calibre plexus within the septum that did not directly communicate with the ligament vasculature. It was not possible to determine if these fine vessels were terminal arterioles or capillaries. Their arrangement suggested that they were arterioles,whereas their diameter was such that they could also be capillaries.

THE PERIODONTAL LIGAMENT

The periodontal ligament, if considered alone, has a complex three-dimensional morphology (figure 27). It was found that the vascular architecture of the ligament differed according to region. There were two different patterns of ligament vasculature associated with the following regions:

(1) interradicular septum

(2) buccal and lingual walls, and interdental septum

(1) The Interradicular Septum

The portion of the periodontal ligament that encompassed the interradicular septum was quite a large proportion of the whole. It had a unique anatomical relationship by virtue of the fact that it was enclosed by the roots of the molar. As a result, that part of the ligament over the septum was principally self-contained as far as the vascular pathways were concerned. The remainder of the ligament vessels could communicate with

other vessels at the apex or in the gingivae whereas the interradicular vessels had no outlet through the gingival tissues. Consequently,the arrangement of the vessels reflected this situation (figures 28, 29, 30). The arteriolar supply came from the medulla and once the terminal arterioles entered the ligament they either enlarged to, or joined with, vessels which were approximately 15 to 30 micrometres in diameter that coursed occluso-apically (figures 28, 29, 30, 31). These vessels were postcapillary venules and they formed a complex vascular plexus within the ligament. There was not a capillary bed between the arteriolar supply and the venous side of the circulation. This arrangement could be classed as an arteriolo-venular bridge as proposed by Forsslund (1959).

The postcapillary venules did not run all the way from the apex to the crest of the septum (figure 18). Instead they coursed for a distance of anywhere between 100 to 400 micrometres within the ligament. The vessels followed a sinuous path and had a few branches that ramified with adjacent vertically oriented vessels,but more branches communicated with the medullary plexus. The apical extremities of each of these short venular segments re-entered the medulla and coursed via the shortest route to the small collecting veins, enlarging in diameter as they went. The coronal extremity of each segment turned at an acute angle,to form a short loop before penetrating the socket wall and entering the medulla. The coronal extremity also appeared to be venular in nature and the short loop drained into a large postcapillary venule or a small collecting venule.

The arteriolar supply usually joined somewhere in the middle of the segment and a segment was often supplied by more than one arteriole. Frequently, branches from the middle of the segment were venular rather than arteriolar. As a consequence,the communications between the ligament plexus and the network in the medulla were predominantly venular.

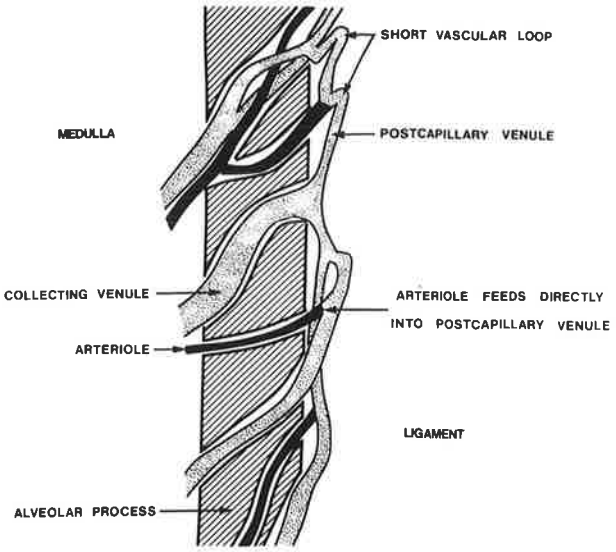


Figure 18: Schematic representation of the ligament vasculature over the vertical walls of the interradicular septum.

At the crest of the interradicular septum the vessels were arranged slightly differently (figure 32). In the ligament itself the vessels did not have any definite orientation rather they tended to have a random pattern. The venular drainage from the region was via vessels with a greater diameter than those draining the sides of the septum, and larger venous vessels (up to 60 micrometres in diameter) were also seen in the ligament area itself. The postcapillary venules from the top of the vertical portion of the septum drained into the venous vessels within the medulla,but also anastomosed with other venules on the horizontal part of the crest. This latter part of the septum was predominantly supplied by venules which had a random venule-venule anastomosing pattern. Because of this,it was difficult to determine the direction of blood flow. Arterioles were seen coursing to this region from the medulla,but their final pattern of ramification with the venules at the crest of the septum was difficult to see. It appeared that the arteriolar supply to the region was less than to the vertical portion of the septum. Some of the casts showed a circumferential invagination that resembled the imprint one would expect from a venous valve. The actual cause of this type of

imprint could not be determined.

(2) The Buccal and Lingual Walls and the Interdental Septum

The vessels around the perimeter of the socket and over the interdental septum were arranged in similar patterns to each other, except the vessels in the interdental region were somewhat more dense (figure 33). Vessel diameters ranged from 6 to 20 micrometres and the majority of vessels had oval or round endothelial cell nuclei imprints (figures 34,35). This suggested that the vessels of small luminal diameter were capillaries (perhaps venous capillaries) and the larger diameter vessel lumina represented postcapillary venules. On the buccal and lingual sides of the socket the vessels were grouped together in tracts with from three to six vessels in each tract. Occasionally, adjacent tracts merged and infrequently a single vessel from one tract coursed obliquely to join another tract. Each tract generally had vessels of two sizes. The smaller vessels were around 10 micrometres in diameter and the larger vessels averaged 20 micrometres in diameter. Often branches between vessels in the same tract occurred, but these were usually between vessels of similar diameter. The predominant feature was that the vessels could be traced, essentially uninterrupted, from the apex to the gingival plexus.

Most of the larger vessels did not exhibit any branching. Those that did branch divided into two, of equal size, just below the gingival plexus (figure 35). Occasionally, further down the ligament, these vessels would merge. The smaller vessels showed quite a variable arrangement and often horizontal branches between adjacent vessels could be seen. Also, these vessels were more likely to have branches that penetrated the socket wall, to anastomose with the medullary plexus.

Although both smaller and larger vessels anastomosed with the gingival plexus, many of the smaller vessels formed a U-shaped loop just below the

gingival crevice, but still within the ligament (figure 36) and the vessels re-entered the bone rather than join with the gingival vessels. The loops were formed with one arm closer to the cementum, the other arm closer to the alveolar bone and the bottom of the loop pointing coronally. Thus the arms were oriented, one behind the other, along a radius of the socket. The arm closer to the tooth was the coronal extremity of a vessel coursing from the apex,and the arm on the alveolar bone side coursed back downwards for 50 to 100 micrometres before penetrating the socket wall.

The smaller 6 to 10 micrometre diameter vessels would branch and intertwine around the 15 to 20 micrometre diameter vessels,but would rarely anastomose with them. Some of the larger vessels in the tracts did not come from the gingival plexus,but from arterioles that penetrated the socket wall from the medulla and bent through 90 degrees to course apically, transforming immediately into postcapillary venules (figure 35). This arteriolar supply usually occurred in the coronal third of the ligament. The exact nature of the communications with the buccal and lingual medullary spaces could not be determined because a satisfactory specimen sectioned in the coronal plane was not obtained. In the interdental septum, postcapillary venules in the ligament sent branches into the medulla,in a similar manner to the postcapillary venules,over the interradicular septum. These venules then joined other venules from adjacent regions and ultimately drained into the collecting venules,which collected blood from the surrounding alveolar process.

At the interdental septum the vessels were generally approximately 20 micrometres in diameter and almost all anastomosed with the vessels in the col (figures 37,39). Over the crest of the septum the vessels from adjacent sockets coalesced. In the apical interdental region the vessels were arranged in a somewhat finer plexus,with a number of loops that extended towards the cementum surface of the root (figure 40). Single loops

extending toward the cementum were seen infrequently in other sites around the perimeter, but they occurred generally in the coronal half (figure 38). The loops at the apex extended for approximately 100 micrometres along a radius of the socket with one arm above the other. Each arm was approximately 15 micrometres in diameter and arose from separate, but adjacent, ligament vessels and were at right angles to the other ligament vessels. The loops were comprised of vessels that were most likely postcapillary venules.

At the coronal extremity of the periodontal ligament the gingival plexus was observed to overhang the ligament vessels below it (figure 36). Although it is difficult to determine precisely from corrosion casts, it was presumed that the gingival plexus closely approximated the crown of the tooth and that the most apical vessels of the gingival plexus, running circularly around the tooth, had a close association with the cemento-enamel junction. Having made this assumption, it followed that the ligament vessels were closer to the alveolar bone than the tooth root. The vessels within the ligament were not arranged in a flat plane and were randomly situated within an annular segment, approximately 50 to 100 micrometres wide adjacent to the alveolar bone.

THE GINGIVAL CREVICE

The gingival crevice is defined in this project, as that part of the periodontium adjacent to the enamel of the tooth that extends from the base of the attachment epithelium up to the crest of the free gingival margin. The crevicular surface measured, on average, 300 micrometres from the crest to the attachment. The vascular plexus supplying this region exhibited the greatest complexity of any of the periodontal structures (figure 41).

Two major vascular arrangements made up the crevicular plexus. The first was a flat two-dimensional plexus that extended over the entire

crevicular region. The boundaries of the crevice, that is the attachment area and the free margin, were each outlined by a single vessel and the flat plexus extended between these two vessels. The vessel at the attachment area was continuous,whereas at the free margin some discontinuity was apparent (figure 19). The plexus comprised vessels approximately 8 micrometres in diameter,which were supplied by arterioles from the gingival tissues proper. Drainage was via a large number of vessels with a diameter of 20 to 30 micrometres,with very distinct round endothelial cell nuclei imprints. Most of these vessels were in the 30 micrometre diameter range and classified as collecting venules. The vessels of smaller diameter were categorized as postcapillary venules and were fairly short and usually drained into a collecting venule.

The predominant vessels below the flat plexus were the collecting venules, some of which anastomosed with the periodontal ligament vessels (figure 41). In addition it was observed that the flat plexus frequently drained directly into a vessel from the periodontal ligament (figure 41), rather than anastomose via the collecting venule. The more coronal half of the plexus drained into vessels from the gingiva proper and the more apical half drained into vessels from the periodontal ligament. The vessels of the flat plexus had indistinct endothelial cell nuclei imprints,but their arrangement and diameter seemed to indicate that they were true capillaries fulfilling primarily nutritive and metabolic roles. The pattern of branching was an open meshwork subjacent to the epithelium. The openness of the mesh did not appear to reflect any functional requirement.

The second major vascular arrangement of the crevice arose from within the flat plexus and consisted of a row or band of twisted vascular loops occurring along the whole length of the crevice (figure 41). In general, this row of loops was sited midway between the epithelial attachment and the crest of the free gingival margin. In those instances where there

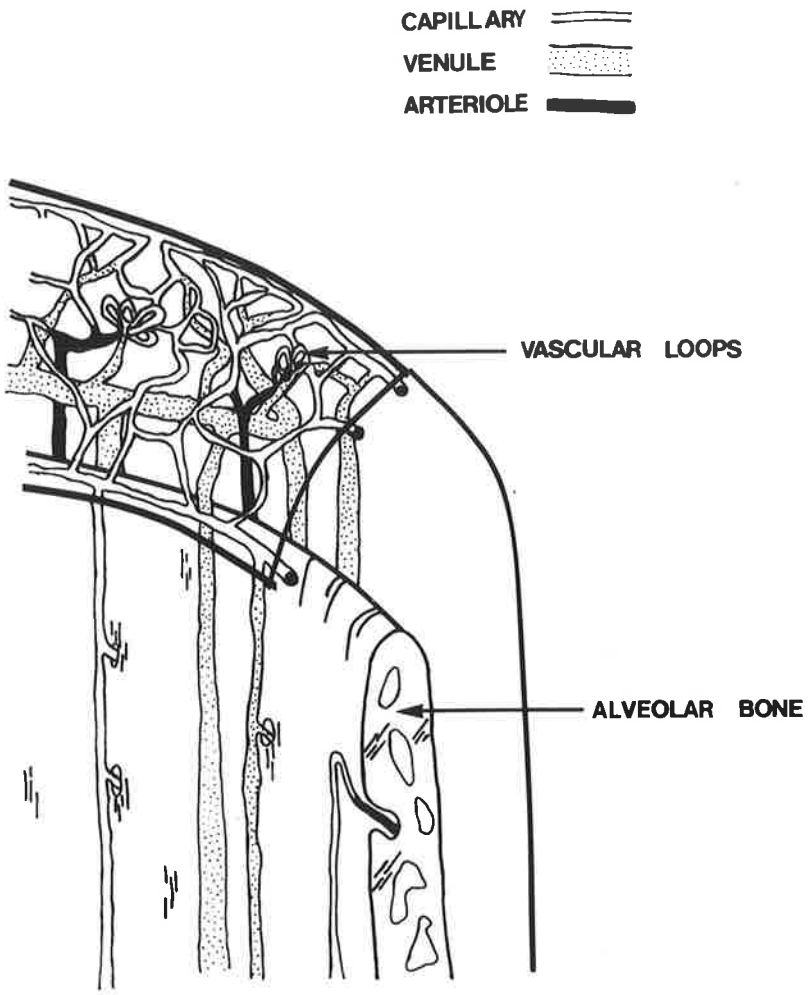


Figure 19: Schematic representation of the vascular architecture at the gingival crevice and coronal third of the ligament.

was a broad band of loops, the band was sited along the apical half of the crevice. Each loop generally consisted of two arms (figure 42). The smaller arm was a branch of an arteriole from the gingival tissues and it extended above the flat capillary plexus toward the crown of the tooth. This arm was approximately 6 micrometres in diameter and can thus be categorised as a true capillary. In many instances this capillary branched once and, less frequently, twice.

The capillary usually did not follow a straight course,but wound around itself and the postcapillary venule which drained the loop. Where branching had occurred, the capillaries twisted around each other and they enlarged as they extended toward the tip of the loop. The height of each loop was approximately 100 to 150 micrometres and the tip of the loop was formed by the capillaries doubling back through 180^{o}. At this stage the vessels were 10 to 12 micrometres in diameter and could be classified as postcapillary venules. As they coursed back toward the flat capillary plexus, these venules coalesced and enlarged further to 20 to 25 micrometres and ultimately joined a large vessel of 25 to 30 micrometres in diameter below the crevicular plexus. These last vessels were classified as collecting venules and provided a large calibre venous drainage away from the vascular loops.

THE INTERPROXIMAL COL

This region was a continuation of the buccal and lingual gingival crevice and had essentially the same vascular arrangement with certain differences, due mainly to the morphology of the region and a major modification in relation to the arrangement of the vascular loops. The flat capillary plexus from the buccal and lingual continued, but was reduced to the apical half of the crevice adjacent to the attachment (figure 43). The coiled vascular loops were present,but these structures were larger and much more complicated. Whereas, in the crevice the coiled loops

occurred in a band, in the col the major proportion of the vascular supply was made up by these loops (figures 44,45). The vascular architecture of these structures resembled that of kidney glomeruli and the term glomerular shall be used in this report to describe the tightly coiled vessel arrangement of the loops found in the col.

The region obtained its arterial supply from the gingival tissues on the buccal or lingual (figure 47). The glomerular structures arose from branches that were 8 to 12 micrometres in diameter. These terminal arterioles extended up toward the apex of the wedge shaped col region and commenced to twist and coil around themselves just below the epithelium. In addition, the vessels branched and these branches also contributed to the coiled glomerular arrangement. As the vessels branched, their diameters became narrower and were classified as capillaries. The capillaries continued to twist and coil but ultimately coalesced to form larger vessels of 10 to 15 micrometres in diameter that flowed into a larger vessel. These larger vessels in turn coalesced until eventually the whole arrangement was drained by a single collecting venule 30-40 micrometres in diameter. This vessel was generally centrally situated.

In most instances the capillary branching began close to the flat plexus,but in others it occurred only at the most coronal one third of the structure. Overall the glomerular arrangements were approximately 100 micrometres high and 30 micrometres wide.

A few hairpin loops,which were very similar to those occurring in the buccal or lingual gingival crevice,could be seen but they were much simpler in arrangement (figure 46). These simple capillary loops were situated either in a row just above the base of the crevice that continued right across the col area,or they were interspersed alongst the glomerular structures. The row of loops arose from the flat plexus continuous with

the gingiva on the buccal and lingual. The lesser number of dispersed loops originated from vessels deeper in the col.

The majority of vessels beneath the glomerular arrangements were horizontal vessels from the gingival papillae on either side,that coursed to and from the glomerular structures. There was also a substantial anastomosis with the periodontal ligament vessels,although this was located (as in the buccal or lingual crevice) more in the region adjacent to the circular vessel at the base of the crevice (figure 39). Below the flat plexus was a collecting venule (30 micrometres in diameter) that coursed right across the col,providing a communication between the buccal and lingual venous plexuses (figure 44).

THE MASTICATORY AND VESTIBULAR MUCOSA

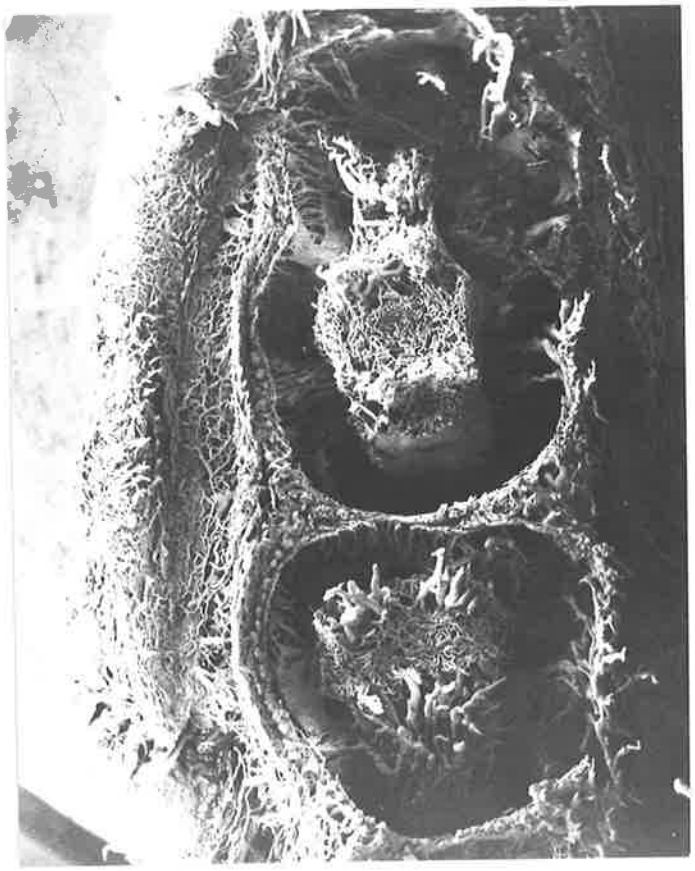
The masticatory mucosa consists of the keratinized epithelium of the gingiva and hard palate. The arrangement of vessels in the palate can be seen in figure 48. The arrangement of the plexus between the rugae was somewhat different to that on each side of the rugal crest. At the bottom of the interrugal valley the vessels were 8 to 10 micrometres in diameter and were arranged in a fairly random plexus. They were supplied by arterioles coming up from the deeper connective tissue at right angles to the epithelial surface. The branches from arterioles were at right angles and often up to half a dozen branches radiated out from the top of each arteriole,resembling the spokes of a wheel. These branches were classified as capillaries and joined with the branches of adjacent arterioles to form a fairly open meshwork. The capillary plexus was drained by vessels 25 to 30 micrometres in diameter,which were classified as postcapillary venules. These vessels branched from the capillary plexus perpendicular to the epithelial surface. The postcapillary venules coalesced to form collecting venules which carried the blood into the deeper connective tissues.

On either side of each individual rugal crest, the capillary plexus consisted of a series of parallel rows running in a sagittal or anteroposterior direction. They anastomosed with the more random capillary plexus about 200 to 250 micrometres away from the ruga. It appeared that the flow of blood was away from the crest of the ruga toward the bottom of the interrugal valley. Whereas the random capillary network of the valley was flat and ran parallel to the surface epithelium, the vessels on the side of the rugae followed a sinuous path with the plane of the curves perpendicular to the epithelial surface. Many postcapillary venules branched off at right angles to these sinuous vessels,to carry blood away to the deeper connective tissues.

The crest of the ruga ran in the coronal plane and was characterized by a vascular spine,running at right angles to the direction of the rows of the sinuous capillary plexus. This spine consisted of a very dense arrangement of capillary loops (figure 49). The arteriolar supply to this particular region has not been clarified due to insufficient time available to obtain good quality sectioned specimens. However, it is presumed that the plexus received its supply from arterioles within the ruga itself,rather than from arterioles of the interrugal valley. The capillaries formed a large number of hairpin shaped loops clustered together under the peak of the rugal crest. These loops drained into either postcapillary venules that coursed back into the rugal connective tissue or into the sinuous capillary vessels going into the palatal soft tissues.

The vestibular mucosa on the lingual side of the alveolus of the mandibular molars resembled parts of the palatal masticatory mucosa (figure 50). The vessels were arranged in rows running anteroposteriorly corresponding to the connective tissue projections into the epithelium. These rows contained a much more complex vascular arrangement than the simple rows on the sides of the rugae in the palate. Here, the rows were

approximately 60 micrometres apart and projected upwards approximately 30 to 40 micrometres toward the epithelial surface. At the top of each row was a very sinuous capillary arrangement,with usually only one or two capillaries coursing uninterrupted anteroposteriorly along the row. At regular intervals postcapillary venules branched off at right angles, but perpendicular to the surface, and drained into the deeper connective tissue joining with adjacent branches to form collecting venules. The rows were supplied by small diameter terminal arterioles,which came from the deeper connective tissues and divided at the base of the projection into the papilla,to send branches anteriorly and posteriorly along the row.



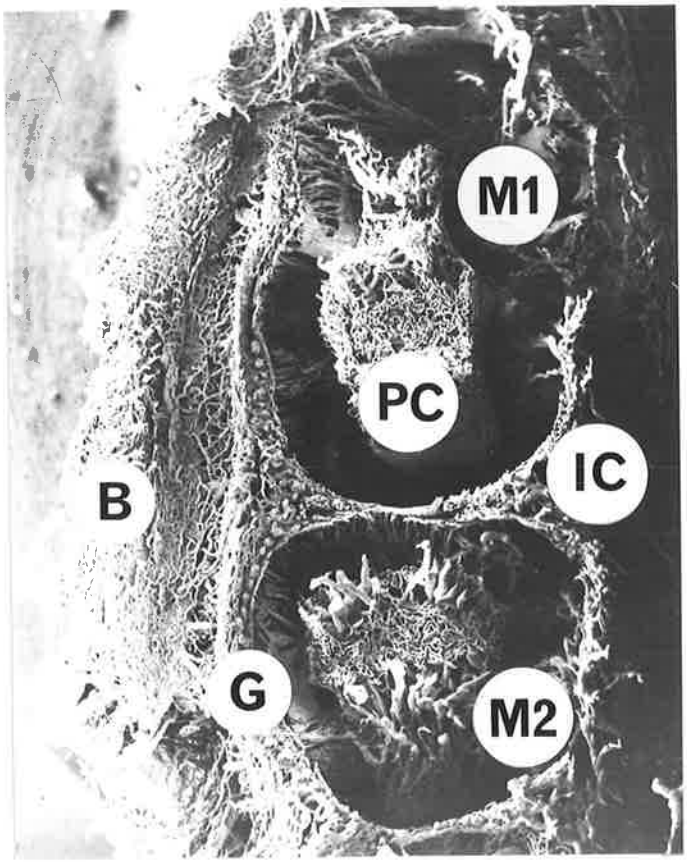


Figure 20

Vascular Cast of First and Second Left Mandibular Molars: No soft or hard tissues are present but it is evident that the overall morphology of the region is retained by the cast. The replication is not quite complete on the lingual side. The specimen is viewed looking down from the occlusal, buccal is to the left and mesial to the top.

B - Buccal mucosa
G - Gingival crevice
IC - Interproximal col
M_1 - Mandibular left first molar
M_2 - Mandibular left second molar
PC - Pulp chamber

Stereo-pair 6^{o} tilt (x 20)
Bar = 100 micrometres

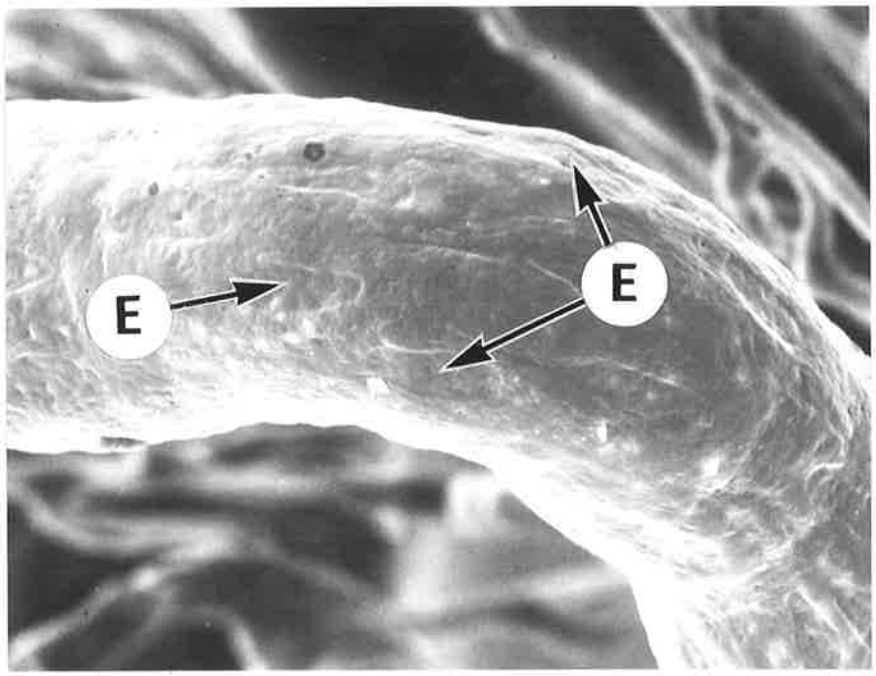


Figure 21

Arteriole: This vessel, approximately 55 micrometres in diameter, has elongated endothelial cell nuclei imprints oriented along the long axis of the vessel, that are typical of arterioles. The direction of blood flow is from right to left. This vessel is in the pulp but typifies the appearance of arteries in the remainder of the periodontium.

E - Endothelial cell nuclei imprints.

Stereo-pair 6^{o} tilt (x 800)
Bar = 10 micrometres

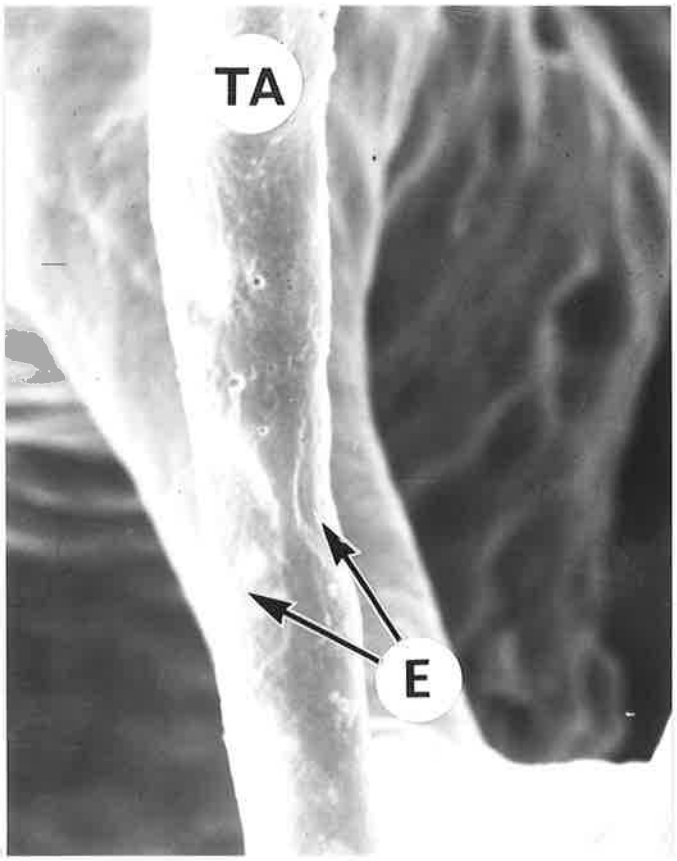


Figure 22

Terminal Arteriole: This vessel is approximately 12 micrometres in diameter and has elongated endothelial cell nuclei imprints typical of arterial vessels. The imprints are oriented along the long axis of the vessel and the direction of blood flow is from bottom to top. This example was found in the interradicular septum between the roots of the mandibular right second molar.

E - Endothelial cell nuclei imprints
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 1300)
Bar - 10 micrometres

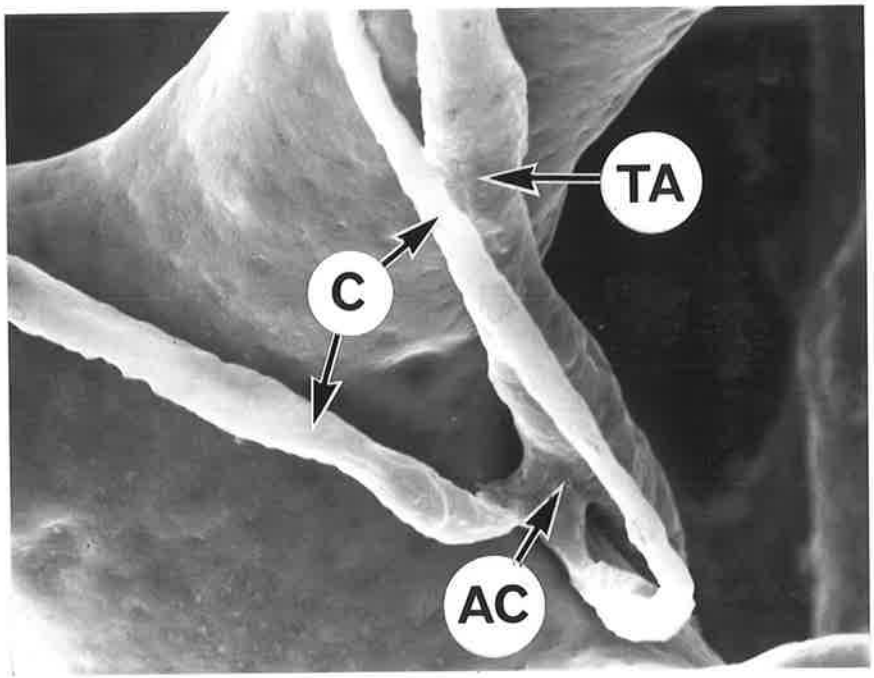
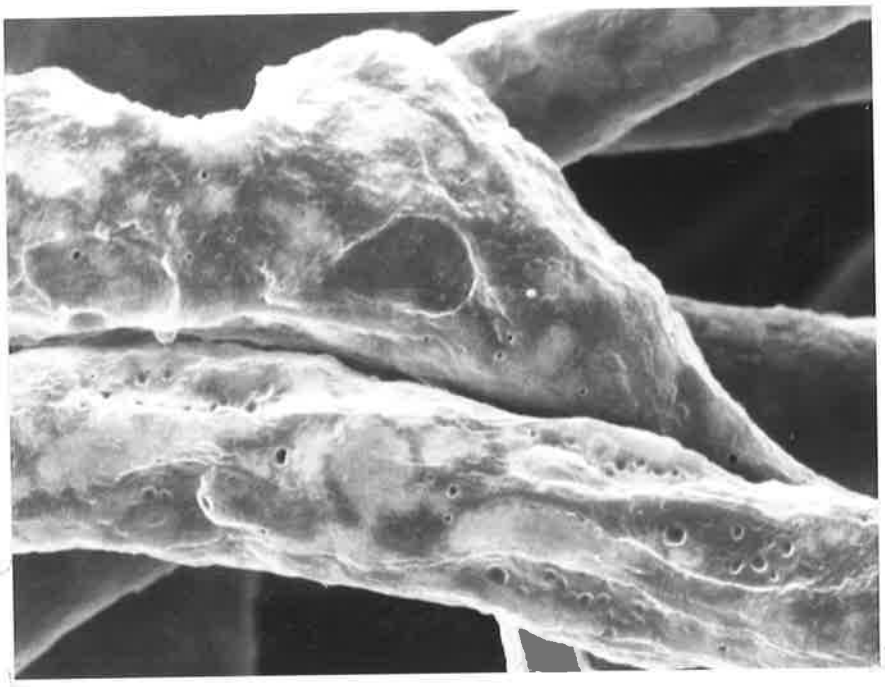


Figure 23

Capillary: The two small diameter vessels with indistinct endothelial cell nuclei imprints are capillaries and are seen here branching from a terminal arteriole. These vessels were found in the interradicular septum of the left mandibular second molar.

AC - Possible arterial cushion
C - Capillary
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 1350)
Bar = 10 micrometres



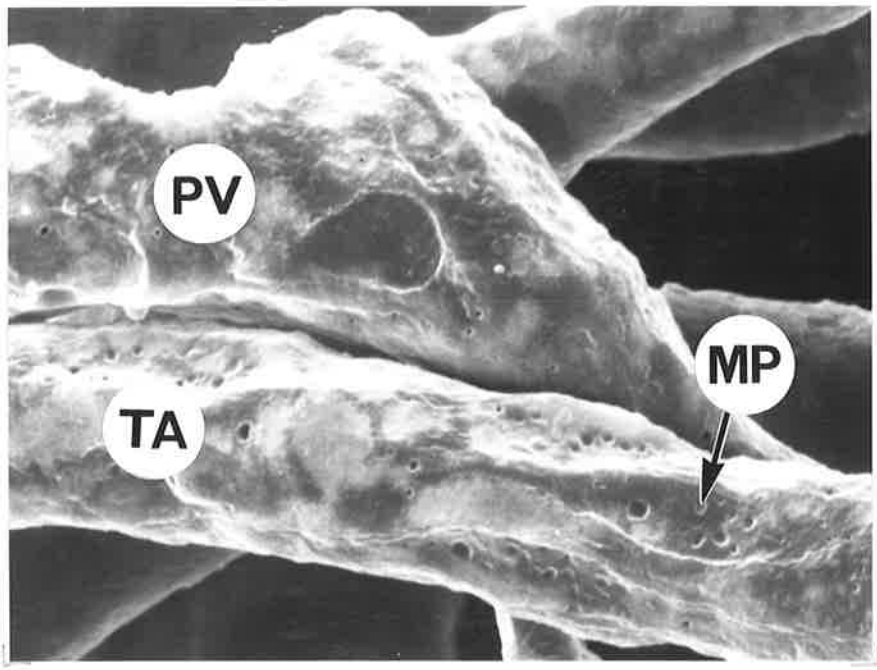
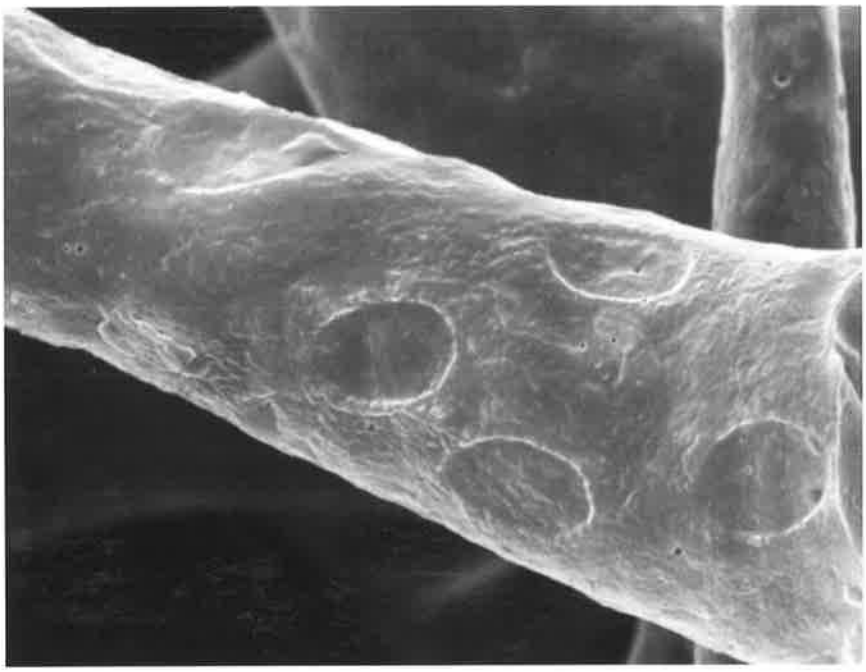


Figure 24

Postcapillary Venule and Terminal Arteriole:
These two vessels display the contrasting endothelial cell nuclei pattern between arterioles and venules. The elongated, spindle shaped imprints are characteristic of arterioles whereas the oval, randomly oriented imprints are indicative of venules. Microvillous protrusions can be seen on the floor of the arterial imprint but not the venous. The direction of blood flow is from left to right in the arteriole but from right to left in the venule.

MP - Microvillous protrusions
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 1350)
Bar = 10 micrometres



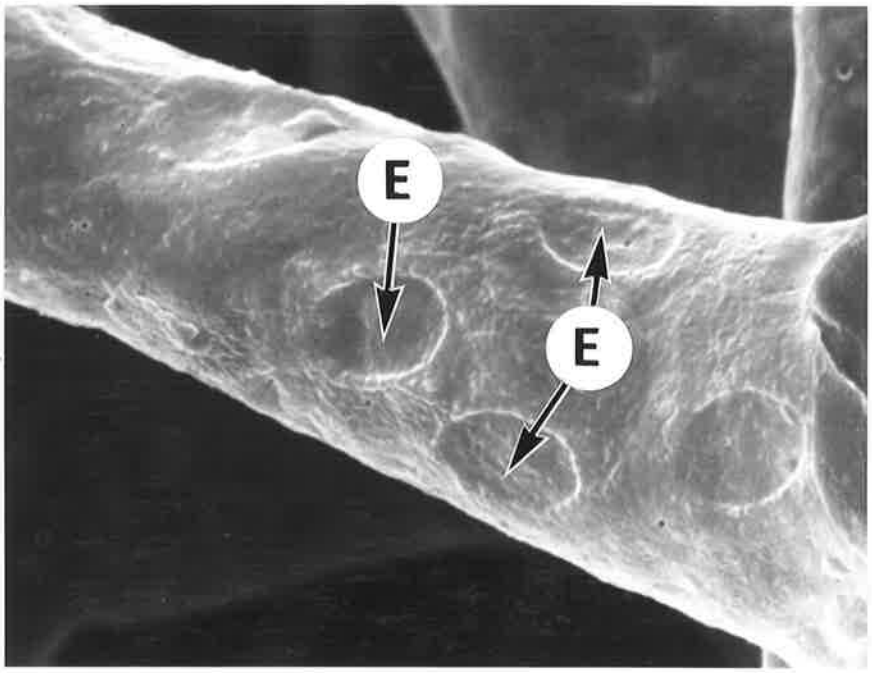
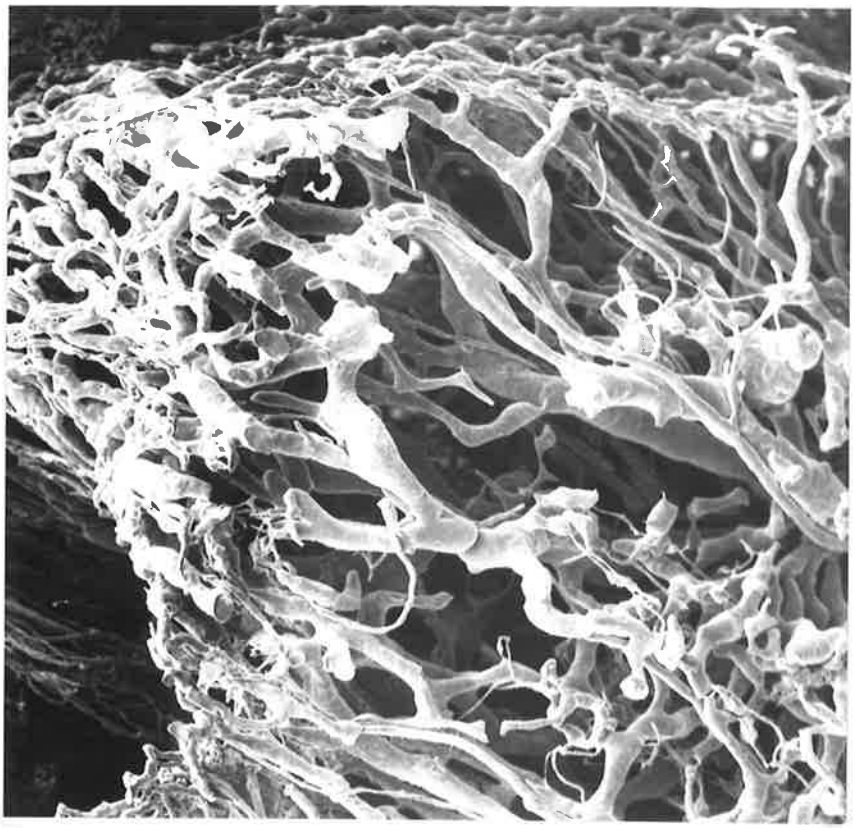


Figure 25

<u>Collecting Venule:</u> The oval-shaped, randomly oriented endothelial cell nuclei imprints that are characteristic of venules are clearly depicted. On the right of the photomicrograph the diameter of the vessel is 35 micrometres and indicates that the vessel can be classified as a collecting venule. The direction of blood flow is from left to right.

E - Endothelial cell nuclei imprints

Stereo-pair 6^{o} tilt (x 1350)
Bar = 10 micormetres



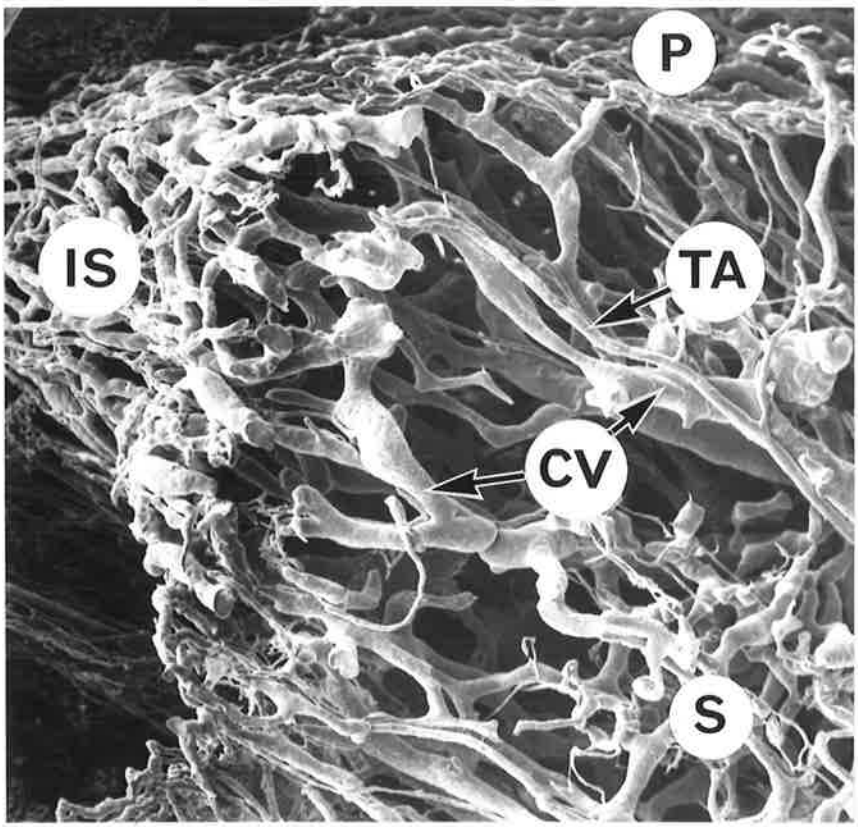
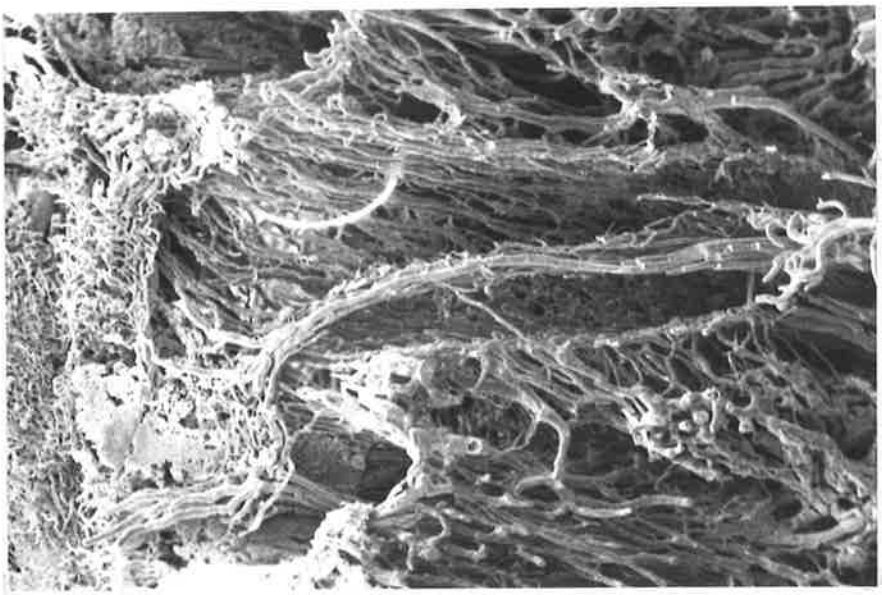


Figure 26

Interradicular Septum: The vasculature of both the bony septum and the overlying periodontal ligament is included in this photomicrograph. The specimen is the buccal portion of a sagittal section through the mandibular right second molar. The occluso-apical axis is horizontal, and mesial is to the bottom.

CV - Collecting venules
IS - Periodontal ligament plexus at the crest of the interradicular septum
P - Periodontal ligament plexus
S - Sinusoid vessels
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 75)
Bar = 100 micrometres



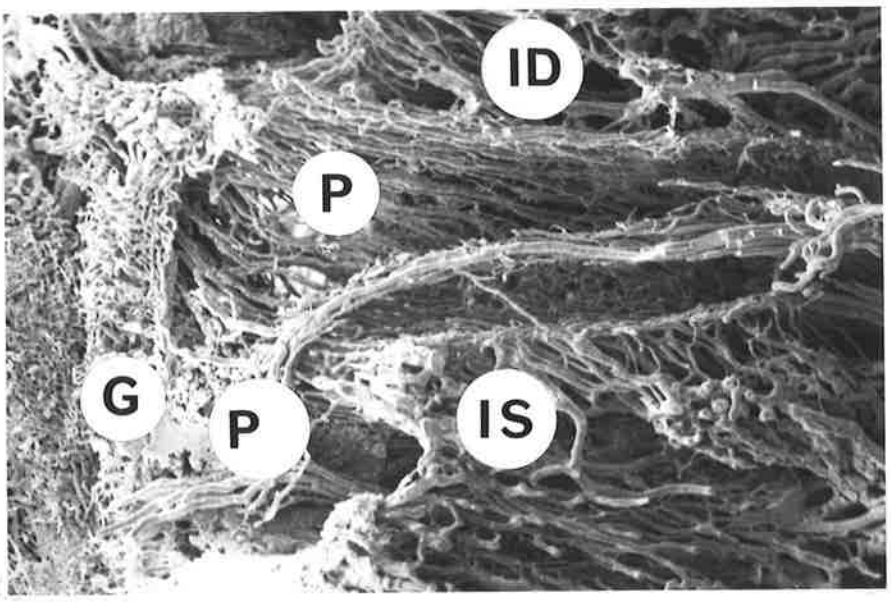
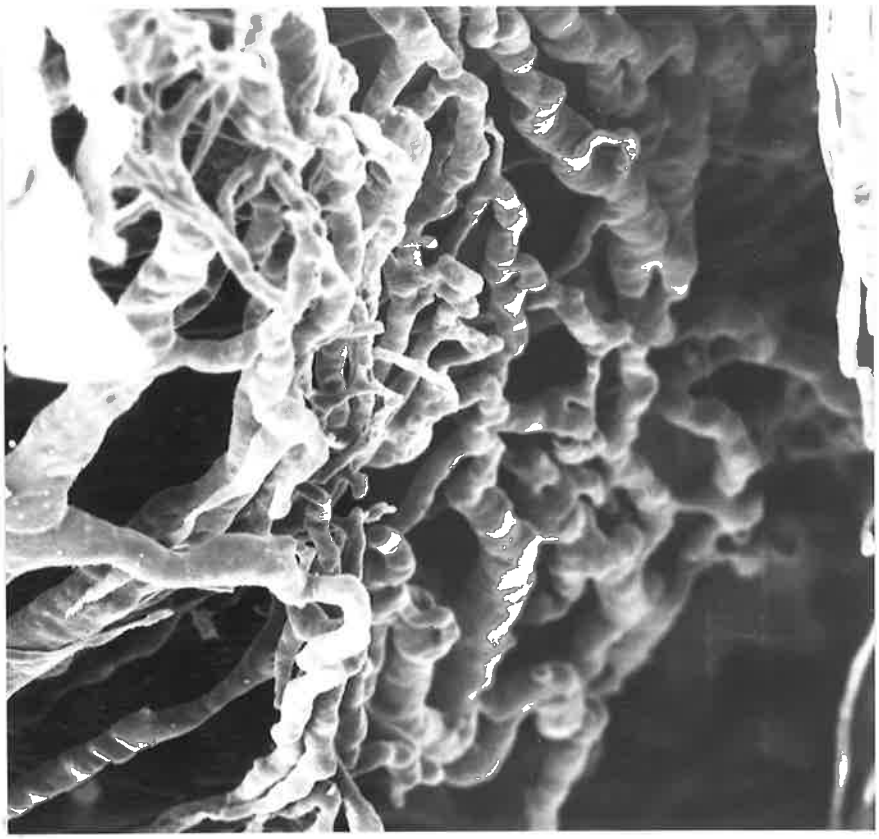


Figure 27

Periodontal Ligament: Replica of the vasculature of the socket of the distal root of the right mandibular first molar. This is the buccal half of a sagittal section and the occluso-apical axis is horizontal. Mesial is to the bottom of the photomicrograph.

G - Gingival crevice
I - Interradicular septum
ID - Interdental septum
P - Periodontal ligament plexus
PU - Pulpal vessels

Stereo-pair 6^{o} tilt (x 30)
Bar = 100 micrometres



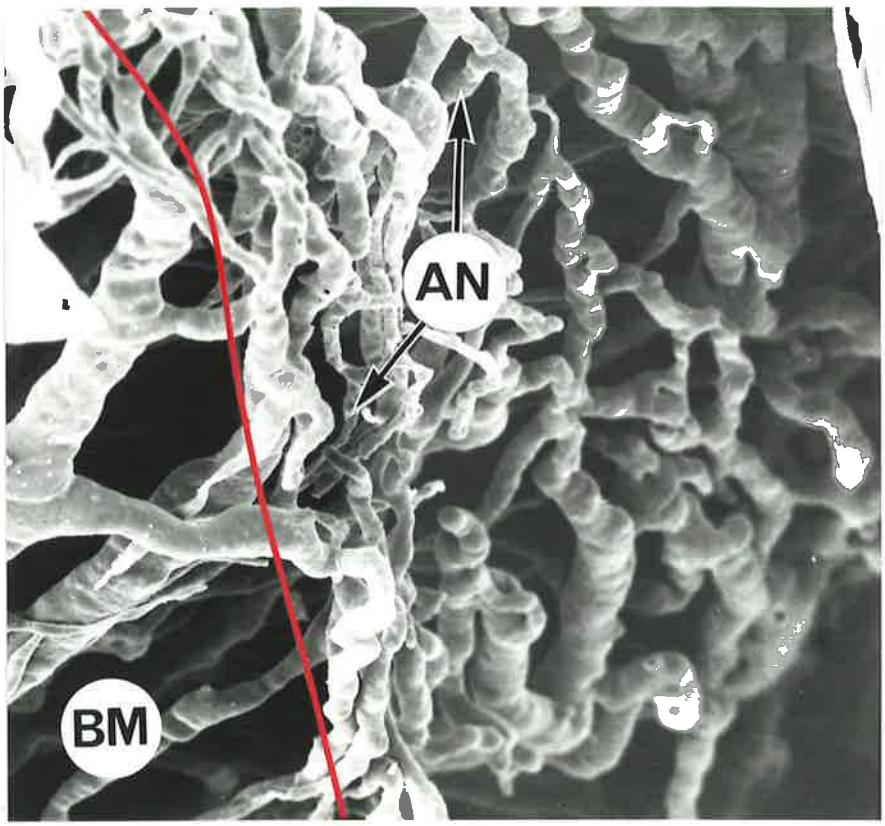
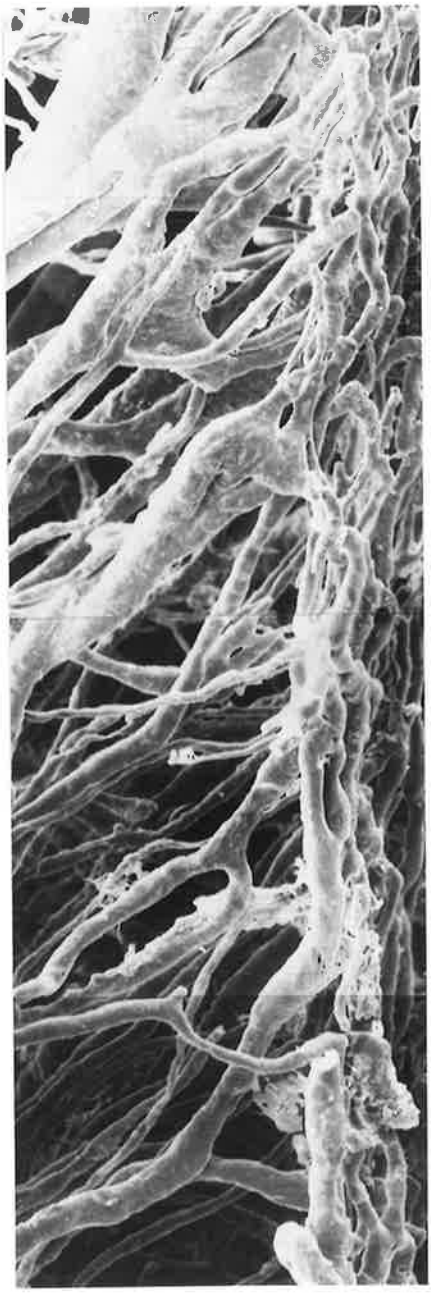


Figure 28

<u>Periodontal Ligament:</u> Showing the arrangement characteristic of the mesial and distal sides of the interradicular septum. This view is looking apically down the mesial side of the distal root socket of the right mandibular second molar. The red line indicates the approximate outline of the alveolar bone, the occluso-apical axis is vertical and the mesial is to the left.

AN - Anastomosis between medullary and ligament vessels
BM - Bone medulla

Stereo-pair 6^{o} tilt (x 225)
Bar = 100 micrometres



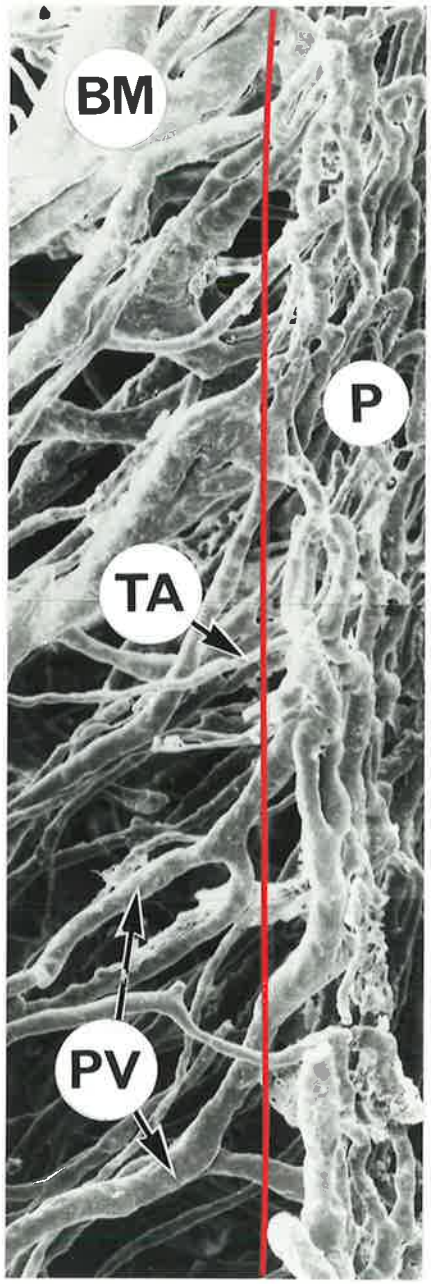
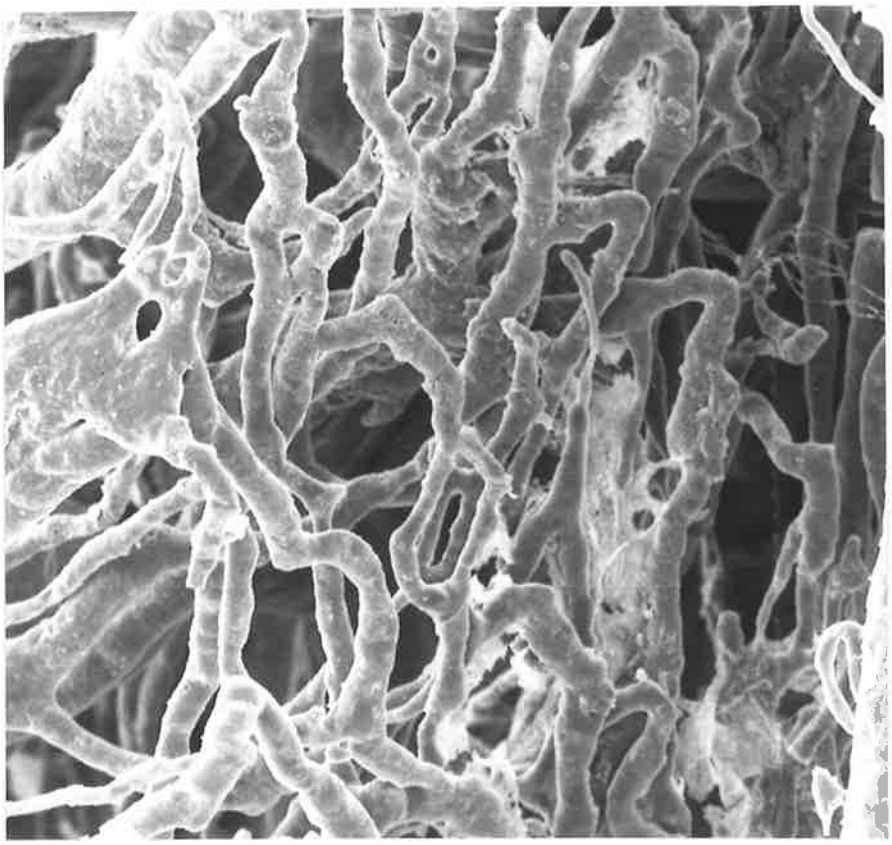


Figure 29

Periodontal Ligament/Bone Medulla Interface:
The extensive communications between the ligament and medullary vessels are evident in this photomicrograph. The specimen is a sagittal section through the middle third of the mesial aspect of the distal root of the left mandibular first molar. The red line shows the approximate outline of the socket wall. The occluso-apical axis is vertical and the mesial is to the left.

BM - Bone medulla
P - Periodontal ligament plexus
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair Montage 6^{o} tilt (x 155)
Bar = 10 micrometres



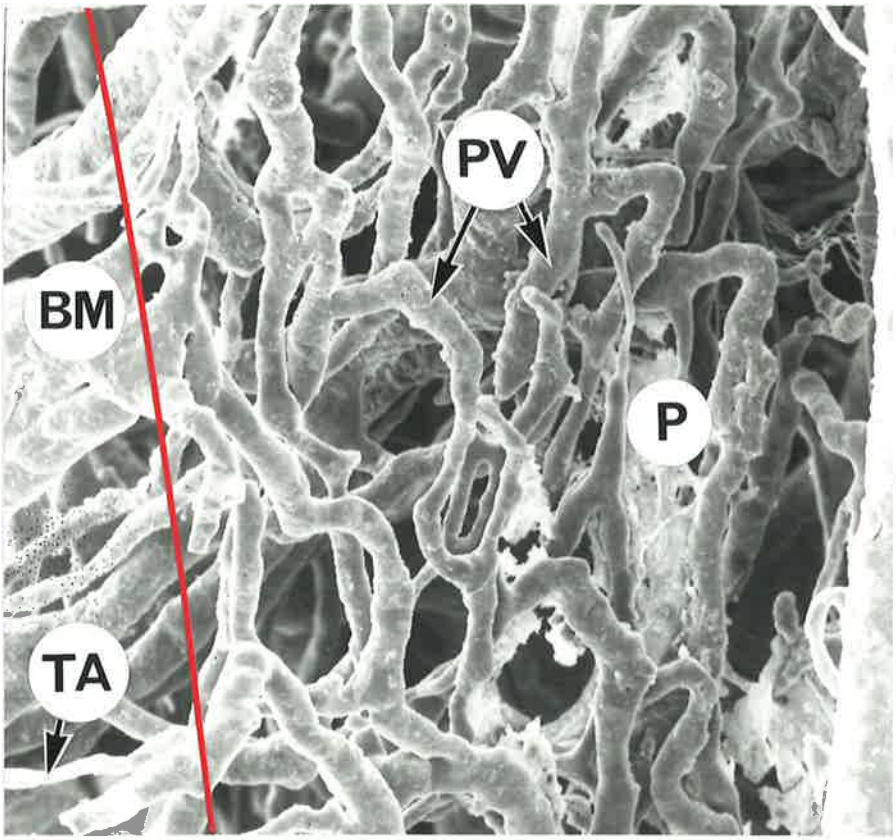
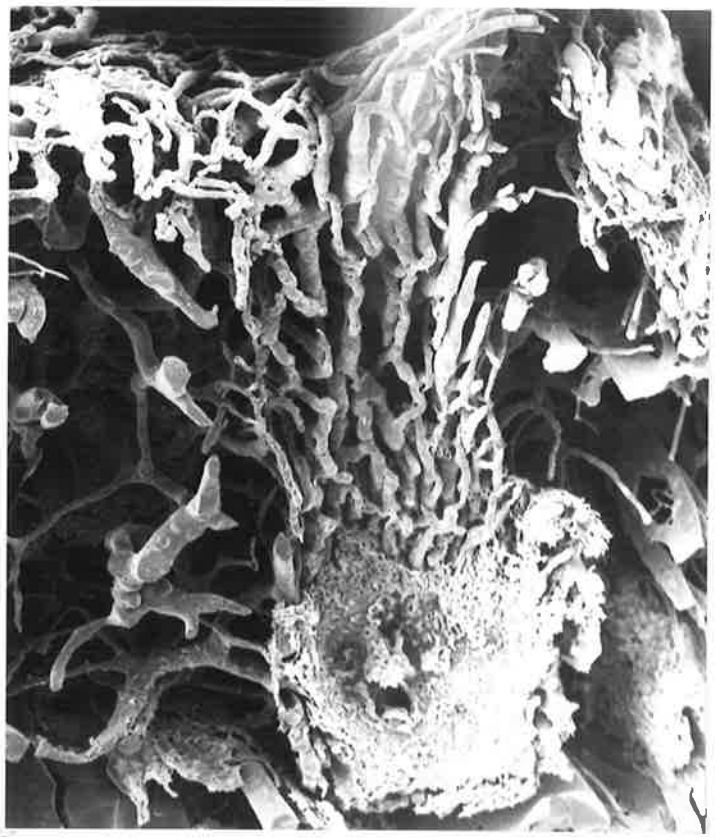


Figure 30

Periodontal Ligament: The above region is a small portion of the previous photomicrograph, viewed from a different angle. A few of the ligament vessels are incompletely cast. The direction of view is from the distal looking mesially. The red line indicates the approximate outline of the alveolar bone.

BM - Bone medulla
P - Periodontal ligament plexus
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 245)
Bar = 100 micrometres



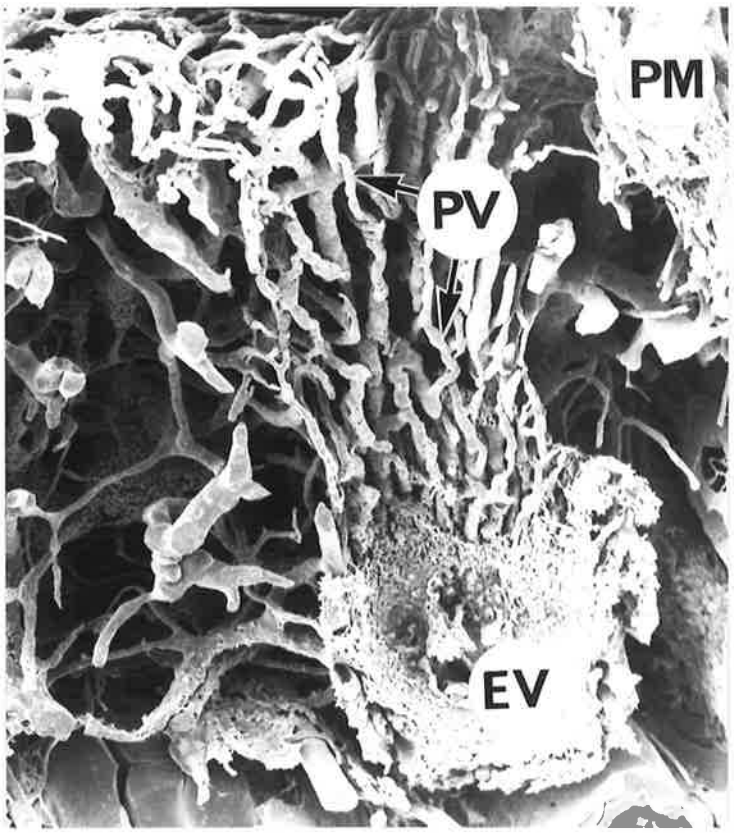
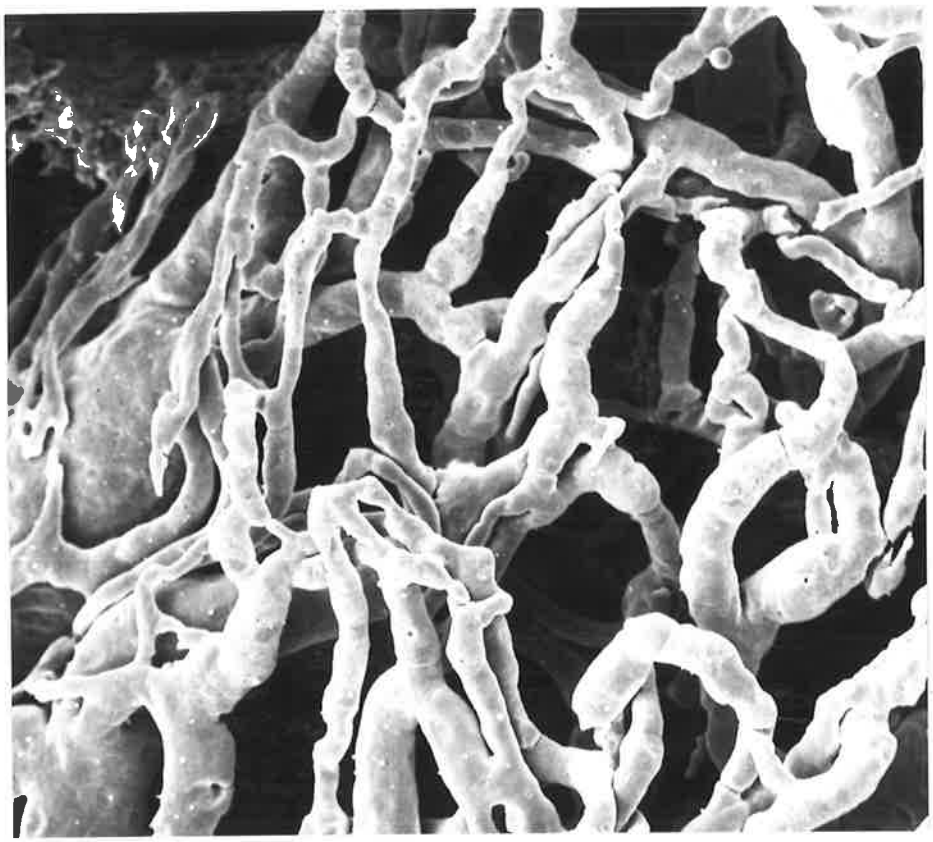


Figure 31

<u>Periodontal Ligament/Bone Medulla:</u> This coronal section of the right maxillary second molar shows a similar, although a slightly less dense, vascular pattern than the mandibular region. There is some extravasation of resin at the apex of the disto-buccal root and also within the bone medulla. The occluso-apical axis is vertical and the palatal is to the right.

EV - Extravasation
I - Interradicular septum
PM - Palatal plexus
PV - Postcapillary venules

Stereo-pair 6^{o} tilt (x 60)
Bar = 100 micrometres



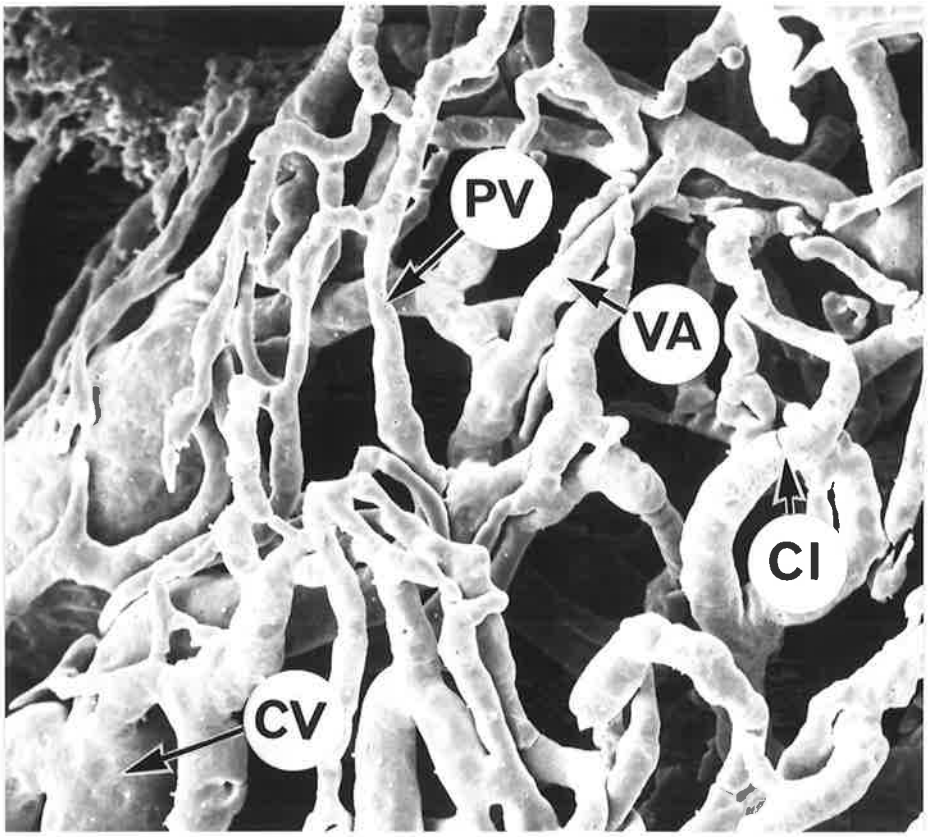
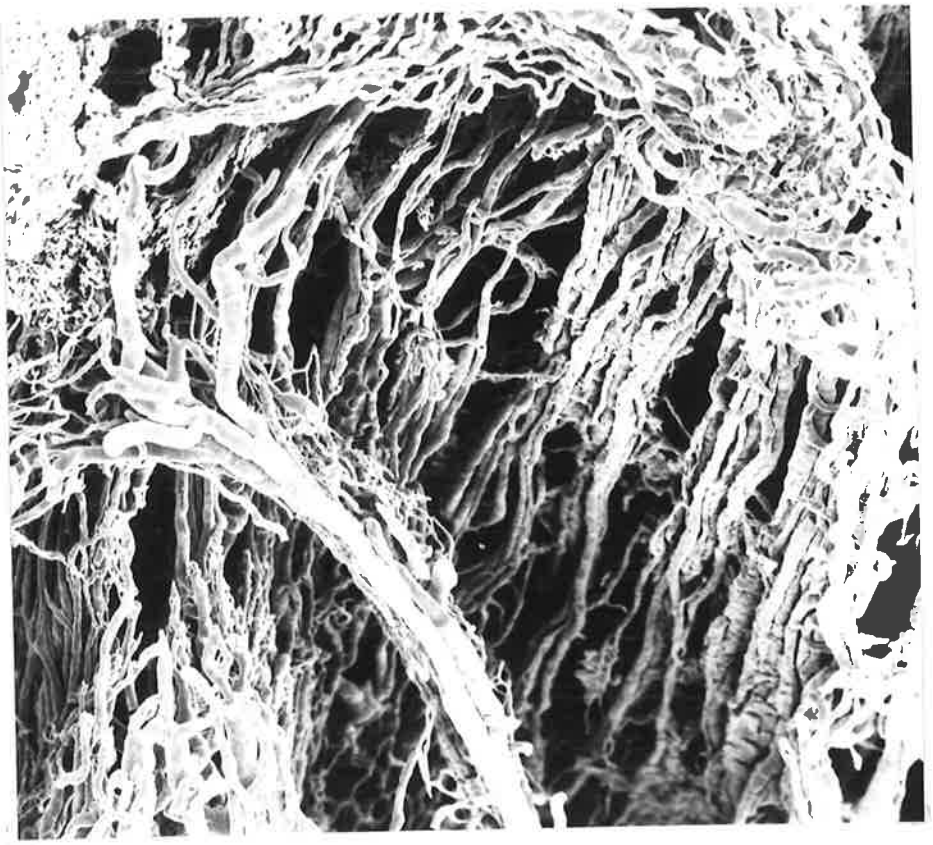


Figure 32

Periodontal Ligament at the Crest of the Interradicular Septum: The arrangement of vessels in this region is more random than the sides of the septum. The vessels are viewed looking down on the crest of the interradicular septum between the mesio- and the disto-buccal roots of the right mandibular second molar. Buccal is to the top and mesial is to the left.

CI - Circumferential invagination
CV - Collecting venule
PV - Postcapillary venule
VA - Venous - venous anastomosis

Stereo-pair 6^{o} tilt (x 200)
Bar = 10 micrometres



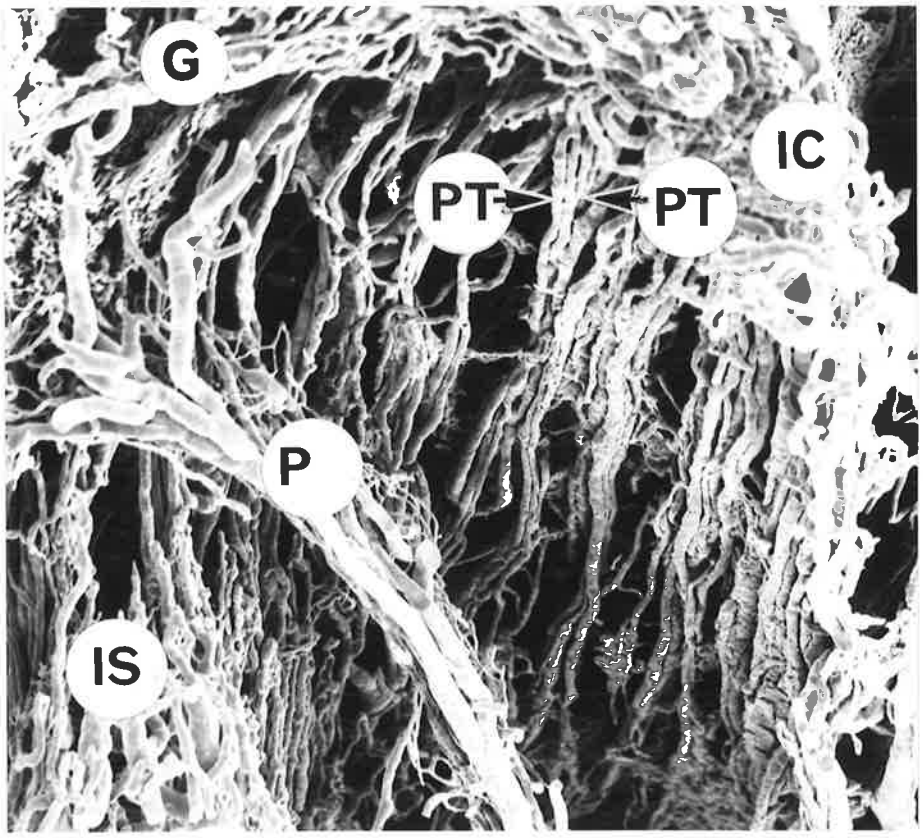
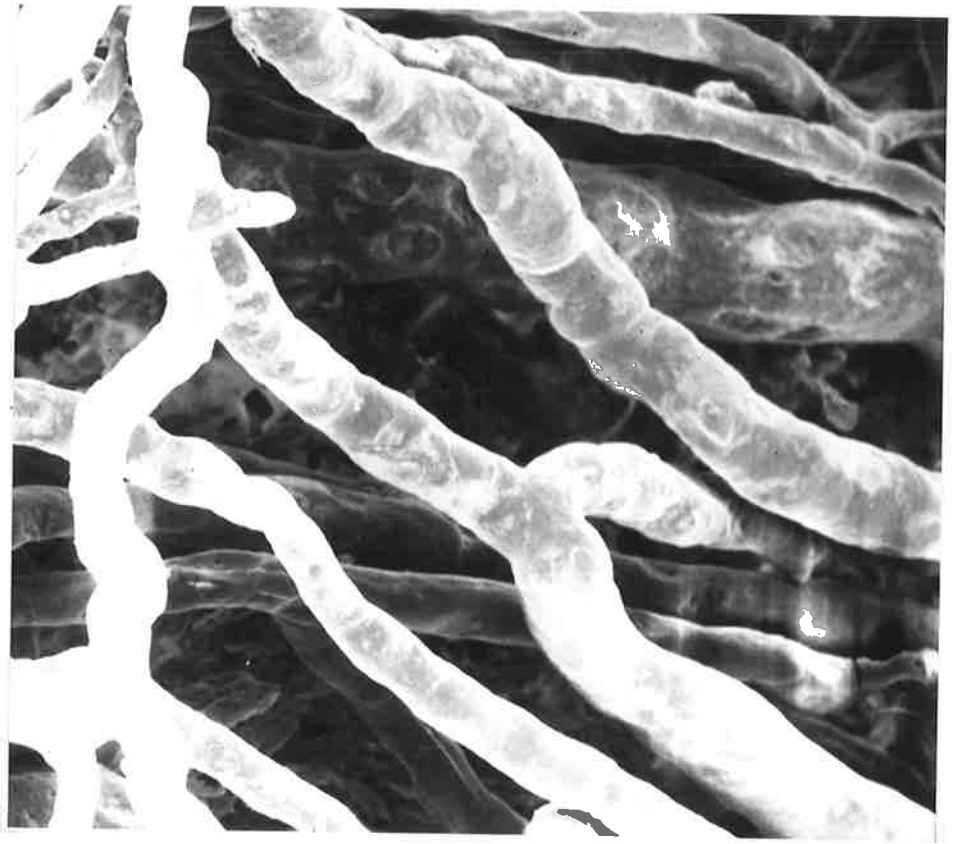


Figure 33

Periodontal Ligament: The palisade arrangement of vessels around the perimeter of the socket is depicted in this photomicrograph of the distobuccal aspect of the right mandibular first molar. The occluso-apical axis is vertical.

G - Gingival crevice
IC - Interproximal col
IS - Periodontal ligament vessels at the crest of the interradicular septum
PT - Periodontal ligament vessel tract
PU - Pulpal vessels

Stereo-pair 6^{o} tilt (x 80)
Bar = 100 micrometres



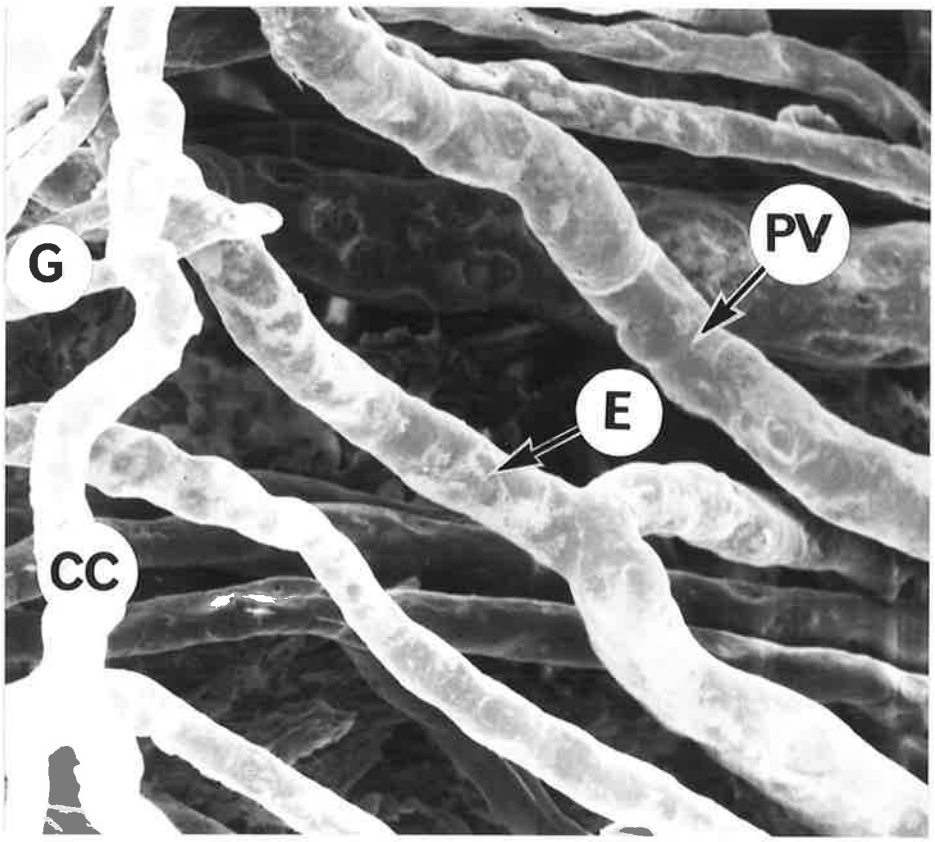


Figure 34

Periodontal Ligament: A higher magnification of the ligament vessels immediately below the gingival crevice at the disto-buccal aspect of the left mandibular second molar. The anastomosis with the capillary plexus of the gingival crevice is obvious. The occluso-apical axis is horizontal.

CC - Circular capillary at the bottom of the gingival crevice
E - Endothelial cell nuclei imprints
G - Gingival crevice
PV - Postcapillary venule

Stereo-pair 6^{o} tilt (x 650)
Bar = 10 micrometres

5.48

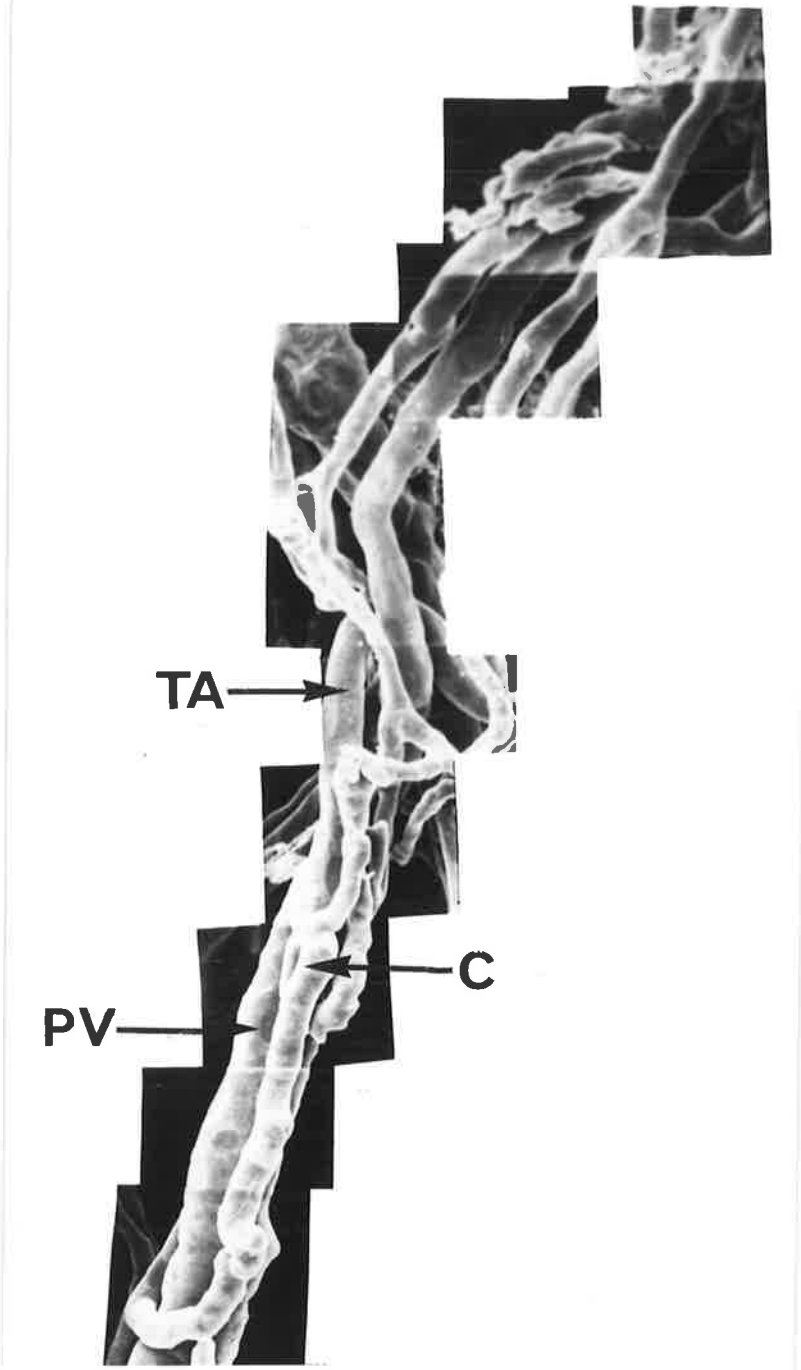
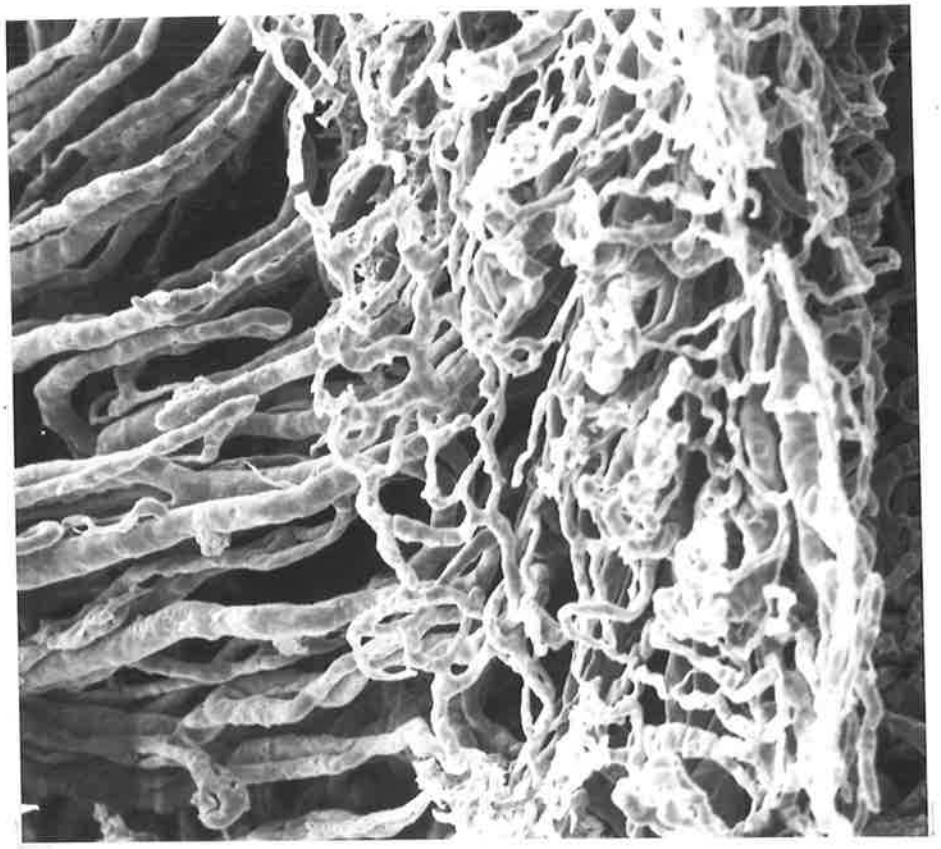


Figure 35 Periodontal Ligament: This montage follows vessels in one of the tracts around the perimeter of the socket. Within the region there is evidence not only of incomplete replication but also extravasation. The region in this photomicrograph is at the disto-buccal aspect of the right mandibular first molar. The occluso-apical axis is vertical.

C - Capillary
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair Montage 6^{0} tilt (x 300)
Bar = 10 micrometres



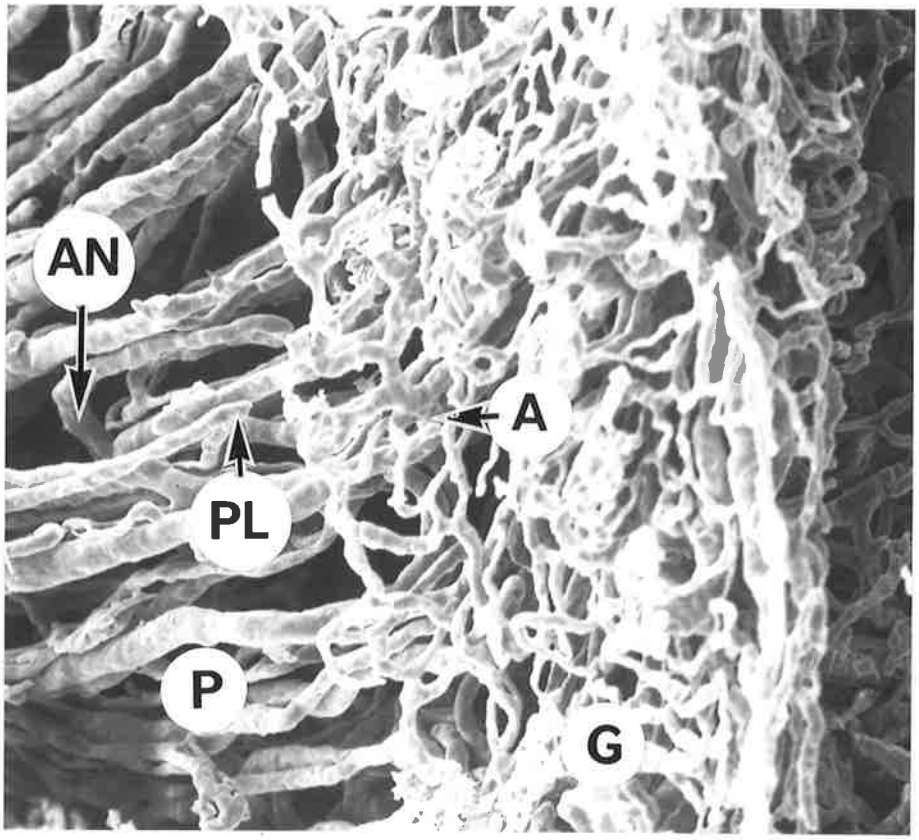
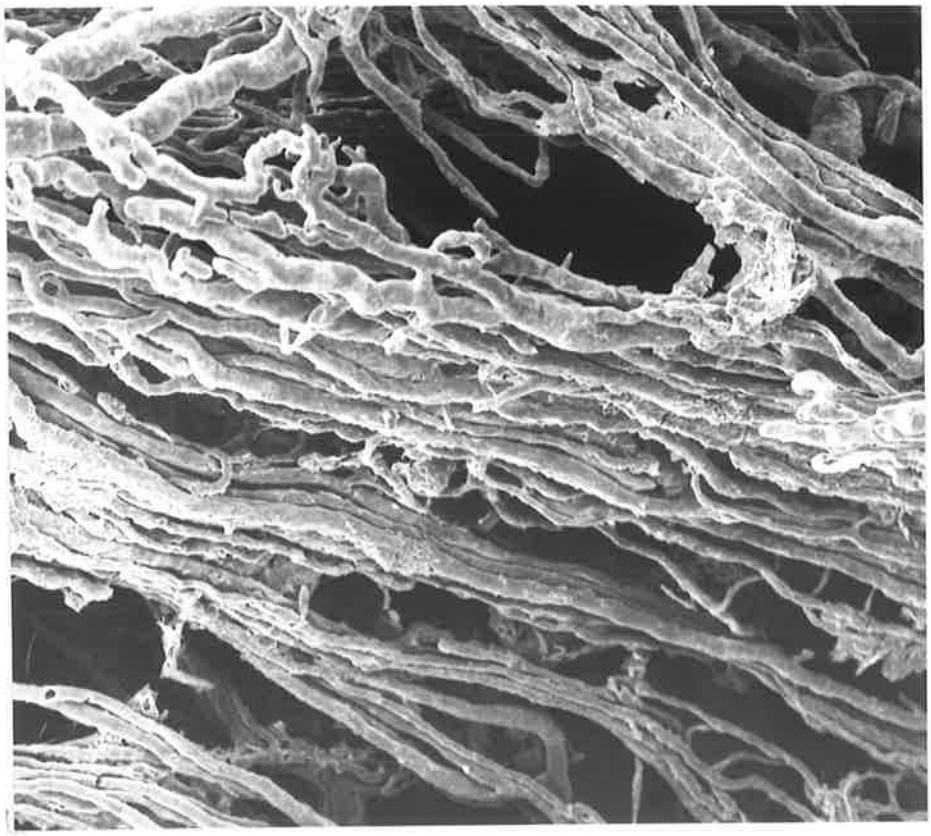


Figure 36

Periodontal Ligament and Gingival Crevice: The inner aspect of the gingival crevice from the free margin to the attachment can be seen. In addition the anastomosis between the gingival and periodontal vessels is readily apparent. The buccal portion of the socket of the left mandibular second molar is depicted here. The occluso-apical axis is horizontal, buccal is to the right and mesial is to the bottom.

A - Anastomosis between gingival and periodontal vessels
AN - Anastomosis between medullary and ligament vessels
G - Gingival crevice
P - Periodontal ligament plexus
PL - Periodontal loop

Stereo-pair 6^{o} tilt (x 185)
Bar = 100 micrometres



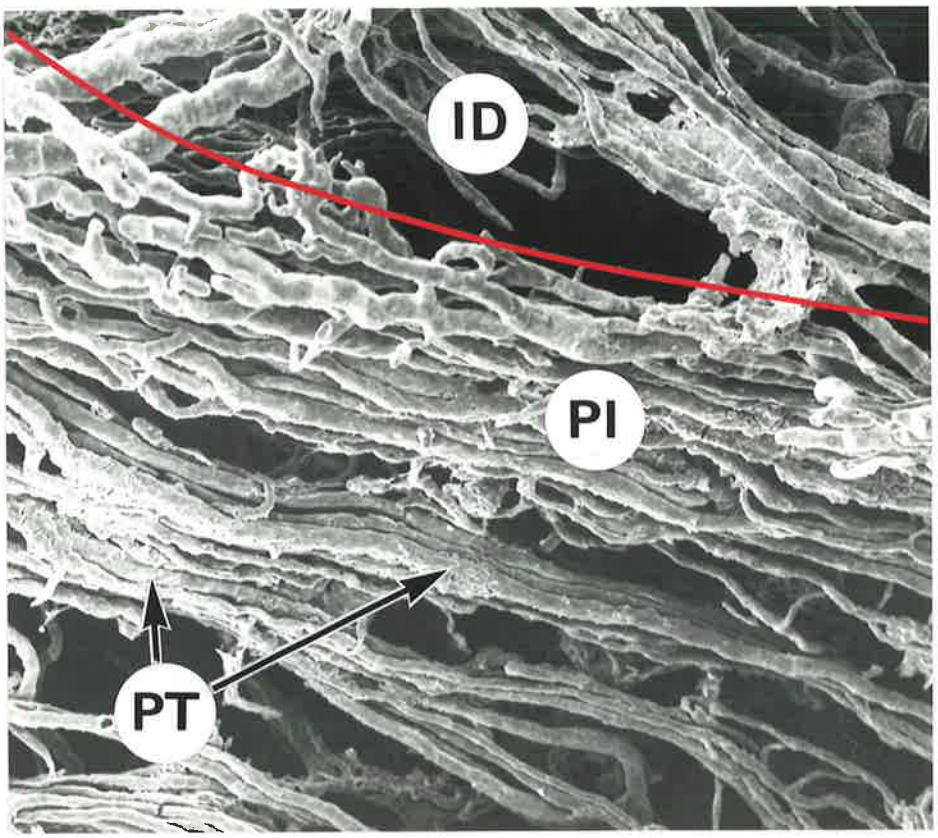
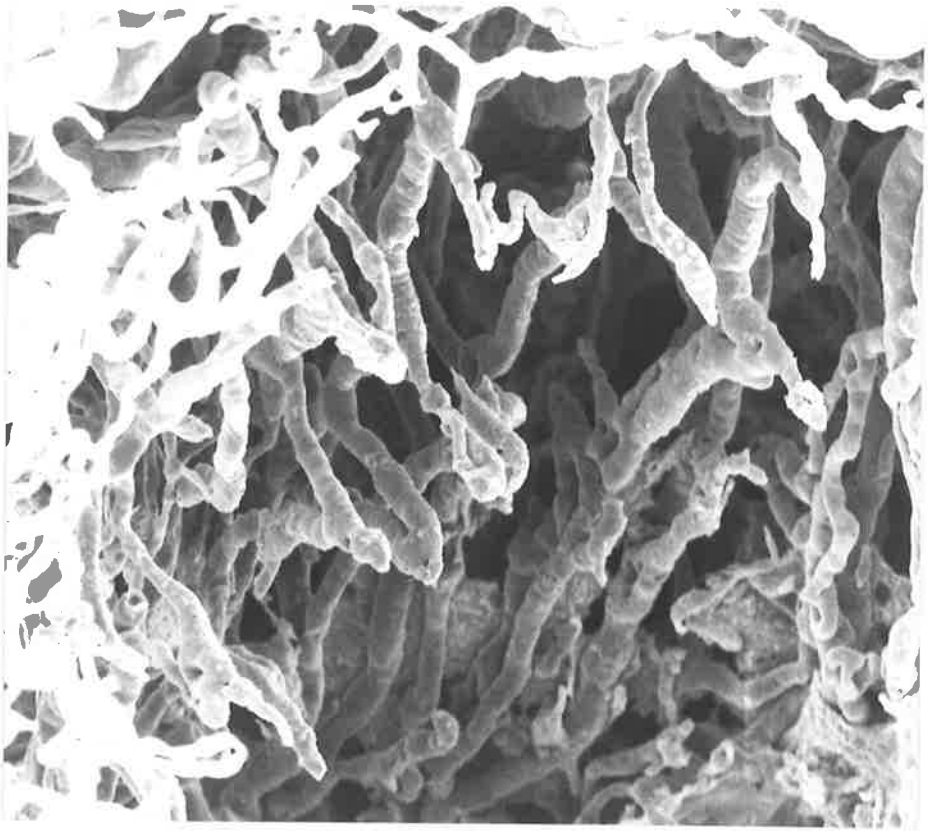


Figure 37 Periodontal Ligament: Detail of vessels at the interdental septum between the left mandibular first and second molars showing the more dense vascularity of this region compared with the vascular tracts around the perimeter of the socket. The occluso-apical axis is horizontal.

ID - Interdental septum
PI - Periodontal ligament vessels over the interdental septum
PT - Periodontal ligament vessel tract

Stereo-pair 6^{o} tilt (x 140)
Bar = 100 micrometres



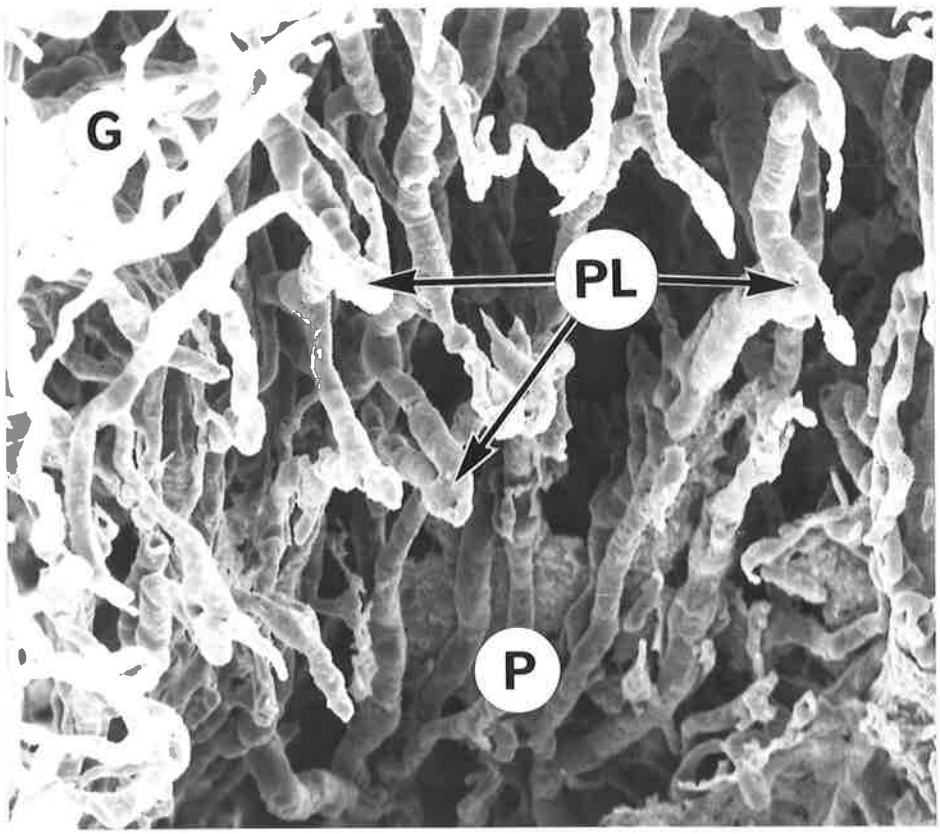
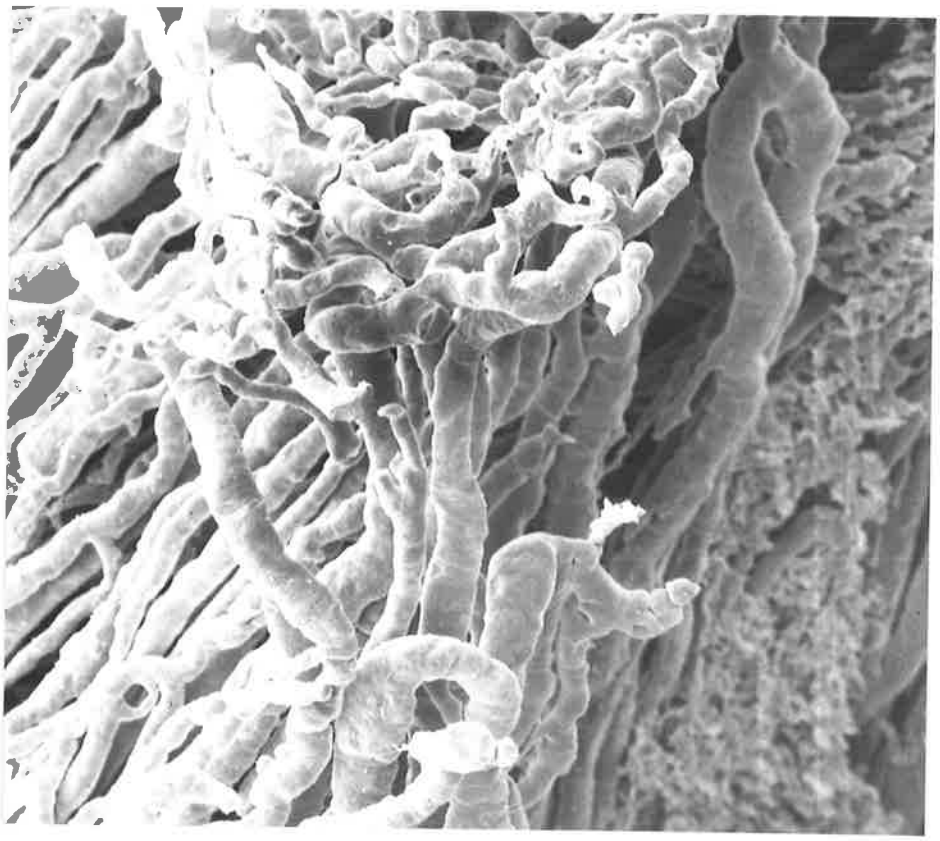


Figure 38

Periodontal Ligament: Showing a number of vascular loops in the periodontal plexus. These loops are more numerous in this specimen than in the others examined. The region in this photomicrograph is the mesiobuccal aspect of the maxillary right second molar. The occluso-apical axis is vertical.

G - Gingival crevice
P - Periodontal ligament plexus
PL - Periodontal loops

Stereo-pair 6^{o} tilt (x 240)
Bar = 100 micrometres



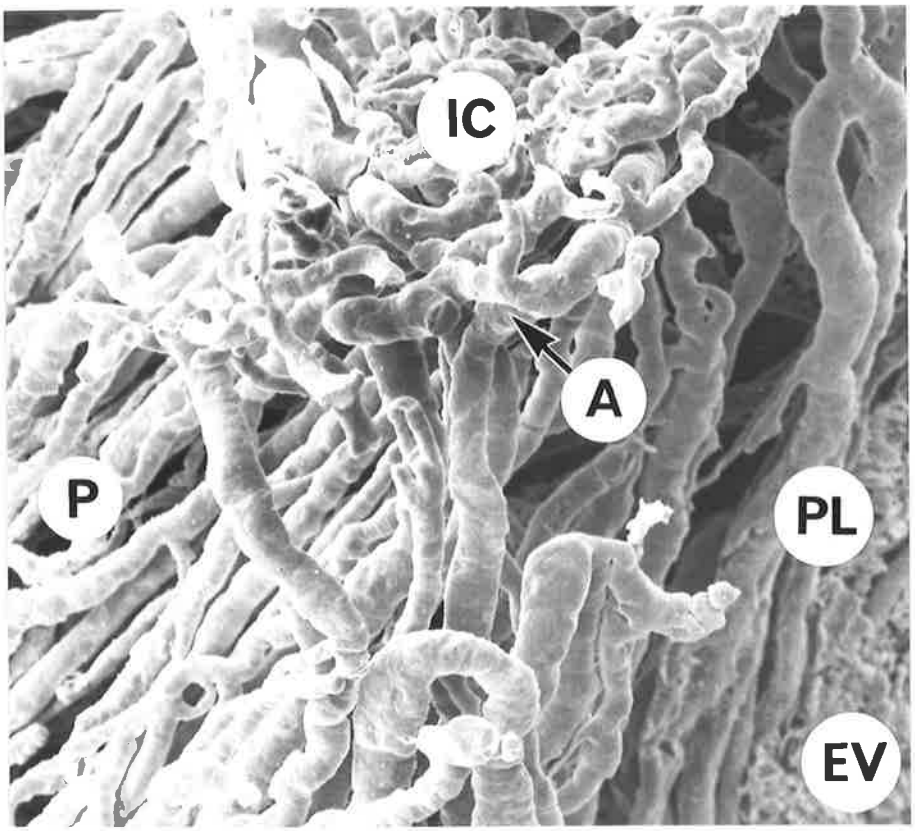
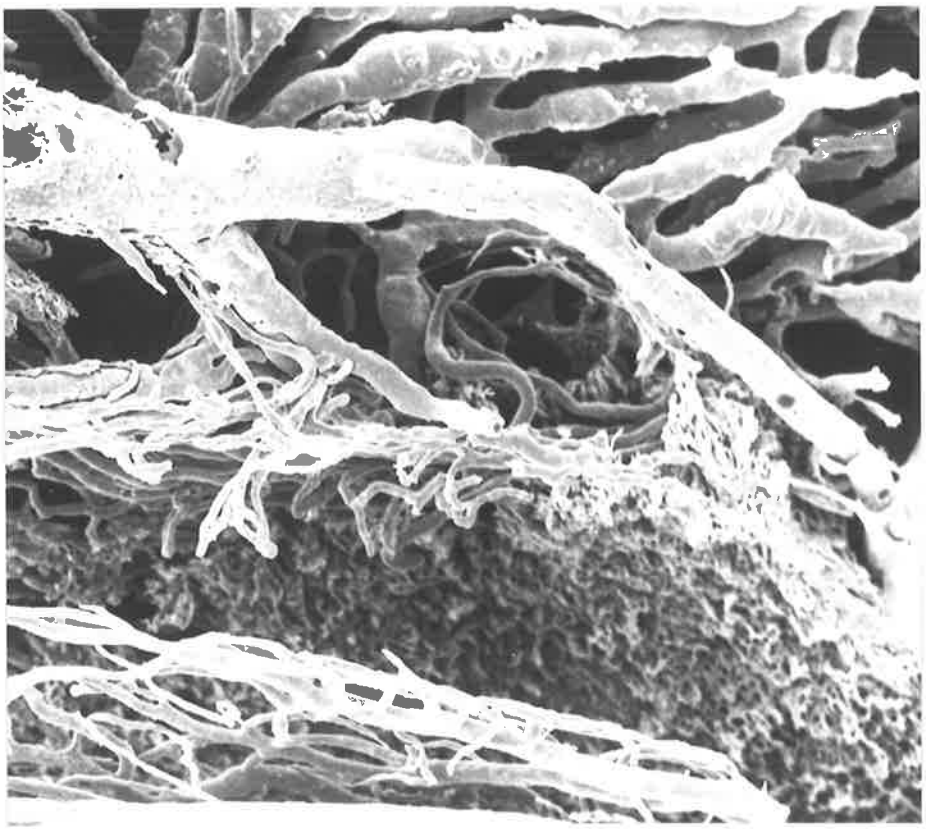


Figure 39

Interdental Septum: This sagittal section through the interproximal col between the right mandibular first and second molars shows vessels from the periodontal ligament on each side of the interdental septum coalescing over the top of the alveolar bone just below the gingival tissues. A periodontal loop can also be seen as can the anastomosis between the gingival and periodontal vessels. The occluso-apical axis is vertical and mesial is to the left.

A - Anastomosis between gingival and periodontal vessels
EV - Extravasation
IC - Interproximal col
P - Periodontal ligament plexus of distal wall of first molar
PL - Periodontal loop

Stereo-pair 6^{o} tilt (x 200)
Bar = 100 micrometres



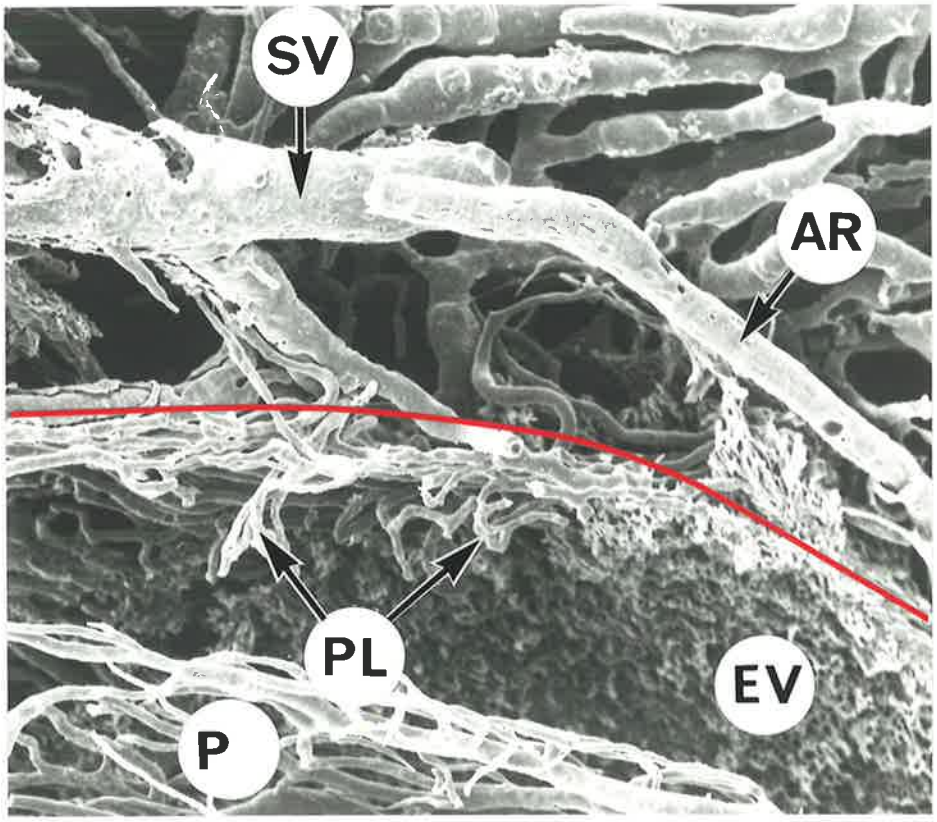
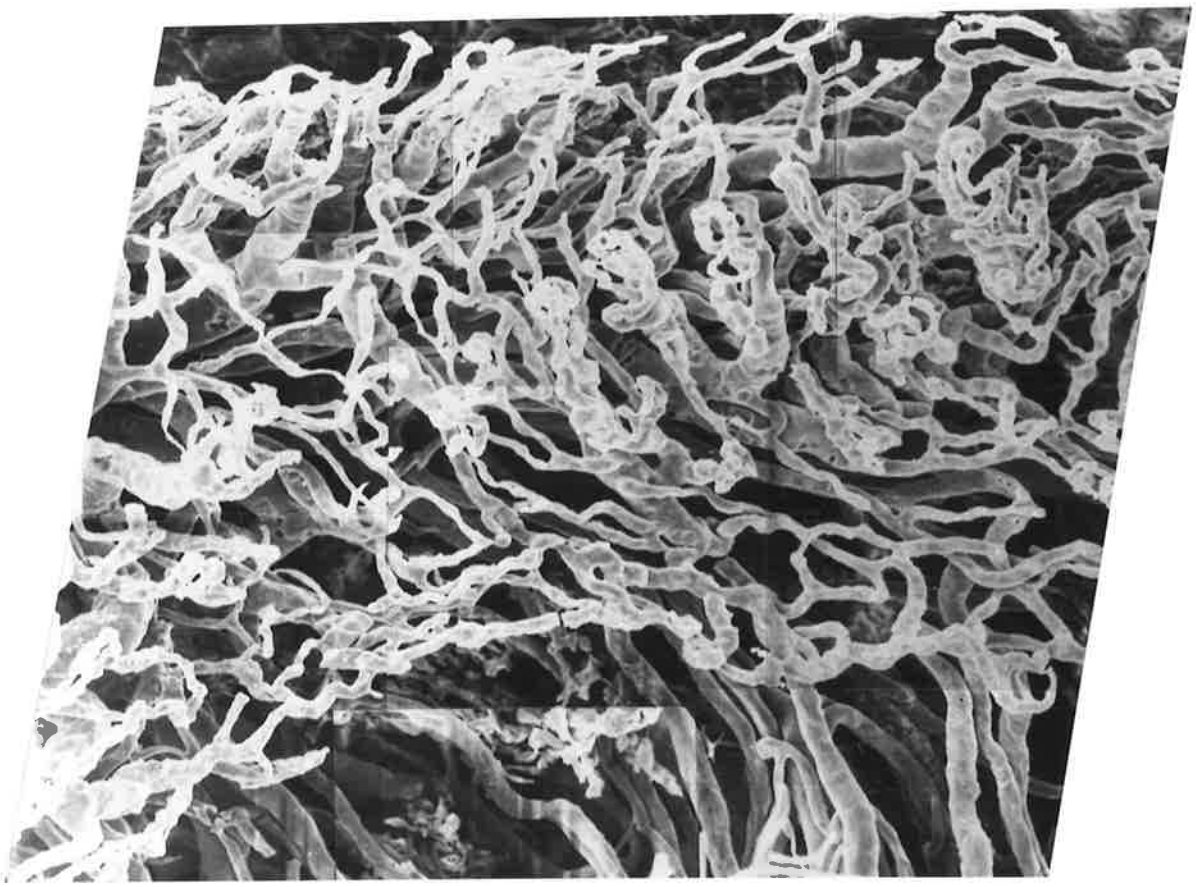


Figure 40

Periodontal Ligament: Periodontal loops also occur in the apical region as depicted in this photomicrograph. The specimen is a sagittal section through the distal root of the right mandibular first molar. The occluso-apical axis is horizontal and mesial is to the bottom.

AR - Arteriole
EV - Extravasation
PL - Periodontal loop
PU - Pulpal vessels
SV - Small collecting vein

Stereo-pair 6^{o} tilt (x 140)
Bar = 100 micrometres



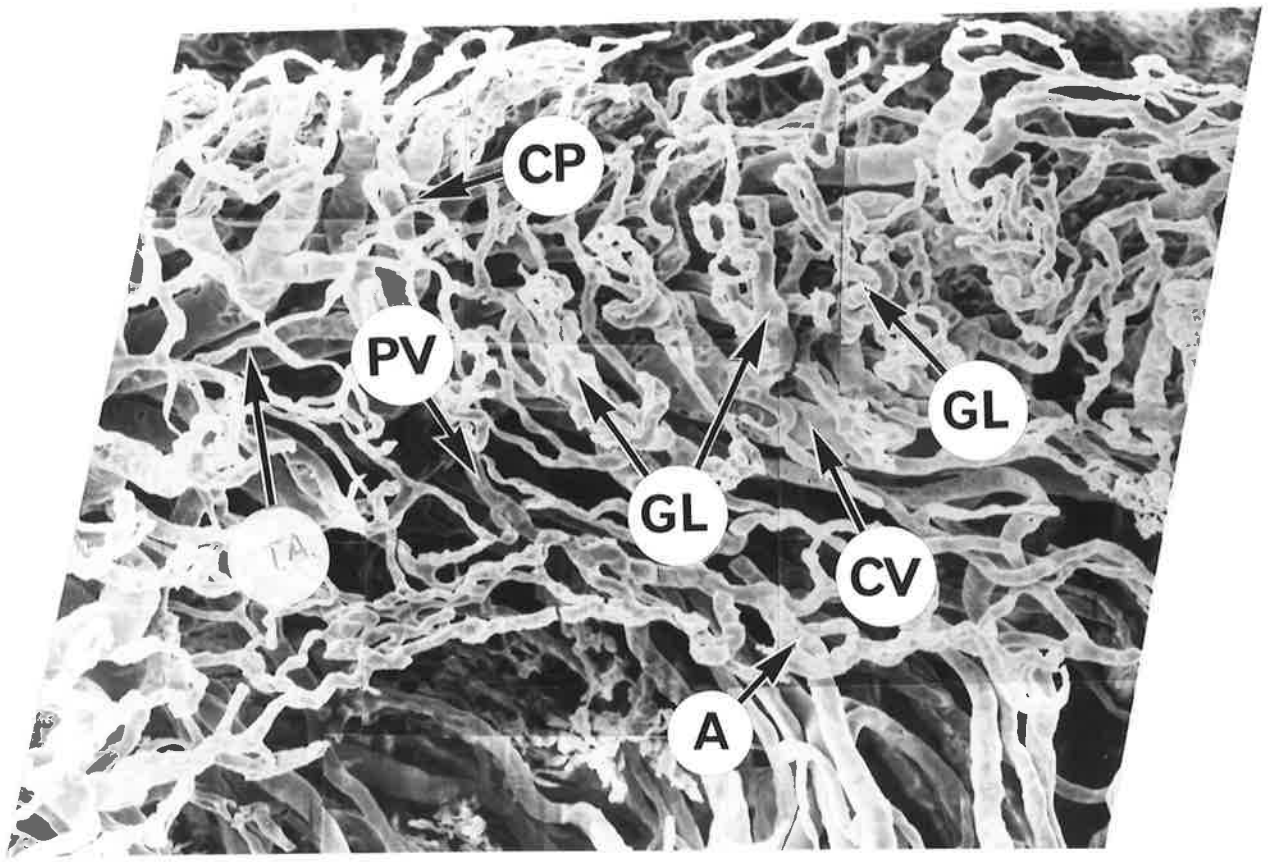
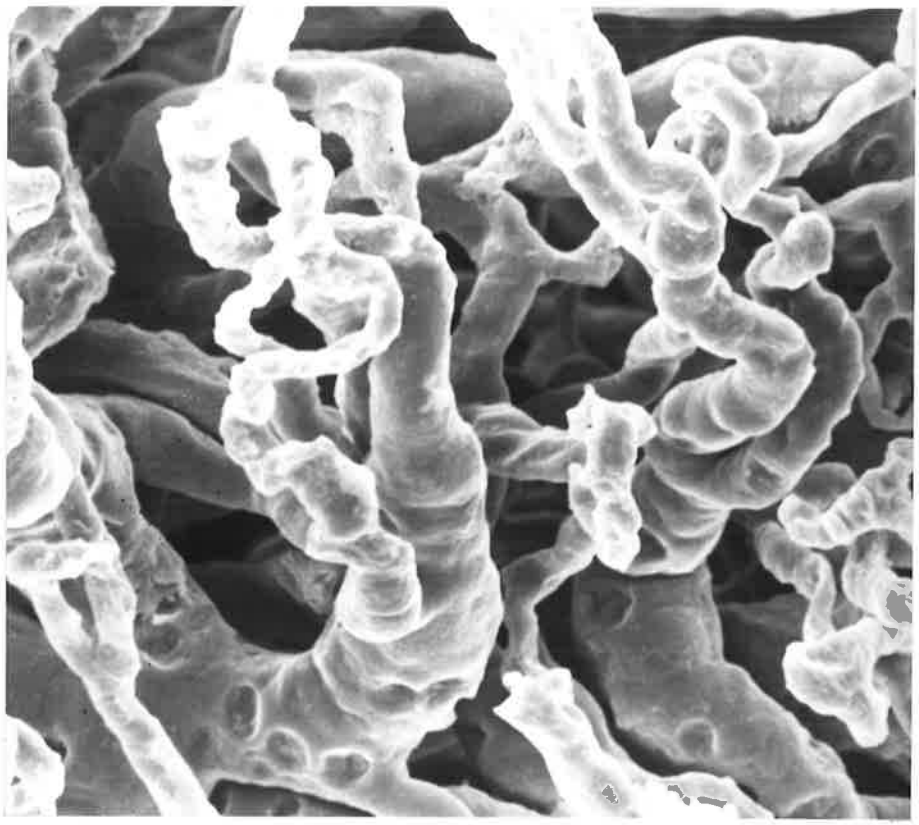


Figure 41

Gingival Crevice: The internal aspect of the gingival crevice from the apex of the unattached gingiva down to the epithelial attachment can be seen. There is a flat open capillary network extending over the whole of the crevice and in the middle third is a row of twisted vascular loops. The region depicted in this photomicrograph is the buccal aspect of the mandibular left second molar, looking down from the occlusal. The buccal is to the top and mesial is to the right.

A - Anastomosis between gingival and periodontal vessels
CP - Flat capillary plexus
CV - Collecting venule
GL - Gingival vascular loop
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair Montage 6^{o} tilt (x 200)
Bar = 10 micrometres



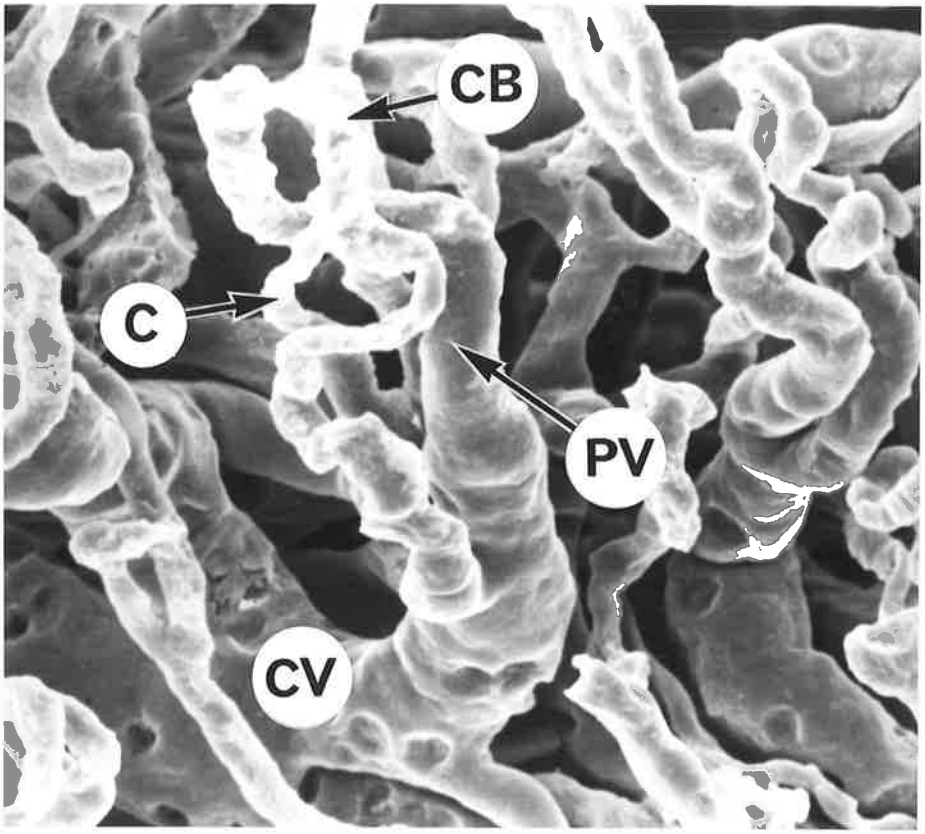
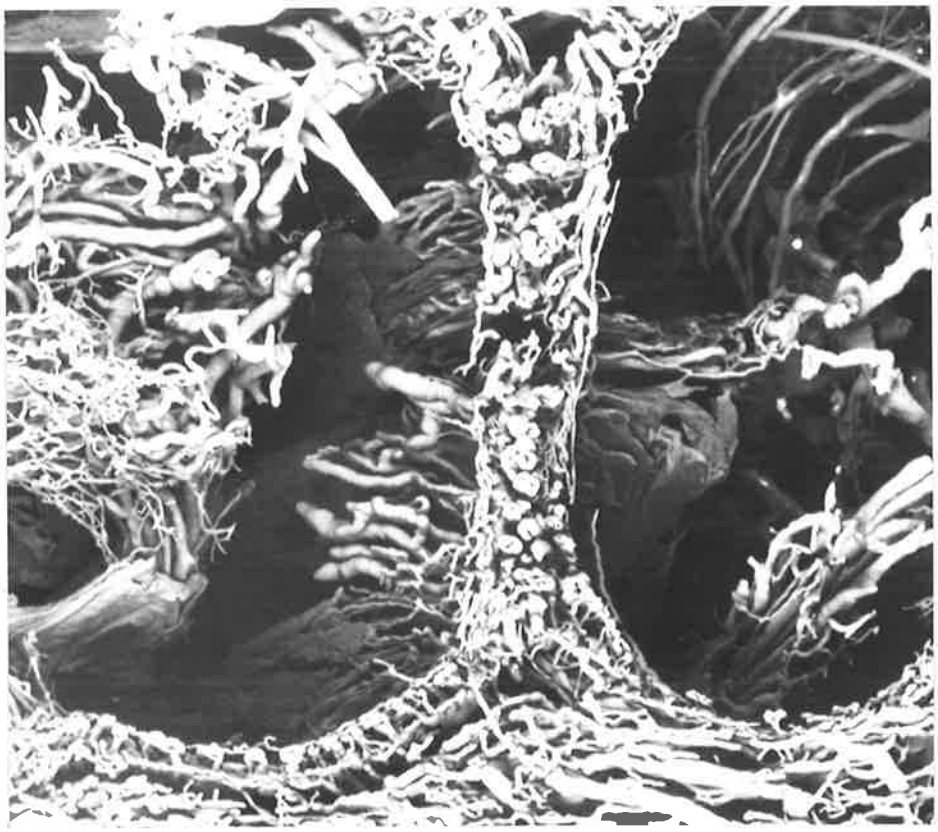


Figure 42

Gingival Crevice: A vascular loop from the previous photomicrograph can be seen under higher magnification. The rather complex vascular architecture is obvious.

C - Capillary
CB - Capillary branching
CV - Collecting venule
PV - Postcapillary venule

Stereo-pair 6^{o} tilt (x 600)
Bar = 10 micrometres



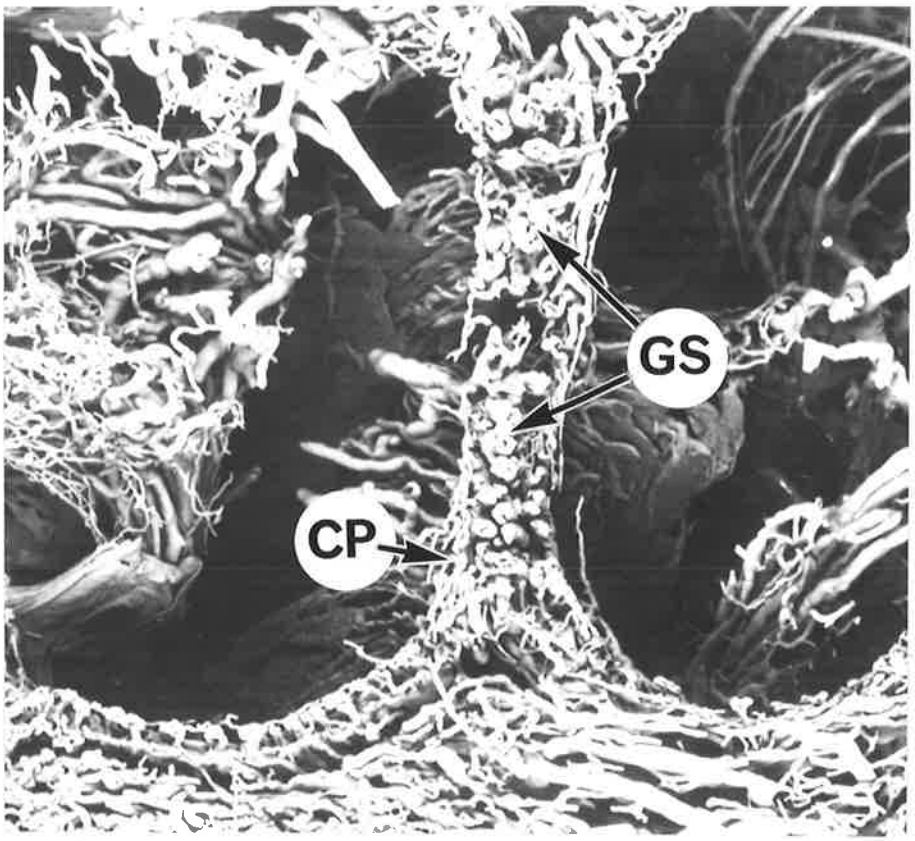
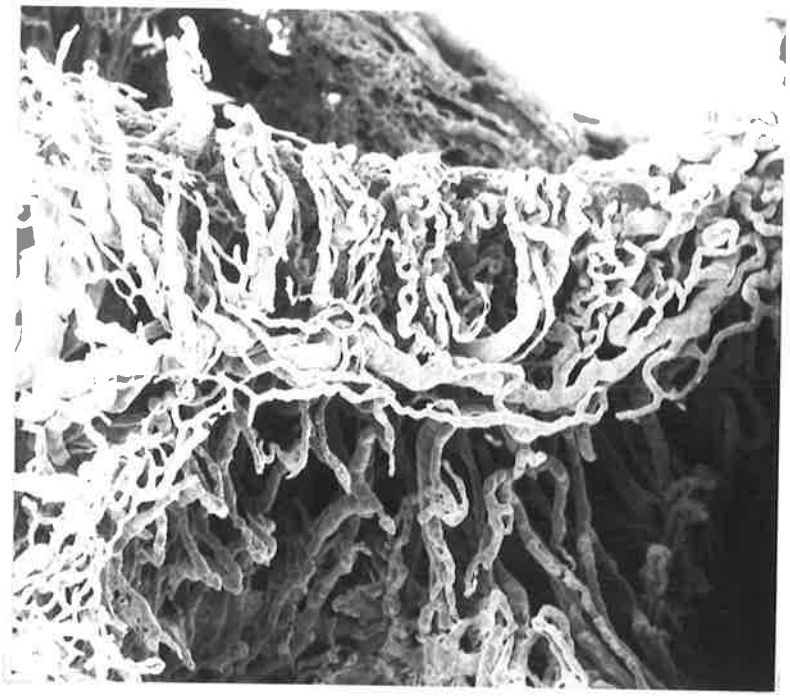


Figure 43

Interproximal Col: Almost the entire interproximal region between the maxillary left first and second molars is shown in this photomicrograph. The region mainly consists of the complicated vascular loops. The broad, flat capillary plexus of the buccal or lingual gingival crevice has condensed to a narrow band at the apical portion of the crevice in the col. The specimen is viewed looking down from the occlusal. The palate is seen at the bottom and mesial is to the left of the photomicrograph.

CP - Flat capillary plexus
GS - Glomerular vascular arrangements

Stereo-piar 6^{o} tilt Backscatter electron imaging (x 40)
Bar = 100 micrometres



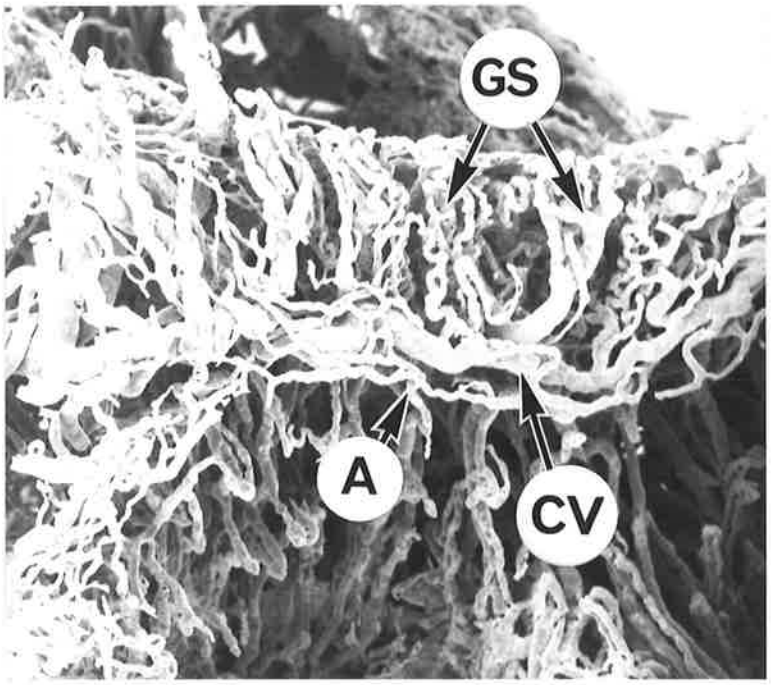
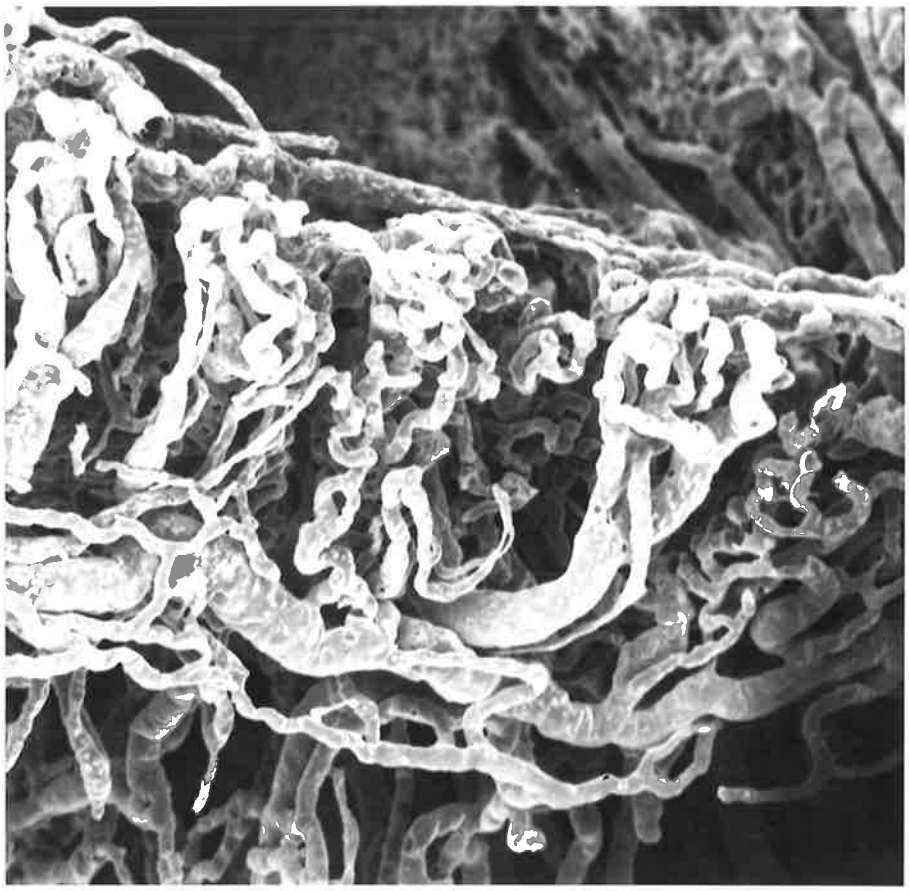


Figure 44

<u>Interproximal Col:</u> The density of the glomerular structures in the col can be clearly seen in this photomicrograph. A prominent feature is the large venous vessel coursing at the base of the crevice from the buccal to the lingual. Extensive anastomoses between the gingival and ligament vessels are obvious. The area depicted is between the maxillary right first and second molars, looking down from the occlusal at right angles to the internal aspect of the crevice. Buccal is to the left and anterior is to the top of the photomicrograph.

A - Anastomosis between gingival and ligament vessels
CV - Collecting venule
GS - Glomerular vascular arrangements

Stereo-pair 6^{o} tilt (x 100)
Bar = 100 micrometres



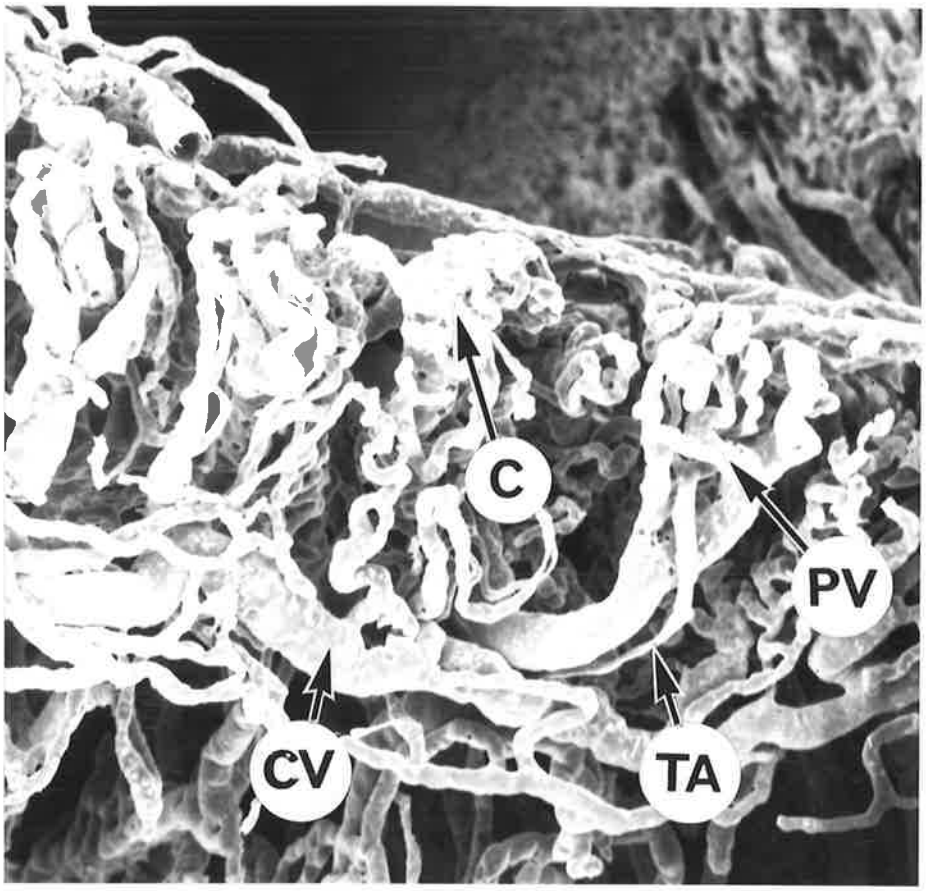
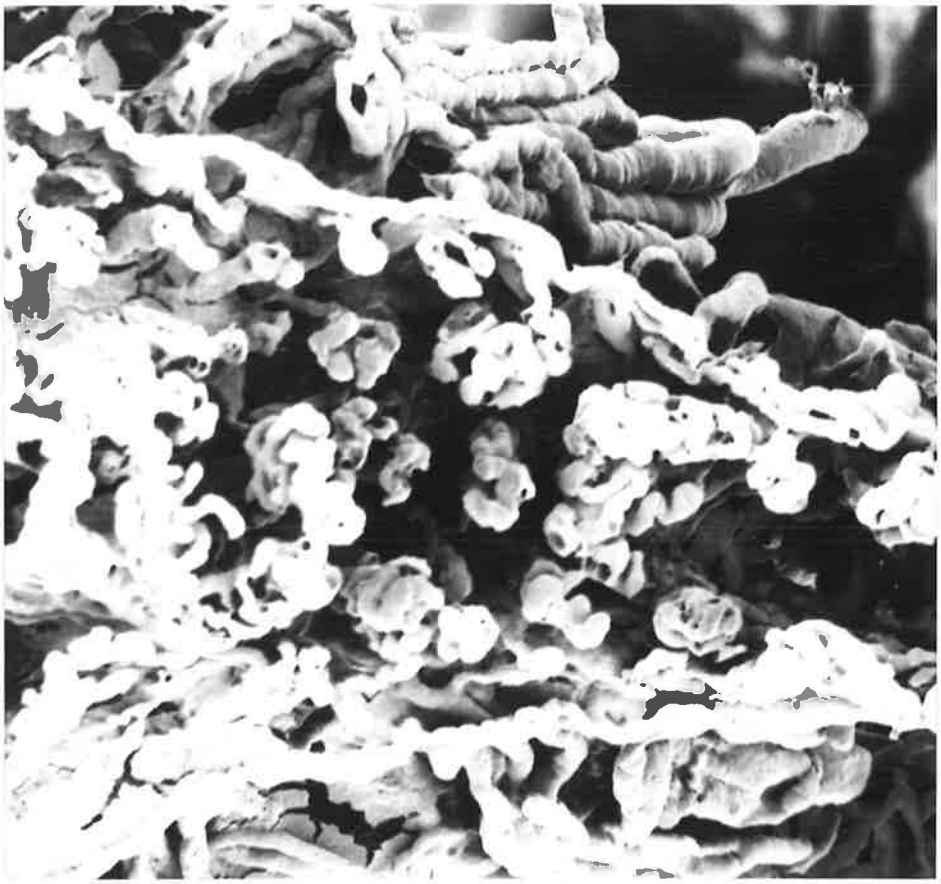


Figure 45

Interproximal Col: The detail of the glomerular arrangements is more readily observed in this higher magnification of the previous photomicrograph.

C - Capillary
CV - Collecting venule
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 200)
Bar = 10 micrometres



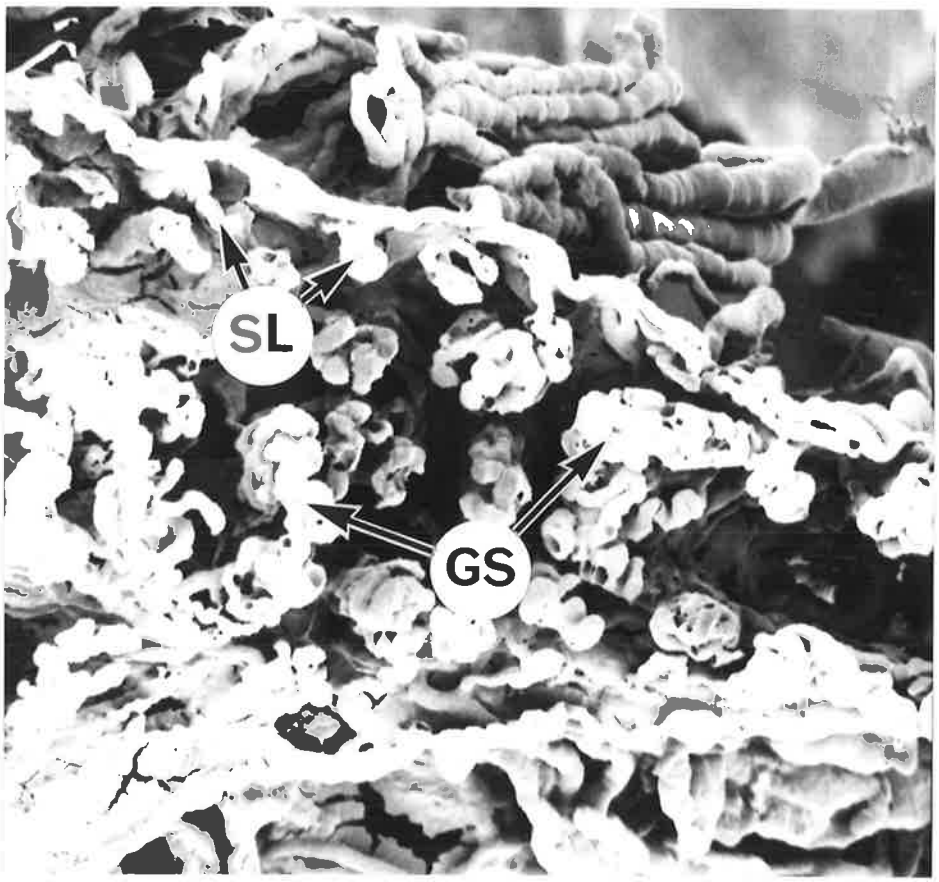
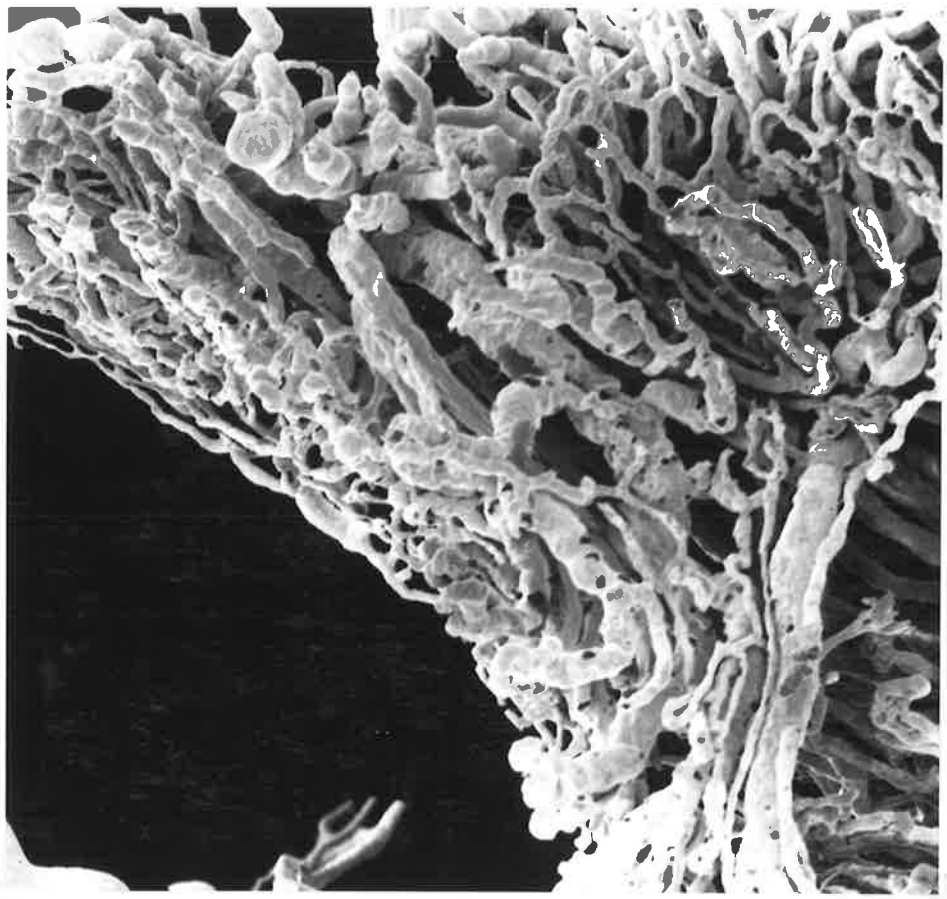


Figure 46

Interproximal Col: In addition to the glomerular vascular arrangements a row of simple loops can be seen at the base of the crevice. The region is the buccal portion of the col between the right mandibular first and second molars, looking down from the occlusal. Buccal is to the left and the anterior is to the bottom.

GS - Glomerular vascular arrangements
SL - Simple Loops

Stereo-pair 6^{o} tilt (x 160)
Bar = 100 micrometres



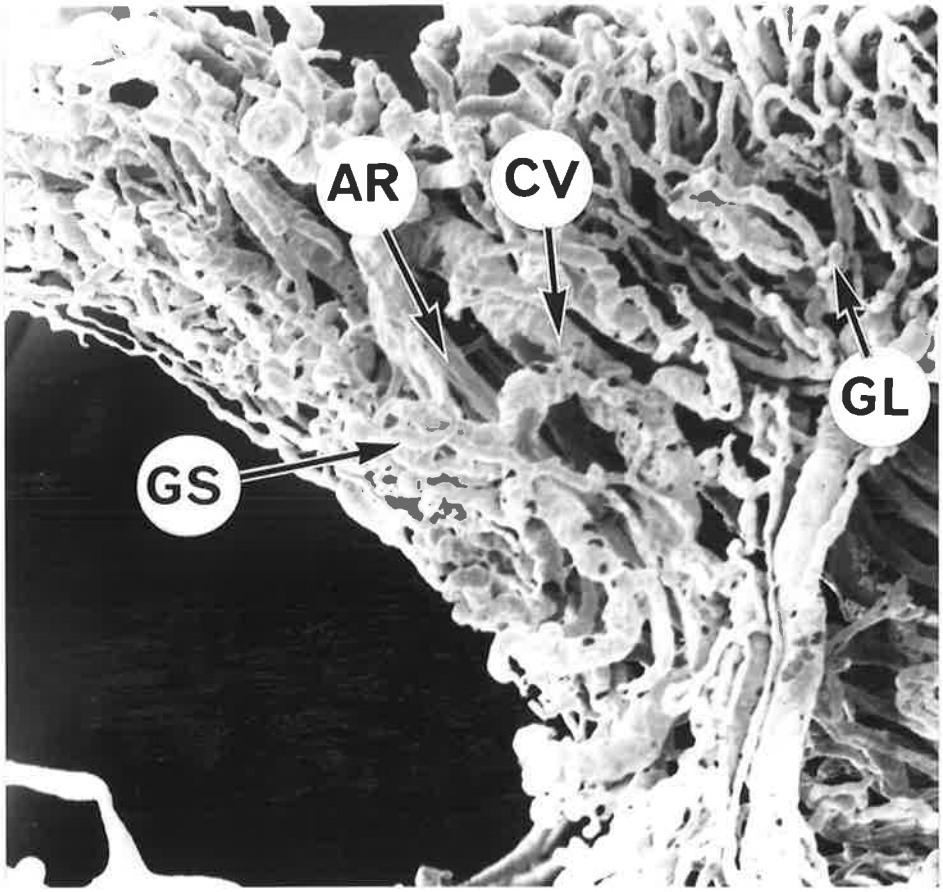
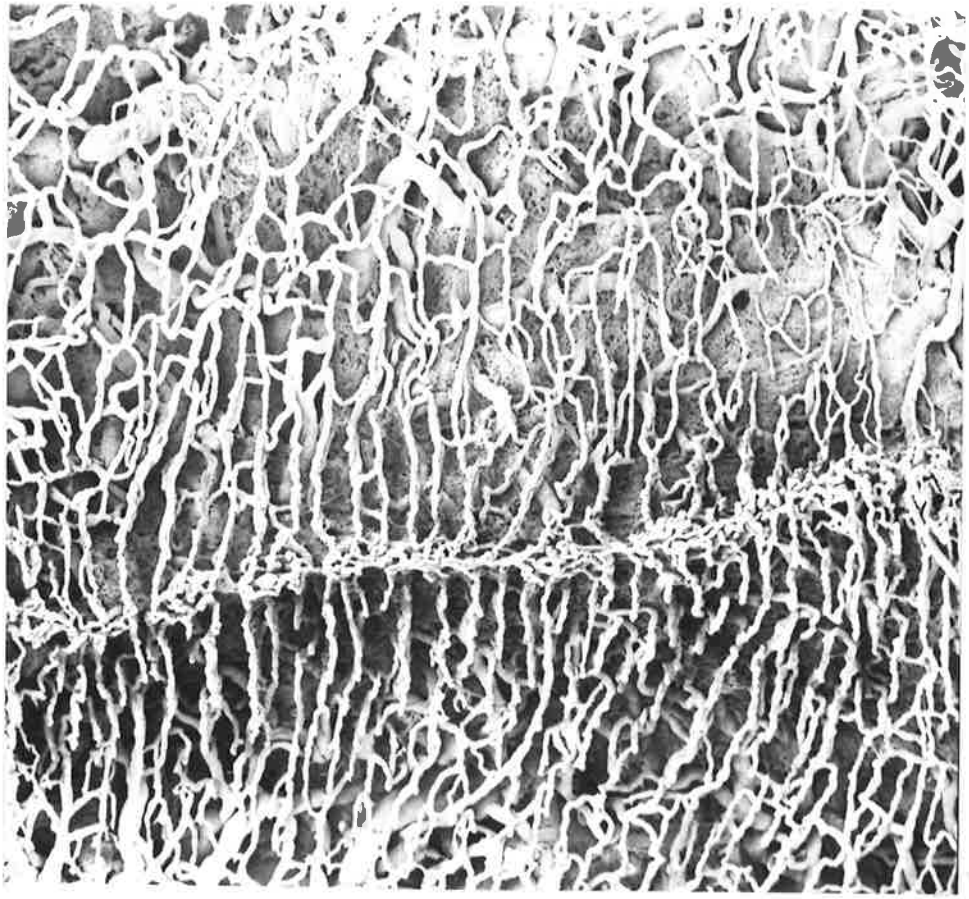


Figure 47

Interproximal Col: The arterial supply and the venous drainage of the col via the interdental papilla can be seen in this specimen. The region is the buccal papilla between the right maxillary first and second molars, looking down from the occlusal at right angles to the internal aspect of the crevice of the first molar. Buccal is toward the top.

AR - Arteriole
CV - Collecting venule
GL - Gingival vascular loop
GS - Glomerular vascular arrangements

Stereo-pair 6^{o} tilt (x 145)
Bar = 100 micrometres



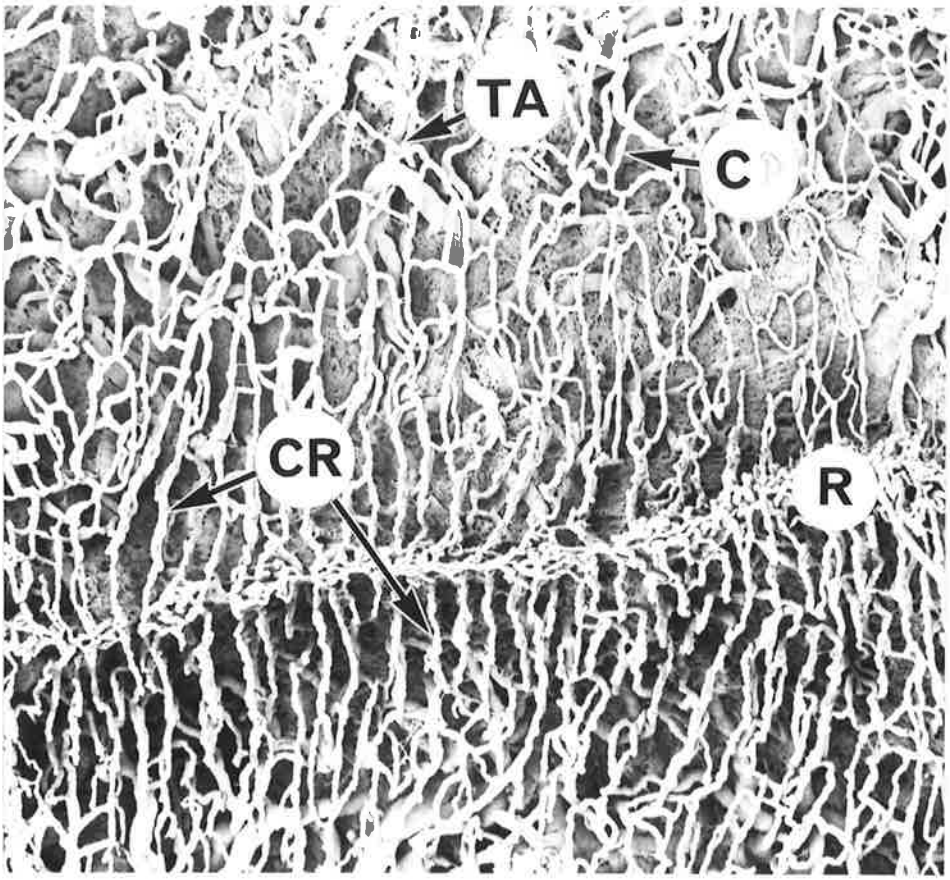


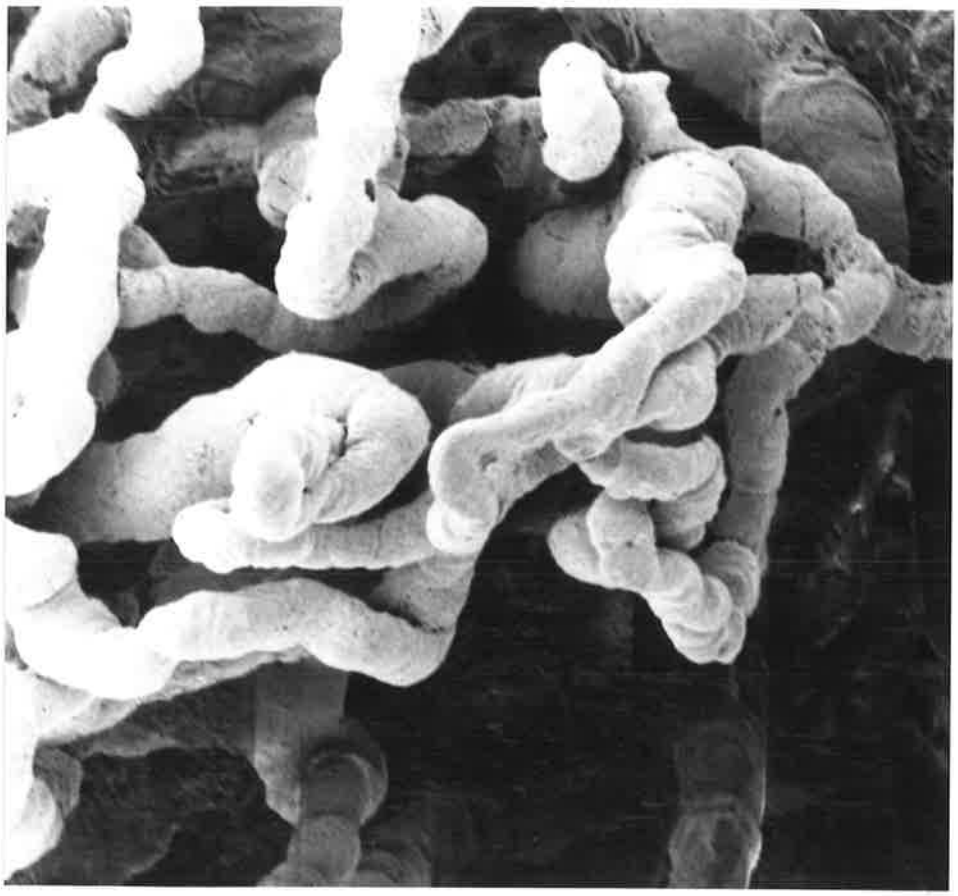
Figure 48

Palatal Mucosa: The capillary plexus of the palate and the vascular arrangement along the ruga is shown. The random capillary arrangement becomes organised into rows on the slopes of the ruga. The region photographed is at the level of the first molars immediately to the left of the midline. The top of the photomicrograph is anterior and the left molars are out of view to the right.

CP - Flat capillary plexus
CR - Capillary rows
R - Ruga
TA - Terminal arteriole

Stereo-pair 6^{o} tilt (x 85)
Bar = 100 micrometres

5.76



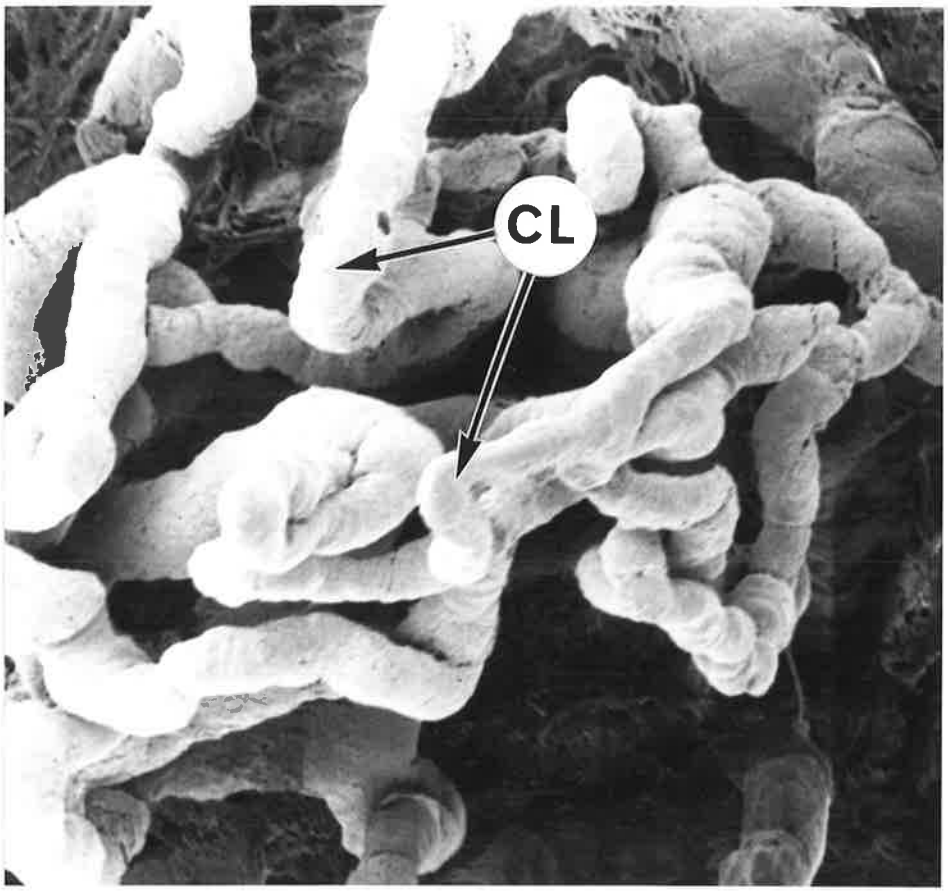
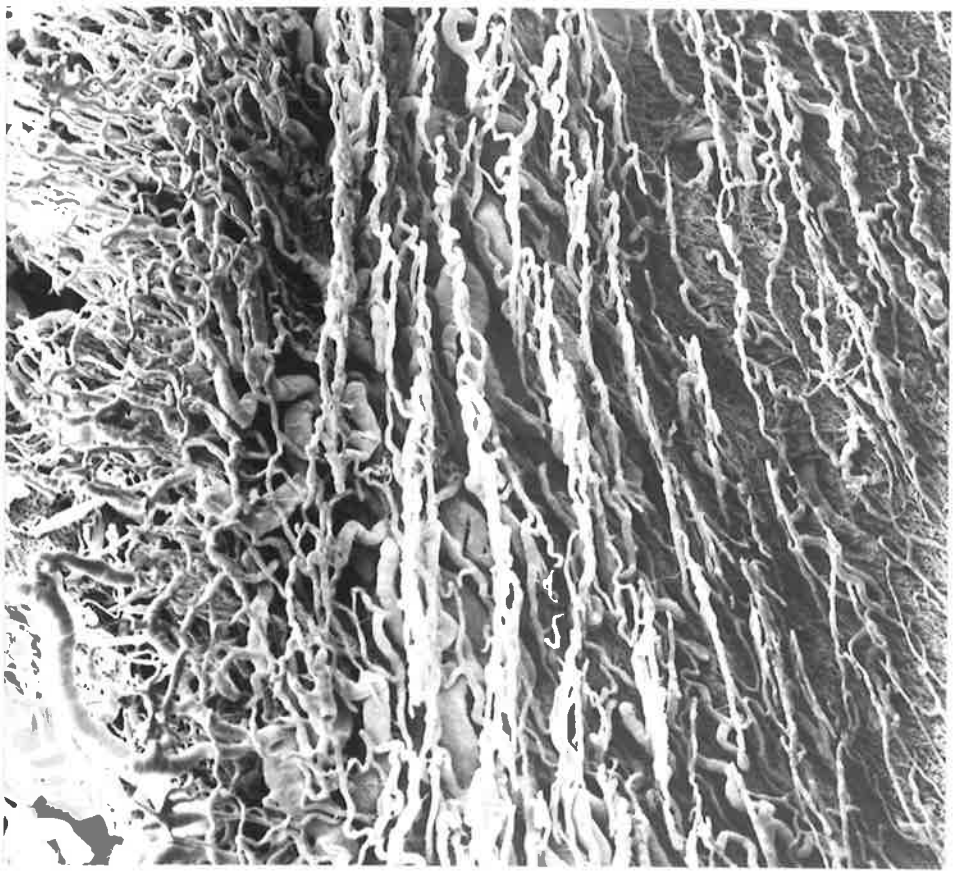


Figure 49

Palatal Mucosa: This photomicrograph is a higher magnification of the previous figure and shows the arrangement of the capillaries at the crest of the ruga. A number of simple loops can be seen.

CL - Capillary loop

Stereo-pair 6^{o} tilt (x 105)
Bar = 10 micrometres



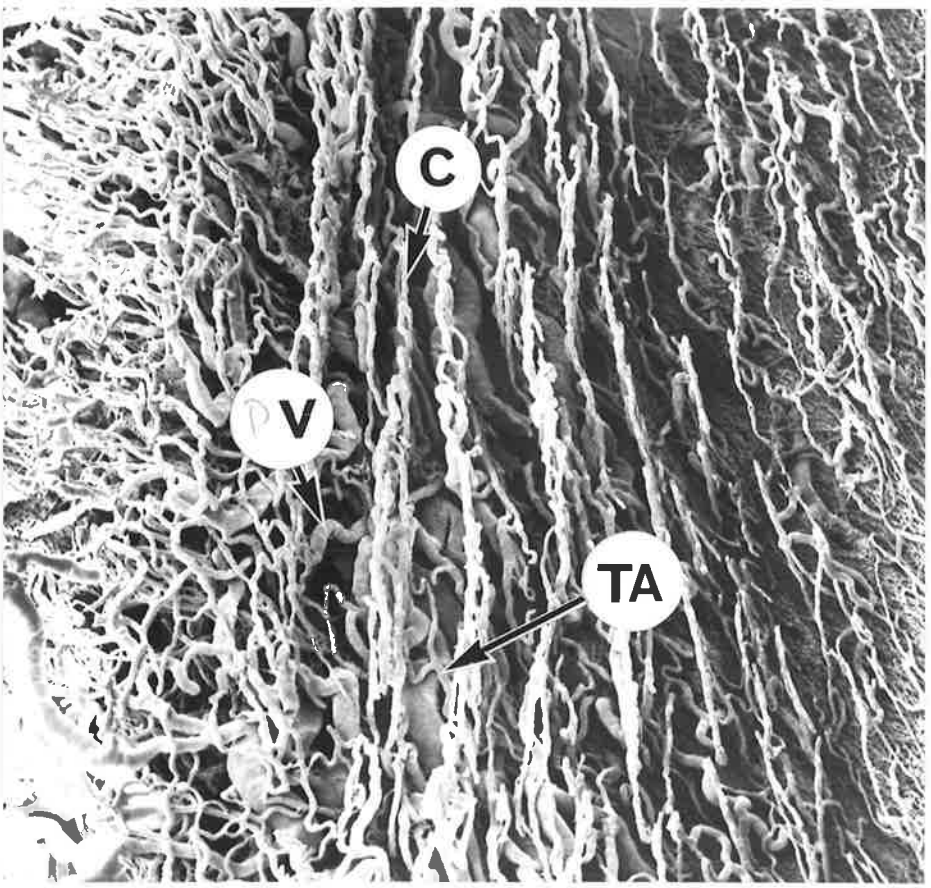


Figure 50

Lingual Mucosa: The vascular arrangement in this region is a series of parallel rows probably corresponding to the connective tissue projections into the epithelium. The regularity of these rows is very similar to that on either side of the rugae of the palate except in this region the rows have a slightly more complicated arrangement and are further apart. The specimen photographed is taken from the left mandible adjacent to the first molar viewed at right angles to the lingual alveolar mucosal surface. The molar is at the left, just out of view and the top of the photomicrograph is to the anterior.

C - Capillary
PV - Postcapillary venule
TA - Terminal arteriole

Stereo-pair 6^{0} tilt (x 105)
Bar = 100 micrometres

SECTION 6

DISCUSSION

TECHNICAL CONSIDERATIONS

The methyl methacrylate vascular casting technique has not before been used to examine the periodontal ligament of the rat molar. It was found that a complete vascular casting of this region was very difficult to obtain. Several workers have used the technique to view regions of the head and neck (Murakami 1975, Ohtani 1980) and have obtained complete replications of the brain and other organs. In this project, however, the brain was consistently not completely replicated,although the other soft tissues appeared completely cast. The bone is an area that is not easily perfused (Gannon 1981, Ohtani 1981) and in addition recent research into bone marrow vasculature has shown that the preparation of the casts often results in loss of some small vessels (Draenert and Draenert 1980).

The reason that the bone medulla is difficult to perfuse with methyl methacrylate is unknown. It may be that most bones are supplied by only a small number of nutrient arteries which anastomose within the medulla,but do not have extensive communication with arteries of the surrounding soft tissues. Consequently, if the nutrient arterial supply is obliterated for some reason,then there is no chance that the vascular bed will be kept filled through anastomosing connections.

Methyl methacrylate is a metabolic poison (Miles 1982) and produces severe skeletal muscle spasm,when it is introduced into the vasculature. It is likely that such spasm would interfere with the blood supply to the region. Most methyl methacrylate casts reported in the literature have been made of the viscera and the problem of vascular obstruction due to skeletal muscle contraction is not very great. In the head and neck region,however,the

vascular network is surrounded by skeletal muscle and so one would expect that the casting might be incomplete in the areas of spasm. A large amount of the bone medulla is supplied by arteries penetrating the cortical bone at sites of muscle attachment. Thus perfusion of bone medullary vasculature becomes difficult,due to the muscle spasm. During the preliminary experimentation,a curare-like drug (Tubocurarine 0.2 ml of 20 mg/2 ml) was used to paralyse the musculature. This did prevent muscle spasm on introduction of the resin but since it takes some time to cannulate the animal after the introduction of the curare,the death of the animal would mean that undesirable changes in the vascular bed could occur. As it turned out,no acceptable castings were obtained by animals perfused in this way, as some muscular spasm occurred on the introduction of gluteraldehyde which was being used at that time in an attempt to gently fix the blood vessels in order that they remained patent during resin perfusion.

The response of the vascular smooth muscle to the methyl methacrylate is poorly understood and little discussion of this aspect has occurred in the literature. The introduction of papavarine into the blood washout solution was designed to cause vascular dilation by smooth muscle relaxation. In this project the actual state of vascular smooth muscle contraction could not be determined. It was suspected that the papavarine was not completely effective in ensuring that the vascular walls were not subject to spasm of the smooth muscle. It is suggested that an alternative drug be used in future trials and a nitro-glycerine derivative could prove most useful (Gannon 1982).

The question of cast completeness and whether the casting represents a physiological norm is always difficult to answer. The present author is nevertheless satisfied that the vascular casts are sufficiently complete so as to enable a description of the entire vasculature of the regions of interest. As for the exact physiological status that the replica represents,

this cannot be precisely stated and is an area where further research is indicated. The vasoactivity of the methyl methacrylate is the main reason for caution in interpretation in this regard. Hodde (1981) discussed this factor and suggested that general vasodilatation may occur due to interruption of the pulsatile flow during the injection procedure. However, Olson (1980) has shown that vessels can be replicated while under the effect of various vasomotor drugs and this appears to overrule the vasomotor reactions to the injection-perfusion and/or the resin contact with the vessel wall.

In relation to other studies into periodontal vasculature utilizing different techniques,it appears that the observations made in the present project are essentially consistent with the earlier works and the major difference is that a large amount of extra information is made available by the improved technique. Thus, whether or not the physiological status is altered, if the basic information confirms earlier work,then the indication is that either no physiological alterations have occured, or the previous investigations produced similar physiologic changes.

Another problem confronted in this project was the presence of material on the specimen that had a disordered morphological appearance. This was first thought to be either uncorroded tissue or extravasation. The methodology used, however, precluded the suggestion that it was uncorroded material,because repeated corrosion and ultrasonic cleansing did not show any improvements. In addition, the location of this occurrence seemed to be fairly consistent. Generally the very apex of a socket was affected and frequently the gingival crevice was also involved. In some specimens the involvement was so general as to render the specimen useless for observation of the blood vessels.

Extravasation of resin can occur at high perfusion pressures when a frank blow-out of the vessels is the result. The morphology of the resin

that escaped from the blood vessels had a striking appearance that brought to mind the architecture of the tissue fluid spaces between the fibre bundles in the periodontal ligament. The work of Casley-Smith and Vincent (1978) showed that interstitial tissue channels can be replicated in the rat and rabbit by the methyl-methacrylate vascular casting technique. It is tempting to suggest that the morphological appearance of the extravascular resin in this project reflected interstitial tissue channels in the periodontal ligament. The limited areas in which this resin was found could reflect that the blood vessels in this region have increased permeability,which in turn could be related to a functional requirement. Barker (1980) found intraligamentous vessels in human tissue which he classified as lymphatics. It could be that the observations in this report show the beginning of a pathway through the interstitial tissues from blood vessels to lymphatics. This hypothesis warrants further research.

Methyl-methacrylate is a highly charged macromolecule and recent research has indicated that diffusion of these large, flexible highly charged molecules occurs readily across membrane barriers (Weibkin 1982). Consequently,the extravascular resin may be a result of this type of diffusion rather than be anatomically related.

MORPHOLOGICAL CONSIDERATIONS

The findings in this project have provided more information on the morphology of the rat molar periodontal vasculature than has been previously published. This is due to a number of methodological improvements. Viewing vascular casts in a scanning electron microscope has enabled the study of a complicated three-dimensional structure at high magnification with a great depth of field (compared to the light microscope). Stereo-pair photography has allowed visualization of three-dimensional images which greatly enhances the information available from the photomicrographs. Only two other investigations into periodontal vascular architecture of molars have taken

advantage of these methodological improvements (Ichikawa, Watanabe and Yamamura 1976, Kishi and Takahashi 1977),although three-dimensional imaging using stereo-pairs was not described. The present project, therefore, represents a significant addition to the information available on the vascular architecture of this region of the rat.

Vessel Classification

Valuable information revealed by this project has been not only greater detail of the vascular architecture,but also a preliminary classification of the blood vessels present. Categorization of the types of vessels that exist in the periodontal ligament in particular has not previously been comprehensively reported.

The classification of vessels provided in the present report cannot be taken as definitive. Only an ultrastructural survey can give positive identification to vascular structures. Nevertheless, the vascular casting technique enables a sound estimate of vessel types to be made that is accurate from the point of view of providing information on basic vessel categories,without being able to determine details of vascular fenestrations, cell junctions, pericytes, muscular layers etc. that allow fine classifications to be made. The following vessel types could be identified

(1) arterioles
(2) terminal arterioles
(3) true capillaries
(4) postcapillary venules
(5) collecting venules
(6) small collecting veins
(7) sinusoids

The classification criteria were not refined sufficiently to be able to delineate unequivocally such entities as precapillary sphincters.

metarterioles, venous capillaries etc.

An important finding in the present project was the absence of certain vessel types from various regions of the periodontium. The alveolar bone, particularly in the interradicular septum, was devoid of a capillary bed except for a small region around the sinusoids deep within the medulla. The periodontal ligament itself did not have a fine capillary plexus and it also lacked networks of arterial vessels. Forsslund (1959) stated that the capillary bed of an organ is thought to fulfill the organ's circulatory needs exactly. The significance of a non-existant fine capillary bed is not understood,but must be related strongly to the functional and nutritional requirements of the particular region.

Most other investigations have not commented on this feature,or have found that no particular vascular element was lacking. In the rat, Kindlova and Matena (1962) described both an arterial and a venous plexus in the ligament and observed a fine capillary plexus anastomosing between the arterial vessels. In contrast, these two workers described only a venous network in the alveolar bone and failed to mention the existence or otherwise of arterial or capillary networks. These findings are diametrically opposed to the present observations and appear to be too great to be explained away by species difference (Kindlova and Matena used male Wistar rats whereas the present project used male Sprague-Dawley rats). Kindlova and Matena's latex casts were examined by light microscopy and their published photomicrographs do not adequately support the descriptions provided. In this project,the scanning electron microscope has provided improved imaging at high magnification and so the photomicrographs, being of superior quality, provide a sounder basis for description. Consequently, the differences between the two projects are considered to be due to interpretations of a complex structure limited in the earlier project by difficulties of resolution. This aspect will be enlarged upon in the discussion of

vascular architecture.

Several ultrastructural studies have been conducted on periodontal tissues but,even so,only limited information is available. Gavin and Trotter (1968) found capillaries and a few small arteries in the gingival tissues of dogs and cats,but did not mention the presence of any venular or arteriolar elements. Kindlova and Plackova (1973) found two types of capillaries in the gingivo-dental junction of rat molars. The type of capillaries were essentially of the same classification as those found by Gavin and Trotter (1968). No discussion of any other vessel type was provided. Mohamed, Waterhouse and Friederici (1973) found capillaries, postcapillary venules, muscular venules and terminal arterioles in rabbit gingival tissues from molars and incisors. In this project it was possible to identify arterioles, true capillaries, postcapillary venules and collecting venules in the gingival tissues. It is surprising, considering the abundance of these vessels in the region, that more mention has not been made of them by the previous workers.

Gavin and Trotter (1968) found only 3% of the gingival vessels to have fenestrations. Kindlova and Plackova (1973) showed that those vessels beneath the crevice (subcrevicular capillaries) had fenestrations and pores,whereas the subjunctional vessels did not. The presence of fenestrations in 30% of rabbit gingival capillaries was established by Mohamed, Waterhouse and Friederici (1973). The presence or absence of pores could not be confirmed in this project. However,in many specimens it was found that extravasated methyl methacrylate was confined to either the gingival crevice region or the apical region. Although extravasation was observed to occur in other regions, it was most consistently found in the just-mentioned areas. This tempts one to suggest that the extravasation might reflect an increase in vascular permeability,rather than frank disruption of the vessels due to high pressure.

As discussed on page 6.4, Casley-Smith and Vincent (1978) investigated

interstitial tissue channels in rat and rabbit tissue and found extravasated resin of similar morphological appearance to that occurring in this project. The amount of extravasated resin found in this project was very much greater than that found by Casley-Smith and Vincent and may reflect an increased vascular permeability in the apical and gingival regions.

This observation would be consistent with the postulated functions of the particular components of the periodontium in relation to such requirements as an energy dissipating mechanism (Bien 1966) and crevicular exudate (Brill and Bjorn 1959),for example. In the gingival area the presence of resin in the interstitium complements the report of fenestrations by Gavin and Trotter, and Kindlova and Plackova.

Ultrastructural investigations limited to the periodontal ligament vasculature are rare in the literature and classifications of vessels are virtually non-existent.

Bevelander and Nakahara (1968) provided the first ultrastructural description of human ligament vessels and,although they did not categorize the vessels definitively,they observed thin walled vessels of varying calibre that resembled capillaries. Rygh (1972) mentioned the existence of arterioles, capillaries and venules in the rat molar ligament,but he did not discuss his criteria for classification or the architectural arrangement of the vessels except to say that they were observed alone or in groups.

Gilchrist (1978) and Barker (1980, 1982) classified vessels from a small segment of human periodontal ligament. Neither author found arterial vessels, which is consistent with the findings in the present investigation. Gilchrist (1978) found venous capillaries, postcapillary venules and collecting venules. Barker (1980) found only small or large vessels in the ligament, the former he classified as pericytic venules and the latter were not classified as blood vessels. Instead they were tentatively categorized as lymphatics. In the human alveolar bone,Barker(1982) identified only

two types of vessels. They were either pericytic venules or lymphatics and bore a close association with each other to the extent that the pericytic venules could be called "vasa lymphorum". The lymphatic vessels extended through foramina into the ligament to terminate in blind endings.

In this project arterioles were seen to supply the vessels in the ligament,but did not course within the ligament for any distance. The majority of vessels were postcapillary venules. There was also a lesser number of capillaries (probably venous capillaries) around the perimeter of the socket. Thus it seems that the vessel types found in the rat closely parallel the types observed in human periodontal ligament tissue. Kishi and Takahashi (1977),observing vascular casts of dog periodontium in the SEM,found arterioles, capillaries and venules. They did not mention the specific criteria of classification for each of these vessel types,but apparently based their descriptions on vessel diameter. Thus it seems that there is a species difference between dog, and human or rat tissue, with more arterial elements being present in the dog. The canine dentition has no lateral masticatory excursions and a different TMJ apparatus. These variations,plus the vascular variation just described,would most likely be a reflection of the different functional requirements placed on the canine dentition due to the carnivorous nature of the normal diet.

Vascular Architecture

Rat

The most comprehensive dissertation on rat molar vascular architecture was published more than twenty years ago (Kindlova and Matena 1962). A number of publications have appeared since and have updated this early information,but have done so only for a few specific areas rather than the periodontium as a whole (Kindlova 1967, 1968, 1970). The present findings partly support and partly conflict with the model provided by Kindlova and Matena and also highlight an area where similar morphological

arrangements have been interpreted differently.

Within the ligament, Kindlova and Matena (1962) described both arterial and venous elements with a fine capillary plexus interconnecting the arterioles. They described blood flow occuring in two directions. The arterioles carried blood from the apex to the coronal portion of the socket and the venules and veins drained blood in the opposite direction from the gingival tissues down to the apex. Both sides of the circulation had many communications with vessels of the alveolar bone.

In this present project,the demarcation of ligament vessels into an arterial side and a venous side was not so clear. Around the perimeter of the socket the vessels ran occluso-apically with very few horizontal connecting branches. The endothelial cell nuclei imprints and luminal diameters indicated that the vessels were either capillaries or postcapillary venules. Arterioles were not found coursing in the ligament itself,although vessels with the characteristic arteriolar endothelial cell nuclei imprints were observed to enter the ligament from the alveolar bone but immediately turn apically and transform into a postcapillary venule or capillary. It appeared that the direction of blood flow for those vessels located at the perimeter of the socket was from the gingival to the apical. Further evidence was provided by the presence of veins draining the crevicular plexus,either coursing down into the mucosa on the buccal aspect of the alveolus,or draining directly into the ligament.

Thus the venous arrangement of the ligament plexus described by Kindlova and Matena was confirmed,but the arterial arrangement was not found.

The vascular architecture over the interradicular septum was poorly described by Kindlova and Matena. In addition, the arrangement of vessels in this region was shown by Heulke and Castelli (1965) in their figure 7, but they did not provide a comprehensive description in the text. These

latter two authors merely mentioned a "concentration of venous channels". The present project, however, was able to provide a great deal of detail about the interradicular septum. The interesting observation was that the direction of blood flow appeared to be opposite to that occurring around the perimeter of the socket. This was difficult to determine precisely and the arrangement could even reflect the possibility of blood flowing either in two directions or tidally, that is, ebbing and flowing according to functional needs. This reversal of blood flow has been noticed in dog gingiva (Hock and Nuki 1971) and so is not an unusual feature of some vascular arrangements.

The blood, rather than flow from the gingival to the apical as seen in the perimeter, instead coursed from the apical up toward the crest of the interradicular septum. This is understandable because the septum is essentially enclosed by the roots of the molar and obviously the major portion of the blood supply must come up from the apical region. The only blood flowing downward toward the apex would be through anastomosing branches of the periodontal ligament entering the region through the furcation from the buccal or lingual sides. Blood could enter the ligament from the bone and then drain downwards,but the arrangement of the ramifications of the arterioles suggests that blood could flow in either direction, up or down. The arteriole supply is more dense on each side of the septum and the venous elements are more prevalent over the crest of the interradicular septum. This last fact indicates that the overall direction of blood flow in that portion of the ligament is predominantly from apical to gingival,but there is the potential for blood flow to be reversed.

Within the alveolar bone itself,the arteriolar supply and the venular and venous drainage are quite well defined,and the arrangement is notable for the absence of a fine calibre capillary plexus,except for a dispersed

open network associated with the sinusoid vessels deep within the medulla. It appears that the vessels are present largely to service the periodontal ligament because the arrangement does not appear to be what one would expect to see if it was catering for the nutrition and function of the bone and bony spaces. The course of the vessels is to and from the ligament by the shortest route and the exchange vessels are predominantly in the ligament and not the bone.

The dual arterial and venous supply to the ligament has only been reported by Kindlova and Matena (1962). Garfunkel and Sciaky (1971) briefly mentioned two parallel but inter-connected networks; one close to the root, the other external to it. Neither of these arrangements have been confirmed in this project, nor in the literature published in the intervening years. The majority of studies describe "blood vessels" coursing occluso-apically closer to bone than cementum (Boyer and Neptune 1962, Bernick 1962, Cernavskis and Hunter 1965). Heulke and Castelli (1965) briefly mentioned that the arteries to the periodontium were multiple tortuous small vessels which, after reaching the membrane, divided into abundant capillaries. These last workers also described concentrations of venous channels about the apices of the molar teeth. Carranza, Itoiz, Cabrini and Dotto (1966) reported vessels that ran parallel to the long axis of the root and gave out irregular branches that intertwined forming a plexus around the root.

All authors generally agreed that there were frequent anastomoses between the ligament vessels and the medullary vessels via foramina in the socket wall (Bernick 1962, Boyer and Neptune 1962, Carranza, Cabrini, Itoiz and Dotto 1966, Garfunkel and Sciaky 1971) and present findings substantiate this.

Around the perimeter of the socket,the vessels coursed in irregular tracts and were predominantly of two sizes,representing capillaries and

postcapillary venules. The capillaries tended to intertwine around the larger vessels,but still coursed occluso-apically. They did not form a fine horizontal plexus as in previous reports (Kindlova and Matena 1962, Heulke and Castelli 1965, Garfunkel and Sciaky 1971, Cernavskis and Hunter 1965). As mentioned previously, species difference may account for these conflicting observations,but it may be more likely related to technique. Except for Kindlova and Matena (1962) the other authors all examined cleared sections to view blood vessels rendered visible by filling with opaque material. The light microscope was used to examine the essentially two dimensional specimens. Consequently, vessels that could be branches extending obliquely above or below a vessel could have been misconstrued to be connecting branches in the same plane,purely because the techniques did not allow three-dimensional imaging. In actual fact, the horizontal vessels may very well have been branches anastomosing with medullary vessels,as was observed in this project.

Hairpin shaped loops were found in this project,either at the apical region of the ligament,or occasionally in the coronal third. Bernick (1962) described similar loops which were related to areas of formation of cellular cementum. Kindlova and Matena (1962) also described periodontal capillary loops,but the present author believes that they were actually describing vascular arrangements associated with the gingival tissues,rather than the periodontal ligament.

The gingival plexus found in this project bears similarities to the illustration provided by Kindlova and Matena in 1962,but those authors interpreted this vessel arrangement as being part of the periodontal ligament plexus and not the gingival crevicular plexus. In a later paper, Kindlova (1965) described the arrangement of vessels in the gingival crevice of the monkey and the findings of the present project more closely resemble this description of monkey gingival vasculature than the description of

the rat.

In addition, Kindlova and Matena (1962) described a horizontal arterial circulus formed by the periodontal ligament vessels. In this project horizontal vessels were observed at the base of the gingival crevice and anastomosed freely with the ligament vessels. The present author considers that these two descriptions are of the same morphological arrangement. The capillary loops arising from the horizontal circulus, as proposed by Kindlova and Matena (1962), are most likely the vascular loops arising from the crevicular plexus as found in the present project. The detail and morphology of these vascular structures has been more completely described by virtue of the more recent methodologies applied in this project.

These crevicular vascular loops do not solely comprise capillaries and, in fact, the majority of the vascular structure consists of postcapillary venules. This fact is consistent with De Almeida and Bohm (1979) who also found vascular loops with a diameter of 10 to 25 micrometres in the rat gingival crevice.

Egelberg (1966a) did not classify the vessels that he found in dog gingival crevice, but suggested that they were predominantly capillary or venular. Nuki and Hock (1974) showed that, with gingival inflammation, vessel loops replaced the entire network of capillaries, precapillary arterioles and postcapillary venules of crestal gingiva and much of the superficial networks underlying the crevicular and buccal gingival surfaces. The loops consisted of narrowed arterial capillaries and postcapillary venules. These venules were from the body of the connective tissue and subjacent to the crevicular epithelium and were dilated to 2 to 3 times their usual size.

It is apparent that the loops of the gingival crevice of dogs and

rats are mainly made up of exchange vessels. It is tempting to suggest, then, that they play a major role in the production of crevicular exudate.

Several workers have suggested that coiled vascular arrangements in the gingival crevice are associated with gingival inflammation (Egelberg 1966a, Nuki and Hock 1974. Hock and Nuki 1975). The question arises as to whether the formations found in this project, and those found by Kindlova and Matena (1962), are in fact representative of the normal healthy gingiva. In this project no detailed examination for marginal gingivitis was conducted on the experimental animals. However, the rats used were kept under normal laboratory conditions with normal diet and so it is considered that the gingival condition reflected the usual status of a normal healthy young adult animal.

De Almeida and Bohm (1979) stated that clinically healthy gingiva was in fact the seat of inflammation that could be detected only by microscopy. In addition, De Almeida and Bohm investigated vascular permeability in the rat gingiva and found that those vessels beneath non-keratinized epithelium showed an increased permeability to intravenously injected carbon. These workers related the increased permeability to the presence of chronic inflammation beneath the non-keratinized epithelium, and in the rat this type of epithelium occurs adjacent to the dentogingival attachment and also over the interproximal col and provides protection from noxious stimuli. This reflects the exact position of the vascular loops in the present investigation. As De Almeida and Bohm point out, it is not yet known if the loops exist only in the presence of an inflammatory process, or whether they are a normal feature of the area.

In the dog, Nuki and Hock (1974) have correlated histologic evidence of chronic inflammation to the presence of vascular loops and have described how the flat plexus of the gingival crevice actually changes into a plexus

with many coiled loops. These workers have also shown that a healthy gingiva can have a looped vascular pattern which has persisted from an earlier experience of chronic inflammation. Such an experience is particularly prone to occur during the eruption of a tooth. This fact supports the variability of vascular patterns in dogs as reported by Kindlova and Trnkova (1972). Kindlova (1968) found that a similar episode of chronic inflammation during the eruption of teeth in rats resulted in the formation of capillary loops. These loops persisted after the subsidence of the inflammation and became a characteristic of normal non-inflamed gingiva.

It appears that chronic gingivitis in dogs is strongly related to the formation of coiled vascular loops in the crevicular region. This association has not yet been clearly established in the rat and further research is necessary to clarify the issue.

The very dense glomerular structures in the interproximal col region have not previously been reported in the rat, although Kindlova and Matena (1962) observed that this region consisted solely of periodontal capillary loops. Glomi or glomeruli have been described in the gingival and periodontal tissues of man and monkey and will be discussed in the next section.

Other Animals

Investigations into periodontal vascular architecture have been conducted on humans, monkeys, dogs, cats, mice, hamsters, opossums, guinea pigs and rabbits. The majority of publications have used the human, monkey or dog models and this discussion will be limited to these animals. Investigations into other animals are rare and have not had sufficient follow-up research to be substantiated. Also, they are less suitable for comparison to the human situation. Thus, even though the findings

may be of general interest, it was decided to exclude these animals from the discussion.

Information about the architecture of the blood vessels in the human was published in 1932 by Hayashi and has not been updated since. However, the description was purely of arterial supply and the venous architecture was ignored.

Recent work by Gilchrist (1978) and Barker (1980, 1982) has shown that only capillary or venular elements exist in the small portion of ligament from the buccal surface of human premolars which they studied. Thus, the schematic arterial supply provided by Hayashi may be inaccurate in the light of these recent findings.

The arterial arrangement in the human did not have a parallel in the rat as found in this project. In the monkey, Kindlova (1965) inferred that there was an arterial supply that ran occluso-apically parallel to the long axis of the tooth. However, this arterial network was not fully described in the text,although the network was drawn in a diagram. Folke and Stallard (1967) could not verify vessels running parallel to the long axis of the tooth with their technique and these workers suggested that this was because the vessels were major representatives for the venous return. The arterial supply to the ligament was shown by Castelli and Dempster (1965) to immediately break into a capillary plexus on entering the ligament space. When considered together, all these observations point to the fact that the predominant vessel types in the monkey periodontal ligament are either capillaries or venular elements. This inference complements the findings in the present project that show a paucity of arterial elements in the ligament of the rat.

The monkey periodontal ligament plexus was reported to be an essentially two layered plexus with a flat capillary network situated closer to the

tooth than the plexus of main vessels from which it arose (Castelli and Dempster 1965, Kindlova 1965). This arrangement was not found in the rat but it was similar to the architecture reported for ligament vessels in the dog (Kishi and Takahshi 1977).

The periodontal ligament of the dog was reported to have arterioles coursing within it (Ichikawa, Watanabe and Yamamura 1976, Kishi and Takahashi 1977). However, these arterioles supplied a capillary plexus and in general they did not run for any great distance in the ligament. The exception to this was the coronal one quarter of the ligament where circularly oriented bundles of thick vessels existed, each containing a venule and one or two arterioles. So, in general, the dog ligament contained mainly capillaries, venules and veins, but in certain regions there was an increase in arteriolar supply. This is contrary to the present findings in the rat and may be a reflection of the different functional requirements of each species. Insufficient research has been conducted on dog periodontal vasculature, however, to confirm the architectural arrangement of the microvessels.

Glomerular structures have been reported to occur in the periodontal ligament of man (Wedl 1881, Schweitzer 1907, Provenza, Biddington and Cheng 1959, Provenza, Biddix and Cheng 1960, Griffin 1965, Bouyssou, Bader, Lodter and Duffaut 1970) and monkey (Folke and Stallard 1967). These structures have not been seen in the ligament of the rat in this project although the gingival tissues, particularly interdentally, do contain these glomerular-like arrangements. Where these structures were reported in the healthy human periodontium, they were found solely at the cervical or coronal portion of the ligament. The interpretation of the work of Wedl (1881) and Schweitzer (1907) suggests that these authors were describing vascular arrangements actually in the gingival soft tissues. This is unclear, however, since one has to rely on other authors' translations

and interpretation of these early works which were reported in German. Where the human tissues were unhealthy,the glomerular structures were more predominant apically and over the interradicular septum (Provenza et al. 1960).

In the monkey, Folke and Stallard (1967) found the "so-called" glomeruli-like structures,but these workers did not mention the localization or numbers of these structures. Kindlova (1965) described structures which resembled glomeruli at the coronal extremity of the periodontal membrane. Much discussion has occurred regarding the role of these coiled vascular structures in the ligament. The present author's interpretation of the literature in relation to these arrangements in the healthy animal is that they occur in that part of the periodontium in the vicinity of the alveolar crest and this is supported by Kindlova's discussion (1965). Consequently, it is considered that they are essentially a component of the gingival tissues and not the ligament itself. This conclusion in in agreement with the present findings for the rat,where no coiled structures were found in the ligament, whereas in the gingival crevice region coiled vascular loops were in abundance. In the diseased state however, coiled vascular structures have been reported in the periodontal ligament (Provenza, Biddington and Cheng 1959, Provenza, Biddix and Cheng 1960). This finding has not been supported by further research and provides a fruitful area for future investigation.

Only Soloviev (1970) has reported glomerular structures in the periodontal ligament of the dog, and this report also remains unconfirmed.

The gingival crevice vasculature has not been examined in the human. Vital microscopic studies have, however, been conducted on the gingival tissues on the outside of the alveolus up to the crest of the free gingival margin,but have not included the crevicular region (Forsslund 1959, Kamijo, Suzuki, Takahashi, Wakatsuki, Maeda and Takeishi 1964). Only a few studies on monkey crevicular vasculature have been reported (Kindlova

1965, Folke and Stallard 1967),whereas quite a number of investigations have been completed on the dog,including examination of the sulcular (or crevicular) vasculature (Egelberg 1966a, Hock and Nuki 1971, Nuki and Hock 1974, Hock 1975).

In the monkey, Kindlova (1965) described structures resembling glomeruli in the coronal extremity of the periodontal membrane, and further occlusally, a second type of capillary arrangement with tenuously looped capillaries and clearly coiled arterial parts in the vicinity of the epthelial attachment. Such an arrangement was not exactly duplicated in the rat in the present findings. However, the results of this project showed a more complicated vascular arrangement than that presented by Kindlova and Matena (1962) and the complexity was similar to the description for the monkey. Kindlova (1965) reported that the coiled capillaries in the monkey were numerically greater interdentally,which is supported by the observations on the rat in this project.

Studies on the marginal gingiva of the dog have shown that,in the absence of inflammation,the sulcular plexus was essentially a flat regular network (Nuki and Hock 1974, Hansson, Lindhe and Branemark 1968). Where inflammation occurred the vascular arrangement changed. The plexus adjacent to the free margin became a series of characteristic loops. The longer loops were twisted or spiralled (Hansson et al. 1968, Nuki and Hock 1974, Hock 1975). The regular plexus continued apically to the base of the gingival crevice,but was characterized by wide tortuous capillaries and venules which had a close relation to the crevicular epithelium (Hansson et al. 1968). Egelberg (1966a) agreed,but also showed, however, that vascular loops could occur all the way down to the base of the crevice in some of his specimens. This vascular arrangement differed from that found in this project.

Previous reports of the same region for the monkey and the rat showed that a regular plexus extended from the free gingival margin down to the base of the crevice and that vascular loops arose from a region either at the base of the crevice (Kindlova and Matena 1962, Kindlova 1965) or, as in this project, in a circular band around the middle third of the crevice. This arrangement seemed to be a morphological feature of healthy tissue and was not related to the presence of gingivitis,although this point has some qualifications as has been discussed on page 6.15.

Kindlova and Trnkova (1972) found similar morphological arrangements of sulcular vasculature in dogs to previous workers,but noted a variability of vascular patterns that was not related to the degree of infiltration. Thus, it seems that dog gingival tissue does not exhibit quite the same morphological arrangements as the rat, nor does it react in the same way in the presence of inflammation. As a consequence, inferences about human tissue drawn from dog or rat tissue must be guarded. The obvious functional difference between dog and human periodontium would partly explain some variation in morphology between the two animals. Rat molar function, however, more closely parallels that of the human molar and so one would expect that morphological arrangements would be similar to man.

The col region has not been examined extensively in any of the other animal models. Kindlova (1965) showed that the coiled capillaries of the crevice were most numerous interdentally. Folke and Stallard (1967) described a vascular arrangement for the area that was randomly oriented, where there was an increased tendency for coiling of the microvessels. No investigations into the human col vasculature were found. The intricate vascular architecture of the col,as seen in the rat,may also be present in the human and the monkey. The arrangements are so distinctive that they must have a unique function and further research into the vasculature of this region would be worthwhile.

As with the rat, information on the blood supply to the alveolar bone of man, monkey and dog is sparse. Castelli and Dempster (1965) described a dense venous plexus at the apex of each alveolus in the monkey as well as drainage of blood via a central efferent venous plexus or channels that ran in the bone marrow of the septi. This was not unlike the venous drainage found within the alveolar bone of the rat in this project. Castelli (1963) described the human situation only vaguely and did not provide any more information than Hayashi (1932), who only described an arterial supply. Barker (1980, 1982) discussed vessel types occurring within human alveolar bone medulla, but did not provide information on architecture. Barker found no arterial elements in his small specimens of buccal compact bone, whereas, in the interradicular and interdental septi of rat, the arterial supply was quite prevalent.

FUNCTIONAL CONSIDERATIONS

Nutrition

Sims (1980) reported that the periodontal ligament of mouse mandibular molars had a vascular proportion averaging 17%, while the microvascular cross-sectional area in human mandibular premolars averaged 11%. Sims considered that such vascular proportions were beyond the limit of that required for nutritional purposes alone and in fact greatly exceeded vascularity of human tendon (Casley-Smith, Sims and Harris 1976), which was used as a normal connective tissue to compare with periodontal ligament connective tissue.

Although a quantitative volumetric analysis was not undertaken in this project, it seemed that the proportion of the ligament taken up by blood vessels approached the proportions found by Sims (1980) for man and mouse. This increased vascular proportion most likely reflects the varied functional requirements placed on the periodontal ligament (Sobin and

Tremer 1977).

The metabolic requirements of the periodontal ligament have been found to be greater than for other connective tissues, probably because of the need for the ligament to maintain its integrity under the severe functional assault to which it is subjected. Sodek (1977) stated that the rate of synthesis of collagen in periodontal ligament was twice as fast as attached gingiva, four times as fast as skin corium used as the reference tissue, and six times as fast as alveolar bone. These findings tend to support the statement that the unique function of the ligament necessitates a high collagen turnover. Therefore, one would expect the vascular supply to reflect this requirement in its arrangement. Certainly the predominance of postcapillary venules in the ligament indicates that a great deal of fluid and nutrient exchange occurs.

Not only does the periodontal ligament have a very rich vascularity, but the gingival crevice also has an abundant blood supply, particularly in the interproximal region. The arrangement of vessels in this region is more elaborate than exists in plexuses that serve purely nutritive purposes. The type of arrangement found here more closely resembles the plexuses in the kidney glomeruli or the villi of the intestine. These structures have a definite function that is unrelated to nutrition. It is suggested that the arrangements in the crevice may also have a function unrelated to nutrition and this view point will be discussed in later sections.

Crevicular Fluid

In dogs, crevicular fluid accompanies the appearance of increased vascular permeability and is not the result of a physiological mechanism (Cimasoni 1974). Instead the vascular permeability was found to be related to inflammation (De Almeida and Bohm 1979) and more particularly to acute situations (Egelberg 1966b). Chronically inflamed, but resting, gingiva

did not produce crevicular exudate,except after mechanical or chemical intervention (Egelberg 1966 c,d). The vascular arrangement in healthy gingiva differed from the chronically inflamed gingiva by the absence of vascular loops which accompanied the appearance of inflammatory cells in the gingiva (Egelberg 1966a, Hock and Nuki 1975, De Almeida and Bohm 1979). Kindlova (1965) suggested that the arrangement of vessels in the healthy gingival crevice of monkeys may be related to crevicular fluid exudate. The work of Egelberg (1966a) and De Almeida and Bohm (1979) could not relate vascular permeability to the presence of loops specifically,although it is apparent that inflammation, crevicular loops, increased vascular permeability and the appearance of crevicular fluid all occur at the same time. The role of the capillary loops remains unknown.

Countercurrent Exchange

Lanciault and Jacobsen (1976) suggested that the minimal anatomical requirement for a countercurrent exchange mechanism is the presence of parallel blood vessels in which blood is flowing in opposite directions. This requirement is met in the vascular loops found in the gingival crevice in this project and indicates that,within this region,not only can there be countercurrent exchange of electrolytes, but also the absorption of fluids from the interstitium into the blood vessels. This latter function is directly contrary to the postulated source of the crevicular fluid and is an area that warrants further definitive research.

The countercurrent exchange of electrolytes may be necessary to maintain the appropriate tissue tonicity in the presence of the range of hyper, hypo and isotonic substances that are ingested. The question arises, however, as to why the other mucous membranes do not show similar structures. It may be that the accumulation of plaque in the gingival crevice necessitates the presence of countercurrent exchange mechanisms to provide an electrolytic and osmotic buffer in order to maintain tissue integrity against the noxius

stimuli provided by the plaque.

Bien (1966) hypothesised that the vascular loops found in the gingival tissues provided a countercurrent exchange mechanism serving a diffusion barrier function to control gas cavitation,in order to prevent bone resorption from occurring during normal masticatory function. Bien also stated that an efficient diffusion exchange was necessary to service respiratory needs, waste disposal and temperature regulation. As a consequence,the vascular loops in the gingival region probably are related to the maintenance of homeostasis in this region.

Interestingly, Bien (1966) suggested that the normal pattern of vascular loops in the gingiva changed to an anastomosing pattern in the presence of inflammation. This changing pattern led to a loss of the mechanism for maintaining the diffusion barrier and so gas cavitation ensued with resulting resorption of alveolar crestal bone. As was discussed in the previous section, it is apparent from work conducted on dogs (Hock and Nuki 1975) that the normal gingival vascular architecture is a flat anastomosing plexus that changes to a plexus of vascular loops during inflammation. This more recent research contradicts Bien's hypothesis that the loss of vascular loops can result in bone resorption. Nevertheless, the presence of vascular loops during gingival inflammation is still most likely related to a countercurrent exchange process that is necessary for the body's defence mechanisms against the cause of the inflammatory response.

Bone Resorption/Repair

The vascular corrosion casts were not able to provide any information on the occurrence of bone or cementum resorption because these hard tissues were removed during preparation. However, within the ligament plexus there were a number of simple vascular loops oriented at right angles to the main occluso-apically directed vessels and these loops extended radially towards the root of the tooth. They were situated mainly at the

apex of the root or dispersed randomly in the coronal one third of the socket. Similar loops were found in the rat molar by Bernick (1962) and he observed that they were associated with the formation of cellular cementum and were necessary to provide nutrients for this process. It is suggested, therefore, that these loops are involved in reparatory processes occurring at the cementum border. Bernick found that these vessels were deeply embedded in the cementum of old rats whose roots were hypercementosed. This project used only young adult rats and an interesting extension to this research would be to take corrosion casts of the vasculature of old rats.

Vignery and Baron (1980) measured the area and amount of bone resorption and deposition that occurred during the physiologic distal drift of rat molars. They inferred that as the distal surfaces of the socket were undergoing resorption the mesial surfaces were subjected to bone deposition processes at a rate matched with the resorption such that the volume of the ligament and the perimeter of the alveolar wall remained constant. The interdental and interradicular septi would be undergoing the most remodelling during this distal drift. In the present project,it was found that these areas had vascular arrangements different from those over the buccal and lingual walls of the socket where the remodelling would be less extensive. No conclusion regarding bone resorption or deposition being related to vascular architecture can be drawn,except to repeat the observation that the predominant vessel type was the postcapillary venule and this type of vessel is known to be strongly related to the exchange of fluids, nutrients and proteins (Majno, Palade and Schoefl 1961). This exchange is obviously necessary in bone remodelling processes.

It was shown in this project that the vessels were more dense over the interdental septum. As mentioned, this region is where a large amount of bone deposition and/or resorption is occurring. Therefore, it could be speculated that the actual density of the vasculature, rather than the architecture might be related to the processes of remodelling during distal drift. Although precise

measurements were not taken, a general observation was that the vasculature over the interradicular septum, as well as having a unique architecture,also had a slightly greater density than the vasculature around the perimeter.

Complementary to this observation about vascular density, Trueta (1963) related the processes of bone resorption and deposition to blood flow rate. Goldhaber (1963) suggested that local variation in oxygen tension may comprise a basic regulatory mechanism for bone resorption and further suggested that variation in oxygen tension could be accomplished by alterations in the local blood supply. Sims (1983) has provided ultra-structural evidence for a proprioceptive mechanism to control the vascular function of the periodontal ligament in his demonstration of an intimate relationship between oxytalan fibres, nerve endings and blood vessels. This type of arrangement may fulfil a dynamic autoregulatory role,or it may have purely a proprioceptive function. Further research is required to elicit more information,but it becomes apparent that within the periodontal ligament there exist means whereby the vascular supply could be closely related to bone resorption and deposition by virtue of both its architecture and mechanisms that might control the local blood supply to influence oxygen tension,as proposed by Goldhaber (1963).

Recent literature has suggested that the resorptive cells were blood born (Jee and Kimmel 1976) and as a consequence resorptive patterns can be influenced by the vascular architecture. Brown (1982) pointed out that the number of Sharpey's fibres attaching to the cementum surface was three times greater than attaching to the alveolar bone. Thus the interstitial volume between the bundles was much less at the ligament/cementum interface than at the ligament/bone interface. This attachment means that the blood vessels have to exist closer to the bone because this is the only area where there is sufficient space for them to course. Brown highlighted the fact that any blood born resorptive cells would be selectively precluded from

the cementum surface by virtue of the lack of room to attack it. Since the bone was closer to the blood vessels anyway, he therefore postulated that more resorptive cells would reach the bone than the cementum and this viewpoint is supported by the fact that bone is resorbed more readily and more extensively than cementum. In the apical area of the tooth,the insertion of Sharpey's fibres was less dense and so the cementum can be more readily attacked in this region. The vascular loops mentioned in the first paragraph of this section obviously are able to exist in the regions of greater interstitial volume. Bernick (1962) related this type of vascularity to cementum formation,but since the apex of a tooth is more readily resorbed than elsewhere,it becomes apparent that the blood vessels are likely to be related to root resorption as well.

The predominantly venous pool of the ligament is composed of a type of blood vessel known to have a high permeability for the exchange of nutrients, gases and cells. Furthermore these vessels are closely associated with those areas that show most resorptive/reparative activity (viz. the alveolar bone and the root apex). Finally a possible mechanism for local vascular control embracing the regulation of oxygen tension and blood flow rate has been found. Therefore one can conclude from these facts that the blood supply to the periodontal ligament plays a significant role in the resorption and deposition of hard tissues.

Dissipation of Occlusal Forces

The mechanisms for withstanding occlusal forces are far from clear. The periodontal vasculature has been implicated in playing a role and this project does provide some information which is relevant to the various hypotheses presented,but by no means provides answers to the questions.

The actual arrangement of the vessels in the ligament, predominantly occluso-apical, has not been given any special significance. Ng, Walker,

Zingg and Burke (1981) found that the application of high forces to a dog premolar produced a change in the vascular resistance of 10% or less. These workers then suggested that one reason for this finding was that the vessels were well protected from occlusion although they felt it more likely that only a small fraction of the measured flow went to the ligament and the remainder was directed towards the pulp, alveolar bone or gingiva which remained unaffected by the application of force. Nevertheless, the force required before periodontal pulsation ceased was 200 times greater than Parfitt (1960) found in man. In addition, Walker, Ng and Burke (1978) found that pulsation in a dog tooth persisted under loads as high as 500 grams and cited the work of Ng (1977) who found pulsation even when the load was 4 kilograms. Walker et al. (1978) hypothesized that the force required to prevent pulsation of a tooth was dependent upon the degree of vascularity of the periodontal ligament and the architecture of the vascular bed.

It is suggested by the present author that the occluso-apical arrangement of vessels within the periodontal ligament allows large heavy loads to be placed on the tooth without occluding the vessels. The mechanism is unclear,but the following postulate is proposed. The fibres of the periodontal ligament travel essentially obliquely across the ligament space and at rest they are a certain distance apart. As the tooth intrudes under function the fibres come closer together. If any vessels were running horizontally between these fibres they would be squashed by the approximation of the fibres as the tooth moves apically. When looked at from the occlusal aspect the fibres radiate out from the tooth to the bone and do not move closer to each other as the tooth intrudes. Only fibres above and below come closer. Thus any vessel running vertically would not be subjected to any constrictions during function.

Muhlemann (1967) produced a diagram showing the effect of horizontal

movement on the periodontal ligament and indicated the partial or total occlusion of horizontally oriented vessels. Picton in 1969 discussed this phenomenon and commented that,on intrusion of the tooth within its socket,the width of the ligament did not change except at the apical region. He stated, however, that the collagen fibres on straightening out would pinch the small vessels intertwined within the bundles. His diagram showed horizontal vessels.

The argument presented here, albeit on teleological grounds, is that the vertical or occluso-apical orientation of blood vessels within the periodontal ligament of the rat molar is best suited to withstand the effects that tooth intrusion has on the patency of the vessels. The question of the presence of horizontal vessels mentioned by several authors has been discussed previously and the present author considers that horizontal vessels per se are uncommon in the periodontal ligament of all species so far investigated. In fact, the teleological basis for the above conclusion is supported by the observation that close examination of all results presented in the literature reveals a predominance of the occluso-apical orientation of blood vessels.

Whether or not this arrangement actually has a greater significance in withstanding occlusal forces has not yet been adequately established and is another area open to future research.

Parfitt in 1967 suggested three modalities which were involved in providing support for a tooth,subjected to functional loading. When a load was applied to a tooth he noticed that initially there was an elastic displacement,followed by recoil on removal of the load. This elastic response was assumed to be associated with the collagen fibres. If the load application continued,the response changed and became similar to that observed in a viscoelastic material and Parfitt attributed this response

to the ground substance. Slow intrusion was observed after prolonged load application and the tissue fluid was thought to be responsible for this response. The role of the vasculature was ignored in this discussion.

Bien, however, in his 1966 paper theorized that functional forces were dissipated firstly by a squeeze film effect, then by the formation of cirsoid aneurisms as the collagen fibres tightened around the randomly oriented blood vessels, and finally by loss of kinetic energy as fluid diffused out of the blood vessels at high pressure through tiny openings in the vascular wall. Bien also suggested that the rete present at the apical and gingival regions provided an efficient diffusion exchange mechanism,as well as a mechanism for maintaining steep pressure gradients across very thin membranes. Without these mechanisms, gas cavitation would occur and ultimately result in bone resorption.

In the present project,the ligament vessels were not randomly oriented but, as discussed earlier,they were arranged in such a manner that their occlusion by collagen fibre tightening would be unlikely. As a consequence, the cirsoid aneurysm hypothesis cannot be supported. Bien also postulated that the gingival arterial rete found by Forsslund (1959) in dog, Kindlova and Matena (1962) in rat and Kindlova (1965) in monkey fulfil the requirements for a countercurrent exchange mechanism, as well as a diffusion barrier, without which gas cavitation would occur,with bone resorption being the ultimate result. The vascular loops in the gingival crevice and the glomerular structures in the interproximal col found in the present project do not contradict the hypothesis. However, these structures were comprised of mainly venular elements,rather than arterial. Nevertheless, the role of postcapillary venules in fluid and metabolic exchange is well known (Majno 1965) and does not detract from Bien hypothesis.

Bien's theories have not been supported by any direct evidence. A number of studies have been conducted since Bien's work. Some workers have

directly implicated the vasculature in the dissipation of occlusal forces,whereas others have denied that a significant role is played by the blood vessels.

Muhlemann (1967) suggested that the periodontal ligament acted as a shock absorbing system and one part of the mechanism was the redistribution of intra-vascular fluid. Slatter and Picton (1972) provided support for a significant role played by the vasculature when they showed that a reduction in the vascular supply substantially reduced the mobility of a tooth to light loads. Wills, Picton and Davies (1976) postulated that,in the early stages of intrusion,blood has an energy dissipating function providing a major viscous component to the displacement and recovery of the tooth.

This suggestion was the first to implicate the vasculature as having a viscous behaviour. Previous discussion attributed an elastic, cushion-like role to the blood vessels and implicated the tissue fluid, ground substance or cellular components in the viscous response. The role of the tissue fluid was subjugated by the findings of Walker, Ng and Burke (1978), who showed that,contrary to Bien's postulates, the major source of tooth support did not come from the free fluids of the ligament,but were provided by the collagen and the ground substance acting in conjunction with the periodontal vasculature. Picton and Wills (1978) reiterated that the viscoelastic properties of the ligament were attributable to the displacement of blood in the vessels, tissue elements and distortion of large polymer molecules which comprise much of the soft connective tissues. They also indicated that the actual flow of blood, rather than the mere presence of blood, through the periodontal vessels contributes to the viscoelastic properties. However, Myhre, Preus and Aars (1979) found that vascular forces and the microcirculation played no role in determining the response of the rabbit incisor to intrusion by small loads,although these

workers acknowledged that the vascular forces were important in setting the actual position of the tooth in the socket.

The connection between the periodontal vasculature and the collagen and ground substance of the ligament acting in parallel to provide tooth support was emphasised by Walker (1980). He suggested, however, that the vasculature did not play the major role in tooth support. Nevertheless, Ng, Walker, Zingg and Burke (1981) suggested a very strong connection between the periodontal vasculature and tooth support,where the blood vessels play a direct and significant role.

The publications to date implicate the periodontal vasculature in playing an important role in the dissipation of functional forces. However, the specific mechanism for this activity has not been delineated. The present project lends support, by inference, to a number of the previous findings and the technique of vascular casting may be an excellent medium for providing further information on the manner in which the vasculature supports the tooth. Of particular interest would be the morphology and function of the vascular tree under such circumstances as axial loading, horizontal loading, the influence of vasoactive drugs, and other factors.

SECTION 7

CONCLUSIONS

(1) SEM examination of internal replica casts is a satisfactory method of obtaining information on the vascular architecture of the rat molar periodontium.

(2) The technique has provided for a classification and reassessment of the type of blood vessels that exist in the microvascular bed of the periodontium.

(3) No separate networks exist in the periodontium. The vasculature of each component enjoys a rich anastomosis with the vascular bed of neighbouring components.

(4) The periodontal ligament has a varied vascular arrangement. The variation reflects the different anatomy of each region within the ligament,as well as the different functional demands placed upon these regions.

(5) Very few arterial elements exist in the microvascular bed of the periodontal ligament. Consequently the vasculature contains principally vessels suited to free exchange of fluids, nutrients and other substances.

(6) The ligament vessels are arranged in an occluso-apical orientation and this concurs with the theory that the vessels so arranged are less likely to be occluded by movements in the Sharpey's fibre system,as the tooth experiences functional loads.

(7) Arrangements in the interproximal col resemble the vascular architecture of both the vasa recta around the loop of Henle in the kidney and also the microcirculation of the intestinal villi. These structures, together with the more simple vascular loops in the gingival crevice, form basic morphological units that would be able to provide an efficient countercurrent exchange mechanism.

(8) The distribution of ligament vessels around the perimeter of the socket is consistent with the theories of the mechanisms of bone deposition and resorption that are active during the distal migration of the rat molars.

(9) Further research is required to clarify the following areas

(a) the physiological status of the perfused vessels, in particular the vasoactive effects of methyl methacrylate

(b) the presence of interstitial tissue channels within the periodontal ligament and the relationship to lymphatic pathways

(c) reasons for the apparent lack of both a capillary bed and an arterial plexus in the periodontal ligament

(d) the relationship between the presence of chronic gingivitis and coiled vascular loops

(e) the function of these coiled structures whether in diseased or healthy tissue with particular emphasis on countercurrent exchange and the absorption of interstitial fluids

(f) the vascular architecture of aged animals

(g) the regulation of the blood supply by the nervous system via autoregulatory phenomena including proprioceptive feedback

(h) the relationship between vascular architecture and the dissipation of functional forces

(i) the effect of orthodontic tooth moving forces on the vasculature of the ligament, bone and gingival tissues

(j) the role of the blood vessels in bone and tooth formation and resorption

(k) the relationship between blood vessels and interstitial tissue fluids.

SECTION 8

APPENDICES

APPENDIX 1

Criteria for an ideal vascular casting medium.

(1) A sufficiently low viscosity or particle size to completely fill tubes of less than 5 micrometres diameter.

(2) Polymerization without shrinkage or distortion.

(3) Must not change the dimensions or distribution of the system being cast as a result of chemical or mechanical effects of the casting mixture.

(4) Must be able to withstand clearing procedures.

(5) Must be visible in the light microscope in cleared specimens.

(6) Must be able to withstand dissection procedures.

(7) Must remain intact after removal of surrounding tissue.

(8) Must retain its original configuration during drying.

(9) Must be rendered conductive.

(10) Must withstand electron bombardment.

(Adapted from Nowell and Lohse 1974, Gannon 1978, Hodde and Nowell 1980).

APPENDIX 2

Phosphate buffer

(1) Stock Solutions

(a) 0.2 M solution of monobasic sodium phosphate (NaH_2PO_4).
Add 27.58 gm. to 1.0 litre of water.

(b) 0.2 M solution of dibasic sodium phosphate ($Na_2HPO_4.2H_2O$)
Add 28.38 gm. to 1.0 litre of water.

(2) To obtain buffer with pH6 add 12.3 ml. of solution (b) to 87.7 ml. of solution (a). Dilute to a final volume of 200.0 ml. to obtain a 0.1 M concentration.

APPENDIX 3

0.1N Hydrochloric Acid

Add 100.0 ml. double distilled water to a glass screw-top bottle.

Slowly add 3.64 ml. concentrated HCl. Use as needed.

APPENDIX 4

TRIS Buffer

For 0.05M Tris-HCl add 0.601 gm. to 100 ml. double distilled water.

To this add 0.005M $CaCl_2$ (0.109gm. to 100 ml. double distilled water.)

Adjust pH to 7.4 by adding conc. HCl if necessary.

SECTION 9

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