A D D E N D U M

Page 77 line 7 to continue:

The marmosets were anaesthetized with 0.2 ml ketamine hydrochloride (Ketalar, Parke Davis Pty. Ltd., Caringbah, N.S.W.) given intramuscularly. When a sufficient depth of anaesthesia was obtained, the chest cavity was opened, the heart removed and the animal decapitated. The head was immediately placed in 10 percent formal saline and stored there for several weeks.

E R R A T U M

Page 52 line 7 throught should read thought.

A MORPHOMETRIC EXAMINATION OF THE PERIODONTAL LIGAMENT VASCULATURE OF THE MARMOSET MOLAR

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

by

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SUMMARY

Although many investigators have studied the vascular morphology of the animal and human periodontal ligament, there is an absence of a definitive systematic assessment of the periodontal vascular distribution. Descriptions of the macroscopic and microscopic vascular architectural arrangements of the mandible and periodontal ligament have tended to be qualitative rather than quantitative.

A light microscopic morphometric investigation was undertaken to establish and evaluate the periodontal ligament vascular distribution of the marmoset monkey mandibular second molar to obtain further species information.

Eight marmoset mandibles were histologically processed and serially sectioned horizontally at 8 microns from the cervical to the apical region of the second molar. These animals had been subjected to various diet regimes over varying periods. In the pilot study, 20 vertical sections per molar root were sampled for two second molars from two animals. As a result of a statistical evaluation of the pilot study, it was determined that six equidistant sections per molar root was an adequate sample for the major study. These sections were stained with Miller's, Verhoeff's iron haematoxylin or Pollack's trichrome. Two hundred and thirty photomicrographs were projected onto a digitizing tablet of a semi-automatic tracing device at a magnification of 6,100x to compute vascular and periodontal ligament areas. Appropriate standard

descriptive statistics were employed using the B.M.D.P. (Biomedical) statistical software package to determine the vascular distribution of the periodontal ligament. In addition, a general linear mixed model on a logit scale was used to determine a stylized periodontal vascular distribution model for each of the three lateral thirds of the periodontal ligament. Ten randomly selected photomicrographs were re-analysed to test the repeatability of the method.

The findings demonstrated that the existence of statistical differences between lateral thirds of the periodontal ligament necessitated the analysis of these regions separately. No significant difference at the one percent level was found for any of the variables examined to test the repeatability of the method.

The lateral vascular distribution was such that the middle third had the greatest vascular volume (13.1 percent), followed by the alveolar ligament third (8.9 percent) with the least to be found in the ligament third near tooth root (2.6 percent). The overall periodontal vascular volume for the marmoset mandibular second molar obtained from the data was 8.3 ± 0.4 percent (mean ± 2 standard errors) whereas that derived from the stylized periodontal ligament model was 7.5 percent. These two overall percentage vascular volume figures were so similar that it was reasonable to conclude that the statistical model of the vascular distribution was an acceptable representation of the data.

The vertical vascular distribution was zone dependent. However, in general, an increase was demonstrated from the cervical

to the apical regions. A consistent finding for all lateral thirds of the ligament, at any depth of the ligament and in all quadrants, was that the percentage vascular volume for the tooth on the right side of the mandible was greater than that on the left.

In zone 1, the alveolar ligament third, quadrants 1, 2, 3 and 4 were found to be statistically different from each other at the one percent level with the lingual surface having an appreciably greater vascular volume. The remaining three quadrants were similar in their vascularity, although the vascular volume on the buccal was greater than that on the mesial which, in turn, was greater than that on the distal.

A detectable statistical difference between mesial, distal or single roots was not evident, nor was there any statistical evidence of differences between animals due to their respective diets.

The intraspecies and interspecies vascular configurations and distributions are discussed with particular emphasis placed on the possible functions of the periodontal ligament vasculature. Some of the previous periodontal vascular distribution findings reported in the literature have been supported by the present observations.

SIGNED STATEMENT

The report contains no material which has been accepted for the award of any other degree or dimploma in any university. To the best of my knowledge and belief, this report contains no material previously published except where due reference is made in the text of the report.

ISAIA DOUVARTZIDIS, B.D.S.

ACKNOWLEDGEMENTS

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CHAPTER 1:

INTRODUCTION

Many investigators have studied the vascular morphology of the animal and human periodontal ligament. However, the majority of the research has viewed this intricate complex as a static system. One possible explanation for this view could be that the experimental techniques implemented failed to reveal the dynamic nature of this versatile organ and tended to concentrate on a two-dimensional perspective thus limiting the ultimate perception of the function of the periodontal ligament and its vasculature.

Of particular concern was the absence of a definitive systematic assessment of the vascular distribution of the periodontal ligament. The most comprehensive work on the human periodontal vascular distribution based on histological research was published by Götze in 1965 and 1976. He reported a vascular volume of one to two percent for premolar teeth. Wills, Picton and Davies in 1976 mathematically derived a periodontal vascular volume of one half to one percent which supposedly accounted for 30 percent of the displacement of anterior monkey teeth under load. Parfitt in 1967 stated that the volume of blood in the periodontal tissues was two percent of the total volume of the ligament space. Sims (1980) reported localised periodontal vascular volumes of 17 percent for mouse mandibular molars and 11 percent for human mandibular premolars.

Thus, a marked discrepancy exists in the percentage figures quoted by various authors as being representative of the periodontal ligament vascular volume. As a direct result, the proportional role that the vasculature plays in the overall tooth support mechanism is difficult to determine.

CHAPTER 2:

AIMS OF THE INVESTIGATION

The purpose of this project was to establish and evaluate the periodontal vascular distribution of the marmoset mandibular second molar using a morphological light microscopic investigation.

The specific aims of the investigation were:

1. To undertake a pilot statistical study to derive quantitative information regarding the distribution of the periodontal ligament vasculature.

2. To use the information obtained from the pilot study to collate the data for the major study.

3. To statistically analyse the major study data.

4. To determine the repeatability of the method employed.

5. To provide information regarding the vascular distribution of the periodontal ligament with particular reference to

   (i) the vertical vascular distribution,

   (ii) the lateral vascular distribution,

   (iii) the relevance of mesial or distal roots,

   (iv) the significance of tooth surface,

   (v) the occurrence of effects due to the respective side of the mandible, and

   (vi) the influence of dietary patterns on vascular distribution.

6. To obtain an average percentage vascular volume figure for the marmoset mandibular second molar.

7. To discuss the significance of the findings with regard to the vascular function within the periodontal ligament.

CHAPTER 3:

REVIEW OF THE LITERATURE

The vascular system of the periodontal ligament has been investigated by a multitude of authors to elucidate its structure, distribution and role in tooth support. The microscopic anatomy of the vascular pattern has been explored in a variety of animals, using numerous laboratory techniques. This literature review gives an account of the studies which have a direct bearing on the microscopic anatomy and function of the periodontal ligament vasculature.

3.1 Macroscopic Vascular Anatomy of the Mandible

The blood vessels which supply the mandible in human and non human primates have been described by Hayashi (1932), Perint (1949), Cohen (1959a, 1959b), Castelli (1963), Castelli and Dempster (1965), Kindlova (1965), Bishop and Dorman (1968), and Cutright and Hunsuck (1970).

Investigations in this area on animals other than primates have been described by Cernavskis and Hunter (1965), and Hellem and Östrup (1981a, 1981b). Reviews of the vasculature of the periodontal ligament and its environs have been written by Saunders and Röckert (1967), and more recently by Edwall (1982).

The blood supply of the mandible is derived from the inferior alveolar artery, which is a branch of the maxillary artery. Castelli (1963) studied the arterial system in the

mandibles of adult human cadavers by injecting Teichmann's paste solution into both common carotid arteries. The alveolar dental branches of the inferior alveolar artery supplied the periodontal ligament, alveolar bone and dental pulp of each tooth.

The inferior alveolar artery terminates in the region of the second premolar by dividing into the mental and incisive branches. The latter supplies the alveoli, pulp, periodontal ligament and gingivae of the canine and incisor teeth. The mandible also receives its circulation from the sublingual branch of the lingual artery, the buccal and masseteric arteries.

Castelli and Dempster (1965) described marked similarities in the origin of the periodontal vasculature in Macaca rhesus and man, except for the maxillary and mandibular anterior teeth. They reported that the arterial supply in the incisor region of the monkey was derived solely from the sublingual artery, through a terminal branch in the symphyseal region. However, Kindlova (1965) reported that the periodontal ligaments of the mandibular anterior teeth in the macaque monkey were supplied by branches of both the inferior alveolar artery and the lingual artery.

Hellem and Östrup (1981a, 1981b) studied the normal and retrograde blood supply to the body of the mandible of dogs using selective coloured arterial perfusates. Under normal conditions, the inferior alveolar artery was found to be the sole source of arterial supply to the mandible body, subperiosteal vessels, attached gingiva and a portion of the buccal mucosa. The interradicular alveolar bone was supplied by branches of the

inferior alveolar artery, which then anastomosed within the periodontal and gingival tissues. In the alveolar process, the medullary and periosteal territories were found to anastomose and appeared frequently as a periodontal periosteal communication. Thirty days after bilateral vascular blocking of the inferior alveolar artery, the cortical and medullary perfusion was regarded as normal, due to the collateral blood supply from the facial artery via its sublingual branch.

One of the problems encountered during Hellem and Östrup's investigation was the difficulty experienced in differentiating between arterial and venous perfusion. Microfil and Colorpaque did not perfuse the entire vascular tree. Sectioning the mandible prior to clearing may have contributed to the difficulty of tracing the vessels from the arterial to the venous side.

Cohen (1959b) described the venous drainage of the human mandible, by radiographing the distribution of injected Micropaque into the cancellous bone of the mandibles of human cadavers. The author observed that the inferior dental veins drained into the anterior facial and maxillary veins. Following this, venous drainage continued upwards to the pterygoid plexus via the inferior dental veins, and downwards to the facial and external jugular veins. The main venous drainage was attributed to the large surface area of the veins of the periosteum, which passed to the facial veins.

Castelli (1963), and Castelli and Dempster (1965) detailed the venous drainage of the periodontium in both monkey and man.

They described two directions of drainage, i.e. toward the dental apex, and to networks within the bone marrow. Periodontal veins were found to increase in diameter as they approached the apex of the tooth. The veins of the periodontal ligament and the alveolar bone joined one another, and then drained into the veins in the interalveolar and interradicular septi. Castelli (1963) observed that the veins had a separate course and did not follow the arteries. Castelli and Dempster (1965) reported the presence of a vascular reservoir belonging to both the periodontium and the marrow space. It was located on each side of the cribriform socket wall, with blood vessels (veins and arterioles) transmitting blood and tending to equalize pressure.

3.2 Microscopic Vascular Anatomy of the Periodontal Ligament

3.2.1 Techniques

A variety of investigational techniques has been employed in an attempt to visualize and understand the purpose of the vascular system of the periodontal ligament. A brief resume of methods used to render credence to this abstruse information follows.

A. Use of Histological Sections

Kindlova and Matena (1959, 1962), Kindlova (1965, 1967), Macapanpan, Weinmann and Brodie (1954), Carranza, Itoiz, Cabrini and Dotto (1966), and Cohn (1972) have used this method. This technique's inadequacy lies in its two-dimensional representation.

However, using stereometric analyses, it is possible to derive a three-dimensional model.

B. Use of Corrosive Specimens

Such techniques have been employed by Kindlova (1965, 1967, 1968, 1970), Kindlova and Matena (1959, 1962), Kindlova and Trnkova (1972), Lenz (1968), Gannon (1981), Wong (1983) and Weekes (1983). The two major problems are firstly of distortion and dimensional change and, secondly, of not being able to represent the vessels in relation to other surrounding tissues.

C. Use of Perfusion Techniques

Investigators using this method include the following: Bevilacqua (1958), Bernick (1960, 1962), Castelli and Dempster (1965), Boyer and Neptune (1962), Egelberg (1966), Cutright and Bhaskar (1967), Turner, Ruben, Frankl, Sheff and Silbertstein (1969), Cutright (1970), Garfunkel and Sciaky (1971), Hellem and Östrup (1981a, 1981b), Weekes (1983), Wong (1983), Maher (1984) and Shore, Berkovitz and Moxham (1984). Reinhold, Hopewell and Van Rijsoort (1983) published a recent paper on a revised Spalteholz method of perfusing blood vessels. However, this method does not provide the minute details obtained with latex application.

D. Use of Microspheres

This method has been used by Folke and Stallard (1967), Kennedy and Zander (1969) and Moore, Gewertz, Wheeler and Fry (1981).

E. Use of Micro-angiographic Techniques

Investigators using these methods include Cohen (1959a, 1959b, 1960), Castelli and Dempster (1965), Cernavskis and Hunter (1965) and Koivumaa and Lassila (1971). The problems encountered include not being able to follow the entire course of the vasculature, and the difficulty in rendering images so that the vessels do not appear superimposed.

F. Use of Impression Techniques

In the study by Birn (1966), it was assumed that each perforation in the alveolar wall was indicative of the presence of a blood vessel. This technique, and the assumptions drawn from it, have not been substantiated by Kishi and Takahashi (1977) and Barker (1980).

G. Plethysmography

Packman, Shoher and Stein (1977) have used this method. Unfortunately, light absorption also depends on other factors and, therefore, the technique is not exclusive in its interpretation.

H. Vital Microscopy

Forsslund (1959), using this technique, could only observe superficial blood vessels due to the limited depth of field. It has since been used by Hansson, Lindhe and Branemark (1968), Hock and Nuki (1971), and Gaengler and Merte (1983).

3.2.2 Classification of Vessels

Bennett, Luft and Hampton (1958) proposed a simple classification of vertebrate blood capillaries based on the presence or absence of a variety of structural features. The morphological criteria were the existence or absence of a continuous basement membrane, the degree of pericapillary cellular investment and the nature of the endothelial cell. This classification should be used with caution, as marked structural alterations can occur within the same capillary, depending on its physical environment.

Forsslund (1959) morphologically classified the capillaries of the human gingiva into four categories consisting of arteriolo-venular bridges, true or non-muscular capillaries, arterio-venous anastomoses and sinusoids.

Rhodin (1967) studied the microvasculature of the fascia of rabbit medial thigh muscles, in vivo, using light microscopy and electron microscopy following fixation. He subdivided arterioles according to the number of smooth muscle layers of the media and proposed a classification of arterioles, terminal arterioles and precapillary sphincters based on vessel diameter, the presence of smooth muscle and other characteristics of wall structure.

Subsequently, Rhodin (1968) described the venous drainage system of the subdermal (fascial) microvessels of rabbit skin using identical materials and methods as in 1967. The classification of the segments of the venular drainage system was

based on the general preponderance and combination of structural characteristics. The criteria used included the internal luminal diameters, the number of cell layers surrounding the endothelial tube and the ultrastructure of these cell layers. The various segments of the venous microvascular bed were described as venous capillaries, postcapillary venules, collecting venules, muscular venules and small collecting veins.

Wolff (1977) chose to describe the ultrastructure of only terminal arterioles, capillaries and venules because of the poor definition that existed between the segments of the arterial and venous microcirculatory beds. This lack of definition was attributed to the variation that was evident from one organ to another, the differences in interpretation and classification between authors, and that transitions along the terminal vascular bed occurred gradually, without the presence of definitive boundaries.

Wiedeman (1962 and 1963) measured the lengths, diameters and numbers of arterial and postcapillary vessels in the wings of unanaesthetized bats using an eyepiece micrometer. She observed the existence of a linear relationship in the calculated total cross-sectional areas formed by the vessels measured. This finding differed from the long accepted concept of small increases in total area, from arterial vessels to arterioles, followed by major increases in area in the capillary network. The studies, showing non-linear increases in cross-sectional areas of vessels, recorded measurements of vessels in fixed preparations. A

disagreement regarding the diameter of capillaries may have contributed to this discrepancy. In living animals, the capillary diameter was measured at 4 microns, whereas, previous literature estimated it at 8 microns. The distortion in the size of blood vessels subjected to histologic preparations, and the difficulty experienced in classifying vessels in the absence of blood flow are two other factors adding to the dilemma.

The greatest representation in the vascular bed examined was the venules. The venous vessels contained 80% of the total blood in the peripheral vascular bed. Wiedeman was of the opinion that vessels should be classified and named according to their position and function, and not according to their diameter.

Gilchrist (1978) experienced difficulty in attempting to classify human periodontal blood vessels according to Bennett, Luft and Hampton (1958), and Rhodin (1967, 1968). This was a result of insufficient material. He postulated that the vessels were derived from the venular microcirculatory system. Gilchrist found no evidence of smooth muscle cells in the restricted sample of vessels examined. However, Avery, Corpron, Lee and Morawa (1975) studied the blood vessels in the mouse periodontium and observed vessels with smooth muscle, some precapillary vessels with an incomplete muscular coating, and even occasional myoepithelial junctions between endothelial and smooth muscle cells.

3.2.3 Distribution of Vessels

According to Gottlieb and Bernhard in 1926 (cited in Orban, 1929), the teeth were considered to be in constant physiologic movement mesially and, as a result, the distal fibres of the periodontal ligament were stretched and the fibres on the mesial aspect of teeth were relaxed. These authors inferred that the blood vessels on the distal would have elliptical shapes, and those on the mesial would be round in shape.

Orban (1929) observed that blood vessels traversed from bone into the periodontal ligament and also in the opposite direction. He found that both blood vessels and nerves could be traced from the apex of the alveolus to the entrance and were not nodular or rounded as stated by Box in 1924 (cited in Orban, 1929).

Hayashi in 1932 (cited in Saunders and Röckert, 1967) provided the first definitive study on periodontal blood supply. Human jaws were injected with carmine gelatin and then examined microscopically following serial sectioning. The dental arteries at the base of each alveolus branched into inter-alveolar rami, which then ran coronally to surround the alveolus on both the labial and lingual aspects. Each dental artery, on entering the periodontal ligament, gave off further side branches which ascended ray-like within the alveolus to surround the tooth root. The inter-alveolar rami gave off side branches which entered the periodontal ligament by perforating the side wall of the alveolus. These vessels coursed coronally, anastomosing with one another and

also with those periodontal branches which arose directly from the dental artery to form longitudinal periodontal arteries. Hayashi described the alveolar bone and periodontal ligament of the maxilla to be richer in its blood supply than the mandible. He reported that in both jaws, the lingual aspect of the alveolar bone was more vascular. In the mandible and maxilla, the premolar and molar teeth possessed more periodontal vessels on the lingual, than the labial side. However, the reverse was true of anterior teeth.

Bevilacqua (1958) used white rats injected with India ink in order to study the vascularization of teeth and their supporting structures. He discovered branches from the alveolar arteries, ascending branches from the apical region and descending branches from the gingiva, all of which ended in pericementum. The vessels followed a tortuous course and formed a basket-like network around the tooth roots. It was proposed that the mechanical or hydraulic network made possible the functional movement of teeth.

Cohen (1960) investigated the vascular architecture of cat mandibles by injecting carmine gelatin via the common carotid artery and then examining histological sections. He found that the blood supply of the periodontal ligament was derived from the apical region, the alveolar bone and the gingival tissue.

Bernick (1960) studied the vascular supply to the developing teeth of rats using the India ink - gelatin perfusion method. He described the periodontal vessels as being parallel to the bony surface of the periodontal ligament, thus confirming their

longitudinal orientation (i.e. occluso-apical). Additional evidence supported the presence of perforating blood vessels entering from openings in the alveolar bone and anastomosing with the periodontal vessels passing gingivally. The periodontal vessels lay almost exclusively on the alveolar half of the periodontal ligament, with the cemental side being devoid of blood vessels. With age, the periodontal ligament was found to narrow, thus shifting the blood vessels nearer to the cementum.

Boyer and Neptune (1962) found a basic similarity between the patterns of blood supply to the teeth of the rat, rabbit, opossum and hamster. The principal source of vascularity came from either periosteal vessels, medullary vessels, vessels supplying the adjacent vasculature, or the intrinsic arteries.

Kindlova and Matena (1962) injected the right common carotid artery of rats with latex and then examined the mandibular corrosive specimens. The afferent arteries entered the periodontal ligament of mandibular molars by perforating the alveolar bone at the apex and through the lateral socket walls at various levels. In the periodontal ligament, they ran axially to the cervical region of the tooth and were interconnected by a fine capillary network, which also supplied the periodontal tissues above the interradicular septa. From the alveolar crest, the vessels were linked and passed axially in arcades and, thus, a horizontal vascular circulus was formed. Free coiling capillary loops branched off from the horizontal circulus, to encircle the whole circumference of the tooth. The marginal periodontium was found to have two separate

vascular networks, one of which supplied the gingiva and the other serviced the periodontal ligament. There were, however, communications between gingival capillaries and periodontal veins.

Venous drainage commenced at the alveolar crest and collected blood from the periodontal loops and the gingival plexus as illustrated in Figure 1. The veins then either entered bone or continued in the periodontal ligament, running axially toward the apex where their calibre increased. Those veins in the periodontal ligament formed a basket-like structure, which encircled the apex, before leaving either by perforating the alveolus below the apex, or the apical part of the interradicular septum. Venous vessels that penetrated the peak of the interradicular septum to join the venous plexus found here, then drained into large veins which either left directly, or joined the veins from the apex of the socket.

Zaki and Van Huysen (1963) examined the early histologic changes in the periodontium of the molar teeth of rats, following the application of force. The periodontal blood vessels on the tension side of the ligament were found to be of large size and situated in the centre of the periodontal space. Those vessels on the pressure side of the tooth movement were, in comparison, smaller in size and located closer to the alveolar bone surface undergoing resorption.

Kindlova (1965) investigated the blood supply of the monkey marginal periodontium using corrosive specimens and histological sections. The vessels of the periodontal ligament

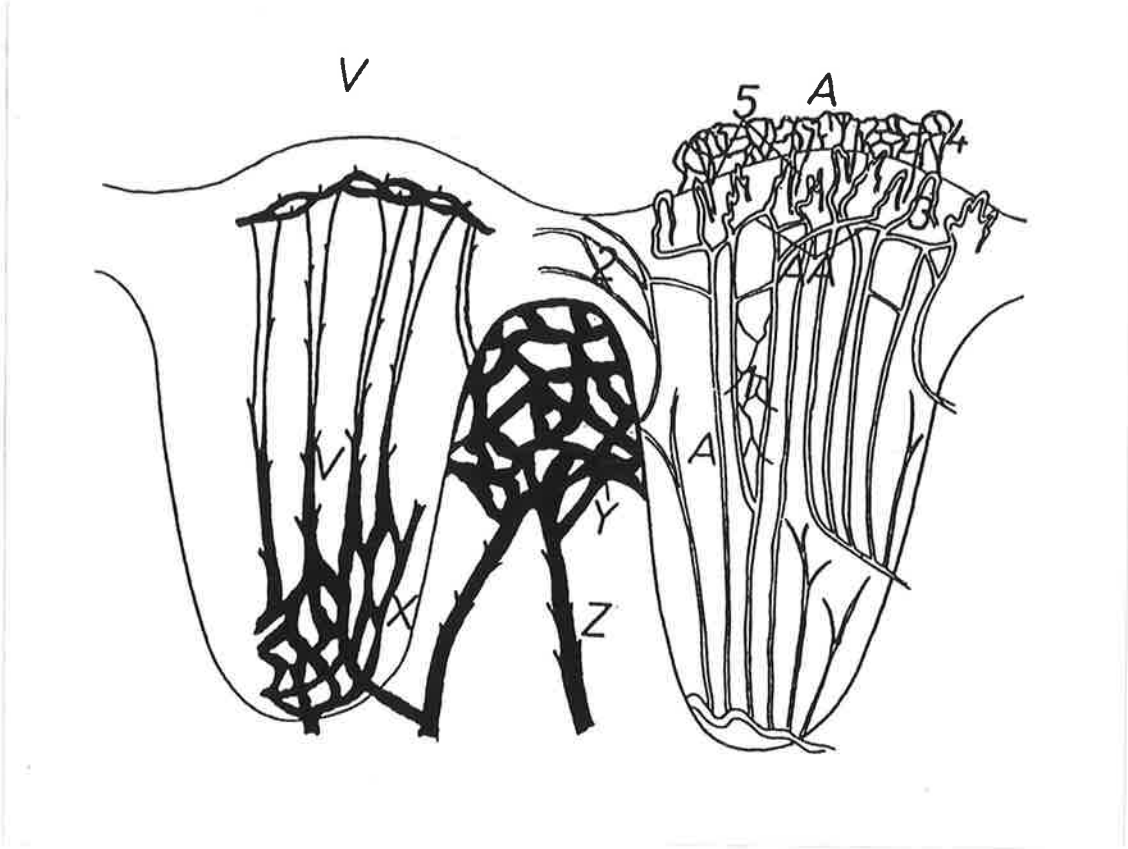


Figure 1. Details of the arterial and venous arrangement of the rat molar periodontium. (from Kindlova and Matena, 1962)

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 = gingival and periodontal capillary communications

were situated adjacent to the bony socket wall, and even running in grooves in the alveolar bone. These main vessels, which ran parallel to the long axis of the tooth, gave off branches towards the tooth, thus forming a flat network of capillaries. There were fewer afferent arteries in the interradicular area of multi-rooted teeth, and the single capillaries which branched from them anastomosed with a venous-like plexus. Kindlova reported incomplete casting between the periodontal veins and the plexus of vessels.

In the marginal part of the periodontal ligament, the capillary network formed a narrow band from which single capillaries ran a coiled course, and then returned to their site of origin, thus forming structures resembling glomeruli. Looped capillaries with coiled arterial parts which encircled venous limbs were found in the region of the epithelial attachment. These venous limbs provided drainage. The main vessels of the periodontal ligament, and those supplying the gingiva, anastomosed in this region. Two or three rows of straight slender loops with equal lengths of arterial and venous limbs extended from the marginal periodontal ligament to the crest of the free gingiva, where they anastomosed with the capillary loops supplying the epithelium of the tissues facing the oral cavity.

Birn (1966) noted a gradual increase in periodontal vascularity further posteriorly in the arch, being least for the lateral incisor and greatest for the second molar. In single-rooted teeth, the blood supply was least in the middle third, greatest in the gingival third and in between these two extremes

in the apical third of the ligament. In multi-rooted teeth, he found the blood supply to be equal in the middle and apical thirds. There were minor differences in magnitude of blood supply between the four tooth surfaces, but these were not found to be significant. For mandibular molars, the blood supply to the periodontal ligament of the distal root was less than that for the mesial root. The majority of blood vessels supplying periodontal ligaments were small in size (less than 150 microns). The larger blood vessels (i.e. perforations greater than 150 microns) were found mostly in the gingival and apical thirds of the alveolus, and they were reported to increase in number from the incisors towards the molars.

Carranza, Itoiz, Cabrini and Dotto (1966) used Wachstein and Menzel's histochemical technique for the demonstration of adenosine-triphosphatase activity to identify and investigate the periodontal vasculature of the rat, mouse, hamster, guinea pig, cat and dog. The periodontal blood vessels ran longitudinally and closer to the alveolar bone than to the cementum. This observation confirmed the findings of Boyer and Neptune (1962), and Kindlova and Matena (1962). However, in areas of active enamel formation (i.e. guinea pig molar), there was a second plexus of blood vessels close to the enamel, running obliquely to the long axis of the tooth and being less dense than the plexus of blood vessels adjacent to the alveolar bone. Vascular connections between the gingival and periodontal ligament vessels were rare. This finding was in contrast to that of Cohen (1960).

Egelberg (1966) perfused dogs with a carbon-gelatin mixture to study the arrangement of blood vessels at the dento-gingival junction. He proposed that the vessels of the crevicular plexus were venular in type, mainly because their diameter was greater than 7 microns.

Folke and Stallard (1967) injected plastic microspheres (15 ± 5 microns) into the right external carotid artery of monkeys to investigate the vascular pattern of the periodontal ligament. Blood vessels from the cribriform plate were seen to enter the periodontal ligament in a perpendicular direction to the long axis of the tooth. Within the periodontal ligament the vessels were reported to form a polyhedric pattern located closer to the bone than the cementum. This study would not verify the findings of Kindlova (1965) and Carranza et al. (1966), that the major periodontal vessels ran parallel to the long axis of the tooth.

Kindlova (1967) used both the corrosive latex cast method and histological sections to compare the vascular supply of the periodontal tissues in rat molars with, and without, periodontitis. A normal healthy periodontal ligament contained vessels, which coursed axially, were interconnected and arranged in palisades. The capillary networks supplied three distinct zones of the periodontium: the periodontal ligament, the epithelial attachment and the gingiva with its epithelium facing the oral cavity. In those animals with periodontitis, the capillaries which had previously exhibited glomerulus-like formations, became more dense, dilated and had varicosities.

Cutright and Bhaskar (1967) demonstrated the vascularity of the monkey periodontal ligament by using a silicone rubber perfusion technique. The periodontal blood supply of posterior teeth originated from the inferior alveolar artery via several channels. Intra-alveolar and apical arteries supplied the apical portion of the periodontal plexus. The middle part of the periodontal ligament was supplied by the intra-alveolar arteries and the cervical portion was derived from the vascular plexus of the gingiva, and the intra-alveolar arteries. Free anastomoses occur between the gingival and intra-alveolar arteries.

Saunders (1967) studied the periodontal and dental pulp vessels in monkeys and man by using micro-angiographic techniques. He showed that the blood supply of the unerupted and erupted tooth was shared. A continuity between periodontal and gingival vessels was revealed with unerupted monkey teeth. This finding contrasts with that of Kindlova (1965).

Kindlova (1968) examined the origin of the vessels of the capillary net to be found in the region of the epithelial attachment, and in the oral aspect of the gingiva. The development of these vessels was studied in rat molars using histologic specimens in addition to the corrosive latex cast technique. The vasculature of the periodontal ligament spread apically with root development, and the arrangement of the main vessels and capillaries was completed prior to tooth eruption.

The vessels supplying the cervical part of the enamel organ gave rise to the vascular network to be found in the region of the epithelial attachment. It was concluded that the coiled capillaries of the epithelial attachment region did not develop from either the vessels of the periodontal ligament, or from the gingival vessels, but were derived independently, to erupt with the tooth as vessels of the enamel organ.

Lenz (1968) investigated the periodontal vasculature of monkeys by injecting Plastoid into the arterial and venous systems and then macerating the jaw. He did not provide any new data.

Hofmann (1968) electronically recorded periodontal pressure pulsations and concluded that the results of his experiments confirmed the belief that an uneven distribution of periodontal blood vessels existed.

Ando (1969) reported three types of periodontal vessels originating from pulp vessels at the root apex, vessels that perforated through alveolar bone and those branching from the gingiva. The larger thicker vessels were positioned closer to the alveolar wall, whereas the smaller slender vessels were found nearer to the cementum. In old adults, this division into two layers became obscure, and only a single layer of vessels was noted. There was an increase in the number of vessels that perforated alveolar bone before entering the periodontal ligament as one moved from the anterior single-rooted teeth to the posterior multiple-rooted teeth. Bundles of vessels were found at the apex

of the tooth socket and on the mesial and distal aspects of the cervical region of the periodontal ligament.

Turner, Ruben, Frankl, Sheff and Silberstein (1969) showed that the blood supply to the periodontal ligament was derived from the apical vessels, the gingiva and from the surrounding bone on the buccal, lingual and inter-dental septa regions.

Cutright (1970) described the morphogenesis of the vascular supply to the permanent teeth of monkeys. The vessels of the developing permanent tooth and those of the periodontal ligament of the deciduous tooth were directly connected. Neighbouring permanent teeth were also found to have vascular connections via their periodontal ligaments.

Kindlova (1970) again reported on the development of the vascular bed of the marginal periodontium of rat mandibular molars. Histological sections and corrosive latex casts were employed to study the vasculature of this region. She attributes the derivation of the periodontal vasculature of rat molars to the vasculature of the alveolar mucosa, the vascular network of the enamel organ and from vessels which form the bed of the periodontal ligament. The circular vessel encircling the cervical region of the enamel organ described by Kindlova in 1968, is assumed to be venous in her 1970 publication. The basic order of events described by Kindlova in this 1970 publication remains unaltered from her 1968 article.

An abstract of a paper by Soloviev (1970) described the vascular network of the periodontal ligament of dogs as being better developed in the mesial and distal regions. The majority of vessels that entered the periodontal ligament, did so from the inter-alveolar septa. Arteries exhibited a large number of anastomoses, and vascular glomeruli consisting of networks of capillaries and epithelial cells were found in the periodontal ligament. The precis did not outline the method used for this investigation.

Garfunkel and Sciaky (1971) reported that, in rat molars, a hammock-like network of blood vessels occupied the periodontal space, with the major vessels running parallel to the long axis of the tooth. These major vessels were joined perpendicularly by finer vessels. The blood vessels of the inter-dental papilla originated within the periodontal ligament and proceeded to connect the buccal and lingual sides. Two parallel, interconnected, periodontal blood vessel networks were discovered, one being close to the alveolar wall and the other close to the cementum surface. Vascular interconnections, through alveolar bone, were noted between the roots of a single tooth, the roots of two adjacent teeth, and also, between the roots of the molars and the incisors. The periodontal network was described as having close links with the blood vessels of the pulp, the alveolar bone, the marrow spaces and the supraperiosteal capillary network of the gingiva.

Kindlova and Trnkova (1972) studied the vascular bed beneath the crevicular and attachment epithelium of the adult,

mongrel dog using latex corrosive casts and histologic specimens. The degree of variability between the vessels located in these areas was related to the degree of inflammatory cell infiltrate found in these regions. The capillary arrangements associated with a small degree of infiltration were the looped and flat capillary networks. This arrangement was described by Egelberg in 1966 as being typical of gingiva without infiltration, however, variations were common. With intermediate degrees of infiltration, some cases had a vertical arrangement of capillaries in the apical segment, while others had oblique capillaries with few anastomoses. In areas with a high degree of inflammatory infiltrate, there appeared a network of coiled capillary loops beneath the entire crevicular epithelium. This variability in the arrangement of capillaries was also observed in the vasculature beneath the oral part of the gingival epithelium, where no inflammatory infiltration existed. Possible explanations proposed were that, the vascular variability may have been a feature of dog periodontium, or that the observed changes were a result of the irritations that the tissue had undergone.

The vascular supply to the marmoset monkey periodontal ligament was described by Levy, Dreizen and Bernick (1972b) as being provided by the dental branches of the alveolar arteries via the periapical area. Mesial, distal, buccal and lingual root surfaces were traversed by apical branches, which passed gingivally, and anastomosed with perforating branches from the inter-alveolar arteries. The blood vessels were reported as

travelling closer to the alveolar bone half of the ligament, even though arterioles and capillaries branched off towards the cementum. The branches arising above the alveolar crest, from the periodontal ligament, palate and alveolar mucosa, supplied the gingival area. It was also reported that the terminal branches of the trans-septal network of blood vessels supplied the interproximal gingiva.

Söderholm and Egelberg (1973) used photographs of the buccal gingiva of dogs taken before and after the development of gingivitis, to study the vascular changes in cases of established gingivitis. The vascular morphology was found to alter in two characteristic ways; widening of the vessels and alteration in the course of vessels. Wider vessels appeared to be located in the venular segments of the vascular bed, and not in the arterial distribution. The alteration in the course of vessels that connected the afferent and efferent aspects of the terminal vascular systems occurred after widening of the vessels. New vascular loops were formed on the efferent side with increasing time. By sixteen days, all vascular systems had an altered architecture. These authors expressed the view that the loop formations seen in this study may have been present in the gingival areas with chronic gingivitis, and that the twelve weeks of tooth cleansing prior to the commencement of the experiment may not have converted the loop formations into vascular networks.

Corpron, Avery, Morawa and Lee (1976) reported on the ultrastructure and distribution of terminal vessels in the mouse

molar periodontium using electron microscopy. They attributed the function of this microcirculatory bed to the exchange of nutrients and gases.

Kishi and Takahashi (1977) used a scanning electron microscope to investigate the methyl methacrylate vascular casts of the periodontal vasculature of mongrel dogs. In order to differentiate between the vessels of the periodontal ligament and those of the alveolar bone, the soft tissues were digested away and the alveolus left intact. The arterial branches of the periodontal ligament originated in the infraorbital artery for the maxillary teeth, and the inferior alveolar artery for mandibular teeth. The periodontal vasculature was classified into four groups according to their derivation: i.e from the vessels supplying the pulp; via openings in the alveolar wall; from the periosteal arterioles of the crevicular epithelium; and from the periosteal arterioles of the alveolar crest.

Both superior and inferior vascular networks were contained in the apical quarter of the periodontal ligament. The superior layer of vessels was found closer to the root of the tooth and ran parallel to the long axis of the tooth. Numerous anterio-venous arterio-arterio and venous-venous anastomoses were observed. The arteries of the inferior vascular layer emerged from oval openings in the alveolar wall at intervals of 200 to 400 microns. Both arterial capillaries and venules were observed to traverse the alveolar pores. The middle part of the periodontal ligament contained the two layers of vessels as described for the apical

quarter. In addition, as one approached the coronal end of the alveolus, bundles of thick vessels were observed to traverse circularly.

In the most coronal part of the periodontal ligament, the two vascular networks were still present, but the superior layer gave off hairpin-shaped vascular loops of 80 microns in length. The inferior layer contained circularly or longitudinally oriented bundles of thick vessels, each of which contained a thick venule and one or two arterioles, and ran obliquely upwards along the alveolar wall. Anastomoses occurred between the circularly oriented vessels of the inferior layer, and the superior network of vessels. The vascular bundles of the inferior layer emitted branches, which coursed parallel to the long axis of the tooth, to join the vascular network of the superior layer.

The superior and inferior layers fused together immediately above the crest of the alveolar ridge, to form a vascular plexus different from those found in other areas. The vessels in this plexus originated infrequently from the vessels in the subepithelial plexus of the internal margin of the gingiva and, in the main, from periosteal arteries. Numerous hairpin-shaped loops arose from the large networks of principal vessels, to project upwards, and surround the tooth root.

Kishi and Takahaski's description of the microvasculature of the dog periodontal ligament agreed with that of the rat periodontal vasculature carried out by Kindlova and Matena in 1962, particularly in regard to the presence of capillary loops in

the region of the gingival attachment. Birn's 1966 assumption that all perforations of the alveolar wall contained blood vessels, was only partially substantiated by this research because, although the majority of alveolar pores contained vessels, some smaller openings did not. The study by Ando (1969) described two layers of vessels in the periodontal ligament as reported by Kishi and Takahashi.

Nakamura, Kiyomura, Nakamura and Hanai (1983) perfused rats to determine the structure of periodontal blood vessels of molars, using light and scanning electron microscopy. The vessels ran parallel to the long axis of the tooth with a basket-shaped arrangement apically. An abundance of blood vessels was described with a preponderance closer to alveolar bone.

Wong (1983) examined the periodontal vascular morphology of the mouse molar using pre-polymerised methyl methacrylate and a corrosion cast technique. The vessels in the molar gingival region were composed of an outer and inner circular vessel system. Loop-like capillaries, which joined together the outer and inner circular vessel systems, were arranged radially at the level of the neck of the tooth.

Periodontal ligament vessels were seen to connect with the inner single circular vessel from its apical side. Glomerular-like structures originating from the molar base of the crevicular region and extending into the gingival epithelium, formed part of the inner circular vessel system. The vessels of the periodontal ligament were larger on the mesial and distal sides than the buccal and lingual sides of the socket. They ran parallel to the

long axis of the tooth, with lateral vascular branches linking periodontal ligament vessels to each other, and to the medullary vessels. A large venous plexus was found around the root apex and in the interradicular regions. Wong concluded that the periodontal ligament vasculature was a predominantly venous structure.

In the cervical third of the molar root socket, the internal diameter of the periodontal vessels was reported to be 20 microns. The vessels ran occluso-apically and were found lying in indentations on the socket wall. Polygonal anastomoses accompanied by an intertwining capillary network were a common feature.

In the middle third of the molar root socket, the periodontal vessels had similar connections and orientations as has been described for the cervical third. However, arterial vessels were found running parallel to, and in close approximation with, the venous vessels. Capillary-like loops connected the arterial and venous vessels. A large elongated venous reservoir was located in the interradicular region. Branches from the buccal and lingual gingival vessels, and from the periodontal ligament vessels anastomosed with the huge venous vessel. Arterial vessels were occasionally observed to join directly with this venous reservoir.

In the apical third of the molar root socket, a hammock-like network was demonstrated around the root apex. The pulp vessels projected occlusally from this apical network. The type

of periodontal vascular anastomoses found in the apical third were similar to those found in the cervical third, except that the venous vessels were larger (internal diameter averaging 25 microns) and the spaces inside the network were smaller.

Weekes (1983) investigated the vasculature of the rat molar periodontium and revealed a predominantly occluso-apical orientation within the periodontal ligament. The majority of vessels were postcapillary venules with arterioles supplying the vessels of the ligament, but not travelling within the ligament for any distance. A closer relation of blood vessels to alveolar bone than tooth root was described, with a greater density over the interdental septum.

It was suggested that due to the complexity of the periodontal ligament vascular morphology, the varied vascular arrangements depending on location, and the high proportion of blood vessels (approaching those found by Sims, 1980), the role of the periodontal ligament could encompass both nutrition and resistance to occlusal loads. These findings were summarized in an abstract by Weekes and Sims (1983).

3.2.4 Vascular Volume

Götze (1965) examined histological sections of human lower second premolars with surrounding bone intact, in order to determine the volume of vascular and fibrous tissues comprising the periodontal ligament. The eight cases aged from 60 to 82 years had a smaller proportional periodontal vascular volume, a

reduced percentage volume of fibre bundle sections and larger interfascicular spaces than the eight cases aged from 26 to 58 years. He reported a figure of 1 to 2 percent as being representative of the vascular volume in the human periodontal ligament.

Parfitt (1967) used both clinical measurements and animal experiments in order to investigate and identify the structures responsible for tooth support. Both intermittent and maintained loads were applied to the long axis of the tooth, and the resultant movement of the tooth, as well as the applied force, were measured by means of transducers. The three types of movements that were recognized included an immediate displacement and recoil, an elastic after-effect following both displacement and recoil, and a slow continuous intrusion into the socket with maintained force. When a load of 200 gm was applied, the tooth was displaced 0.01 mm. Parfitt considered that the elastic reaction which occurred as soon as the load was applied to the tooth, and the slow displacement seen with a maintained load, were two discrete systems acting in series. The elastic system, he attributed to collagen and the slow intrusion was associated with the hydrodynamic action of tissue fluid. The volume of blood in the periodontal tissues was reported to be 2 percent of the total volume of the ligament space. The author proposed that an increased blood pressure could result in dilation of the vessels, which would cause extrusion of the tooth in its socket by increasing the volume of the periodontal space.

Wills, Picton and Davies (1976) investigated the role of fluid systems within the monkey periodontium by injecting angiotension, noradrenaline and physiological saline, and by allowing some animals to die by exsanguination. Angiotension was injected into a leg vein causing vasoconstriction, an increased arterial blood pressure, and reduced displacement of the tooth with applied load. Noradrenaline was injected submucosally, resulting in a transitory rise in blood pressure and reduced tooth displacement with applied load. Physiological saline was injected submucosally over the root of the test tooth and resulted in an increased movement of the tooth. These authors attributed the latter movement to an increased extracellular fluid volume and the possible extrusion of the tooth. There was reduced tooth movement and actual tooth intrusion with exsanguination mainly as a direct result of the loss of blood and extracellular fluid. When the animal's thorax was compressed manually, the movement of the tooth increased, and this was presumed to be due to the blood being pumped back into the periodontal ligament.

Wills, Picton and Davies inferred that 30 percent of the displacement was due to changes in blood volume, and that 10 percent could be attributed to changes in the extracellular fluid volume. They considered the blood to have an energy dissipating function when smaller loads were applied.

Using a mathematical derivation, these authors found the volume of the ligament to be directly proportional to its thickness. Having assumed that the tooth could be represented

as a right circular cone whose semi angle was 14° 30', the ligament thickness of the macaque monkey was 100 to 200 microns, and that the vasoconstrictor action affected all blood vessels in the periodontal ligament, then a figure of 0.5 to 1 percent was derived to represent the volume of the ligament occupied by displaceable blood.

Götze (1976) reported on the vascular volume of the periodontal ligaments of human anterior and premolar teeth. In both anterior and posterior teeth, the labial/buccal and lingual regions exhibited a greater volume of vessels than did the mesial and distal area. The following table of figures has been adapted from his article:

	No. of teeth	Mesial Vol. %	Distal Vol. %	Labial/Buccal Vol. %	Lingual Vol. %
Upper anterior tooth	26	1.63	1.67	2.24	2.58
Lower anterior tooth	11	1.67	1.67	3.03	3.44
Upper Premolar	12	1.97	1.94	2.71	2.84
Lower Premolar	7	1.86	1.86	2.82	3.50

Table 1. Percentage vascular volumes. (from Götze, 1976)

When Götze compared the volume percentage of blood vessels in the coronal, middle and apical thirds of the periodontal ligaments of upper and lower anterior teeth and premolars, he found that there was an increase as one progressed from the coronal to the apical thirds, of all four surfaces of the teeth. The graphs detailing these data have been adapted from Götze's article and presented as Figures 2 and 3.

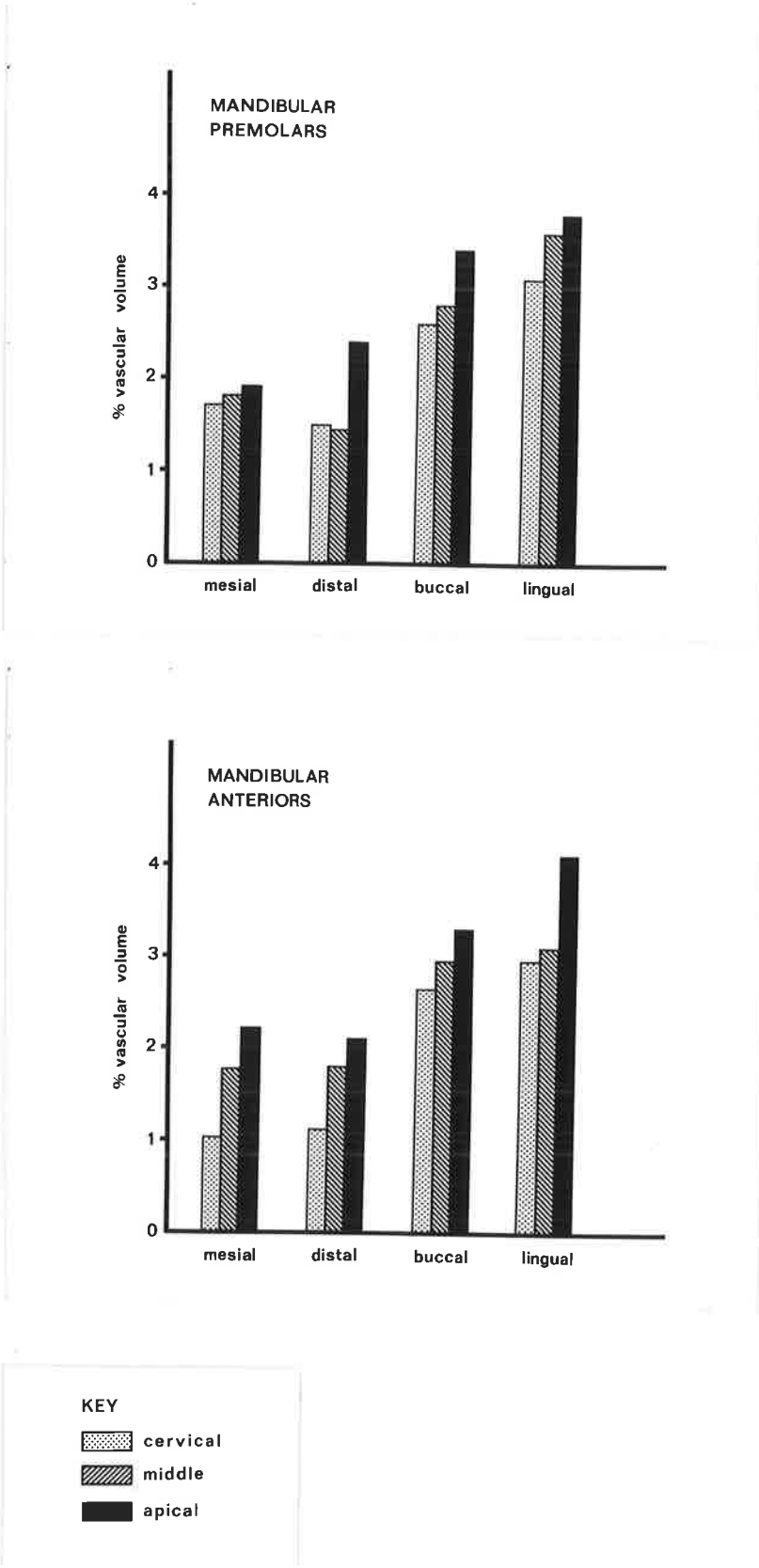


Figure 2. Graphical representation of the periodontal ligament percentage vascular volume for human mandibular anterior and premolar teeth.
[Adapted from Götze, 1976]

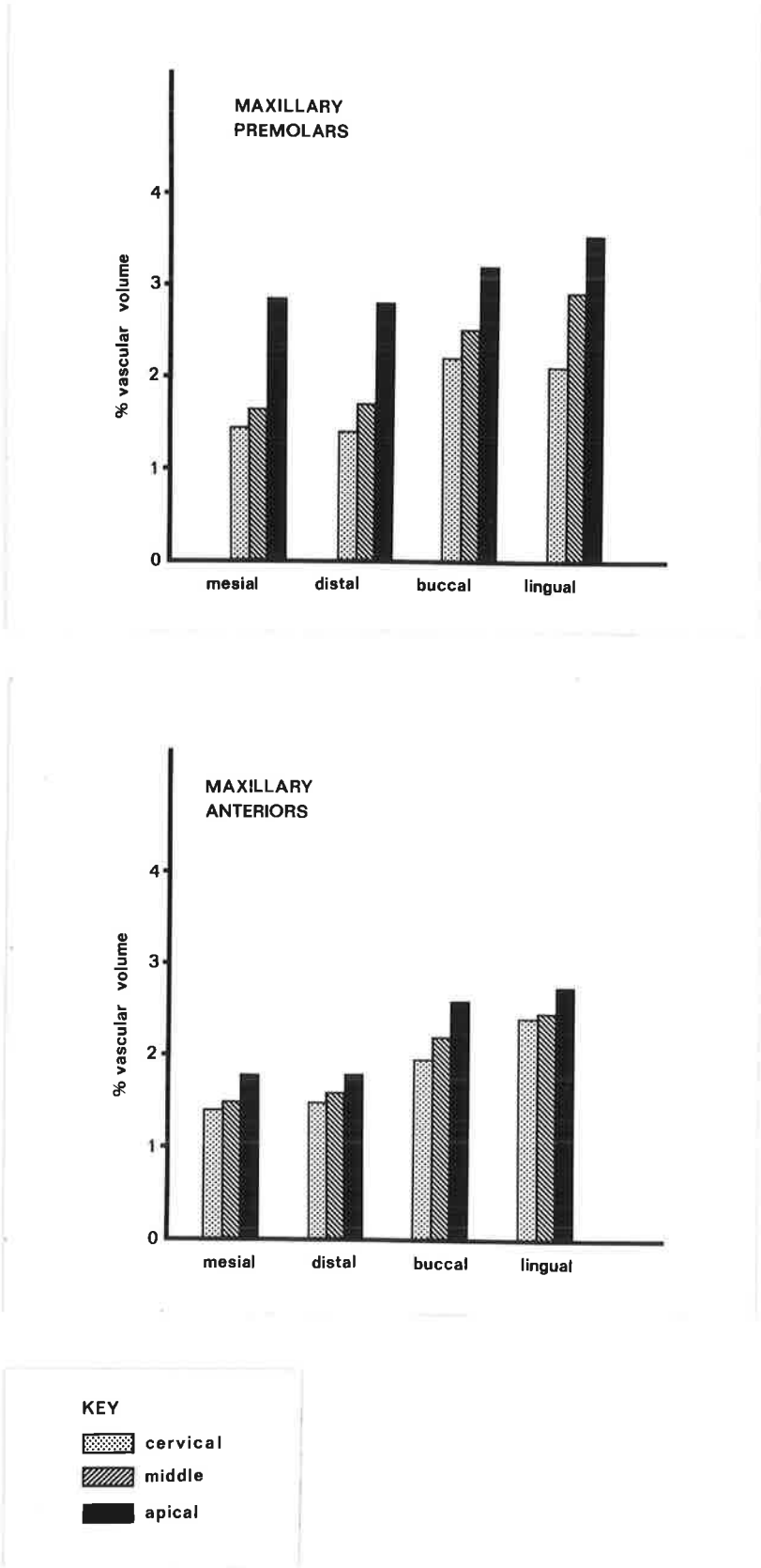


Figure 3. Graphical representation of the periodontal ligament percentage vascular volume for human maxillary anterior premolar teeth.
[Adapted from Götze, 1976]

Götze (1980) compared the volume percentage of fibre bundle sections and blood vessels on the lingual aspect of the periodontal ligaments of anterior teeth, to their percentage volume in the area of remaining ligament. A relationship was found to exist between the high percentage of fibre bundle sections, and the high proportional volume of vessels, on the lingual aspect of the periodontal ligament of anterior teeth.

Sims (1980) reported that mouse mandibular molars had a periodontal vascular proportion of 17 percent, and that human mandibular premolars had a micro-vascular cross-sectional area of 11 percent. The buccal aspect of maxillary premolars in adolescent male and female humans was found to have a periodontal vascular volume of 20 percent in some locations. These figures are in obvious contrast to the relatively conservative vascular volume percentages proposed by Götze.

3.2.5 Arteriosclerosis and the Vasculature of the Periodontal Ligament

Due to the fact that the marmosets used in this study had been exposed to three diets composed of polyunsaturated fats, saturated fats, or a colony diet, it is pertinent to this literature review to explore the area of arteriosclerosis, and its influence on the vessels of the periodontal ligament.

Arteriosclerosis has commonly been described as "hardening of the arteries" with a thickening and loss of elasticity of arterial walls. Robbins (1968) referred to three distinctive

morphological variants of this pathological process. These were atherosclerosis, Monckeberg's medial calcific sclerosis and arteriolosclerosis. Lobstein (1833) (cited in Sabine, 1977) coined the term arteriosclerosis and Marchand (1904) (cited in Sabine, 1977) introduced the word atherosclerosis to define a particular intimal involvement of the more generalised arteriosclerosis.

The pathogenesis of atherosclerosis is multifactorial. Various theories which are not necessarily mutually exclusive, have attempted to explain the aetiology of this disease state. These theories have been reviewed by Ross and Glomset (1976) and the major risk factors by Stamler (1978).

Atherosclerotic vascular changes within the periodontal ligament, alveolar bone and gingiva have been reported by Stahl and Fox (1953), Quintarelli (1957), Quart, Stahl and Sorrin (1960), Stahl, Witkin and Scopp (1962), and Grant and Bernick (1970) (cited in Severson, Moffett, Kokich and Selipsky (1978)).

The effect of nutrition, in general, on the periodontal ligament has been reviewed by Nizel (1981) and Ferguson (1982). The difficulty has been to differentiate between changes due to the biological mechanisms of aging, and those due to pathology, particularly where older specimens have been used for research. The strict adherence to specific diets has played an important role in the clarification of this distinction.

In order to ascertain the manner in which fatty acids influence the metabolism within the periodontium, Rao, Shourie and Schankwalkar (1965) fed three groups of rats three diets varying in fat content (i.e. high, adequate and no fat) for a period of seven weeks, and then examined the periodontium histologically. The "high fat" group with a 30 percent dietary fat content was the most affected, and exhibited an irregular fibre arrangement with an accompanying proliferative fibrosis within the periodontal ligament. The bone and cementum were characterized by fibrotic resorption areas, which were associated with dilation and haemorrhage of blood vessels. An inflammatory trend with highly cellular and extensively dilated vascular elements was displayed within the periodontal ligament of the "no fat" group. Resorption of bone and cementum associated with a dilated vascular element occurred concurrently. However, these resorption areas differed from the "high fat" group in that they were fewer, localized and uniform in character in the "no fat" group.

Rao, Shourie and Shankwalkar described degenerative changes with excessive fat intake and inflammatory signs with fat deficient states. It is of interest to note that the fat used in this study was safflower oil which is dominated by a 77 percent content of the polyunsaturated essential fatty acid, linoleic acid (Cole and Eastoe, 1977) which cannot by synthesized by animals. Linoleic acid derivatives are precursors of prostaglandins which have been implicated in the contraction of smooth muscle and dilation of certain vascular beds (El Attar, 1978). Increased levels of crevicular fluid prostaglandin E have been correlated

with periodontal disease severity (Offenbacher, Odle, Gray and Van Dyke, 1984). However, El Attar (1978) described a complex relationship between prostaglandins and the inflammatory response, attributing both anti-inflammatory and inflammatory effects depending on the relative ratios of the different prostaglandins present.

Kennedy and Zander (1969) investigated the effects of unilateral ischemia in the periodontal tissues of squirrel monkeys. The histological changes observed were directly related to the duration of ischemia, but were not associated with histopathologic alterations reported previously with periodontal disease. Hyperemia followed ischemia, with the epithelial attachment being the least affected, due to its collateral circulation with the vessels of the periodontal ligament.

Kennedy (1969) determined the vascular effects of experimental ischemia in monkeys using microspheres followed by India Ink perfusion. The methods of re-establishing the vascular supply included the opening of pre-existing vessels which bypassed the occluded ones, and by the formation of new vascular structures in the latter stages through endothelial proliferation.

Bernick, Levy and Patek (1969) reported arteriosclerotic changes of the periodontal and gingival arterial vessels of older marmosets, including intimal thickening and elastic hyperplasia. These authors recognized the significance of aging to the prevalence of periodontal disease, but did not list the ages of the animals, nor any details regarding their diets.

Grant and Bernick (1970) used human material from three individuals aged 55, 72 and 76 years. Arteriosclerotic changes included intimal thickening and narrowing of the lumen, which resulted in a decreased blood flow to the area. Other observed features were calcification of vessel walls and hyalinization of the adventitia and media. Unfortunately, no mention of previous illnesses or dietary patterns that may have contributed to the evidence of arteriosclerotic vascular disease was made by these workers. This study also lacked a comparative basis with younger individuals.

Prout and Tring in 1971 (cited in Ferguson, 1982), fed rats a fat-free diet deficient of essential fatty acids and noted a more vascular molar periodontal ligament, with an irregular and disorientated collagen figre arrangement.

Dreizen, Vogel and Levy (1971) produced atherosclerosis of the arteries and arterioles supplying the lips, tongue, periodontium, oral mucosa and salivary glands of young rabbits confined to a cholesterol-supplemented hyperlipaemic diet for two to six months. Even though the lumens of many small arteries and arterioles were almost completely obliterated by atheromatous incursions, there was no roentgenographic or microscopic evidence of ischemic changes in the oral structures. This was attributed to the plentiful collateral circulation and the absence of complete ischemia. The lack of any reported evidence of necrosis, calcification, haemorrhage or thrombosis does not necessarily preclude a reduction in organ function.

Dreizen, Levy and Bernick (1972) induced osteomalacia in marmosets given a diet composed of 5 percent cholesterol, 23 percent coconut oil and essential nutritional elements. The control animals did not histologically exhibit a narrowing and disruption of the cortical and cribriform plates, nor radiographically display a partial to complete loss of lamina dura, as did those with the malabsorption syndrome. No mention was made of any resultant periodontal vascular effects. This was surprising considering that coconut oil contains short-chain saturated fatty acids with very little linoleic acid.

Gresham (1976) illustrated differences that occurred between species and within species. In man, the bulk of plasma cholesterol is carried by the β-lipoproteins or low density lipoproteins, whereas in the marmoset, which he describes as the least susceptible primate to naturally occurring atherosclerosis, there is an equal percentage of cholesterol carried by α- and β-lipoproteins. Squirrel monkeys are highly susceptible to the development of atherosclerosis, even though they exhibit the same lipoprotein situation as the marmoset.

Dreizen, Stern and Levy (1978) fed rabbits a cholesterol supplemented diet and a cholesterol unsupplemented diet, on alternate months for two years, in order to examine arteriopathies in the aorta and oral vasculature. Control animals were given a nonatherogenic diet for the same period. Intimal and medial arteriopathies were manifested by the labial, gingival, palatal, periodontal and alveolar arteries. The vessels exhibiting the

most involvement were the lingual and coronary arteries respectively. Although lesions which reduced the calibre of the lumen were present in these vessels, there was an absence of complete occlusion, infarcts, ulceration, haemorrhage or thrombosis.

The vulnerability of the lingual artery to atherosclerotic changes has been demonstrated in the rabbit by Dreizen, Vogel and Levy (1971), in the marmoset by Dreizen, Levy and Bernick (1976) (cited in Dreizen, Stern and Levy, 1978), in the rhesus monkey by Mostofi and Goepp (1981), and in man by Dreizen, Levy, Stern and Bernick (1974), and Dreizen, Levy and Stern (1976) (cited in Dreizen, Stern and Levy (1978). Evidence of atherosclerosis of the lingual artery is now viewed as an early warning sign of arteriosclerotic changes further down the oral vascular tree. Similar claims have been made for the inferior dental artery by Bradley (1975) and for the facial artery by Miles, Craig, Langlais and Wadsworth (1983).

Mostofi and Goepp (1981) studied the induction and regression of atherosclerotic vascular lesions in the rhesus monkey tongue. In either the induction or regression diet phases, it was revealed that the larger arteries (luminal diameter greater than 200 microns) exhibited both a higher susceptibility to the development of atherosclerosis, and a faster regression of the disease state than the smaller sized arteries (luminal diameter less than 200 microns).

Glagov and Ozoa in 1968 (cited in Mostofi and Goepp, 1981) concluded from their study on atherosclerosis of the pulmonary.

renal and mesenteric arteries that these vessels, which offered a low resistance to blood flow, were relatively uninvolved by atherosclerotic lesions. Based on this statement, Mostofi and Goepp proposed that the reason for the frequent involvement of the vessels of the tongue with atherosclerosis could be attributed to the high resistance to blood flow within the active, muscular tongue. This proposal could be adapted to the periodontal ligament vasculature.

Sabine (1977) alluded to the difficulty encountered in being able to distinguish between age-associated intimal changes and those due to the pathologic state of atherosclerosis. He did not advocate correlating data collected from experimental animals, with the atherosclerotic syndrome seen in man, due to the wide variation in susceptibility observed between animal species, the artificial state of dietary and hormonal alterations, and the production of experimental lesions over a relatively short period of time.

Johnson (1984) published an abstract which attempted to implicate atherosclerosis as a contributory factor in periodontal disease. Rhesus monkeys were fed an atherogenic diet for 54 months and then blood flow in the right posterior regions of the maxilla and mandible was measured using radiolabelled microspheres. Control animals were fed a normal diet. Results showed that the mean blood flow in the atherosclerotic animals was 45 percent less than that of the control group. The association between the signs of periodontal disease and reduced blood flow was only significant

in the maxillary arch, which appeared unusual unless it was a reflection of the different vascular trees in each jaw.

It is of relevance to note that previous studies (Dreizen, Vogel and Levy, 1971, Dreizen, Levy, Stern and Bernick, 1974) which have shown histologic evidence of atherosclerotic changes in oral vessels, have not found compromised organs or structures supplied by these atheromatous vessels. Johnson, however, reported a marked reduction in blood flow, which if maintained over a long period of time could result in pathologic tissue changes, since even a collateral supply would become involved.

Arteriosclerosis has been described as a disease of the arteries, and yet investigations centering on the periodontal ligament have neglected to take into account its predominantly venous vasculature.

3.2.6 Aging and the Vasculature of the Periodontal Ligament

The aging process has been described as an insidious decline characterized by the death of cells and the loss of functions to which the organism cannot adapt. The changes that occur with chronological age are an intrinsic part of the nature of all multicellular organisms. However, the mechanism of aging may be influenced by an interaction between inheritance, environment and nutrition.

Investigations which have reported the effects of aging on the periodontal ligament have been cited by Severson, Moffett,

Kokich and Selipsky (1978) and reviewed by Thomas (1946) and Edwall (1982).

Bernick (1962) investigated the effects of aging on the vasculature of the periodontal ligament of the molar teeth of rats, aged from one to eighteen months. The blood vessels were demonstrated by the saline-India-gelatin perfusion method. With increasing age, there was a reduction in the number of perforating vascular branches which entered the periodontal ligament from openings in the alveolar bone. This phenomenon was attributed to a progressive thickening of bony trabeculae which resulted in the production of fused dense bone. The restriction of vessels to the bony half of the periodontal ligament was a constant feature irrespective of the age of the animal. In the older animals, the presence of cellular cementum in the apical third of the root was associated with vascular terminals, whereas, the superiorly located acellular cementum was devoid of blood vessels. Bernick did not consider the potential influences on the periodontal vasculature of periodontal disease or a dietary imbalance. He attributed the changes to aging alone.

Grant and Bernick (1972) histologically examined periodontal specimens obtained from human maxillae and mandibles of three males aged 55, 62 and 76 and one female aged 92 years. The periodontal ligament in all cases exhibited an increased fibrosis and a reduced cellularity with hyalinization of ligament fibres. Hyalinized areas were associated with a decreased number of blood vessels and the formation of cartilage. Although a reduced number

of blood vessels was observed in the bone and periodontal ligament, all specimens displayed arteriosclerotic vessels. Grant and Bernick (1970) interrelated arteriosclerosis, the resultant relative ischemia, and age changes within periodontal tissues.

Periodontal disease was evident in all the human specimens studied by Grant and Bernick in 1972, however, the disease was not quantified nor were any changes within the ligament ascribed, even partially, to this inflammatory state. Due to the recurrence of similar periodontal changes between different aging human specimens, these workers argued that senescence and not environment was responsible for their morphological observations.

Roper, Knerr, Gocka and Stahl (1972) found no statistically significant correlations between the level of inflammatory periodontal disease and increasing age. This was not surprising considering the deficiencies and omissions encountered when reviewing their article. A longitudinal study would have been of greater benefit. The epidemiologic research by Marshall-Day, Stephens and Quigley in 1955 (cited in Roper, Knerr, Gocka and Stahl, 1972) reported an increase in periodontal disease with advancing age.

Levy, Dreizen and Bernick (1972a) examined the effects of aging on the marmoset monkey periodontium by obtaining histological specimens from mature and aged animals. The fibrous, vascular and osseous components of the marmoset periodontium were affected by the aging process. The degenerative arteriosclerotic type of changes seen in the periodontal vasculature of aged animals were not evident in the mature marmosets. These arteriopathies

manifested as thickening and degeneration of vessel walls, with regional arterioles assuming a hyalinized appearance. Some arterioles exhibited medial calcification, while others had an "onion-skin" configuration.

Severson, Moffett, Kokich and Selipsky (1978) selected 80 periodontal ligaments of maxillary bicuspids and first molars from 24 human cadavers with an age range of 20 to 90 years. This comparative histologic study revealed well organized periodontal ligaments of younger specimens, and irregularly structured with reduced cellular and fibre content for older specimens. The small vascularized interstitial spaces of young specimens were surrounded by numerous periodontal fibres, whereas, in older specimens, the vascularized interstitial spaces of the periodontal ligament enlarged. Older samples contained a large number of blood vessels in the interdental and interradicular bone.

No evidence of arteriosclerotic changes was found in this study which contrasts with the reports of Grant and Bernick (1970), and Levy, Dreizen and Bernick (1972a). Severson et al. expressed the opinion that ischemia would not cause aging or pathologic changes of any clinical significance in periodontal tissues because of their rich, anastomosing vascular supply. This is contrary to the views of Grant and Bernick (1972).

Ketterl (1983), in his review article, deemed age-induced changes as unavoidable, physiological processes. The age-induced vascular changes of the periodontal ligament were similar to those in the pulp, but to a lesser extent. Bennett, Kelin and Biddington in 1965 (cited in Ketterl, 1983) reported a progressive reduction

in the number of arteries supplying the apical region with increasing age. From the age of 40 years, the arterial intima thickened, the adventitia calcified and the veins attained a more tortuous course.

Franke in 1974 (cited in Ketterl, 1983) observed that aged periodontal ligaments no longer had any vascular bundles of Wedl and thus concluded that the hydraulic function of the ligament was less likely to occur.

To regard aging as a natural physiological process is concomitant to discounting all the influences that promote or retard its progress. The attribution of certain features purely to the aging process discounts the influence of the environment. The difficulty lies in separating one from the other.

3.3 Function of the Periodontal Ligament Vasculature

Marshall Hall (1831) (cited in Sobin and Tremer, 1977) expressed the opinion that the number and distribution of vessels would be characteristic of the vascular function within that tissue. Thus, nutrition was deemed the function of simple circulatory networks with few vessels. It was expected that a modified vascular system, with an increased number of vessels and a complex distribution, would be present in tissues where nutrition was not the only purpose of the vasculature.

As early as 1833, Joseph Fox (cited in Picton, 1969) suggested that the blood vessels in the periodontal ligament may act as a shock absorbing system.

Thoma in 1893 (cited in Rodbard, 1970) proposed that mechanical forces modified the vascular architecture. He found that vascular calibre varied with the rate of blood flow, and that vessel length was dependent on the tension exerted by the tetherings of the vessels. According to Thoma, an increase in capillary pressure induced the formation of new capillaries, and vessel wall thickness varied with the tension on its wall.

Boyle (1938) investigated the tooth suspension system of human and guinea pig incisors. The apical portion was supposed to function as a hydraulic chamber, which was designed to rapidly dissipate hydraulic pressures of a smaller magnitude than those functional occlusal stresses dealt with in the incisal part of the periodontium. It was proposed that, once the maximum occlusal force had been attained, then the hydraulic pressure became zero and the occlusal force was then transmitted ultimately as tension on the collagenous periodontal fibres. Boyle implicated the periodontal vascular system in the tooth support mechanism.

McCauley and Gilda (1943), having injected dogs with radioactive phosphorus, proposed that the cementum and periodontal ligament possessed a nutritive function. The amount of radioactive phosphorus deposited in normal teeth was found to decrease with increasing distance from circulatory vessels. Similar studies showing the passage of substances from the vessels of the periodontal ligament to the hard tissues of the tooth were performed by Wasserman, Blayney, Groetzinger and De Witt (1941) and Stüben and Spreter von Kreudenstein (1960).

Waerhaug (1960) outlined the concepts concerning the mode of attachment of the epithelial cells to the tooth surface based on the histological experiments using celluloid strips in the gingival crevice. It was proposed that the blood pressure assisted in keeping the epithelial cuff in close approximation to the hard tissues of the tooth. In this way the vasculature was throught to play a physical role in the maintenance of the dento-gingival junction.

Parfitt (1960) devised a machine which utilised rectilinear transducers and was capable of measuring the force applied to human maxillary incisors, as well as their axial tooth movements. When a continuous axial force of 500 gm was maintained, the tooth moved apically with the axial displacement bearing a linear relationship with time. Upon removal of this force, recovery again occurred in two phases as was reported with the use of an intermittent force. The extrusion observed during the second phase of recovery was a logarithmic function of time, and the first phase of immediate recoil was dependent only on the force applied. Parfitt proposed that two fluid systems were responsible for the support of a tooth. The collapse and recovery of smaller blood vessels of the periodontal vasculature was believed to be involved with loads below 15 gm, whereas, the tissue fluid tide was implicated for higher loads.

Bien and Ayers (1965) observed the period and amplitude of the oscillation of the rat right, maxillary, central incisor in its socket. In the living animal, when a load was placed on the

incisal edge for up to one minute, the tooth intruded, but on removal of the load it returned to, or approached its original position. In the sacrificed animal, the experiment was repeated and an increased amplitude and period of vertical oscillation of the tooth in the socket was noted. The tooth also remained further intruded after the load was removed.

The pressure exerted on the periodontal ligament exceeded the capillary blood pressure, and Bien and Ayers proposed that three independently acting, but interacting fluid systems comprised the complex hydrodynamic periodontal ligament. These three major systems included the circulatory system of blood and lymph, the viscoelastic system and the interstitial fluid. These authors suggested that the characteristic of hydraulic damper was a direct result of the blood flow in the periodontal ligament.

Kindlova (1965) divided the periodontium into three zones on the basis of blood supply. These were firstly, the periodontal ligament, secondly, the gingiva facing the oral cavity and thirdly, the gingiva facing the tooth surface and lying coronal to the periodontal ligament. She attributed the characteristically arranged blood vessel networks in these areas to the metabolic requirements of these tissues. It was postulated that the presence of the coiled capillaries in the connective tissue, in the vicinity of the alveolar bone crest, was associated with the nutritive requirements necessary to maintain the junction of hard and soft tissues.

Bien (1966a, 1966b) published two articles in the same year following on from the work of Bien and Ayers in 1965. The existence of a biphasic response to axial loading and the involvement of two fluid-damping effects in the tooth support mechanism was proposed. The first damping effect implicated a squeeze film effect involving the replenishment of interstitial fluid, which only occurred if the applied forces were low. As the squeeze film mechanism became exhausted the second damping effect of the periodontium came into play. This was slower than the initial mechanism and was thought to involve vascular changes in the periodontal vessels. It was proposed that the creation of cirsoid aneurysms acted as minute springs, replenished the squeeze film, and dissipated the kinetic energy by forcing the fluid through the vessel wall. The response was considered to be due to a single Maxwell element.

Wills, Picton and Davies (1976), who disputed the theories of Bien (1966b), proposed that for forces below 1.0N the fluid systems played an important energy dissipating function. Whereas, at higher loads, the fibrous components of the periodontal ligament were directly associated with the transmission of force.

Folke and Stallard (1967) found that the periodontal vessels were located closer to bone than cementum. The clinical significance of this observation, proposed by these workers, was that the vascular arrangement allowed the blood to flow in various directions, thus equalizing the pressure and providing a vascular hammock during mastication. These authors supported the views of

Castelli and Dempster (1965) that the periodontal vasculature maintained a nutritive role.

Parfitt (1967) was not of the opinion that teeth were supported by the tension placed on inelastic periodontal fibres - attached to root and bone. He recognised the presence of a multifactorial tooth supporting mechanism, which was dependent on the physically independent tissue components in the periodontium.

Contrary to the close association between autonomic nerve fibres and the blood vessels of the periodontal ligament described by Bernick and Levy (1968), Aars and Linden (1982) proposed that interference with the vascular component of the periodontal ligament had little direct effect on the mobility of the canine tooth of the cat. This interference with the periodontal vasculature took the form of increasing the activity of the sympathetic nerves, which resulted in intrusive and transverse movements of the tooth. The transverse movements of the actual tooth crown were not measured, however, the relative displacement of the piezo-electrical crystals attached to the tooth and maxilla were recorded. Similar studies have been performed on the rabbit incisor (Aars 1983a, 1983b).

Mühlemann (1967) reviewed the experimental data on tooth mobility to that time. He placed major emphasis on the responses of a tooth to horizontal loading and concluded that the bony socket, the collagen fibre system, the intravascular and extravascular fluid systems and the interfascicular connective tissues all contributed to the mechanism of tooth support.

However, the theory Mülhlemann favoured was the cybernetic model proposed by Körber and Körber in 1966 (cited in Mühlemann, 1967).

Dubner, Sessle and Storey (1978) in their resume on the location and distribution of mechanoreceptors in the periodontal ligament, reported their presence in the apical region and largely on the lingual aspect of the tooth. This work followed on from that of Sessle and Hannan (1976), who indicated that the role of periodontal receptors could be to provide peripheral feed-back to the cortex during the masticatory act. In this manner, a form of positive feedback on jaw closing muscle activity was provided, in addition to inducing powerful inhibitory effects on jaw closing motoneurone activity. The close association between neural elements and the vasculature of the periodontal ligament could possibly indicate a combined protective role in the tooth support mechanism.

Gianelly (1969) examined the relationship between force, vascular patency and alveolar bone resorption by exerting tipping forces on the maxillary canines and incisors of dogs. An association was shown to exist between the presence of a patent periodontal vascular network and force-induced frontal resorption, and vascular occlusion and undermining resorption. The role of the vasculature of the periodontal ligament was described as manifold. Its obvious purpose was to supply essential nutrients and oxygen for the increased energy demands needed to carry out bone resorption. The vasodilation observed in some areas of active bone resorption was a response to the increased energy demands of

the tissue, and the resultant local increase in oxygen tension was said to create a more favourable environment for bone resorption.

Khouw and Goldhaber (1970) demonstrated the changes that occurred in the periodontal vasculature of dogs and monkeys with the application of experimental orthodontic forces. After 24 hours of force application, the compressed side of the periodontal ligament showed partial, or complete, vascular occlusion while on the tension side, the vessels of the periodontal ligament were extended. Two days later there were less pronounced differences, and seven days after the application of force, the tension side exhibited increased vascularity and new bone formation. Simultaneously, the pressure side re-established its vascularity, and bone resorption became evident.

These authors proposed that the increased vascularity resulted in a better exchange of nutrients, gases and metabolic products between the vasculature and the surrounding tissues. They reasoned that conditions for the diffusion of metabolites were more favourable owing to the increased surface area of blood vessels and the close proximity of the vessels to the resorbing alveolar bone and the osteoclasts. In addition, it was argued that the oxygen transported in the blood was primarily a source of energy, and that the surrounding tissue would respond to its presence, reduced levels, or absence, accordingly. Thus, an increased vascularity would make more oxygen available to meet the increased energy demands of the tissues.

Rodbard (1970) reviewed the potential effects that mechanical forces could have on connective and cardiovascular tissues, and the resultant active tissue responses to these forces. The amount of collagen found in blood vessels varied with the product of blood pressure and the radius of the vessel. Blood vessels adapted to the increased tensile stress by depositing greater amounts of collagen where blood pressures were elevated, and where the radius of the vessel was greater.

Körber (1970) amplified periodontal micropulsation of human incisors. The human tooth was seen to possess a small range of movement on either side of its resting position. The pulsatile volume fluctuations of the periodontal blood vessels were transmitted through the whole ligament to the tooth root, thus keeping the tooth in motion. In clinically firm teeth, the average movement was 0.4×10^{-3} mm. It was postulated that if the entire periodontium were exposed to uniform volume fluctuations, then the expected displacement would be along the long axis of the tooth. However, after precise measurements were taken, pulsatile movements of human incisors were revealed to be directed from the neutral position toward the labial side. To explain this phenomenon, Körber concluded that the palatine vessels were larger and more numerous than the periodontal vessels on the labial side, and as a result, the pulsation volume on the palatine side was greater, thus producing the labial direction of pulsation movement of the tooth.

Koivumaa and Lassila (1971) induced hyperfunction and hypofunction for periods of one week to three months in the first molars of rats, and then examined the changes in the vascularization of the periodontal ligament, by vital staining with Aquablack B and Micropaque Powder. Hyperfunction was induced by placing an amalgam restoration 1 mm overhigh in occlusion, in the first right molar of the rat. The vascular changes in the hyperfunction group began early and were inconsistent at this stage. After a longer observation time, both the volume and density of the vascularization of the superficial tissues increased. Changes in the deeper periodontal vasculature were rare over this time span. Hypofunction was induced in the antagonist lower first molar when the maxillary first molar was extracted. The vascular changes in the hypofunction group were more distinct, started later and were graver than those of the hyperfunction group.

Pihlstrom and Ramfjord (1971) used histometric measurements to assess the periodontal effect of non-function on the teeth of monkeys. It was concluded that non-function reduced the width of the periodontal ligament by one half to two thirds during the first three months, and increased the gingival inflammation and the loss of bony support.

Slatter and Picton (1972) measured the intrusive movements of the anterior teeth of monkeys when loads were applied approximately along the long axis of the tooth. The local injection of 1:10,000 noradrenaline reduced the tooth mobility and attained a maximum effect one hour after the injection. Both

phases of tooth movement were affected by the administration of the vasoconstrictor, noradrenaline. These authors proposed that the reduction in size of the lumen of some arterioles within, or supplying the periodontal ligament, would only allow the vessels to refill slowly, thus preventing the tooth from attaining its original rest position. This interference in the slower second phase of recovery was compounded by the insufficient time allowed for the return of blood and tissue fluid to the periodontal ligament. This was a result of the application of thrusts to the tooth with a frequency of 1 every 10 seconds.

The investigation was not clear as to whether the noradrenaline affected the vessels in the periodontal ligament, or those vessels supplying the ligament. It was concluded that the results of this study lent credence to the theory that blood vessels play an important role in the provision of axial tooth support.

Wills, Picton and Davies (1972) applied forces to the incisal edges and down the long axes of monkey central incisors in order to measure and record the motion of each tooth. A force of 2.5N was applied for 1 second and then a period of 1.5 minutes was allowed to lapse in order for the test tooth to return to its original position. After an interval of 10 seconds, a second thrust was applied and the entire sequence was repeated. The displacement of the tooth was measured and expressed as a proportion of the initial displacement, and following the implementation of the "curve stripping" technique, three lines

were extrapolated with displacement expressed logarithmically against a linear time scale.

The resultant relaxation curve was composed of three exponential components. The first phase of recovery was rapid and was responsible for approximately 50 percent of the spring forces. The second phase was not as steep and represented 30 percent of the restoring force. The third phase represented a more gradual return to the equilibrium position, and due to the presence of a large base-line shift, was liable to error. A fourth phase with a very fast component was obtained in one or two cases, and a fifth element was postulated to exist. These authors supposed that the presence of three and possibly five exponential components implied the presence of three and possibly five Voigt elements. They concluded that the periodontal tissues were visco-elastic in nature. A comparison between the results of upper and lower incisors indicated differences in rates of initial relaxation. These were attributed to variations in bone density and blood supply.

Investigations and resultant findings of Wills, Picton and Davies (1972), and Picton and Wills (1978) were contrary to those of Bien (1966b), who described the biphasic response to axial loading as being due to a single Maxwell element. In a Voigt element, the spring and damper are in parallel with the same strain in each, and the stress being proportioned between the two. The spring and damper of a Maxwell element are in series and undergo the same stress but are subjected to different strains.

Griffin and Spain (1972) detailed the vascular supply to the human periodontal ligament mechanoreceptors using light and electron microscopy. The type I receptor appeared to receive its blood supply by diffusion and the type II receptor was supplied directly by a metarteriole. Myo-endothelial junctions were found to be a feature of afferent terminal arterioles, and Rhodin (1967) considered that these junctions probably served as pathways for the exchange of blood borne transmitter substances and other metabolites.

Sims (1973) observed and reported on the distribution of the oxytalan fibre system in the periodontal ligament of mouse molars. The oxytalan network was found distributed from the dentino-cemental junction to the blood vessels located adjacent to the alveolar wall. Distinct regional patterns of fibre arrangements were described. As a result of the close association between the oxytalan network and the walls of blood vessels, it was hypothesized by the author that the fibre behaved as a form of proprioceptive mechanism, contributing to the regulation of blood flow in the periodontal ligament. Sims continued this theme of research in articles on the human oxytalan fibre system in 1975 and 1981. In 1976, he reported changes to the human oxytalan network with the application of orthodontic forces. The mouse molar was studied in 1977, 1981, 1983a, 1983b and Sims used normal and lathyritic mice in 1978 and 1980.

Rygh (1973) demonstrated the ultrastructural alterations in the tissues on the pressure side of the periodontal ligament, when the human premolar teeth were moved buccally using a fixed orthodontic appliance. The light microscopic findings revealed a compression of the periodontal ligament and its blood vessels. The ultrastructural vascular changes included the swelling of endothelial cells, which then allowed communication between the blood vessels and the surrounding tissues. This study indicated that the cellular and vascular changes occurred prior to the fibrillar alterations. However, the initial tissue changes preceding the first observation period of two days were not detailed by this researcher.

Storey (1973) measured the rates of tooth movement in humans and in laboratory animals using orthodontic appliances. The tissue changes associated with the application of heavy and light forces were described. Three biological systems were involved with the study of tooth movement. The bioelastic system supported the teeth and its components included the interstitial fluid which created a squeeze film effect, the architecture of the periodontal ligament which induced the creation of cirsoid aneurysms following the tightening of periodontal fibres, and the viscoelastic properties of the ligament. These viscoelastic properties demonstrated a greater resistance to instantaneous heavy forces, than to light forces exerted over long periods of time. The biphasic changes occurred with the continuous loading of teeth, and bidisruptive deformation resulted if loads became excessive.

Wills, Picton and Davies (1976) advocated that 30 percent of the displacement of the tooth was due to alterations in blood volume, and 10 percent could be ascribed to the extracellular fluid. Even though the volume of displaceable blood was claimed by these authors to occupy 0.5 to 1 percent of the periodontal ligament, any reduction in the vascular volume was shown to have a marked effect on the intrusion of a tooth.

Casley-Smith, Sims and Harris (1976) used electron microscopy to examine the lengths of capillaries, surface areas and intercapillary distances in the tissues of the human knee, the synovial capsule and membrane, fat and tendon. The vascularity of these regions was far less than in muscle tissue, and in other regions of the body that were metabolically active.

Packman, Shoher and Stein (1977) monitored the circulatory changes in the human periodontal ligament revealing that, with the tooth at rest, the pulsatile movements recorded were synchronous with the heart beat. The application of axial and horizontal forces resulted in a decrease in blood volume in the areas of the periodontal ligament under compression. When forces of 90 to 180 gm were applied, an increase in blood volume was observed in those recordings under tension. A biphasic change, with an initial increase in blood volume followed immediately, or after a delay of 2 to 5 seconds, with a progressive decrease in blood volume, was characteristic for forces above the critical 90 to 180 gm level. Using the light reflection technique, it was possible to record the increase in pulse amplitude in areas of tension throughout both phases.

The authors proposed that the observed biphasic change represented evidence of autoregulation of blood vessel tone in the periodontal tissues. The area of the periodontal ligament under tension as a result of applying forces greater than the critical level, would experience increased transmural pressure and vascular tone, which would in turn reduce the oxygen tension in the immediate area and adjacent alveolar bone. The opposite should occcur in areas under compression. Thus, it was suggested that vascular autoregulation altered the local tissue oxygen tension, and as a result played a role in the osseous remodelling of alveolar bone when occlusal or orthodontic forces were applied.

Sobin and Tremer (1977) indicated that in order to understand the control mechanisms of microcirculations, one must first define their three-dimensional architecture. Microvessels were classified according to whether their functions were primarily nutritional or regulatory, homeostatic and operant. Nutritional vascular beds were described as being similar between different tissues or organs, whereas, operant vascular beds tended to determine the skeleton structure of the organ and have a characteristic microvascular pattern. A distinction was made between total microvascular beds which had an open channel complex, and dynamic microvascular beds which were functionally open depending on the selective regulatory mechanisms that acted at a specified time. The importance of this distinction related to the techniques used to demonstrate these vascular systems. The dynamic microcirculation could only be reproduced experimentally

when blood flow and all living processes cease, whereas, the total microvascular bed could only be exhibited when the regulatory mechanisms have been interrupted or abolished during life.

Murata (1978) theoretically considered the effects of a variable filtration coefficient and lymph flow on the fluid exchange across blood capillary walls to the tissue space. In order to analyse these fluid movements and simplify calculations, twelve assumptions were used among which the capillary-tissue system was represented geometrically by a Krogh cylinder and the blood as an incompressible Newtonian fluid. Using a series of derived equations, Murata analysed transcapillary fluid exchange. Lymph flow was found to increase with an increase in venous or arterial pressure, and decrease with an increase in the effective pressure of interstitial fluid. However, under normal conditions, a protective mechanism would exist to keep lymph flow at a constant volume. Therefore, the effects of an increase in arterial or venous pressure would be cancelled out by the increase in effective pressure of interstitial fluid. In many ways, the assumptions made tended to over simplify a multidimensional system and thus lose the intricacies of its effect when in function.

Walker, Ng and Burke (1978) recorded the fluid pressures in the periodontal ligaments of the canine teeth of dogs, in order to discover the contribution made by the free fluids of the ligament to the tooth support mechanism. Using linear regression analysis, these authors could not demonstrate a significant correlation between the resting intraperiodontal pressure and either the

systolic or diastolic blood pressure. With the application of loads up to 500 gm, there was an immediate increase in pressure, which then reduced rapidly having a halving time of less than one second. Removal of the load inversed the observed pattern, producing a reduced peak pressure and a longer halving time. Exsanguination had no effect on the resting intraperiodontal pressure, and even following fluid injection there was no change in this pressure.

These authors concluded that the periodontal tissue fluid was totally isolated from the blood, and that the lymphatics served as the only means of communication between the periodontal ligament fluid and the other body tissues. The tooth support mechanism derived from these studies was not based on the theories of Bien (1966a, 1966b), who attributed the major portion of tooth support to the free fluids of the periodontal ligament. Contrary to this, Walker, Ng and Burke suggested that the solid (collagen fibre), the semi-solid (ground substance) and the periodontal vasculature were responsible for the tooth support mechanism, and that the tissue fluids of the ligament provided a minor amount of viscous support.

Wills and Picton (1978) applied a standard loading regime to the central incisors of monkeys in order to record the displacement of the tooth and its resting position for periods of 1.5 to 5 hours. The loading regime consisted of six initial priming thrusts of 4N, with a loading rate of 4N per second, and intervals of 10 seconds between each thrust. After allowing a

two-minute recovery period, 10 more thrusts were repeated as outlined. The regime was repeated at half hourly intervals for 1.5 to 5 hours. Submucosal injections of water over the root resulted in a rapid extrusion of the tooth followed by a gradual recovery of the resting position of the tooth. Injecting saline over the root produced intrusion of the tooth followed by a more rapid recovery of the resting position of the tooth. The presence of water did not reduce the mobility of the tooth, whereas, in three cases where saline was injected, the displacement of the tooth was reduced.

It was proposed that the extrusion of the tooth observed with the injection of water, resulted from the increased pressure caused by its volume, and by the swelling of the collagen network following the binding of water by the proteoglycan molecules. Because of the low viscosity of the free water the mobility of the tooth would increase. Two opposing effects were evident with the injection of saline. Extrusion resulted from the volume effect of the added fluid, but an opposing intrusive movement of the tooth occurred with the withdrawal of free fluid from the ligament via the periodontal blood vessels. The sodium and chloride ions neutralized the proteoglycans charges which then freed the fluid in the ground substance. An increase in mobility was noticed with the reduction in fluid viscosity. Eventually, however, the resultant intrusion elongated the collagen fibres, and thus reduced the tooth mobility. In the absence of injections, no correlation was observed between the mobility of the tooth and

its resting position. This phenomenon was attributed to the continuous change of fluid flow in and out of the ground substance throughout the periodontal ligament.

Moxham (1979) continuously recorded the effects of vaso-active drugs on the eruption of the rabbit mandibular incisor using a displacement transducer. Hexamethonium (10 mg/kg), guanethidine (3 mg/kg) or hydrallazine (1 mg/kg) were injected intravenously, via the marginal ear vein, and produced an increase in the rates of eruption like tooth movements for the first two hours, and then proceeded to return to their control values. Initially, however, the rabbits injected with guanethidine and hydrallazine showed incisor intrusion with a reduced mean rate of tooth movement occurring in the first 30 minutes and 15 minutes, respectively. Arterial perfusion of noradrenaline (0.003 mg/kg) resulted in an increase in systemic blood pressure and the intrusion of the tooth. When acetylcholine (0.01 mg/kg) was administered, blood pressure decreased, and where there was no prior resorption, an initial rapid extrusive movement was observed. Following the initial effects of noradrenaline and acetylcholine, occasionally there were eruption-like movements at a rate greater than expected.

It was suggested by Moxham that the same processes may have been responsible for the mechanisms of tooth support and tooth eruption, particularly as they are both properties of the periodontal tissues. Alterations in the periodontal tissue hydrostatic pressure based on Starling's hypothesis could be

responsible for the effects of these changes, but because blood flow and/or tissue fluid pressures within the periodontal ligament were not measured, this conclusion was presented with some caution.

Kardos and Simpson (1979) theorized on the existence of the periodontal ligament as a collagenous thixotropic system, whose rheological behaviour had the following three characteristics; pressure alone bringing about an isothermal change in viscosity, a time-dependent recovery system that maintained its contours when the pressure was removed, and a hysteresis loop to represent the shear stress versus the shear rate. Tooth eruption and tooth movement were explained as the slow deformation of the soft tissue gel as a result of the pressure caused by cardiac systole, which developed in the extremely vascular apical area of the tooth follicle. When a critical pressure was attained at the base of the tooth, the temporary bonds in a gel lattice broke down, and eruption was initiated. The rupture of the bonds along the line of shear did not necessitate the expenditure, or the absorption of energy, and resulted in a reduction in viscosity. The critical initiating pressure for tooth movement or eruption was thought to result from the pulsatile nature of capillaries.

Kardos and Simpson (1980) attributed the failings of the present concepts of the collagen system operation within the periodontal ligament to the artificial changes occurring during tissue processing, and the comparison of the properties of this tissue with tendon collagen.

Walker (1980) presented a simple mathematical model to illustrate the known characteristics of the periodontal ligament. Ross, Lear and De Cou (1976) (cited in Walker, 1980) represented the load deflection behaviour of the periodontium with a mathematical model which took into account the linear dashpot and the non-linear spring. The derived equation was as follows:

$$f = (a\,x + k)\;x$$

where f = applied load

x = deflection

a and k = characteristic constants.

Walker proposed the blood vessels as the principal supporters of the teeth, if they could be shown to possess similar mechanical properties. Using internal pressurized, man-made tubing to represent the blood vessels, it was possible to assess the load deflection behaviour when the fluid-carrying cylinders were subjected to transverse forces. Flow rates were also recorded until the tube occluded.

The results indicated that the vasculature did not dominate the tooth support mechanism, even though the equation proposed by Ross, Lear and De Cou was shown to adequately describe the load deflection characteristics of a transversely loaded tube. The ratio, a/k, was found to be several orders less than that for the periodontal ligament. It was concluded that the collagen and/or ground substance accounted for the non-linear load deflection behaviour of the periodontal ligament, and that the tooth support mechanism was represented by three

components of the ligament (i.e. the vasculature, the ground substance and the collagen) acting in parallel. This mathematical model should always be considered just that, a model, and not a representation of the true dynamic nature of the periodontal ligament.

Wills and Picton (1981) applied axial loads to macaque monkey incisors in two sequences, and measured the bone and tooth displacements with a linear variable differential transducer. The first loading sequence involved the application of a load of 4N every 30 minutes at a loading rate of 4N per second. The resting position was assumed to be immediately prior to the applied thrust. The second loading sequence consisted of 16 thrusts, with a 10 second interval between all thrusts, except numbers 6 and 7, when the interval was two minutes. Using a load of 4N the loading rate was maintained at 4N per second. The sequence was repeated every 30 minutes, and the resting positions of the tooth were recorded before the first and the sixteenth thrusts, in any one series.

For the single thrust cases, a strong correlation existed between the slope of the logarithmic displacement versus the logarithmic force curves, and the change of the tooth within the socket. An increase in the slope of the curve correlated with an intruded position of the tooth, whereas, a reduced slope was related to a more extruded position of the tooth. This response did not occur with the second loading sequence where a constant reduction in slope between the first and sixteenth

thrusts was observed. Three positioning mechanisms were postulated to explain this phenomenon. At the start, over the first one or two minutes, a short term recoil and a return of the blood occurred. The second mechamism of passive recovery of the position of the tooth became evident after a series of loadings, and was thought to be due to the re-binding of water molecules and the re-polymerization of the ground substance. In the long term, over a few hours, changes in the fluid content and a metabolic turnover of the collagen fibre network resulted in the active positioning of the tooth in its socket.

Ng, Walker, Zingg and Burke (1981) used dogs to isolate the arterial supply to the periodontal ligament, pulp and gingiva of the mandibular fourth premolar. The blood flow and blood pressure in the mandibular artery were recorded, while occlusal forces of 20N and 35N were applied for 8 seconds, with a 2 minute recovery period. When intrusive forces of less than 350N were applied, the blood flow did not significantly alter. However, loads of 1050N reduced vascular flow, increased vascular resistance by 10 percent, but did not affect arterial pressure. On removal of the load, the increase in blood flow was found to be greater than the reduction observed with load application. The authors suggested that occlusal loading could result in an ischemic periodontal ligament as a result of the impaired blood flow.

Periodontal pulsation of the canine fourth premolar did not cease at 35N, which was 200 times the load applied to human incisors by Parfitt in 1960. By correlating Birn's 1966 findings

that vascular density of the periodontal ligament increased posteriorly, Ng, Walker, Zingg and Burke proposed that functional loading and periodontal vascularity were associated entities. Special differences were acknowledged. It was their considered opinion that the vasculature of the periodontal ligament did directly and significantly participate in the tooth support mechanism. They also indicated that the density and architecture of the periodontal vasculature could, in part, be determined by the functional loads applied to the teeth.

Picton and Wills (1981) exposed a cross-section of the monkey periodontal ligament, produced a silicone rubber impression and a positive replica using a polyimide resin. This replica was examined under the S.E.M. and further replicas were made with the tooth subjected to changing conditions of load. Unfortunately, blood vessels could not be identified from the impressions and the authors suggested that the vessels collapsed as a result of trauma, and not because of the shortcomings of the technique used. It was stated, however, that interpretation of the replicas had to be guarded, mainly due to the observed extrusion of the ligament when the root was not loaded.

Shore, Moxham and Berkovitz (1982) quantitatively assessed the ultrastructure of the periodontal ligaments of rat mandibular impeded and unimpeded incisors. The teeth were unimpeded for a period of 18 days and the results showed changes in function, turnover and biochemical properties without any changes in structure. This investigation did not include data concerning

the periodontal vasculature. However, these authors did acknowledge its implication in the tooth support mechanism.

Ferrier and Dillon (1983) investigated the water binding capacity of the periodontal ligament using scrapings of this tissue obtained from pigs. They concluded that the water binding capacity accounted for the viscoelastic properties of the support function of the periodontal ligament, but only at low stress values. These researchers and their hypotheses lent credence to the findings of Wills, Picton and Davies (1976) that high stress values are supported by the collagen fibre network (also a visco-elastic component) of the periodontal ligament.

Gaengler and Merte (1983) demonstrated the structural and functional independence of the periodontal and gingival blood circulation of the rat incisor, using vital microscopy and various applied loads. Ischemia appeared both in areas of compression and tension, with dilated vessels evident peripheral to these well defined zones. As the force increased so did the ischemic involvement of the larger vessels. The mechanism of functional adaptation within the ligament determined the eventual extent of vessel involvement with applied forces.

Moxham and Berkovitz (1983) reported on the effects of extrusive loads on the lathyritic rabbit periodontal ligament. For both control and lathyritic teeth, these workers recorded biphasic, visco-elastic extrusive and recovery responses, with a significantly greater displacement for lathyritic teeth during all phases of movement. They concluded that the degree of collagen

cross-linking could influence the resistance of the periodontal ligament to displacing forces, however, no mention was made of the effects, if any, of lathyrism on the vasculature of the periodontal ligament.

The definitive function of the periodontal ligament vasculature has yet to be resolved, however, glimpses of its possible roles have been presented in this literature review. Even though various researchers dispute the proportional responsibility allocated to various structures within the periodontal ligament with regard to the mechanism of tooth support, it cannot be denied that the periodontal ligament vasculature plays a major role.

CHAPTER 4:

MATERIALS AND METHODS

4.1 Animal Sample

Eight marmoset (Callithrix jacchus jacchus) monkey mandibles were obtained from the Department of Oral Pathology and Oral Surgery of The University of Adelaide, who originally received the specimens from the Commonwealth Scientific and Industrial Research Organisation. The mandibles were preserved in a 10 percent formal saline solution. The animals were numbered and their sex, age and diet had been determined.

Animal Number	Sex	Date of Birth	Date of Death	Diet
324	Male	24. 9.77	21. 6.82	Colony Diet
24	Male	15.11.80	15. 7.82	Colony Diet
3	Female	21. 7.79	7. 7.82	Colony Diet
28	Male	2.12.80	10. 8.82	Saturated Fat Diet
23	Male	30.10.80	27. 7.82	Saturated Fat Diet
32	Female	27. 4.81	12.10.82	Saturated Fat Diet
19	Male	25. 9.80	19. 8.82	Polyunsaturated Diet
30	Female	16.12.80	31. 8.82	Polyunsaturated Diet

Animal Number	Date of Diet Commencement
324	Colony control (ongoing)
24	Colony control (ongoing)
3	Colony control (ongoing)
28	18.1.82 (Saturated)
23	18.1.82 (Saturated)
32	5.7.82 (Saturated)
19	18.1.82 (Polyunsaturated)
30	18.1.82 (Polyunsaturated)

Those animals not on colony diets, had been fed diets supplemented with 12 percent fat, either as sunflower seed oil (polyunsaturated fat) or mutton fat (saturated fat), over periods ranging from 3 months to 7 months. According to McIntosh (1984), these diets were not intended to produce arteriosclerotic or atherosclerotic changes, but merely to simulate human dietary equivalents in young adult monkeys.

Cardiac function was measured using radio-nuclide angiography. There was no evidence of pathological change in the hearts of this group of animals. However, subsequent experiments with an increased time period on diet supplements, and an elevated percentage of dietary fat content, have begun to show increased cardiac work in both fatty groups with the mutton fat fed animals showing more of an increase. Those animals on subsequent diets had cardiac function tests performed prior to diet commencement, whereas the animals used in the present study did not have this beneficial control measure performed, and were sacrificed on completion of these tests.

Goss (1983) histologically investigated the lingual arteries from the animal sample used in the present study to conclude that this preliminary indicator of atherosclerotic changes exhibited no abnormal signs. Further information concerning these animals has been outlined in Appendix I.

Prior to proceeding with processing, the mandibles were cleaned of excess tissue and muscle attachments by scraping with

a scalpel blade. To avoid dehydration, the tissues were kept continually moist with 10 percent buffered formalin solution.

4.2 Photographic Records of the Mandibles

The mandibles were recorded photographically with a Minolta SRT 10 1b, a Novaflex bellows and a Minolta Auto-Bellows 100 mm Rokkor lens (Minolta Camera Company, Osaka, Japan). After the Kodak Ektachrome 50 Tungsten film was processed, each frame was mounted in Anti-Newton slide binders.

4.3 Sectioning of Mandibles

Each mandible was sectioned into three segments, using a diamond disc at 6,000 rpm and copious amounts of 10 percent formal saline solution to prevent dehydration. Two cuts were made, i.e. one between the right canine and first premolar and the other between the left canine and first premolar. These sections were cut at the expense of the canine roots rather than the premolar roots.

4.4 Radiographic Technique

All three segments of each mandible were radiographed prior to the commencement of demineralisation procedures, in order to set a baseline so that the end point of demineralisation could be determined. The following radiographic technique was used:

1. Kodak periapical ultraspeed film: 22 x 35 mm.
2. Siemens Heliodent machine.
3. Voltage: 50 kV.
4. Current: 7mA.
5. Exposure time: 0.1 sec.

4.5 Demineralisation and Demineralisation End Point Determination

The gross posterior mandibular segments were placed in perforated metal containers, which were suspended from stainless steel hooks in a glass beaker containing the decalcifying solution of 40 percent formic formate (Appendix II.2). Demineralisation occurred at room temperature and the solution was continually agitated with a magnetic stirrer.

Radiographs were taken on the eighth, tenth, thirteenth and fourteenth days, and decalcification was completed on the fourteenth day which was deemed the end point in accordance with the work of Fejerskov (1971).

In Fejerskov's paper, demineralisation end point was ascertained by the following three methods:

1. Mechanical cutting test. The bony tissue was cut with a razor blade and was considered to be demineralised when no undemineralised hard patches were encountered.

2. Radiographic test. A standardized technique was used and demineralisation was complete when all the radiopaque material representing the hard tissue had disappeared.

3. Chemical test. The presence of residual calcium ions in the demineralising fluid resulted in their precipitation on the addition of ammonium oxalate. This test was only employed when nitric acid, or formic acid - sodium citrate were the demineralising solutions.

Of these three methods, Fejerskov obtained satisfactory results with the mechanical cutting test and the radiographic test, but the determination of the demineralisation end point was not as accurate with the chemical test. He stated that the mechanical cutting test would be used on its own as long as the hard tissue components were to be examined histologically.

In addition to the radiographic test, the mechanical cutting test was also employed in this present study to determine the demineralisation end point. Using a new safety edge razor blade for each procedure, the ramus of each mandible segment was cut horizontally being parallel and in line with the occlusal plane of the molar and premolar teeth. Demineralisation was deemed complete when no hard tissue was encountered.

4.6 Neutralization

On the completion of demineralisation, the specimens were suspended in 5 percent sodium sulphate (Na_2SO_4) for two days prior to dehydration and blocking procedures.

4.7 Tissue Processing and Paraffin Embedding

The specimens were processed by the normal Double Embedding Technique (Appendix II.3). Because of the size of the mandible segments, a longer time was required for each stage of this procedure.

The specimens were infiltrated with paraffin wax (Appendix II.3), at 60°C for 40 minutes, prior to blocking in plastic moulds using the "Tissue Tek II Tissue Embedding Centre" (Lab-Tek Products, Division Miles Laboratories Inc., Naperville, Illinois).

The orientation of the mandible segments during blocking was along the long axis of the molar teeth, so as to allow sectioning in the required horizontal plane at right angles to this long axis.

4.8 Sectioning

Serial sections were cut on a Rotary Microtome Model 280 (American Optical Corporation, Scientific Instrument Division, Buffalo, N.Y.). Sections cut with the microtome set at 8 µm proved to be most suitable for staining and evaluation purposes.

Each section was flattened by floating it on a warm water bath, transferred to a gelatinized slide (Apprndix III), and placed on a hot plate. This procedure was followed by an overnight stay in the 37°C oven, and then 40 minutes in the 60°C oven.

For this investigation 8,583 horizontal sections were cut and mounted on 2,861 slides.

4.9 Staining

Pollack's trichrome stain was used, but in a modified form from the technique of Luna (1968). This staining technique has been detailed in Appendix IV. The step involving nuclear staining with Geimsa's solution was routinely omitted in order to enhance the staining of collagen. After staining, all slides were mounted in D.P.X.

According to Burnett (1978), sections stained with Pollack's trichrome for less than 10 seconds had green stained collagenous elements in the periodontal ligament, red stained cells and red stained bone. However, sections stained for more than three minutes still exhibited a green stained periodontal ligament with red stained cellular elements but had a green coloured bony matrix. With this latter time span, some areas of periosteal bone and dentine remained stained red. Sharpey fibres were stained red, even though the surrounding bone was green. Yet, where these Sharpey fibres entered the periodontal ligament, they became green in colour.

Dreyer (1980) could only show a red coloured bone matrix, irrespective of the staining time when using Pollack's trichrome stain. The staining differential between bone and Sharpey fibres, as reported by Burnett (1978), was not consistent with Dreyer's findings. However, red coloured fibres in bone did change to green on entering the periodontal ligament.

Verhoeff's iron haematoxylin stain was used in accordance with Culling (1974) and Miller's stain as detailed by Miller (1971). These staining techniques have been outlined in Appendix V and VI respectively. Both these elastin stains were employed, in order to identify periodontal arterial vessels via their internal elastic lamina, and subsequently search for arteriosclerotic changes within these vessels.

Attempts were made to identify a suitable cholesterol stain. However, because of the following three reasons, this was not pursued further:

1. Culling, Hyde, Inwood, Mellor, Sergovich, Spencer and Thompson (1976) have not found a specific stain or histochemical method for cholesterol. The Schultz method was recommended, but only if one kept its shortcomings in mind.

2. Frozen sections were required for the Schultz method, and all the animal material in this study had already been processed using the double embedding technique.

3. The method recommended by Culling et al. (1976) was described as insensitive, requiring 5 to 25 percent cholesterol to be present for even faint colour development, which would only endure for 30 to 60 minutes.

4.10 Photography of Sections

Photomicrographs of selected slides were taken with an Axiomat N.D.C. Microscope (Carl Zeiss Inc., Oberkochen, West Germany). Kodak Ektachrome daylight professional film, with an ASA rating of 64, was used for the production of 35 mm colour slides.

4.11 The Pilot Study Sample

In accordance with the research by Sims (1983), involving a morphometric analysis of the periodontal ligament vasculature of mice, it was recommended (Personal communication: Sims, 1983, Personal communication: Leppard, 1983) that the sample for the pilot study be 20 sections from two half mandibles, taken from two animals.

These 20 sections represented the periodontal ligament of the second molar thus:

8 from the cervical third of the periodontal ligament

4 from the middle third of the periodontal ligament

8 from the apical third of the periodontal ligament.

The chosen sections were evenly distributed amongst each vertical third of the ligament by virtue of distance.

Zero point was taken as the first indication of periodontal ligament attachment to the tooth and the end point was determined as the section with the final remaining apical section of tooth root.

Original intentions to use the first molar had to be abandoned, due to the radiographic evidence of bone loss and the histologic evidence of a lowered level of periodontal ligament attachment. The presence of periodontally involved first molars was detected in three animals, two of whom were the oldest amongst the sample.

The second molar was selected for investigation, as there was no similar evidence of a disturbed periodontal ligament in any of the animals.

Sample designs for use in stereology have been discussed by Cruz-Orive, Gehr, Müller and Weibel (1980) and Cruz-Orive and Weibel (1981), and optimizing sampling efficiency by Gundersen and Osterby (1980, 1981).

Mayhew and Cruz-Orive (1973) derived mathematical corrections which made morphometric information obtained from biased samples more reliable. Furthermore, Oberholzer, Rohr, Bitterli, Sandoz and Ehrsam (1980) discussed the implications of using a minimal sampling size, whereas Schroeder and Münzel-Pedrazolli (1973) outlined the use of non-randomly oriented

tissue sections in their morphometric study of gingival tissues. Prothero, Tamarin and Pickering (1974) investigated the morphometrics of living specimens by describing a methodology which allowed the three-dimensional study of growing microscopic animals.

4.12 The Major Study

As a result of the quadratic effect exhibited by the vertical vascular distribution down the periodontal ligament of the pilot study sample, Leppard (1983) recommended that six equidistant sections be used for the recording of the remaining data. This routine constituted the major study.

The present study illustrates the use of systematic stratified sections through anisotropic tissue, with structural grading. This method may affect the emphasis placed on the analysis of stereological parameters.

4.13 The Collection of Morphometric Information

4.13.1 Use of Projector

The slides were placed in a Kodak Carousel S Projector (Eastman Kodak Co., Rochester, N.Y., U.S.A.), which was then positioned on a table set at a fixed distance from the screen. The lens on the projector was a Kodak Vario-Retinar 70-120 (Eastman Kodak Co., Rochester, N.Y., U.S.A.). Neither the projector nor the table were moved anteriorly or posteriorly from their allocated positions. Only lateral movements of the

projector were undertaken to allow for the width of the image projected onto the screen.

4.13.2 Use of Digitizer

The semi-automatic tracing device used during this study was the "Manual Optical Picture Analyser" (MOP-1, Carl Zeiss Inc., Oberkochen, West Germany). More advanced computer systems have been described by Hoppeler, Mathieu, Bretz, Krauer and Weibel (1980) and Prothero and Prothero (1982).

The MOP system operated on the magneto-strictive principle. Magnetized steel wires were contained within the measuring tablet. These wires ran horizontally and vertically at regular intervals, and at right angles to each other. The wire grid formed a magnetic field with pulse directions following along the horizontal and vertical wires. The pulses originated alternatively from the two sides of the tablet and became superimposed, resulting in a constant pulse speed. A pulse count was established when a stylus intercepted the magneto-strictive pulses. The measurement of the coordinates with the tablet and stylus relied in the measuring of the time lag between the discharge of the pulse and its reception. The count was transformed into a distance or coordinate point by the microprocessor and, with the use of X and Y coordinates, various geometric magnitudes were established. These values were recorded in absolute millimetres and millimetres squared.

The component parts of the MOP-1 system were:

A. The Display/Keyboard Unit

B. The Measuring Tablet

C. The Stylus

D. The Printer.

A. The Display/Keyboard Unit

The digital display contained a total of 16 digits to indicate values, channels, subroutine codes and other information. The red warning light located on the upper left of the display would light up under the following circumstances:

1. Before a function had been selected.
2. Whenever the stylus was greater than 4 mm from the surface of the tablet.
3. When the active area of the tablet was insufficiently magnetized.
4. If the stylus connecting cable was defective.
5. When the console electronics were defective.

It is possible to substitute a magnification or reduction factor for the absolute millimetre measurement and, in this manner, compensate for the magnification or reduction of an object so that the object's original dimensions could be displayed and printed.

The keyboard was divided into the following four groups of keys:

1. Prime Functions.
2. Process Functions.
3. Channels and Input Codes.
4. Special Inputs.

Detailed information concerning these functions, codes and inputs can be found in Appendix VII.

The function sequence derived from this present morphometric study of the blood vessels of the periodontal ligament was as follows:

1. SET
2. PRINT
3. SUMM.
4. AREA
5. ENTER
6. INPUT : A maximum of six numbers of identification,
   e.g. 235712
   where 23 = animal number
   57 = slide number
   1 = right side of mandible
   2 = distal root of second molar.
7. ENTER
8. NUMBER 0 : Use the stylus and draw the perimeter of the vessel No. 0.

9. ENTER 1 : Use the stylus and draw the perimeter of the vessel No. 1.

10. ENTER vessel areas 2 to 9.

11. PRINT

On the completion of the print out, the memory of the previous vessels is cleared while still retaining instructions 1 to 7 and then one proceeds to enter the next 10 vessel areas.

B. The Measuring Tablet

The measuring tablet was fixed and mounted vertically on a wall. It was connected to the rear panel of the keyboard console. Re-magnetization of the tablet surface was found to be necessary every 10 weeks using a magnetizing bar.

A3 size white paper was attached to the surface of the tablet and the image of the vessels of the periodontal ligament was projected onto this paper. At first, the image was traced using a fine lead pencil and subsequently, using the stylus, all relevant data were traced and then recorded within the confines of the tablet which allowed measurements to be made.

C. The Stylus

The stylus contained a ball-point ink pen which, with the application of firm pressure on the paper over the tablet, resulted in the emission of a high pitched buzzer sound. Once the image had been circumscribed with the pen returning to the

original starting point, a louder signal indicated the termination of the measurement process. The stylus was connected to the keyboard console.

D. The Printer

A movable printing head carrying seven needles moved over the metallised paper by means of an electric motor. Through sliding contact, the needles received pulses of current which burnt the metal coating on the paper leaving an imprint of the measured values. The printer was located on the lower right corner of the keyboard console.

4.13.3 Data Recording Sheets

The relevant information was recorded on data collection sheets as represented in Appendix VIII. The key to this recorded information can be found in Appendix IX.

4.14 Degree of Magnification

Using a stage micrometer with the specimen glass slide in the Axiomat N.D.C. Microscope (Carl Zeiss Inc., Oberkochen, West Germany), the magnification factor of the illuminated specimen on the measuring tablet was determined to be 6,100x the original.

Cruz-Orive (1982) reported that the analysis by light microscopy of a given set of sections, was free from magnification fluctuations. To ensure as constant a projection magnification as

possible, the images were traced within the confines of the tablet surface.

The minimum vessel area recorded was one square millimetre which, at a magnification of 6,100x, resulted in an actual area of 164 square microns. Assuming a circular vessel, its diameter would be 14.46 microns, which in effect excluded the capillary system within the periodontal ligament (Rhodin, 1967, 1968).

In tracing the vessel lumen, there was a noticeable absence of thick vessel walls and, in the majority of instances, the thickness of the pen nib of the stylus represented the magnified thickness of the vessel wall.

4.15 The Error of the Method

Ten randomly selected histological slides were chosen out of the 230 available. These were re-projected, re-traced and areas were recorded again using the same semi-automatic tracing device. All criteria were maintained in the pilot study, in the analysis of the remaining data, and during the examination of the error of the method. This information was then submitted for statistical inspection.

A general logistic model was found to be appropriate to relate the proportion of blood vessels located in the various depths and lateral extensions of the periodontal ligament. The model was fitted using non-linear maximum likelihood techniques, and large sample estimates of the errors of fitted parameters

were calculated using standard methods. Likelihood ratio tests were used to determine the significance or otherwise of the various characteristics. All the calculations were performed using the B.M.D.P. (Biomedical Package) Statistical Software (1981 Edition) text.

CHAPTER 5:

FINDINGS

The vascular volume figures reported in this study represent minimal percentage values, due to the exclusion of the vast capillary system and the measurement of vessel lumen areas, without the inclusion of vessel walls.

5.1 Histological Staining Methods

The three staining methods used have included Pollack's trichrome, Verhoeff's iron haematoxylin and Miller's stain.

5.1.1 Pollack's Trichrome

The Pollack's trichrome stain used was modified from the technique of Luna (1968) due to the omission of the step involving nuclear staining with Geimsa's solution. Sections stained with Pollack's trichrome for 20 seconds exhibited green-stained collagen components of the periodontal ligament, and red-stained bone and dentine as shown in Figure 4.

5.1.2 Verhoeff's Iron Haematoxylin

Verhoeff's procedure constituted staining with an iodine, ferric chloride and haematoxylin mixture, followed by differentiation with ferric chloride to reveal the presence of elastic fibres.

Yellow-stained cytoplasm and muscle and red-stained collagen was achieved, but very little was found in the way of black-stained elastic fibres within the periodontal ligament and the walls of ligament vessels. However, the major arterial supply to the mandible, the inferior alveolar artery, exhibited black-stained elastic tissue with a distinctive wavy course indicating the internal elastic lamina.

5.1.3 Miller's Stain

Miller's stain was developed to demonstrate both coarse and fine elastic fibres. Neither the Verhoeff's iron haematoxylin, nor the Miller's stain, differentiate between oxytalan and elastic fibres.

Histological sections stained with Miller's revealed the presence of yellow-stained cytoplasm and red-stained collagen, with little evidence of black-stained elastic fibres within the periodontal ligament, or in ligament vessel walls. On the other hand, the inferior alveolar artery demonstrated its internal elastic lamina definitively as shown in Figure 5.

At no stage did any of the tissues stained with the three alternative methods reveal evidence of arteriosclerotic or atherosclerotic lesions. This included the inferior alveolar artery.

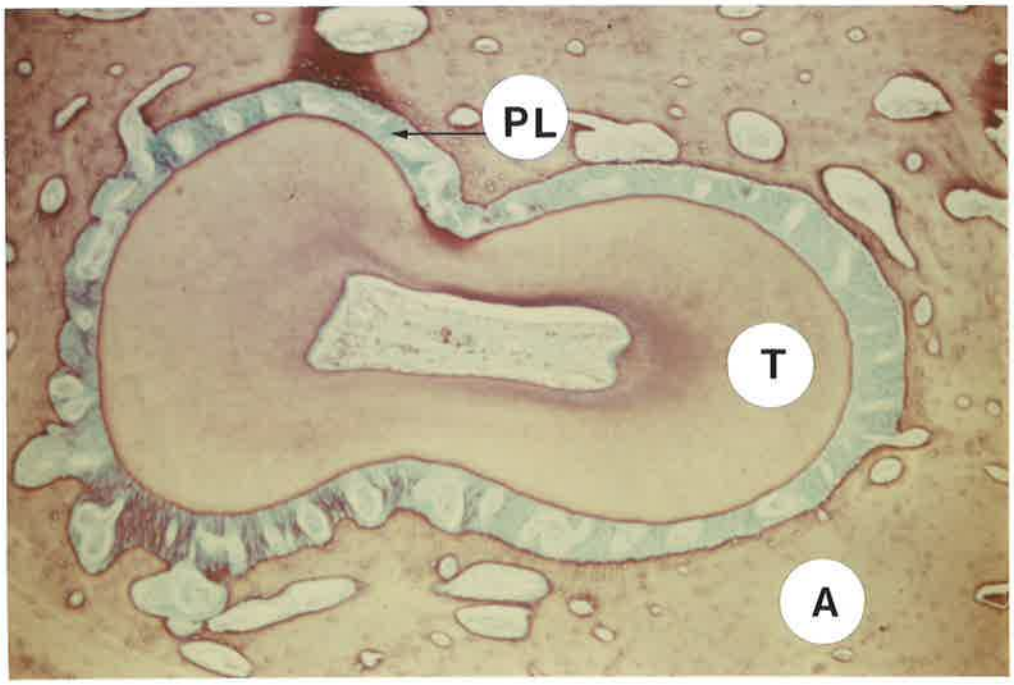


Figure 4. Photomicrograph of a serial section of animal 30, slide 36b, at a depth of 822 microns in the cervical third of the marmoset right mandibular second molar.
Pollack's trichrome x150.

PL - Periodontal ligament
A - Alveolar bone
T - Tooth structure

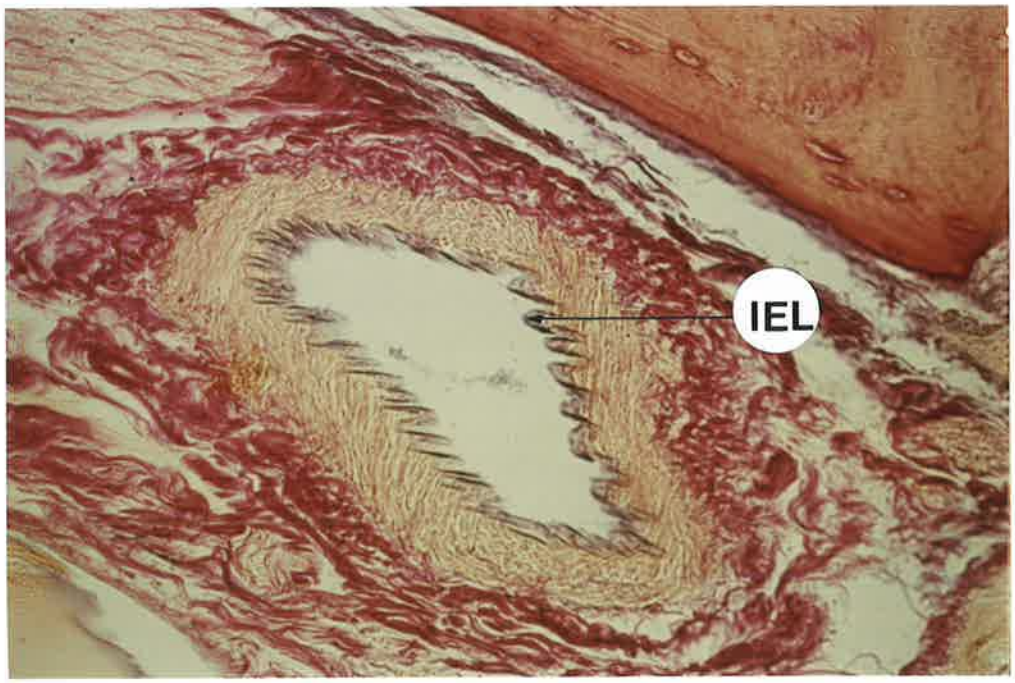


Figure 5. Photomicrograph of a serial section of animal 324, at a depth of 1488 microns showing the right inferior alveolar artery.
Miller's stain x950.

IEL - Internal elastic lamina

5.2 Statistical Analysis

Having collated the data, the variable representing the total area of vessels within a particular zone was transformed to the logit scale and analysed using the regression programme IR (from the B.M.D.P. Statistical Software 1981 Edition). As a result of this analysis, statistical differences between zones were found. Moreover, the relationship of the above variable to animal, depth of the periodontal ligament, right or left side of the mandible, mesial, distal or single root and quadrant was different for each of the three zones. Subsequent investigations involved the analysis of each zone separately.

The detailed analysis of zones involved the use of a general linear mixed model on a logit scale using the 3V programme. By implementing the fitted parameters obtained from this programme, a stylized periodontal ligament vascular distribution model was tabulated for each of the three zones showing the effects of the significant statistical parameters for an animal with average, above average and below average vascularity. This information can be found in Tables 2, 3, 4 for zone 1, Tables 5, 6, 7 for zone 2 and Tables 8, 9, 10 for zone 3 involving quadrants 1, 2, 3 and 4. Tables 13, 14 and 15 show percentage vascular volume changes in zone 1 for quadrants 5, 6, 7 and 8.

5.3 The Repeatability of the Method

Two hundred and forty observations (columns of information) were made over the ten photomicrographs which were randomly

selected and then projected, traced, digitized and tabulated a second time. The information which was tested as part of the variability trial included the following:

Areas of zone 1, zone 2 and zone 3.

The number of measurements for each zone within each octant.

The summation of these vascular areas for each zone within each octant.

The variability data and its counterpart from the major study were tested using a series of paired t tests from programme 3D. This information is presented in Table 11. There was no significant difference, on average, for any of the aforementioned five variables at the one percent level.

5.4 Lateral and Vertical Vascular Distribution

It is of little relevance to describe the vertical vascular distribution of the periodontal ligament without an immediate reference to the zone. Vertical distributions are in effect zone dependent. It is for this reason that these results have been presented collectively.

The pilot study revealed the presence of a vertical vascular distribution with a quadratic effect being the greater in the cervical third, the least in the middle third, and between these two levels for the apical third of the ligament.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	1.84	1.44	2.87	1.59	1.23	0.96	1.92	1.06
200	2.05	1.61	3.19	1.77	1.36	1.07	2.13	1.18
300	2.27	1.78	3.53	1.96	1.51	1.18	2.36	1.30
400	2.50	1.96	3.89	2.16	1.67	1.31	2.60	1.44
500	2.75	2.16	4.27	2.38	1.84	1.44	2.86	1.59
600	3.02	2.37	4.67	2.61	2.01	1.58	3.14	1.74
700	3.30	2.59	5.10	2.85	2.20	1.73	3.43	1.90
800	3.59	2.82	5.54	3.10	2.40	1.88	3.73	2.07
900	3.89	3.06	6.00	3.37	2.61	2.05	4.04	2.25
1000	4.20	3.31	6.47	3.64	2.82	2.21	4.37	2.44
1100	4.53	3.57	6.95	3.92	3.04	2.39	4.71	2.63
1200	4.86	3.83	7.45	4.21	3.27	2.57	5.05	2.82
1300	5.20	4.11	7.95	4.51	3.50	2.75	5.40	3.03
1400	5.54	4.38	8.46	4.81	3.73	2.94	5.76	3.23
1500	5.89	4.66	8.97	5.11	3.97	3.13	6.12	3.44
1600	6.23	4.93	9.48	5.41	4.21	3.32	6.48	3.64

Table 2: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of below average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	2.35	1.84	3.65	2.03	1.56	1.22	2.44	1.35
200	2.61	2.05	4.05	2.25	1.74	1.36	2.71	1.50
300	2.89	2.27	4.47	2.49	1.93	1.51	3.00	1.66
400	3.18	2.50	4.93	2.75	2.13	1.67	3.31	1.84
500	3.50	2.75	5.40	3.03	2.34	1.84	3.64	2.02
600	3.83	3.02	5.91	3.32	2.57	2.01	3.98	2.22
700	4.18	3.29	6.44	3.62	2.80	2.20	4.35	2.42
800	4.55	3.59	6.98	3.94	3.05	2.40	4.73	2.64
900	4.93	3.89	7.55	4.27	3.31	2.60	5.12	2.86
1000	5.32	4.20	8.14	4.61	3.58	2.82	5.53	3.10
1100	5.73	4.53	8.74	4.97	3.86	3.04	5.95	3.34
1200	6.14	4.86	9.35	5.33	4.14	3.26	6.38	3.59
1300	6.56	5.20	9.96	5.70	4.44	3.50	6.82	3.84
1400	6.99	5.54	10.59	6.08	4.73	3.73	7.26	4.10
1500	7.42	5.89	11.21	6.45	5.03	3.97	7.70	4.36
1600	7.85	6.23	11.83	6.83	5.33	4.21	8.15	4.62

Table 3: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	2.99	2.35	4.63	2.58	1.99	1.56	3.11	1.72
200	3.31	2.61	5.13	2.87	2.22	1.74	3.45	1.91
300	3.67	2.89	5.66	3.17	2.45	1.93	3.81	2.12
400	4.04	3.18	6.22	3.50	2.71	2.13	4.20	2.34
500	4.44	3.50	6.82	3.84	2.98	2.34	4.61	2.57
600	4.85	3.83	7.44	4.21	3.26	2.56	5.05	2.82
700	5.29	4.18	8.09	4.59	3.56	2.80	5.50	3.08
800	5.75	4.55	8.77	4.99	3.88	3.05	5.97	3.35
900	6.22	4.93	9.47	5.41	4.20	3.31	6.47	3.64
1000	6.71	5.32	10.19	5.83	4.54	3.58	6.97	3.93
1100	7.22	5.72	10.92	6.28	4.89	3.86	7.49	4.24
1200	7.73	6.14	11.66	6.73	5.25	4.14	8.03	4.55
1300	8.25	6.56	12.41	7.19	5.61	4.43	8.56	4.87
1400	8.78	6.99	13.17	7.65	5.98	4.73	9.11	5.19
1500	9.31	7.42	13.92	8.12	6.35	5.03	9.65	5.52
1600	9.83	7.84	14.66	8.58	6.72	5.33	10.20	5.84

Table 4: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of above average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	2.88	2.88	2.88	2.88	2.21	2.21	2.21	2.21
200	3.40	3.40	3.40	3.40	2.62	2.62	2.62	2.62
300	3.98	3.98	3.98	3.98	3.07	3.07	3.07	3.07
400	4.59	4.59	4.59	4.59	3.54	3.54	3.54	3.54
500	5.22	5.22	5.22	5.22	4.04	4.04	4.04	4.04
600	5.87	5.87	5.87	5.87	4.54	4.54	4.54	4.54
700	6.51	6.51	6.51	6.51	5.05	5.05	5.05	5.05
800	7.14	7.14	7.14	7.14	5.54	5.54	5.54	5.54
900	7.73	7.73	7.73	7.73	6.01	6.01	6.01	6.01
1000	8.26	8.26	8.26	8.26	6.44	6.44	6.44	6.44
1100	8.74	8.74	8.74	8.74	6.81	6.81	6.81	6.81
1200	9.13	9.13	9.13	9.13	7.13	7.13	7.13	7.13
1300	9.43	9.43	9.43	9.43	7.37	7.37	7.37	7.37
1400	9.63	9.63	9.63	9.63	7.52	7.52	7.52	7.52
1500	9.72	9.72	9.72	9.72	7.60	7.60	7.60	7.60
1600	9.70	9.70	9.70	9.70	7.58	7.58	7.58	7.58

Table 5: A stylized periodontal ligament vascular distribution model tabulated for Zone 2 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of below average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	3.85	3.85	3.85	3.85	2.97	2.97	2.97	2.97
200	4.55	4.55	4.55	4.55	3.51	3.51	3.51	3.51
300	5.30	5.30	5.30	5.30	4.10	4.10	4.10	4.10
400	6.10	6.10	6.10	6.10	4.73	4.73	4.73	4.73
500	6.93	6.93	6.93	6.93	5.38	5.38	5.38	5.38
600	7.77	7.77	7.77	7.77	6.05	6.05	6.05	6.05
700	8.61	8.61	8.61	8.61	6.71	6.71	6.71	6.71
800	9.41	9.41	9.41	9.41	7.35	7.35	7.35	7.35
900	10.17	10.17	10.17	10.17	7.96	7.96	7.96	7.96
1000	10.86	10.86	10.86	10.86	8.51	8.51	8.51	8.51
1100	11.46	11.46	11.46	11.46	9.00	9.00	9.00	9.00
1200	11.96	11.96	11.96	11.96	9.40	9.40	9.40	9.40
1300	12.34	12.34	12.34	12.34	9.71	9.71	9.71	9.71
1400	12.59	12.59	12.59	12.59	9.91	9.91	9.91	9.91
1500	12.71	12.71	12.71	12.71	10.01	10.01	10.01	10.01
1600	12.68	12.68	12.68	12.68	9.99	9.99	9.99	9.99

Table 6: A stylized periodontal ligament vascular distribution model tabulated for Zone 2 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	5.14	5.14	5.14	5.14	3.97	3.97	3.97	3.97
200	6.06	6.06	6.06	6.06	4.69	4.69	4.69	4.69
300	7.04	7.04	7.04	7.04	5.47	5.47	5.47	5.47
400	8.08	8.08	8.08	8.08	6.29	6.29	6.29	6.29
500	9.15	9.15	9.15	9.15	7.14	7.14	7.14	7.14
600	10.23	10.23	10.23	10.23	8.01	8.01	8.01	8.01
700	11.29	11.29	11.29	11.29	8.86	8.86	8.86	8.86
800	12.32	12.32	12.32	12.32	9.69	9.69	9.69	9.69
900	13.28	13.28	13.28	13.28	10.47	10.47	10.47	10.47
1000	14.14	14.14	14.14	14.14	11.18	11.18	11.18	11.18
1100	14.90	14.90	14.90	14.90	11.79	11.79	11.79	11.79
1200	15.52	15.52	15.52	15.52	12.30	12.30	12.30	12.30
1300	15.99	15.99	15.99	15.99	12.69	12.69	12.69	12.69
1400	16.30	16.30	16.30	16.30	12.95	12.95	12.95	12.95
1500	16.45	16.45	16.45	16.45	13.07	13.07	13.07	13.07
1600	16.42	16.42	16.42	16.42	13.05	13.05	13.05	13.05

Table 7: A stylized periodontal ligament vascular distribution model tabulated for Zone 2 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of above average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	0.14	0.14	0.14	0.14	0.06	0.06	0.06	0.06
200	0.17	0.17	0.17	0.17	0.08	0.08	0.08	0.08
300	0.19	0.19	0.19	0.19	0.09	0.09	0.09	0.09
400	0.22	0.22	0.22	0.22	0.10	0.10	0.10	0.10
500	0.25	0.25	0.25	0.25	0.11	0.11	0.11	0.11
600	0.28	0.28	0.28	0.28	0.13	0.13	0.13	0.13
700	0.30	0.30	0.30	0.30	0.14	0.14	0.14	0.14
800	0.32	0.32	0.32	0.32	0.14	0.14	0.14	0.14
900	0.33	0.33	0.33	0.33	0.15	0.15	0.15	0.15
1000	0.33	0.33	0.33	0.33	0.15	0.15	0.15	0.15
1100	0.33	0.33	0.33	0.33	0.15	0.15	0.15	0.15
1200	0.32	0.32	0.32	0.32	0.15	0.15	0.15	0.15
1300	0.31	0.31	0.31	0.31	0.14	0.14	0.14	0.14
1400	0.29	0.29	0.29	0.29	0.13	0.13	0.13	0.13
1500	0.26	0.26	0.26	0.26	0.12	0.12	0.12	0.12
1600	0.24	0.24	0.24	0.24	0.11	0.11	0.11	0.11

Table 8: A stylized periodontal ligament vascular distribution model tabulated for Zone 3 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of below average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	0.23	0.23	0.23	0.23	0.10	0.10	0.10	0.10
200	0.27	0.27	0.27	0.27	0.12	0.12	0.12	0.12
300	0.32	0.32	0.32	0.32	0.15	0.15	0.15	0.15
400	0.37	0.37	0.37	0.37	0.17	0.17	0.17	0.17
500	0.41	0.41	0.41	0.41	0.19	0.19	0.19	0.19
600	0.45	0.45	0.45	0.45	0.21	0.21	0.21	0.21
700	0.49	0.49	0.49	0.49	0.22	0.22	0.22	0.22
800	0.52	0.52	0.52	0.52	0.24	0.24	0.24	0.24
900	0.54	0.54	0.54	0.54	0.24	0.24	0.24	0.24
1000	0.54	0.54	0.54	0.54	0.25	0.25	0.25	0.25
1100	0.54	0.54	0.54	0.54	0.25	0.25	0.25	0.25
1200	0.53	0.53	0.53	0.53	0.24	0.24	0.24	0.24
1300	0.51	0.51	0.51	0.51	0.23	0.23	0.23	0.23
1400	0.47	0.47	0.47	0.47	0.22	0.22	0.22	0.22
1500	0.43	0.43	0.43	0.43	0.20	0.20	0.20	0.20
1600	0.39	0.39	0.39	0.39	0.18	0.18	0.18	0.18

Table 9: A stylized periodontal ligament vascular distribution model tabulated for Zone 3 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of average vascularity.

Depth (μm)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
100	0.38	0.38	0.38	0.38	0.17	0.17	0.17	0.17
200	0.45	0.45	0.45	0.45	0.20	0.20	0.20	0.20
300	0.52	0.52	0.52	0.52	0.24	0.24	0.24	0.24
400	0.60	0.60	0.60	0.60	0.27	0.27	0.27	0.27
500	0.68	0.68	0.68	0.68	0.31	0.31	0.31	0.31
600	0.74	0.74	0.74	0.74	0.34	0.34	0.34	0.34
700	0.80	0.80	0.80	0.80	0.37	0.37	0.37	0.37
800	0.85	0.85	0.85	0.85	0.39	0.39	0.39	0.39
900	0.88	0.88	0.88	0.88	0.40	0.40	0.40	0.40
1000	0.89	0.89	0.89	0.89	0.41	0.41	0.41	0.41
1100	0.89	0.89	0.89	0.89	0.41	0.41	0.41	0.41
1200	0.87	0.87	0.87	0.87	0.40	0.40	0.40	0.40
1300	0.83	0.83	0.83	0.83	0.38	0.38	0.38	0.38
1400	0.78	0.78	0.78	0.78	0.36	0.36	0.36	0.36
1500	0.71	0.71	0.71	0.71	0.33	0.33	0.33	0.33
1600	0.64	0.64	0.64	0.64	0.29	0.29	0.29	0.29

Table 10: A stylized periodontal ligament vascular distribution model tabulated for Zone 3 (Q1,2,3,4) showing the effects of significant statistical parameters for animals of above average vascularity.

Variables Tested	Mean Difference	Standard Error	t Value	p Value
Area of zone 1	.24	.63	.37 n.s.	.71
Area of zone 2	-.16	.48	-.33 n.s.	.74
Area of zone 3	1.06	.53	2.01 n.s.	.05
Number of measurements	-.004	.009	-.45 n.s.	.66
Sum of measurements	.08	.16	.5 n.s.	.62

Table 11. Statistical information on the variability trial used to test the repeatability of the method.

	A = Ave. Ligament Area (mm^2)	Standard Error	B = Ave. Vascular Area (mm^2)	Standard Error	Ratio B/A
Zone 1	1318.06	34.03	117.86	5.49	.089
Zone 2	968.21	24.81	117.56	4.17	.121
Zone 3	822.32	22.02	21.83	1.43	.026

Table 12. Average ligament and vascular areas for zones 1, 2 and 3.

Depth (um)	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
	Q5	Q6	Q7	Q8	Q5	Q6	Q7	Q8
100	1.93	1.93	1.93	1.93	1.20	1.20	1.20	1.20
200	2.14	2.14	2.14	2.14	1.34	1.34	1.34	1.34
300	2.37	2.37	2.37	2.37	1.48	1.48	1.48	1.48
400	2.61	2.61	2.61	2.61	1.63	1.63	1.63	1.63
500	2.86	2.86	2.86	2.86	1.79	1.79	1.79	1.79
600	3.13	3.13	3.13	3.13	1.96	1.96	1.96	1.96
700	3.40	3.40	3.40	3.40	2.13	2.13	2.13	2.13
800	3.69	3.69	3.69	3.69	2.31	2.31	2.31	2.31
900	3.98	3.98	3.98	3.98	2.50	2.50	2.50	2.50
1000	4.27	4.27	4.27	4.27	2.69	2.69	2.69	2.69
1100	4.57	4.57	4.57	4.57	2.88	2.88	2.88	2.88
1200	4.88	4.88	4.88	4.88	3.07	3.07	3.07	3.07
1300	5.18	5.18	5.18	5.18	3.27	3.27	3.27	3.27
1400	5.47	5.47	5.47	5.47	3.46	3.46	3.46	3.46
1500	5.76	5.76	5.76	5.76	3.65	3.65	3.65	3.65
1600	6.05	6.05	6.05	6.05	3.83	3.83	3.83	3.83

Table 13: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q5,6,7,8) showing the effects of significant statistical parameters for animals of below average vascularity.

	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
Depth (um)	Q5	Q6	Q7	Q8	Q5	Q6	Q7	Q8
100	2.46	2.46	2.46	2.46	1.54	1.54	1.54	1.54
200	2.73	2.73	2.73	2.73	1.71	1.71	1.71	1.71
300	3.01	3.01	3.01	3.01	1.89	1.89	1.89	1.89
400	3.32	3.32	3.32	3.32	2.08	2.08	2.08	2.08
500	3.64	3.64	3.64	3.64	2.28	2.28	2.28	2.28
600	3.97	3.97	3.97	3.97	2.49	2.49	2.49	2.49
700	4.31	4.31	4.31	4.31	2.71	2.71	2.71	2.71
800	4.67	4.67	4.67	4.67	2.94	2.94	2.94	2.94
900	5.04	5.04	5.04	5.04	3.18	3.18	3.18	3.18
1000	5.41	5.41	5.41	5.41	3.42	3.42	3.42	3.42
1100	5.78	5.78	5.78	5.78	3.66	3.66	3.66	3.66
1200	6.16	6.16	6.16	6.16	3.90	3.90	3.90	3.90
1300	6.53	6.53	6.53	6.53	4.14	4.14	4.14	4.14
1400	6.90	6.90	6.90	6.90	4.39	4.39	4.39	4.39
1500	7.26	7.26	7.26	7.26	4.62	4.62	4.62	4.62
1600	7.61	7.61	7.61	7.61	4.85	4.85	4.85	4.85

Table 14: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q5,6,7,8) showing the effects of significant statistical parameters for animals of average vascularity.

	Percentage Vascular Volume (Right Molar)				Percentage Vascular Volume (Left Molar)			
Depth (um)	Q5	Q6	Q7	Q8	Q5	Q6	Q7	Q8
100	3.12	3.12	3.12	3.12	1.96	1.96	1.96	1.96
200	3.47	3.47	3.47	3.47	2.17	2.17	2.17	2.17
300	3.83	3.83	3.83	3.83	2.40	2.40	2.40	2.40
400	4.21	4.21	4.21	4.21	2.65	2.65	2.65	2.65
500	4.61	4.61	4.61	4.61	2.90	2.90	2.90	2.90
600	5.03	5.03	5.03	5.03	3.17	3.17	3.17	3.17
700	5.46	5.46	5.46	5.46	3.45	3.45	3.45	3.45
800	5.90	5.90	5.90	5.90	3.74	3.74	3.74	3.74
900	6.36	6.36	6.36	6.36	4.03	4.03	4.03	4.03
1000	6.82	6.82	6.82	6.82	4.33	4.33	4.33	4.33
1100	7.28	7.28	7.28	7.28	4.64	4.64	4.64	4.64
1200	7.75	7.75	7.75	7.75	4.94	4.94	4.94	4.94
1300	8.21	8.21	8.21	8.21	5.25	5.25	5.25	5.25
1400	8.67	8.67	8.67	8.67	5.55	5.55	5.55	5.55
1500	9.11	9.11	9.11	9.11	5.84	5.84	5.84	5.84
1600	9.54	9.54	9.54	9.54	6.13	6.13	6.13	6.13

Table 15: A stylized periodontal ligament vascular distribution model tabulated for Zone 1 (Q5,6,7,8) showing the effects of significant statistical parameters for animals of above average vascularity.

Any examination of the data on the lateral vascular distribution of the periodontal ligament would necessitate a separation into zones 1, 2 and 3. This has been highlighted by the information presented in Table 12 which shows that the average summation of vascular areas in zones 1 and 2 are almost the same, whereas their respective average ligament areas are markedly dissimilar.

5.4.1 Zone 1

A. For Quadrants 1, 2, 3 and 4

Zone 1 is that lateral third of the periodontal ligament closest to alveolar bone. Within this zone, variations exist depending on the animal, the side of the mandible, the quadrant and the depth of the periodontal ligament. The only variable not to have a detectable influence in zone 1 was the respective root (mesial, distal or single).

Graphical representations of quadrant, side and depth effects in zone 1 are illustrated in Figures 6, 7 and 8 for average, above average and below average vascularity.

Within zone 1, the following information has emerged:

1. All quadrants are statistically different from each other, with quadrant 3 having an appreciably greater vascular volume than quadrants 1, 4 or 2. These latter three quadrants were similar in their

vascularity although the vascular volume in quadrant 1 was greater than quadrant 4 which in turn was greater than that in quadrant 2.

2. The vertical vascular distribution was least for the cervical third, greatest for the apical third and between these two extremes in the middle third.

3. The periodontal vascular volume of the second mandibular molar on the right side of the mandible, at any ligament depth, in any quadrant, was greater than that on the left side of the mandible. This was statistically significant at the one percent level.

4. The average percentage vascular volume in zone 1 derived from the data was 8.9 percent.

B. For Quadrants 5, 6, 7 and 8

Within zone 1, for quadrants 5, 6, 7 and 8, variations exist depending on the animal, the side of the mandible and the depth of the periodontal ligament. The quadrant effect seen with quadrants 1, 2, 3 and 4 is no longer evident with quadrants 5, 6, 7 and 8. As such, quadrants 5, 6, 7 and 8 express the same percentage vascular volume changes with ligament depth as shown in Tables 13, 14 and 15.

A possible explanation for this finding could be attributed to the redistribution of octants 6 and 7, which together constituted quadrant 3, into quadrants 6 and 7. The relocation of these octants,

which contained a greater volume of vessels than quantified in other regions, dissipated the effect seen in quadrant 3.

All other factors with regard to vertical vascular distribution, right and left molars and average percentage vascular volume figures, remain the same as for quadrants 1, 2, 3 and 4.

5.4.2 Zone 2

Zone 2 is the lateral middle third of the periodontal ligament. There are no detectable quadrant or root effects within this zone. Influences due to the animal, the side of the mandible and the depth of the ligament, were detected. Figures 9, 10 and 11 represent graphically the relationship of these relevant variables in zone 2 with respect to an average, above average and below average vascularity for quadrants 1, 2, 3 and 4.

Percentage vascular volume figures for quadrants 5, 6, 7 and 8 were slightly numerically different from those of quadrants 1, 2, 3 and 4, but this difference was not significant statistically at the one percent level.

The following information is worthy of future discussion:

1. The vascular volume in each quadrant was the same within zone 2.

2. The vertical vascular distribution was least in the cervical third, increasing through the middle third and peaking at the apical third, where a plateau effect was evident.

3. The periodontal vascular volume of the second mandibular molar on the right side of the mandible at any ligament depth, in any quadrant, was greater than that on the left side of the mandible. This was significant statistically at the one percent level.

4. The average percentage vascular volume on zone 2, derived from the data, was 13.1 percent.

5.4.3 Zone 3

Zone 3 is that lateral third of the periodontal ligament closest to the tooth surface. In that there are only animal, side of the mandible and ligament depth effects, and no detectable quadrant or root effects, this zone is similar to zone 2. However, because of the relatively small percentage vascular volume figures for zone 3, it was decided not to represent all of them graphically. Figure 12 illustrates the variation of percentage vascular volume with ligament depth for zone 3 on the right side of the mandible, for an animal of average vascularity in quadrants 1, 2, 3 and 4.

Percentage vascular volume figures for quadrants 5, 6, 7 and 8 were slightly numerically different from those of quadrants 1, 2, 3 and 4, but this difference was not significant statistically at the one percent level.

The following information has been derived from zone 3 data:

1. The vascular volume in each quadrant was the same in zone 3.

2. The vertical vascular distribution was such that the least volume appears in the cervical third, the greatest at the middle third and between these two extremes at the apical third. However, there was no great numerical difference over this range.

3. As in zones 1 and 2, zone 3 shows a predilection for a greater percentage vascular volume on the right side of the mandible in comparison with the left, at any ligament depth, in any quadrant.

4. The average percentage vascular volume in zone 3, derived from the data was 2.6 percent.

Both the pilot and major studies were consistent in their findings regarding the proportional vascular volume found in zones 1, 2 and 3. The greatest vascular volume was reported in zone 2, the least in zone 3, and between these two extremes in zone 1.

5.5 Vascular Distribution According to Quadrant

Because of the absence of a detectable quadrant effect in zones 2 and 3 for quadrants 1, 2, 3 and 4, any discussion on the vascular distribution according to quadrant is only of relevance in zone 1 where

$$Q3 > Q1 > Q4 > Q2.$$

However, there does not appear to be an appreciable difference in the vascularity between quadrants 1, 4 and 2.

From the data, it has been possible to derive a percentage vascular volume figure for each quadrant which is representative of all three zones collectively. These are as follows:

Quadrant 1	7.7%
Quadrant 2	7.6%
Quadrant 3	8.5%
Quadrant 4	9.0%.

These numerical values should not be quoted out of context, since they do not account for the fact that quadrant effects are zone dependent, being relevant and significant only in zone 1 for quadrants 1, 2, 3 and 4.

5.6 Vascular Distribution According to the Side of the Mandible

In all zones, at any depth of the peirodontal ligament, in all quadrants, the percentage periodontal vascular volume of the second molar appeared to be greater on the right side of the mandible than on the left. The pilot study involved the analysis of molars taken from different animals, but from the same side of the mandible, thus any differentiation based on this variable was not possible in the original study.

5.7 Vascular Distribution According to Mandibular Molar Root Conformation

A detectable statistical significance of whether the tooth had a mesial, distal, or was single rooted, was not evident for all three zones of the major study. However, in the pilot study, the mesial root appeared to have a greater vascular volume than the distal root. This phenomenon was consistent between animals, in all octants, zones and ligament depths.

5.8 Vascular Distribution According to Diet

There was no statistical evidence of any detectable effect on vascularity between animals due to their respective diets. Evaluations involved the use of the 3V programme.

The statistical analysis was limited by the disproportionate and small number of animals in each dietary category, and with the variation in the duration that the diets were implemented.

5.9 Total Percentage Vascular Volume

The total percentage vascular volume for each animal was tabulated as follows:

Animal Number	% Vascular Volume
324	5.7
24	9.8
3	7.1
28	6.6
23	11.8
32	5.9
19	7.6
30	7.0

Table 16. Average total percentage vascular volume for each animal.

Using the data accumulated from both the pilot and major studies, the average periodontal vascular volume for the second mandibular molar of the marmoset monkey was 8.3 ± .4 percent (mean ±2 standard errors). The representative figure derived from the stylized periodontal ligament vascular distribution was 7.5 percent.

It is of some interest to note that these values are similar and, as such, the statistical model of the vascular distribution and the original data from which it was derived are reasonable and acceptable representations of each other.

Statistically significant data forms the basis for the major part of any discussion involving biological research today. No matter how reassuring a role this information plays, one must always be aware that a statistical significance may not always equate with a biological significance.

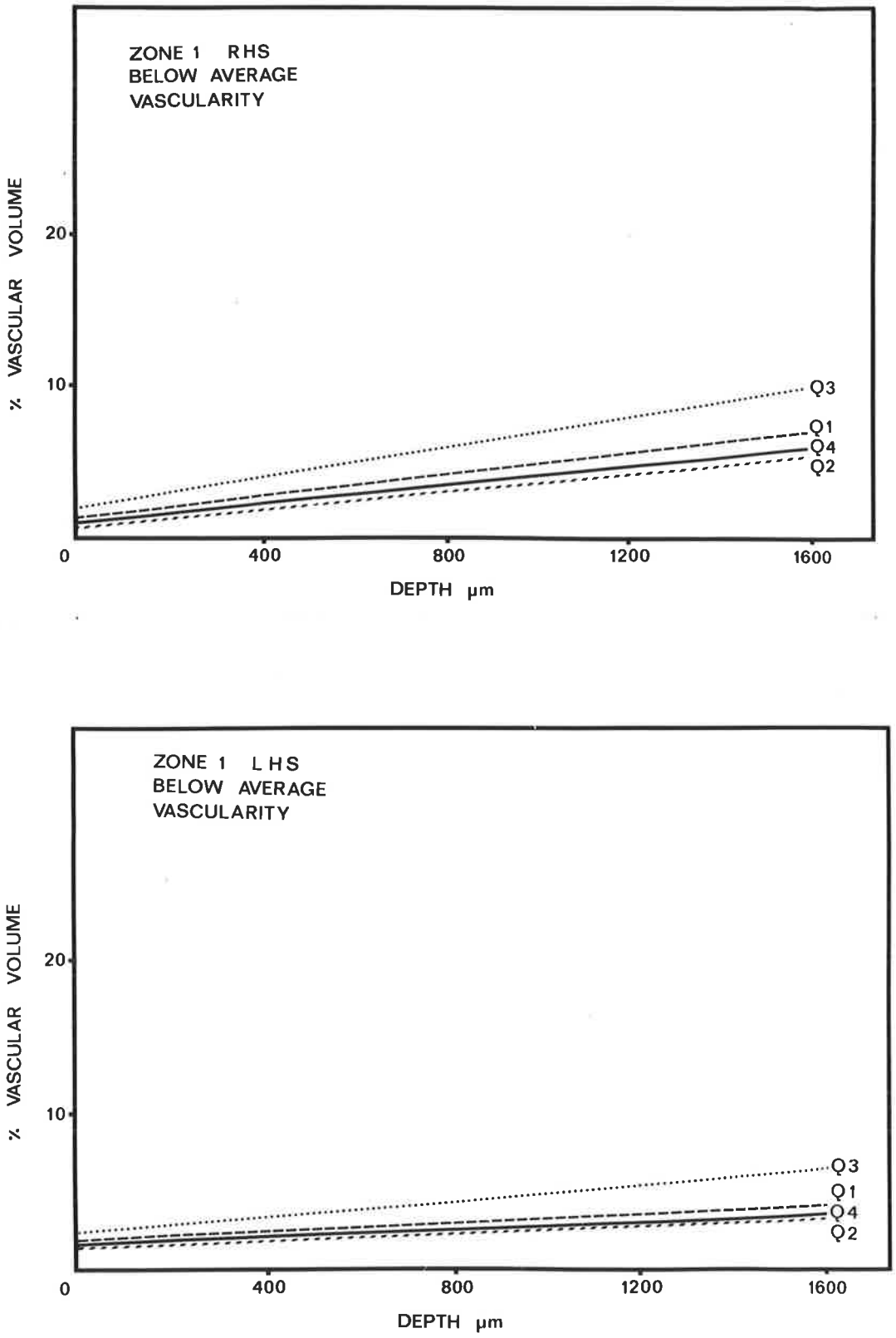
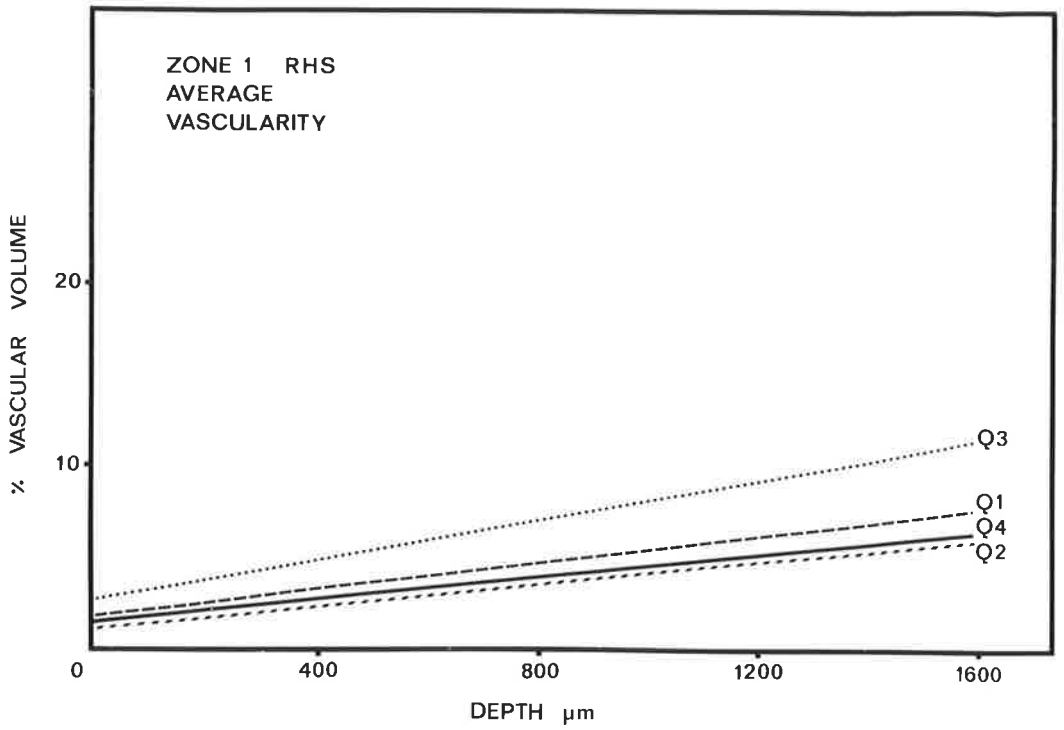


Figure 6. Graphical representation of the changes in percentage vascular volume with ligament depth in zone 1 for a right and left marmoset mandibular second molar from an animal of below average vascularity.



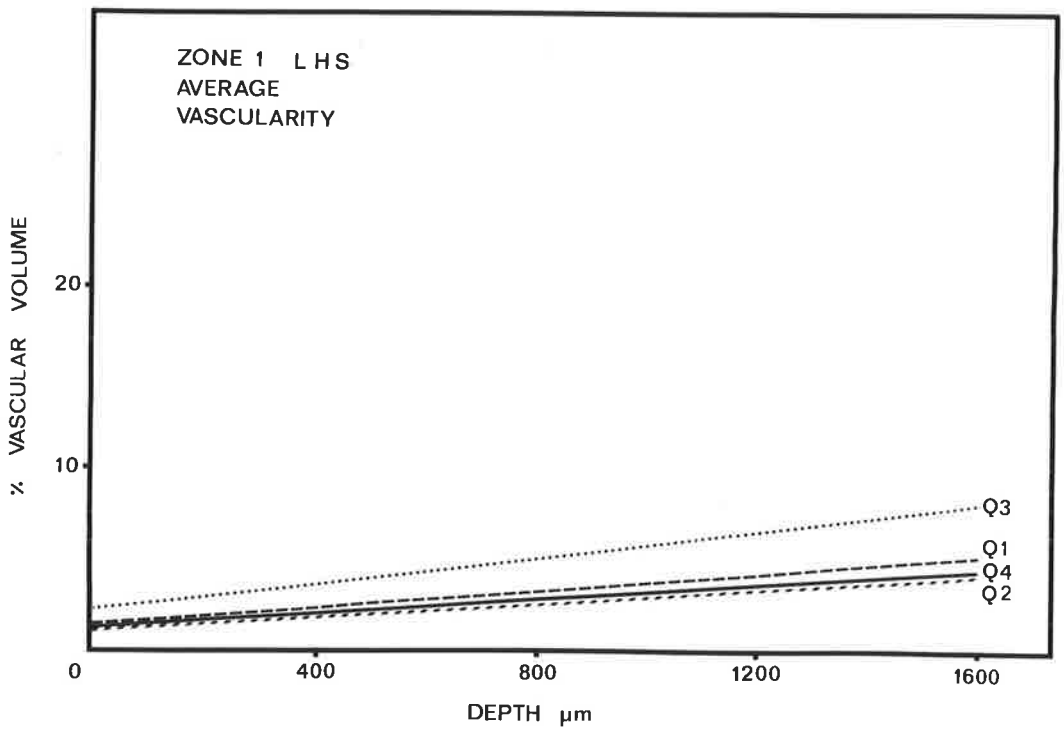
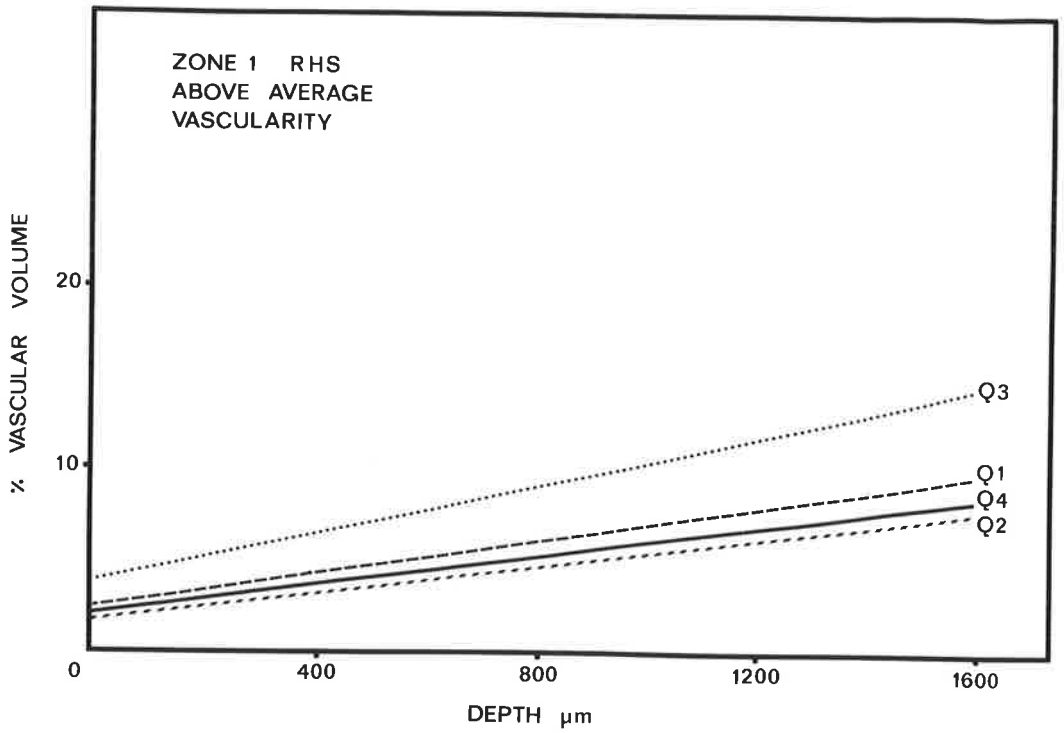


Figure 7 Graphs showing the changes in percentage vascular volume with ligament depth in zone 1 for a right and left marmoset mandibular second molar from an animal of average vascularity.



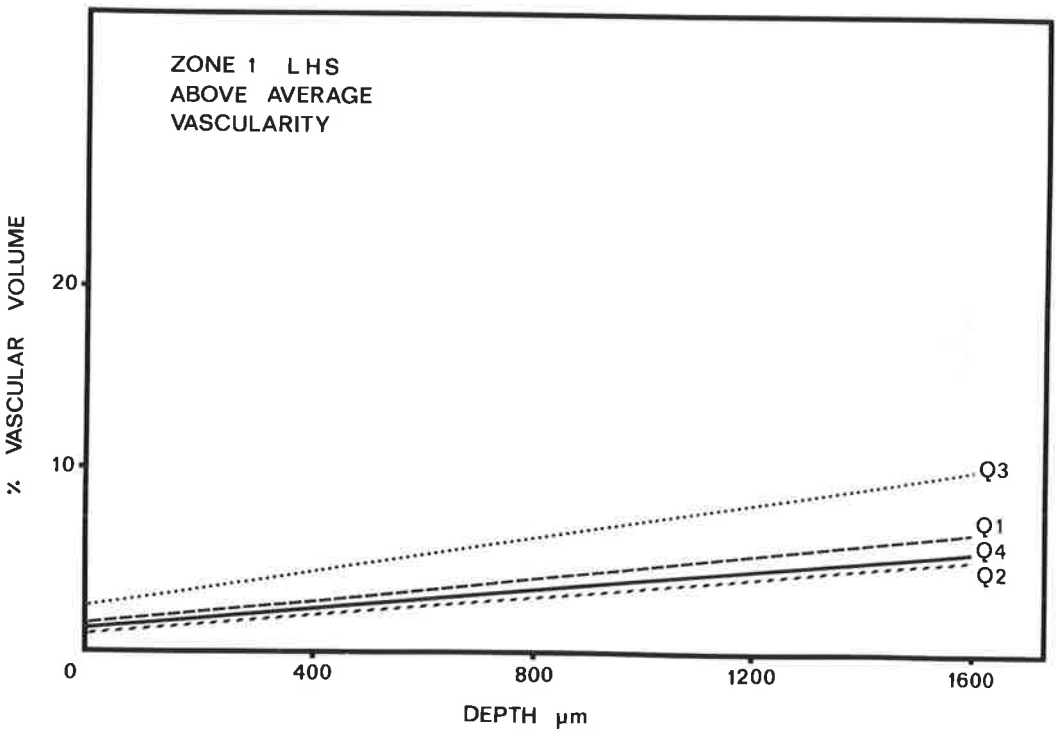
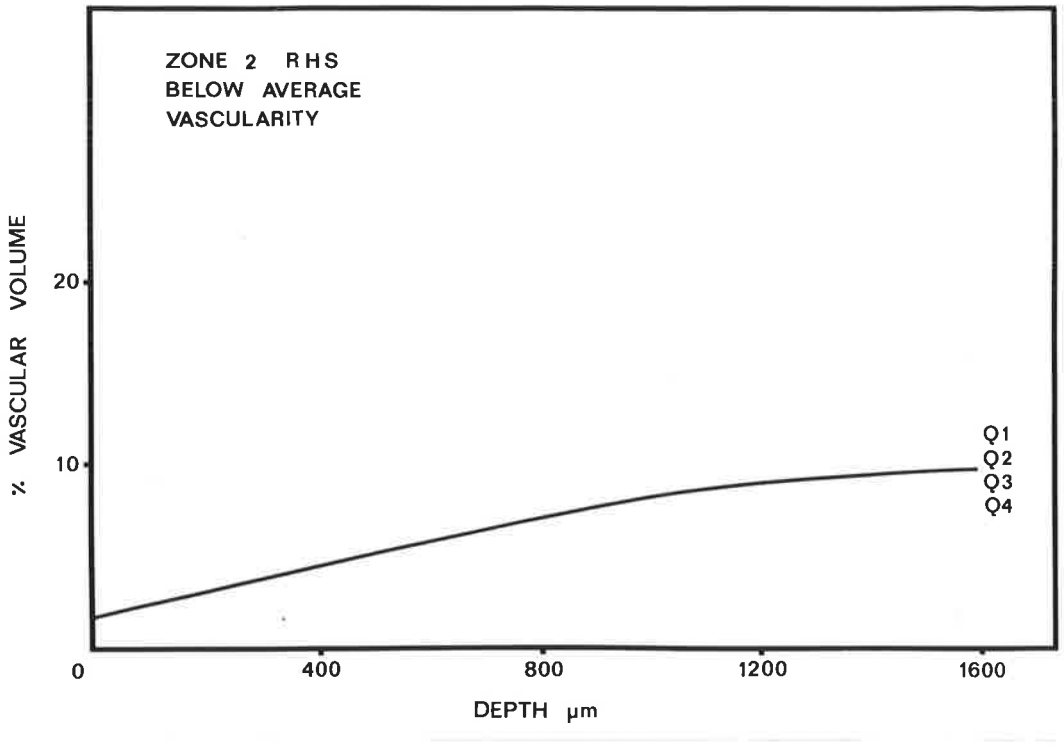


Figure 8. Graphical representation of the changes in percentage vascular volume with ligament depth in zone 1 for a right and left marmoset mandibular second molar from an animal of above average vascularity.



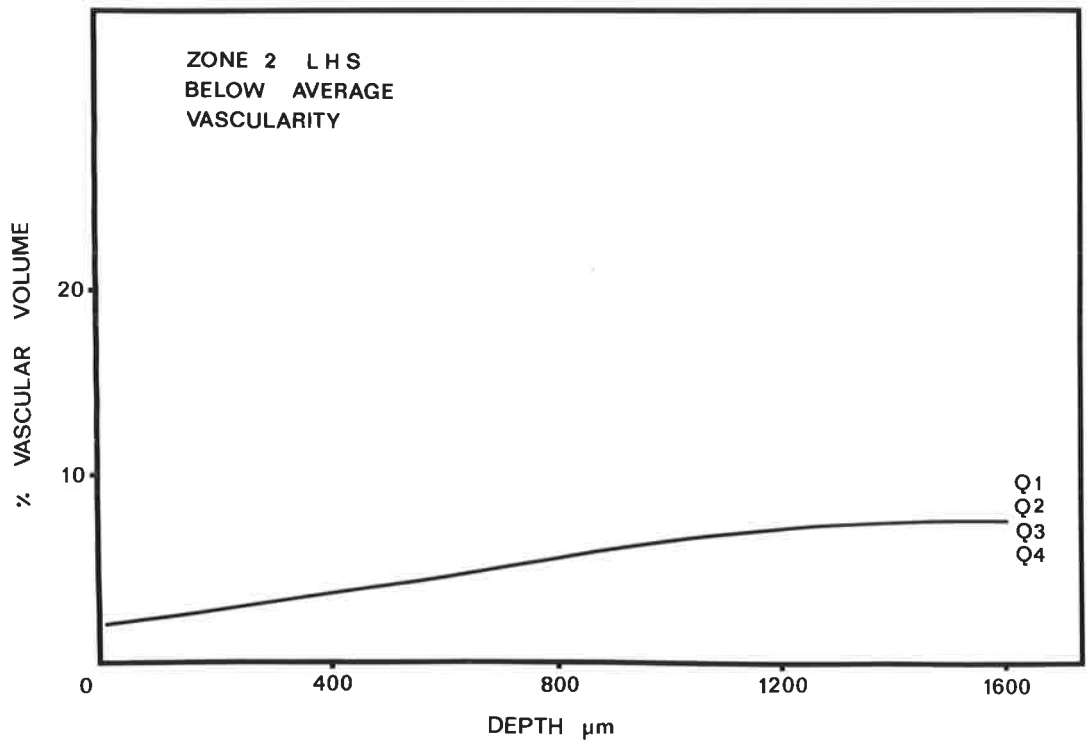
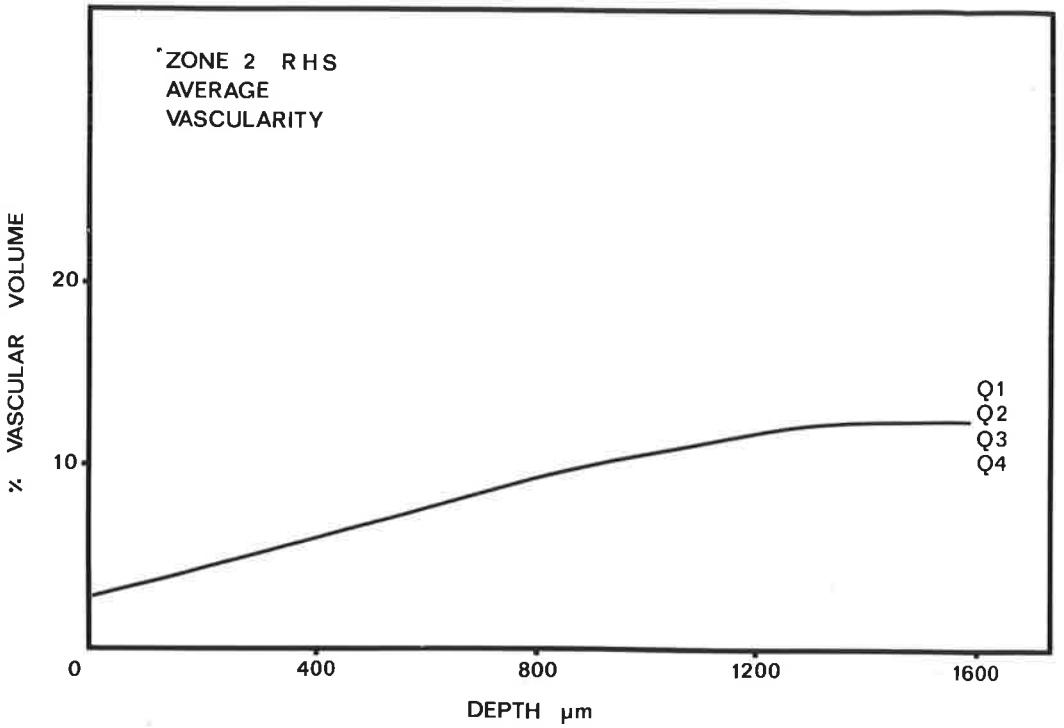


Figure 9. Graphs showing the changes in percentage vascular volume with ligament depth in zone 2 for a right and left marmoset mandibular second molar from an animal of below average vascularity.



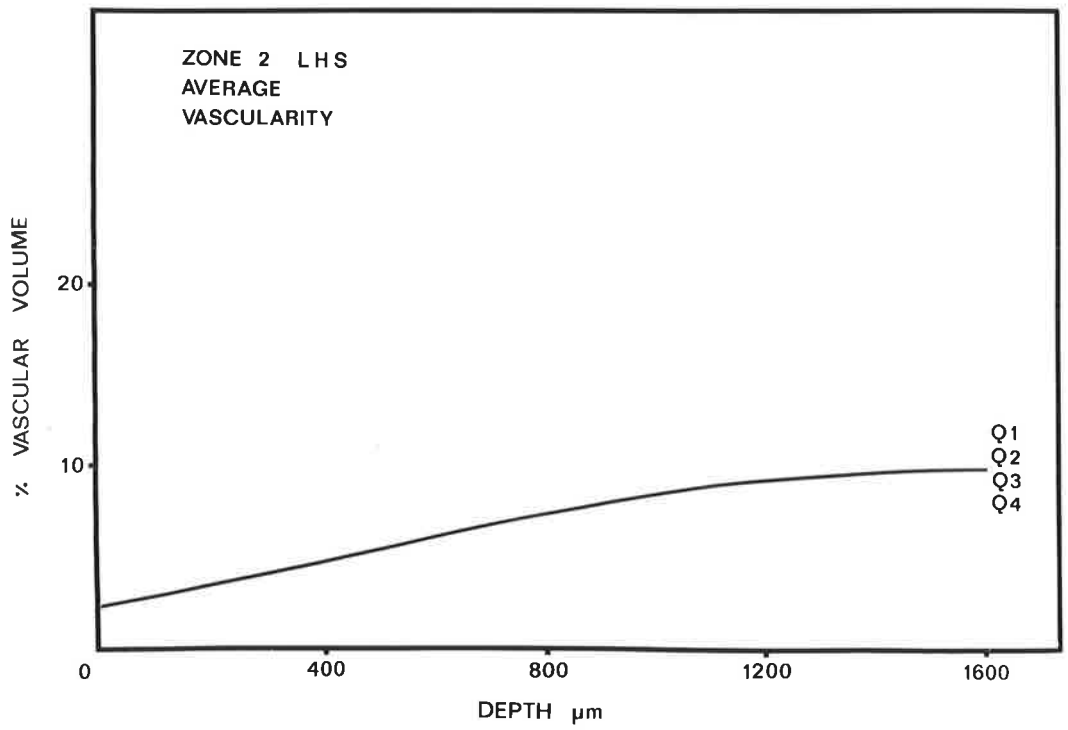


Figure 10. Graphical representation of the changes in percentage vascular volume with ligament depth in zone 2 for a right and left marmoset mandibular second molar from an animal of average vascularity.

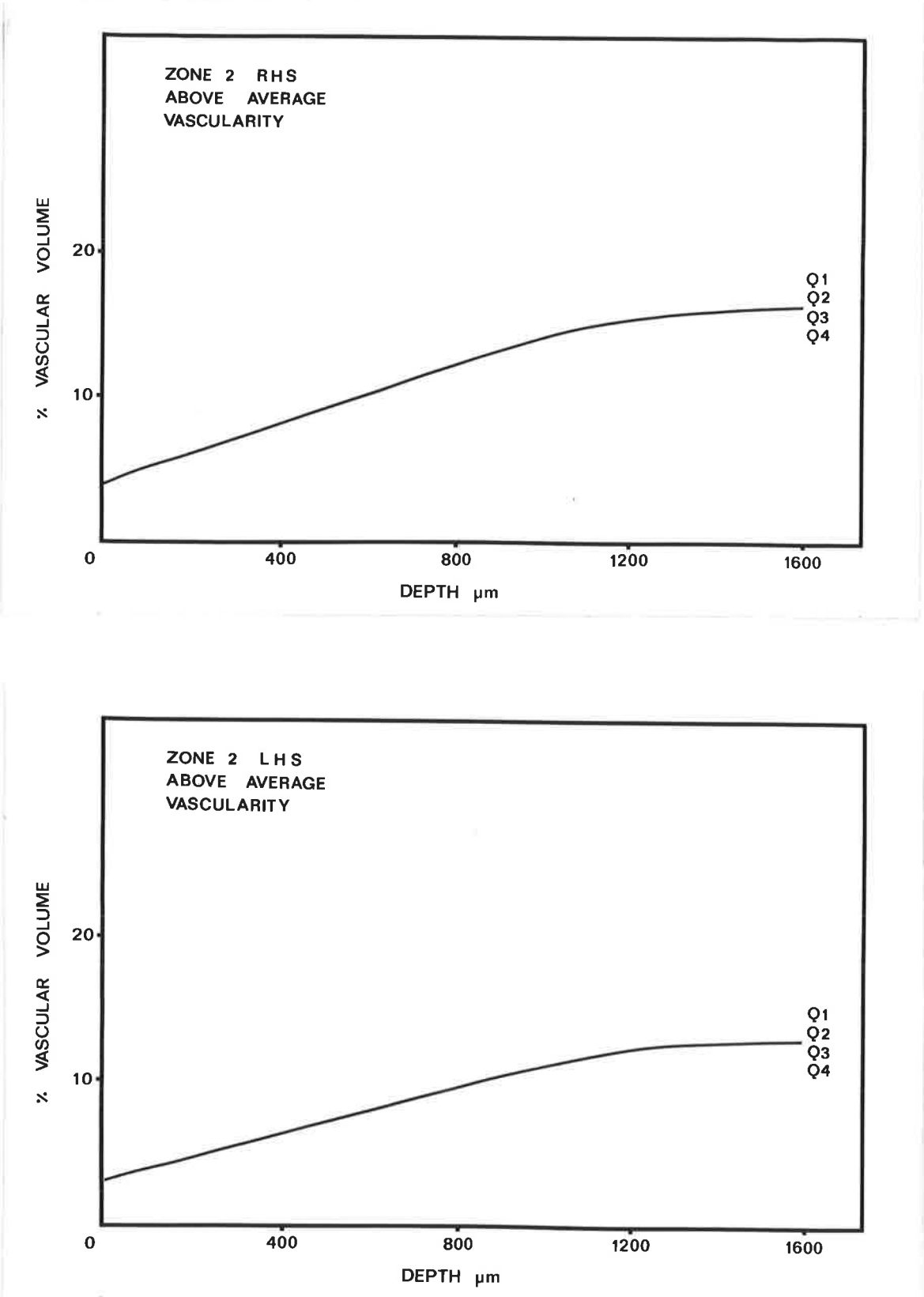


Figure 11. Graphs showing the changes in percentage vascular volume with ligament depth in zone 2 for a right and left marmoset mandibular second molar from an animal of above average vascularity.

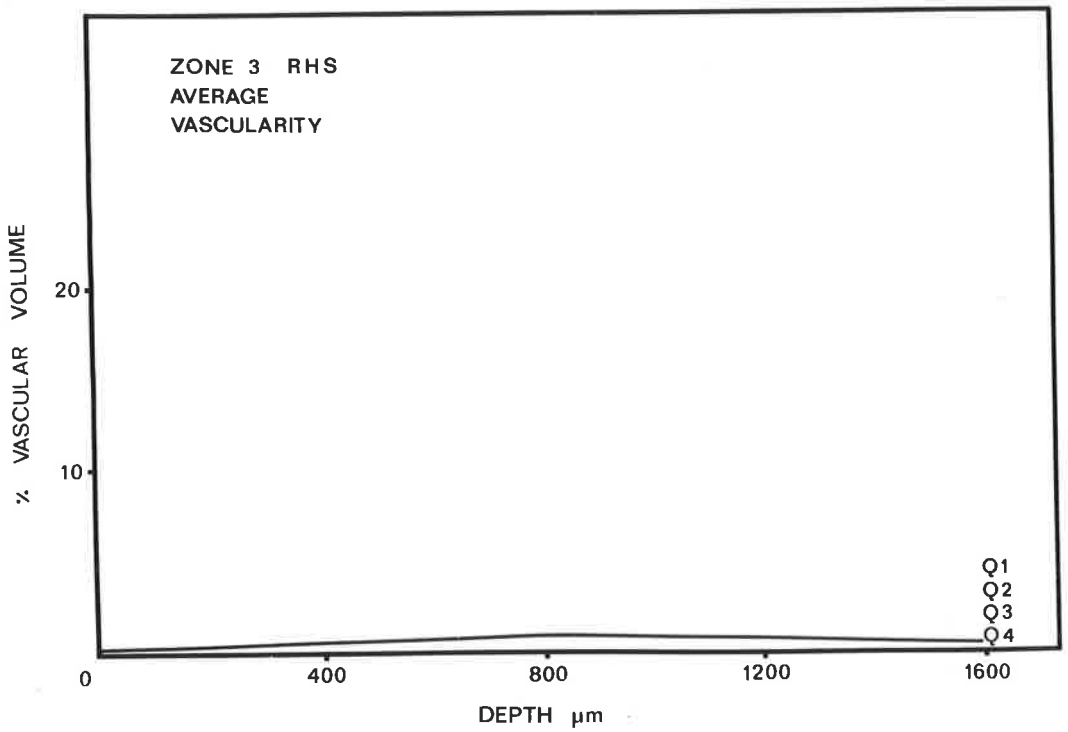


Figure 12. Graphical representation of the changes in percentage vascular volume with ligament depth in zone 3 for a right marmoset mandibular second molar from an animal of average vascularity.

CHAPTER 6:

DISCUSSION

6.1 The Experimental Technique

The possible sources of error which may have influenced the major investigation are considered within the realms of the following discussion. However, action to control or overcome some of these problems was limited due to time constraints.

6.1.1 Errors in Tissue Processing

A. Section Compression

Compression of paraffin sections during sectioning for light microscopy introduces systematic error. Attempts were made during floating to re-expand the sections and thus compensate for this compression. If all the tissue components were affected equally, then the volume or area density estimate should not be in error. However, the overestimation of dimensionless parameters will occur.

The compression factor has been expressed in Weibel's 1979 text as follows:

$$fc = lc/lo$$

where fc = compression factor

lc = compression length of the section following sectioning

lo = original length of the block in the direction of the cutting stroke.

The width of the section has been assumed not to be affected. Surface density was reportedly the major parameter affected and a correction formula was proposed by Loud et al. (1965) (cited in Weibel, 1979).

Steigman, Weinreb and Michaeli (1984) applied computerized histomorphometry to investigate the dimensional changes in rat incisor tissues which were processed histologically and embedded in paraffin wax. These 6 micrometer sections were compared with undemineralized sections of 100 microns and 2 micrometer thick glycol methacrylate embedded sections. Wax caused tissue shrinkage and methacrylate was responsible for swelling of the tissues. However, no statistically significant change was found in the periodontal ligament of wax-embedded specimens. Reductions in tooth size occurred mesio-distally with little alteration in the bucco-lingual width. This difference was attributed to the ovoid shape of the cross sections of rat incisors.

B. Formaldehyde Fixation and Tissue Shrinkage

Bahr, Bloom and Friberg (1957) investigated the effects of formaldehyde fixation on the volume, weight and specific gravity of various tissue specimens. Three stages of volume changes during fixation were reported.

1. A brief period of slight shrinkage depending on the tonicity of the fixative solution. This was found to occur only in very strongly hypertonic media.

2. A period of swelling which could advance to cell bursting. The factors involved have been listed as follows:

    1) Intracellular salt concentrations.
    2) Membrane permeability.
    3) Donnan equilibria.
    4) Inability of the cells to continue active osmotic work.

3. Secondary shrinkage due to a process of slow chemical alterations.

The swelling of tissues fixed in formaldehyde was shown to be inversely proportional to the concentration of this fixative. The aqueous media in which the fixative was dissolved was also found to be responsible for the amount of swelling observed during fixation. Substances of medium and high molecular weight (e.g. sucrose) could diminish and even suppress this phenomenon. Weight changes closely paralleled volume changes. However, histological procedures did not have a pronounced effect on the specific gravity of tissue samples.

Bahr, Bloom and Friberg performed their experiments with wall tissue blocks, and stressed their awareness that the results could not simply be applied to the changes seen in indiviaul components of the tissue examined.

Crawford and Barer (1949) showed reasonably consistent tissue reactions when various specimens were fixed in 10 percent

formaldehyde at 24°C for 72 hours, dehydrated in ethanol for 36 hours, cleaned in xylene and infiltrated with paraffin. They advised caution when applying the concept of isotony to the process of fixation, due to the disturbance in osmotic equilibrium as a result of cell death.

C. Dehydration and Tissue Shrinkage

Shrinkage was also shown to occur during the dehydration procedure. Bloom and Friberg (1956) (cited in Bahr, Bloom and Friberg, 1957) observed that ethanol did not cause as much shrinkage as methanol. Bahr, Bloom and Friberg (1957) upheld this view and also recommended a step-wise dehydration procedure.

Dehydration in ethanol without a stepped procedure resulted in an average shrinkage of 33 percent for formaldehyde fixed material. The implementation of a step-wise dehydration produced less abrupt volume changes.

D. The Effect of the Osmolality of Storage Media

Lindskog and Blomlöf (1982) investigated the effect of different osmolalities and compositions of storage media on the viability and integrity of human periodontal ligament cells and their membranes. Storage in hypotonic solutions caused reversible and irreversible cell membrane damage which was potentiated if the storage medium was bacterial contaminated saliva. Variations in pH (6.7 to 7.3), salt and macromolecular content of milk and sucrose did not influence either cell vitality or membrane integrity

at an isotonic osmolality during the 3 hour storage period. Fixation and preparation for scanning electron microscopy followed the storage of human fibroblast-like cells. However, these authors did not elaborate on whether these subsequent procedures compounded any effects that were observed due to storage alone.

E. The Linear Shrinkage Factor

Tissue shrinkage acts in three dimensions and, although it does not affect volume density, surface density is influenced by this phenomenon. In order to ascertain the original volume, one must first determine the linear shrinkage factor as follows:

$$fs = ls/lo$$

where fs = linear shrinkage factor

ls = a characteristic length in the shrunken tissue block

lo = a characteristic length in the original tissue block.

The original volume can be ascertained from the following formula:

$$Vo = Vs \cdot fs^{-3}$$

where Vo = original volume

Vs = shrunken tissue volume

fs = linear shrinkage factor.

Correction formulae for surface density measurements have been presented in Weibel's 1979 text.

The amount of tissue shrinkage is dependent on the method used and can be dramatic with paraffin sections where fs ~ 0.74.

6.1.2 Possible Errors in the Collection of Morphometric Information

Stereology is a theoretical statistical branch of science found particularly useful in biological morphometry. It has been defined by Weibel (1979, 1981) as follows:

"Stereology

is

(1) a body of mathematical methods

relating

(2) three dimensional parameters defining the structure

to

(3) two dimensional measurements".

A similar definition has been proposed by Little (1974), and the multi-dimensional perspectives of stereology have been discussed by Miles (1972).

Morphometry on the other hand has been described by Elias, Hennig and Schwartz (1971), and Elias (1967), as the commensuration of structure.

A. The Principle of Delesse

Delesse (1847) (cited in Weibel, 1979) was a French geologist who proposed that the fractional volume of a particular component in a solid body could be estimated on ramdom sections

by measuring the fractional area of the component. Mayhew and Cruz-Orive (1973, 1974) derived the Delesse principle by using a concentric sphere model to resemble a cell.

B. The Measurement of Area Density

The measurement of area density by linear integration was introduced by Rosiwal (1898) (cited in Weibel, 1979). The two other methods used to achieve an estimated profile area were planimetry and point counting. These techniques bear a strict analogy to the derivation of the Delesse principle.

C. The Holmes Effect

Holmes (1921) (cited in Williams, 1977), recognized the effect of finite section thickness on stereological estimates. One must keep in mind the fact that stereological techniques are applied onto an observation plane which in reality represents the projection of the total content of a relatively thick slice of tissue specimen. A greater error is incurred in the estimation of both the volume and surface area of the structure, or its components, with an increased thickness of section as a larger amount of enclosed components are projected onto the observation level.

Miles (1976) advocated the use of a set of formulae to correctly estimate stereological parameters from a transmission microscopy study using thick sections.

Franklin and Craig (1978) attempted to minimize the Holmes effect by using a microtome set at 4 μm. However they did imply

that any further reduction in section thickness would be uneconomical in terms of time and effort expended.

D. Loss of Cap Sections

Weibel (1979) described this phenomenon found in thick sections where a particular biological feature may be lost due to processing, or not be visible due to insufficient contrast, and thus reduce the effective section thickness.

The loss of cap sections has been reported to result in a reduction in the value of stereological parameters (i.e. volume or area density). However, due to the Holmes effect, which tends to increase the value of these stereological parameters, Weibel (1979) disregarded these phenomena in that a counter balance was achieved.

E. The Effect of Resolution on Stereological Measurements

Some stereological measurements are affected by the magnification or microscopic resolution for the study. However, it is pertinent to question the level of detail of surface texture which would, in fact, be relevant to a particular stereological analysis.

De Hoff (1981), in his investigation of the stereological meaning of the inflection point count as a means of classifying shapes, expressed the opinion that the level of resolution had to be reported if one wished to determine this parameter.

F. A Comparison of Methods for Planar Image Analysis

Mathieu, Hoppeler and Weibel (1980) and Mathieu, Cruz-Orive, Hoppeler and Weibel (1981) investigated whether computer assisted tracing devices could significantly improve the accuracy and efficiency of stereological analysis. In these papers, the efficiency of a particular method was defined as the precision of the final estimate achieved per unit measurement time on a given set of sections. The precision of an estimate was found to be proportional to the reciprocal of its error variance.

The results showed that standard errors were reduced when an automatic image analysis or point counting with a 100-point grid was used, as opposed to the semi-automatic computer image analysis with a MOP. In addition, however, the use of the MOP considerably increased the accuracy of individual measurements at the expense of measuring time, and its use was justified when an accurate measurement was desired using a few sections only.

G. Tracing Faults

Weibel (1979) expressed the opinion that computer assisted tracing devices had greater precision than manual point counting procedures, and had the added advantage of one tracing operation yielding several pieces of information (e.g. perimeter, diameter). However, he did qualify this statement by stating that the accuracy depended on the precision of tracing which was a difficult task with small profiles, or with features with a complicated outline. The use of one operator for all measurements

would reduce these faults and also help to minimize the human error component. In the present study the image was first traced with lead pencil and then with the stylus.

One must accept the MOP-1 as an image analyser which provides a non-automated means of measuring various parameters. However, the ultimate interpretations of this two-dimensional information remains the responsibility of the investigator.

6.1.3 Future Systems

Hoppeler, Mathieu, Bretz, Krauer and Weibel (1980) described the use of small computer systems for stereology. The use of computers was more efficient in regard to the accumulation and storage of data and for the manipulation, statistical evaluation and graphical representation of this information. Despite the accelerative advances seen in the field of electronic data processing, these authors adhered to the belief that normal point and intersection counting would still be implemented.

Prothero and Prothero (1982) advocated the use of a computer software package called MORPHO for the collection, storage and processing of data. The use of ultrastructural, microscopic, or macroscopic serial sections revealed the presence of three-dimensional structures. Various programs were available in the package which could discover and correct errors, make hard copies of the digitized contours and carry out statistical calculations. The process of microcomputer based three-dimensional reconstruction from serial sections has been pursued by Sundsten and Prothero (1983).

Spence, McConnell, Sloan and Allen (1984) advocated the use of an automatic image analysis system as a quantitative technique for the study of normal and diseased vaculature. The digital video image splitter was introduced by Hogan, Morris and McMurray (1984) for the measurement of vessel lumen diameter and wall thickness. This device is limited to the measurement of vessels aligned vertically on the video image and constitutes a limitation when multiple vessels must be measured in the same field.

These types of computer systems will become more affordable particularly as the technology advances at a rapid rate. Not only will they become justifiable in terms of the more efficient use of available time and effort, but their importance will also be ascribed to the ability to enhance the value of resultant information.

6.2 The Vasculature of the Periodontal Ligament

The majority of the research concerning the vasculature of the periodontal ligament has been carried out on animal models. It is the purpose of this discussion to not only investigate the literature with regard to interspecies animal differences, but also to undertake the description of intraspecies variation found within particular animal models.

6.2.1 Intraspecies Periodontal Ligament Vasculature Differences

A. Anterior Single Rooted Teeth

Even though the major study undertaken by the present author did not detail any observations of the teeth in the anterior region of the mandible, a comparison of molar and anterior teeth is relevant.

Hayashi in 1932 (cited in Saunders and Röckert, 1967), described the periodontal vasculature of single rooted human teeth as having a greater representation on the labial than the lingual side. This finding was consistent irrespective of whether the tooth belonged to the maxillary or mandibular arch. On the other hand, Körber (1970) attempted to explain the labial pulsatile movements of human maxillary incisors by concluding that the palatal periodontal vessels were both larger and more numerous than the labial periodontal vessels. Korber's assumption has not been confirmed histologically.

Götze (1976, 1980) reported an increased vascular volume on the labial and lingual periodontal ligament surfaces for human anterior teeth. A parallel was drawn between the high percentage of fibre bundle sections and the high proportional volume of vessels on the lingual aspect of the periodontal ligament of anterior teeth.

Birn (1966) claimed an increase in periodontal vascularity as one moved further posteriorly in the maxillary and mandibular arches, being least for the lateral incisors and greatest for the

second molars of human cadavers. This improved periodontal vascularity constituted both an increase in number and size of blood vessels. The gradual increase from tooth to tooth, as described by Birn, did not confirm the research of Hayashi (1932) (cited in Saunders and Röckert, 1967), whose investigations revealed a blood supply increase from tooth group to tooth group with a fall inside each group.

The single rooted teeth studied by Birn (1966) exhibited their greatest blood supply in the gingival third, their least in the middle third, and an intermediate level in the apical third of the periodontal ligament. Although mesial and distal surfaces were found to have a better blood supply than the buccal and lingual surfaces, the difference was considered to be small and therefore insignificant.

Ando (1969) investigated the jaws of human cadavers and, like Birn (1966), described a gradual increase in the number of periodontal vessels as one moved from the anterior to the posterior teeth. A reported greater number of vessels entered the periodontal ligament by perforating through the alveolar bone as one progressed posteriorly along the arch. Bundles of vessels were located on the mesial and distal surfaces of the cervical region of the periodontal ligaments of teeth.

Kindlova (1963) observed different vascular patterns for the incisors and molars of rats. The gingival vessels of the molars did not supply the entire interdental papilla whereas

with incisors, the vessels of the interdental papilla were derived from the gingival vessels.

Garfunkel and Sciaky (1971), in their study on rats, reported the presence of vascular interconnections between the roots of a single tooth, the roots of two adjacent teeth and between the roots of molars and incisors. Details concerning the orientation, size and number of these vessels were not established.

Wong (1983), using SEM studies of vascular perfusion casts, contrary to the findings of Kindlova and Matena (1959) who researched the rat incisor, observed no significant differences in the vascular morphology between the maxillary and mandibular incisors of mice. He supported the work of Garfunkel and Sciaky (1971) on the rat that the incisor vessels overlying the cementum increased in diameter towards the coronal region.

Two parallel periodontal blood vessel networks were described by both Garfunkel and Sciaky (1971), and Wong (1983). These two networks, one being closer to the alveolar wall and the other in the proximity of the cementum surface, were interconnected according to Garfunkel and Sciaky.

Although Castelli and Dempster (1965) observed a venous mesh closer to the alveolar wall, they also reported that the capillaries found within the periodontal ligament formed a layer of vessels closer to cementum.

The continuously growing incisors of rodents were researched by Carranza, Itoiz, Cabrini and Dotto (1966). They reported the

presence of a dense plexus of vessels closer to enamel than to bone, and numerous communications between this plexus and the network of vessels closer to bone. These workers related the increased vascularity to the needs of the enamel forming tissue.

Wong (1983) described a separate system of large longitudinally arranged vessels which he termed boundary vessels. The demarcation seen between the regularly arranged, densely packed vessels of the enamel zone and the irregular, less dense arrangement of the vessels in the cementum zone was the responsibility of these boundary vessels.

B. The Molar Periodontal Ligament

A longitudinal arrangement of vessels parallel to the long axis of the tooth has been described by Hayashi (1932) (cited in Saunders and Röckert, 1967), Bevilacqua (1958), Bernick (1960, 1962), Boyer and Neptune (1962), Kindlova and Matena (1962), Kindlova (1965, 1967), Carranza, Itoiz, Cabrini and Dotto (1966), Garfunkel and Sciaky (1971), Kishi and Takahashi (1977), Wong (1983) and Weekes (1983).

Those researchers who observed the periodontal vasculature as being closer to bone than cementum included Schweitzer (1909) (cited in Kindlova, 1965), Cohen (1960), Kindlova (1965), Carranza, Itoiz, Cabrini and Dotto (1966), Folke and Stallard (1967), Khouw and Goldhaber (1970), Levy, Dreizen and Bernick (1972b), Corpron, Avery, Morawa and Lee (1976) and Kishi and Takahashi (1977).

The presence of two layers of longitudinally arranged vessels in the periodontal ligament has been proposed by Garfunkel and Sciaky (1971) in rat molars, Ando (1969) in human cadavers, Carranza et al. (1966) in the guinea pig molar and Wong (1983) in the mouse molar. In the majority of cases, these two vascular networks were parallel with one being closer to the alveolar bone and the other close to the cementum surface. Garfunkel and Sciaky (1971) described an interconnection between the two layers of vessels positioned closer to the alveolar wall and the slender, smaller vessels adjacent to the cementum. These layers fused and became a single layer of vessels in old adults.

This author's present findings revealed a lateral periodontal vascular distribution which was greatest in the middle third, least in the third adjacent to cementum, and between these two extremes in the third of the ligament adjacent to alveolar bone. An analysis quantifying the lateral distribution of vessels has not previously been performed, although qualitative observations have been reported in the literature by Hayashi (1932) (cited in Saunders and Röckert, 1967), Bernick (1962) and Carranza et al. (1966).

The continuously growing guinea pig molar exhibited active enamel formation and Carranza et al. (1966) reported that in addition to the plexus of longitudinally running vessels close to the alveolar bone, a second network of blood vessels existed close to the enamel. This layer of vessels ran obliquely to the long axis of the tooth and was described as being less dense than the plexus of blood vessels adjacent to the alveolar bone. This

arrangement was not evident in the molars of the dog and cat and appeared to be specifically related to the formation of enamel.

Birn (1966) studied the number and size of the perforations in the alveolar walls of human tooth sockets and assumed that each perforation represented a blood vessel. His work was subsequently refuted by the TEM studies of Barker (1980). Nevertheless, some similarities can be drawn between the findings of the present author and those reported by Birn.

In single rooted teeth, Birn observed that the blood supply was least in the middle third, greatest in the gingival third and in between these two extremes in the apical third of the human periodontal ligament. Bearing in mind the preliminary content of this author's pilot study results, the quadratic nature of Birn's observations for single rooted teeth was also noted for the two rooted second molar tooth of the marmoset monkey. This may have been a result of the small sample size used in the initial study, since the subsequent major study revealed a gradual increase in vascular volume from the cervical to the apical regions with no quadratic effect.

For mandibular molars, Birn reported that the blood supply to the periodontal ligament of the distal root was less than that for the mesial root. This author's initial findings supported Birn's view with particular reference to the monkey mandibular second molar. However, the statistical results derived from the major study data disclosed the absence of a root effect. Again,

this difference in results may have been due to the limited sample size for the pilot study.

Various researchers have stipulated a particular periodontal ligament surface predominance of blood vessels. Hayashi in 1932 (cited in Saunders and Röckert, 1967) observed that blood vessels were located closer to cementum than alveolar bone and for molars, were mainly represented on the lingual tooth surface. However, Hayashi in 1932 (cited in Birn, 1966), was reported to have stated that the buccal margin of the alveolar bone, the middle of the mesial surface and the apical region of the distal surface were the regions with the greatest periodontal vascular supply.

Götze (1965, 1976) researched the periodontal vascular volume of human premolars and concluded that the buccal and lingual tooth surfaces were better supplied than the mesial and distal regions. In addition, the recorded vascular volume increased from the cervical third to the apical third of the periodontal ligament.

The resultant findings of the present author are in agreement with Götze's observations regarding the vertical vascular distribution down the periodontal ligament. This author supports the view that a predominance of vessels lie on the lingual ligament surface. The buccal, mesial and distal vascular volumes, although statistically different, appear to be grouped graphically somewhat closer together.

The workers who did not support Götze's assessment and instead attributed a greater qualitative vascular volume to the mesial and distal surfaces of the periodontal ligament included Ando (1969), Soloviev (1970) and Wong (1983) who used human cadavers, dogs and mice, respectively.

Reports concerning a predominance of periodontal vascular volume on a particular side of the mandible have not thus far been encountered. Birn (1966) did not explore this factor, due to the assumption that homologous alveoli on the left and right sides of human jaws were the same.

The present investigation revealed a statistically significant difference between the vascular volume of the second molar ligament on the left and right sides of the mandible. The right was greater than the left in all zones laterally across the ligament, at all depths and in all quadrants. The significance of this statistical difference, which may not be biologically relevant, will be discussed in a later section.

One must account not only for the various animal species, but also for the multitude of experimental techniques, when evaluating differences in the qualitative and quantitative assessments of the distribution of the periodontal vasculature.

6.2.2 Interspecies Periodontal Ligament Vasculature Differences

A. The Mouse Periodontal Ligament Vasculature

The vascular morphology and ultrastructure of the periodontal ligament of the mouse has been investigated by Carranza, Itoiz, Cabrini and Dotto (1966), Avery, Corpron, Lee and Morawa (1975), Corpron, Avery, Morawa and Lee (1976), Sims (1977, 1980, 1981, 1983a, 1983b) and Wong (1983).

Although the vasculature of the mouse periodontium has not been extensively reported upon in the literature, a general concensus exists as to the occluso-apical orientation of vessels located closer to the alveolar bone than to the cementum.

Quantitative data concerning the vascular volume of the mouse periodontal ligament have not been presented in the work of these researchers, and yet Sims (1980) reported that mouse mandibular molars contained regional periodontal vascular proportions of 17 percent. This figure was markedly higher than the more conservative estimates of one to two percent, which were proposed by Götze (1965) in human premolars and Wills, Picton and Davies (1976) in the macaque monkey.

B. The Rat Periodontal Ligament Vasculature

The periodontal ligament vasculature of rat molars and incisors has been researched by numerous workers including Bevilacqua (1958), Kindlova and Matena (1959, 1962), Bernick (1960, 1962), Boyer and Neptune (1962), Zaki and Van Huysen (1963),

Cernavskis and Hunter (1965), Carranza et al. (1966), Kindlova (1963, 1967, 1968, 1970), Garfunkel and Sciaky (1971), Koivumaa and Lassila (1971), Shore, Moxham and Berkovitz (1982), Weekes (1983) and Shore, Berkovitz and Moxham (1984).

Bernick (1962) described a longitudinal periodontal vascular network which resided closer to the alveolar bone, with branches extending toward the tooth where cellular cementum was present and root resorption was occurring. An absence of blood vessels was noted adjacent to acellular cementum.

Garfunkel and Sciaky (1971) observed that the blood vessels of the mandibular and maxillary molars were similar. The microvascular beds comprised a hammock-like apical network, vessels running parallel to the long axis of the tooth, connections between the vessels of the periodontal ligament and alveolar bone and the existence of two parallel but interconnected vascular networks. Although one network was located closer to the root surface, and the other was described as being external to the latter, no definitive position was stipulated for the second vascular layer.

The work of Garfunkel and Sciaky (1971) supported the findings of Carranza et al. (1966) that the blood vessels of the periodontal ligament linked adjacent teeth through the alveolar bone. This observation contradicted the proposal put forward by Kindlova and Matena (1959) that the periodontal blood vessels of the incisors and molars terminated at the apex of the alveolar crest.

In summarizing their research, Garfunkel and Sciaky (1971) and Kindlova (1963) were under the impression that the blood supply of the teeth of man and rats was similar. They held the opinion that the work of Cohen (1960) and Castelli (1963) on human tissue supported and correlated with their studies on the blood supply of rats' teeth.

Kindlova and Matena (1962) described the occluso-apically orientated arterioles as being joined by a fine capillary network which also supplied the periodontal tissues above the interradicular septa. Venous drainage commenced at the alveolar crest, collecting blood from the capillaries of the horizontal arterial circulus, and continued either axially in the periodontal ligament or entered bone. The calibre of the venous vessels increased as the basket-like apical venous plexus was approached. The veins then perforated the alveolus below the tooth apex or the apical part of the interradicular septum and anastomosed with the venous vessels of the bone marrow.

Although the periodontal vasculature of the rat has been studied extensively, an absence of quantitative information regarding vascular distribution has been noted.

C. The Dog and Cat Periodontal Ligament Vasculature

Research on both dogs and cats has been carried out by Perint (1949) and Carranza et al. (1966). It is surprising to note the extensive use of dogs, whereas Cohen (1959a, 1960) was one of the few workers to examine the vasculature of the cat mandible.

The periodontal vasculature of dogs has been investigated by Wasserman, Blayney, Groetzinger and De Witt (1941), McCawley and Gilda (1943), Boehl (1954) (cited in Kindlova and Matena, 1962), Egelberg (1966), Gianelly (1969), Khouw and Goldhaber (1970), Soloviev (1970), Kindlova and Trnkova (1972), Söderholm and Egelberg (1973), Kishi and Takahashi (1977), Walker, Ng and Burke (1978), Ng, Walker, Zingg and Burke (1981) and Hellem and Östrup (1981a, 1981b).

Cohen (1959a) studied the mandibles of adult cats, a dog, a monkey and one human cadaver, in order to draw comparative conclusions. Unfortunately, the discussion centred around the various experimental techniques used rather than the results encountered. It was demonstrated, however, that the cat had only one single venous channel in the mandible. The human venous system investigation proved inconclusive due to the damage caused to the veins and capillaries by the passage of the needle used in inject Micropaque.

In Cohen's 1960 report on the vasculature of the cat mandible, three sources of blood supply to the periodontal ligament were discussed. These were from the apical region of the tooth, from the alveolar bone and from the gingival tissue. It was suggested that the periodontal vessels travelled close to the socket wall in grooves. However, a quantitative analysis of preferred vessel locations both vertically down and laterally across the periodontal ligament was not undertaken.

Egelberg (1966) reported the presence of a flat plexus of blood vessels close to the crevicular epithelium and extending from the gingival margin of healthy gingiva to the base of the crevice. Vascular loop formations similar to those found beneath the oral epithelium of healthy gingiva were not observed under the crevicular epithelium. This thin layer of vessels was similar to the vascular arrangement described by Carranza et al. (1966), and Kishi and Takahashi (1977), of a "plane-like" vascular plexus surrounding the epithelial cuff.

The work of Kindlova and Matena (1962) on the rat, and Kindlova (1965) on the monkey, revealed structures resembling glomeruli and rows of slender loops with equal lengths of venous and arterial limbs in this epithelial cuff region. This latter description differs distinctly from that of Egelberg in 1966.

Kishi and Takahashi (1977) presented a most comprehensive study of the periodontal vasculature of mongrel dogs. They located both an inferior and superior vascular network in the apical quarter of the periodontal ligament. The superior layer of vessels was described as having a "rope-ladder" appearance due to the presence of horizontal connecting vessels at intervals of 120 to 260 microns. This superior network of vessels was found travelling longitudinally closer to the root of the tooth, thus supporting the description of Carranza and his colleagues (1966). The inferior layer of vessels ran at right angles to the superior layer and closer to the alveolar wall. The two vascular networks persisted in the middle part of the periodontal ligament and for most of the coronal region.

At the crest of the alveolar bone, the superior layer gave off hair-pin shaped vascular loops, whereas the inferior layer contained circularly or longitudinally orientated bundles of thick vessels which ran upwards along the alveolar bone. These findings supported the work of Kinklova and Matena (1962) on the rat, particularly in regard to the presence of capillary loops in the region of the gingival attachment.

Ando in 1969 described two layers of vessels in the human periodontal ligament as reported by Kishi and Takahashi (1977) for the dog.

D. The Monkey Periodontal Ligament Vasculature

The monkey has been used as an experimental animal to research the morphology and ultrastructure of the periodontal ligament vasculature. In addition, a better understanding of the function of the various components of the periodontal ligament in the tooth support mechanism has been sought through the investigation of the response of monkey teeth to applied loads.

Some of these workers have included Kindlova (1965), Castelli and Dempster (1965), Folke and Stallard (1967), Cutright and Bhaskar (1967), Lenz (1968), Levy and Bernick (1968), Kennedy and Zander (1969), Cutright (1970), Cutright and Hunsuck (1970), Khouw and Goldhaber (1970), Levy (1971), Pihlstrom and Ramfjord (1971), Levy, Dreizen and Bernick (1972a, 1972b), Slatter and Picton (1972), Wills, Picton and Davies (1972, 1976), Wills and Picton (1978, 1981) and Picton and Wills (1981).

Castelli and Dempster (1965) observed afferent arterioles, 100 microns in diameter, running a sinuous course in the marrow space of the septum. Sometimes the arterioles paralleled the radicular axis of the tooth. Blood vessels entered the middle and apical thirds of the periodontal ligament via cribriform openings in the alveolar wall and immediately formed capillary branches with a polyhedric plexiform pattern orientated parallel to the long axis of the tooth.

The capillary layer was located close to the cementum, whereas the venules anastomosed to form a mesh closer to the alveolar bone than the capillary network. Kindlova (1965), described the presence of a flat capillary network with an irregular meshwork, adjacent and closer to the tooth than the main vessels of the periodontal ligament, which ran parallel to the long axis of the tooth and in the proximity of, and partly within, grooves in the alveolar wall. However, Kindlova did not actually specify the exact location of the capillary layer.

Cutright and Bhaskar (1967), revealed that the blood supply of the labial periodontal ligament of the monkey mandibular anterior teeth was derived from vessels that originated in the labial soft tissues, and penetrated the labial alveolar bone directly to enter the periodontal ligament. Except for this labial area, the periodontal ligament vasculature of the anterior teeth was the same as that for posterior teeth whose vessels were derived apically from intra-alveolar and apical arteries. In the middle third, periodontal vessels arose from intra-alveolar arteries,

and in the cervical portion from intra-alveolar arteries and the gingival vascular plexus. The presence of free anastomoses between the gingival and intra-alveolar arteries was supported by Boyer and Neptune (1962) and Bernick (1960) in rats, and Kindlova (1965) in monkeys.

Khouw and Goldhaber (1970) subjected dog and monkey teeth to experimental orthodontic forces. These authors postulated that the greater reactivity of bone with respect to remodelling, as compared to cementum, was due to the observed richer blood supply in the connective tissue adjacent to the alveolus than found next to the connective tissue lining of the cementum. However, the normal peridontal ligament vasculature was described as having an uneven distribution, with the vessels located approximately in the middle third of the periodontal ligament between bone and cementum. The present author's results supported Khouw and Goldhaber's latter findings as they, too, described a distribution of vessels across the periodontal ligament space, but with a concentration located in the middle third between cementum and alveolar bone.

Kindlova (1965) illustrated the blood supply of the monkey marginal periodontium as a narrow, coronal band, which was condensed from the flat periodontal capillary network described as the main vessels of the periodontal ligament. Further, coronal to the single capillaries which arose from and returned to the narrow band of vessels, Kindlova observed looped capillaries with coiled arterial parts encircling a thick venous limb. Anastomoses

between the main vessels of the periodontal ligament and the vessels supplying the epithelium facing the oral cavity occurred in this region.

The vascular arrangement demonstrated by Kindlova (1965) was of a more intricate nature than that presented by Folke and Stallard (1967). These workers verified the findings of Kindlova (1965), and Carranza et al. (1966), that the major periodontal vessels ran parallel to the long axis of the tooth, but their description of the vessels supplying the oral epithelium was limited, perhaps due to the restrictions imposed on interpretations based on an experimental technique involving the use of microspheres.

The high cost of housing and maintaining monkeys reflects the fact that more research has not been ventured using this animal model, particularly as it represents man's closest analogue. The work that has been performed thus far leaves much information open to conjecture, for vessel types have not been classified and a quantitative analysis of their distribution has not been executed.

E. The Human Periodontal Ligament Vasculature

In this field of research, specimens were obtained from adult cadavers and dead infants. Although the gross anatomical features of the vascular distribution of the human head has been investigated thoroughly, very little information has been available regarding the vascular architecture within the human periodontal ligament.

Attempts have been made to explore this area of limited knowledge by Hayashi (1932) (cited in Saunders and Röckert, 1967), Forsslund (1959), Cohen (1959a, 1959b, 1960), Castelli (1963), Götze (1965, 1976, 1980), Birn (1966), Saunders and Röckert (1967), Ando (1969), Körber (1970), Rygh (1973), Gilchrist (1978), Barker (1980) and Sims (1975, 1976, 1980, 1981).

Hayashi in 1932 (cited in Saunders and Röckert, 1967), has continuously been referred to in the literature since his pioneering publication on the vasculature of the human periodontal ligament of single and multiple rooted teeth. A predominant number of vessels was found on the lingual surface of molars and premolars, and on the labial surface of anterior teeth. According to Hayashi, the periodontal ligament was supplied by the dental arteries which entered at the apical region and by inter-alveolar arteries which perforated the alveolar wall to course coronally. These vessels anastomosed with one another and with those periodontal branches which arose directly from the dental artery to form longitudinal periodontal arteries.

Castelli (1963) reported the presence of eight to twelve main vascular channels which arose from the inferior alveolar artery and supplied the dental pulp, alveolar bone, inter-alveolar septi and periodontal ligaments. These arteries anastomosed with the capillary network of the gingiva. Castelli did not elaborate on the details involving the route by which these vessels entered the periodontal ligament.

Venous drainage was achieved by the anastomosis of the veins of the alveolar bone, periodontal ligament and those found

in the inter-alveolar septi. Castelli observed the separate course of veins and arteries.

Birn (1966) investigated the perforations in the alveolar socket wall and by directly associating the vessel and aperture size, he concluded that the blood supply to the periodontal ligament increased gradually from tooth to tooth toward the posterior teeth. In single rooted teeth the blood supply was least in the middle third, greatest in the gingival third and in between these two extremes in the apical third of the ligament. Multiple rooted teeth had an equivalent blood supply in the middle and apical thirds of the ligament.

Although the mesial and distal surfaces exhibited a better blood supply than the buccal and lingual regions, Birn did not consider the difference to be significant. This finding was in contrast to those of Ando (1969) on human cadavers, Soloviev (1970) on dogs and Wong (1983) on mice, who all described the mesial and distal surfaces as having a greater number of vessels than the buccal and lingual surfaces. Götze (1965, 1976, 1980), however, found an increased vascular volume on the buccal and lingual surfaces as opposed to the mesial and distal regions of the periodontal ligament.

The methods used to carry out these investigations differed, and this could account somewhat for the discrepant results that were encountered. Quantitative analyses of vascular volumes have been attempted by Götze (1965, 1980), whereas the

remaining aforementioned information was based on qualitative observations.

More research needs to be carried out using the human model, however difficulties would be encountered in obtaining suitable specimens. The work and subsequent interpretations based on the use of cadavers may be compromised by the prior establishment of degenerative processes.

6.3 Nutrition and the Periodontal Ligament Vasculature

Although Ferguson (1982) outlined the effects that the deficiency of various dietary factors would have on the periodontal tissues, he also said that a direct cause and effect relationship was, as yet, unproven.

The presence of dietary variations between animals in the present study did not result in arteriosclerotic vascular changes, mainly due to the relatively short duration of the experiment and the low levels of saturated and polyunsaturated dietary supplements. Although statistical methods were employed to show an absence of dietary influence in this group of eight animals, limitations, due to the small size of the sample, make this aspect of the investigation worthy of further research using a controlled study of larger proportions. Such data would be of particular use with the marmoset as the experimental animal, since this primate serves as a valuable analogue to man (Levy, 1971).

The theoretical association, proposed by Glagov and Ozoa in 1968 (cited in Mostofi and Goepp, 1981), between a low blood flow resistance and the reduced presence of atherosclerotic lesions, can be applied to the periodontal ligament. Gaengler and Merte (1979a, 1979b) (cited in Edwall, 1982) reported a capillary flow rate in the rat incisor periodontal ligament of 0.02 to 0.04 millimetres per second, and that of venules to be 0.1 millimetres per second. These figures proved to be ten times smaller than the bone capillary flow rates recorded by Bränemark in 1959 (cited by Edwall, 1982) who studied rabbit tibial blood flow.

The low resistance to blood flow within the periodontal ligament, its rich regional blood supply in conjunction with a predominantly venous vascular nature, may account for its relative immunity from atherosclerotic involvement.

6.4 Periodontal Ligament Vascular Volume and Vascular Function

The investigations of Wills, Picton and Davies (1976), and Götze (1980), have emphasized the role of the vasculature in the tooth support mechanism. Quantitative periodontal vascular volumes have been either tabulated or derived mathematically. It was Marshall Hall in 1931 (cited in Sobin and Tremer, 1977), who expressed the opinion that the number and distribution of vessels were characteristic features of the vascular function within a tissue. Nutrition was considered to be the only function of a structure or organism if the vasculature had a simple arrangement,

and if the vessels were few in number. This is obviously not the case for the periodontal ligaments used in the present study.

It is of some interest to note that in the present study, laterally across the periodontal ligament, the greatest vascular volume lies in the middle third which is also the location of Sicher's (1954) (cited in Gianelly and Goldman, 1971) intermediate plexus. The function of this central anastomosis of periodontal fibres was supposedly to accommodate small tooth movements by the adaptation of fibre connections so as not to reduce the functional efficiency of the tooth. This particular arrangement of fibres has since come under disrepute and is now deemed an artefact resulting from the plane of section (Zwarych and Quigley, 1965 (cited in Gianelly and Goldman, 1971)).

Although the present author does not purport that such a fibre arrangement exists, it is not inconceivable that a congregation of vessels in this same middle third of the ligament would be ideally situated to disperse undue loading.

Conservative estimates of periodontal ligament vascular volumes have been proposed by Parfitt (1967) of two percent, Götze (1965) of one to two percent, and Wills, Picton and Davies (1976) of one half to one percent. Sims (1980) reported localised periodontal vascular volumes of seventeen percent for mouse mandibular molars and eleven percent for human mandibular premolars. The present study propounds the value of 8.3 percent for the periodontal ligament of the marmoset second mandibular molar.

Wills, Picton and Davies (1976) derived a mathematical formula using at least four assumptions. Assumption one dealt with the root of the tooth which was represented as a right circular cone. Unfortunately such a simplistic approach to tooth root morphology cannot be substantiated by classic texts on tooth form, i.e. Wheeler (1969) and Kraus, Jordan and Abrams (1969). The second assumption stated that the tooth was originally displaced 10 microns for a load of 0.5N. Wills, Picton and Davies, in their written resume of materials and methods described the measurement of only the three thrusts which followed a succession of thrusts at 4N per second. The preceding thrusts were applied to the tooth at intervals of 10 seconds, thus not allowing sufficient time for recovery of the tooth. Measurements of tooth movement were taken after a limiting value of movement had been achieved. An explanation regarding the purpose of the preceding thrusts was not ventured upon by these workers. One possible reason could be that Wills, Picton and Davies were attempting to establish a baseline of tooth movement from which any variation could be attributed to the particular solutions injected into the animal.

The third assumption was that the action of the vasoconstrictor reduced the tooth displacement by 40 percent, and that the vasoconstrictor acted upon all the vessels of the periodontal ligament. This assumption negated the ability of the animal to release vasoactive substances as part of its own physiological state. It also presumed that smooth muscle cells or some other contracting mechanism existed in the blood vessel walls of all the vessels of the periodontal ligament. Gilchrist

(1978) found no evidence of smooth muscle cells in the tunica media of the vessels examined in the human periodontal ligament, whereas, Avery, Corpron, Lee and Morawa (1975) described smooth muscle in the terminal blood vessels of the mouse periodontium.

The fourth assumption alluded to the thickness of the macaque periodontal ligament being 100 to 200 microns for central incisors at the mid root region. The variability between the eight teeth measured was over a range of 51 to 336 microns and this was recorded at the mid root region alone. The standard deviation of 74 microns was large and lent this data either to accusations of imprecision, or to an explanation of gross variability such that a mean measurement would be meaningless in the real biological sense.

Wills, Picton and Davies (1976) concluded that their derived vascular volume of 0.5 to 1 percent of the periodontal ligament accounted for 30 percent of the displacement of the tooth. These figures appeared disproportionate. In the light of the vascular volume figure of 8.3 ± .4 percent derived from the present study for the marmoset mandibular second molar, it would appear that the periodontal vasculature plays a greater supportive role than that proposed by Wills, Picton and Davies (1976).

Götze (1965) reported vascular volumes of one to two percent for human second premolars, and yet in 1976 he graphically represented blood volumes of up to 3.5 percent for the lingual surfaces of mandibular human permolars.

Parfitt (1967) did not dismiss the periodontal ligament vascular volume as inconsequential. However, he attributed to it, a value of only 2 percent. No method of derivation of this figure was given. Parfitt proposed conditions under which one could increase the vascular volume. Vessels dilated as a result of increased blood pressure within the ligament, producing an increased vascular volume. However, this increased blood volume did not alter the physical characteristic of the tooth supporting structures.

The clarification of the vascular volume question can only be resolved through further quantitative research. One would then be in a position to review the function and control of the tooth support mechanism.

One of the possible explanations for the different vascular volume figures proposed by various workers may be accounted for by the masticatory variations seen between animal species. Man represents the evolutionary pinnacle of mammalian jaw development, having the widest range of mandibular movements due to the temporomandibular articulation. However, specialized masticatory patterns, such as the gnawing of rodents, the milling of ungulates and the scissor-like action of carnivores, have evolved.

Assuming that the vascular volume and architecture of the periodontal ligament is involved in the tooth support mechanism, then its degree of involvement would be dependent on the particular masticatory pattern and the force vectors it was designed to

withstand. The periodontal ligament as a proprioceptive and supportive organ is a functional, essential part of the masticatory apparatus and, as such, should not be viewed in the isolation of supporting a single tooth. It represents just one part of a complex synergistic apparatus specifically designed to cope with that species' masticatory evolutionary pattern. The vascular volume and architecture of a particular periodontal ligament would thus vary, depending on the demands placed on it at a specific time and for a specific situation. It is not conceivable that such a highly sophisticated arrangement of vessels does not have the ability to fine tune its activities and responses.

Thus, it seems unjustified to attempt to generalize on the vascular volume figure of an idealised periodontal ligament, irrespective of any parameters or constraints. In this light, all periodontal vascular volume quantitative figures may be considered valid. However, one must also recognise the broad range that exists within and between species, and the respective effect of the vascular volume as part of that tooth support mechanism, but not in isolation of its role within the masticatory apparatus.

Chewing in man, and in most other mammals, has been described as being unilateral by Moore (1981), Hiiemae (1978) and Gysi (1921) (cited by Moore, 1981). Anisognathy has been attributed to the lingual inclination of mandibular molars and premolars and the buccal inclination of the corresponding maxillary teeth. Hiiemae (1978) believed that true bilateral

chewing was only possible where the transverse width of the lower jaw was the same as the upper jaw (e.g. rodent), or where the width of the lower jaw was greater than the upper. Gysi suggested that even though the muscles were in action on both sides, chewing occurred unilaterally.

Yurkstas (1965), in his research on the masticatory act, discovered a preference for people to use the right side for chewing when both sides were of equal efficiency and comfort. He attributed this unilateral function to the same neural elements which dictate the use of the right hand or right leg in preference to the left. Yurkstas may, however, have had in his sample, a selection of right handed people.

Nevertheless, it is of some interest to speculate on the biological reason, if any, for a statistically significant greater periodontal vascular volume on the right side of the mandible than on the left.

In the present study, the distribution of the periodontal vascular volume was found to be zone dependent with a quadrant effect being expressed only in zone 1 for quadrants 1, 2, 3 and 4. The lingual surface of the periodontal ligament in the lateral third adjacent to alveolar bone had the greatest percentage vascular volume.

If one considers the anatomy of this second molar region, certain features are encountered which may be of relevance. The mandibular molars have slightly lingually inclined crowns and

consequently buccally inclined roots. The mandibular canal, at its inception, is more medial in comparison to its medio-lateral passage to the mental foramen. In the region of the second molar, which is approximately half way along the mandibular canal, the apices of this tooth would be buccal to the canal.

The expression of a greater percentage vascular volume on the lingual surface may be attributed to either its strategic anatomical position, or to a functional role.

With the action of vertically orientated forces, and in consideration of the lingual inclination of molars, it would not be presumptuous to envisage a resultant force vector which would drive the tooth crown further lingually. Under such circumstances, the location of a major part of the vascular component of the tooth support mechanism on the lingual surface of the periodontal ligament would be advantageous.

The increase in vascular volume with increasing depth of the periodontal ligament is indicative of the importance of the root apex region in tooth support. Previous descriptions of apical basket-like vascular networks have been made by Bevilacqua (1958), Lenz (1968), Garfunkel and Sciaky (1971) and Wong (1983).

Most of the research on the responses of teeth to loading has been conducted on single rooted anterior teeth. These workers have included Parfitt (1960), Bien and Ayers (1965), Gianelly (1969), Khouw and Goldhaber (1970), Körber (1970), Slatter and Picton (1972), Wills, Picton and Davies (1972), Storey (1973),

Wills, Picton and Davies (1976), Walker, Ng and Burke (1978), Wills and Picton (1978), Moxham (1979) and Wills and Picton (1981).

The response of a two or three rooted molar tooth to loading has not been investigated except for hyper- and hypofunction tests on rat molars by Koivumaa and Lassila (1971) which did not represent a quantitative study of the forces applied. There may be a problem of gaining sufficient access to these areas. Nevertheless, conjecturing on the tooth support mechanism, and specifically that of the molar, can only be theoretical until these investigations have been performed.

The concept of periodontal ligament compartmentalization as proposed by Shore, Berkovitz and Moxham (1984) into regions which differ functionally, biochemically and/or structurally is an admirable one, bar for the implication of definitive roles and boundaries to the exclusion of other areas. This author prefers to conceptualize the periodontal ligament as a synergistic model with multi-dependent, interacting and sharing systems, but within which there is room for the recognition of specialized functions.

CHAPTER 7:

CONCLUSIONS

1. The repeatability of the method employed to collate the data is satisfactory with no significant statistical difference at the one percent level for any of the variables tested.

2. The lateral vascular distribution is such that:

zone 1	(alveolar third)	=	8.9 percent
zone 2	(middle third)	=	13.1 percent
zone 3	(tooth third)	=	2.6 percent.

3. The vertical vascular distribution is zone dependent with zone 1 having a steady increase with increasing ligament depth, zone 2 exhibiting a gradual increase with a plateau developing in the apical third and zone 3 showing an increase in the middle third. However, in general, the vertical vascular distribution was found to increase from the cervical to the apical regions.

4. Within zone 1, quadrants 1, 2, 3 and 4 were found to be statistically different from each other at the one percent level with:

$$Q3 > Q1 > Q4 > Q2$$

i.e. lingual > buccal > mesial > distal.

5. All quadrants exhibited the same vascular volumes in zones 2 and 3.

6. Within zone 1, quadrants 5, 6, 7 and 8 failed to exhibit a statistical difference at the one percent level of significance. Thus, the disto-buccal, disto-lingual, mesio-lingual and mesio-buccal surfaces in the alveolar third of the periodontal ligament can be regarded as having the same vascular volume.

7. The percentage vascular volume for a right molar was greater than that of a left molar in all zones, at any ligament depth and in all quadrants. This finding was statistically significant at the one percent level.

8. Although the pilot study showed the mesial root to have a greater vascular volume than the distal root, this was not found to be the case in the major study where no root effect was established.

9. There was no statistical evidence of any detectable effect on vascularity between animals due to their respective diets.

10. The average periodontal vascular volume for the marmoset mandibular second molar obtained from the data is 8.3 ± 0.4 percent (mean ± 2 standard errors). The vascular volume figure obtained from the present study is eight times that derived mathematically by Wills, Picton and Davies (1976) and four times that obtained histologically by Götze (1965).

11. The statistical model of the vascular distribution is an acceptable representation of the data.

12. The resultant findings of the present author are in agreement with Götze's (1965, 1976) observations of a vertical vascular volume increase from the cervical third to the apical third of the periodontal ligament. Götze's conclusion that the buccal and lingual tooth surfaces were better supplied than the mesial and distal regions has been confirmed by the present study. However, the present author supports the view that a predominance of vessels lie on the lingual ligament surface, but only in the alveolar third of the periodontal ligament.

13. Further research is required to quantify the periodontal vascular volume of different tooth types within and between animal species and in particular, that of the human. The theories describing tooth support mechanisms need to be reassessed in the light of tooth loading experiments using molars. It is further envisaged that controlled dietary studies be performed using the marmoset as the experimental animal.

CHAPTER 8:

APPENDIX I

ZOOLOGICAL FEATURES OF MARMOSET MONKEYS

Marmoset monkeys are small New World primates of the family Callithricidae, suborder Platyrrhini. According to Levy, Dreizen and Bernick (1972b), the word "marmoset" is derived from the French "marmouset" which translates as a "grotesque image" or "mannikin".

The four recognized marmoset genera are Cebuella, Callithrix, Sanguinus and Leontopithecus. They are all indigenous to the forested areas of South America from Costa Rica to northern Bolivia and southern Brazil.

The following details provide a description of these animals:

1. General Description

   They are flat nosed with a dolichocephalic head, an elongated body and a long, hairy non-prehensile tail.

2. Behaviour

   In the wild they are highly arboreal, diurnal and omnivourous.

3. Diet

   In the wild marmosets subsist largely on a diet of fruit, tender vegetation, seeds, seed pods, insects, bird eggs

and nestlings. According to McIntosh and Looker (1982) who developed a marmoset colony in Australia, the diet of these animals contained 59.7% wheat, 20% soybean meal, 10% meat meal, 5% skim milk powder, 2% cottonseed meal, 2% blood meal, salt, trace elements and vitamins and a piece of fruit daily.

4. Primary Dental Formula

$$I\ ^{2}/_{2} \quad C\ ^{1}/_{1} \quad M\ ^{3}/_{3}$$

5. Permanent Dental Formula

$$I\ ^{2}/_{2} \quad C\ ^{1}/_{1} \quad Pm\ ^{3}/_{3} \quad M\ ^{2}/_{2}$$

The presence of three premolars and two molars in each jaw quadrant is the distinguishing feature of the marmoset permanent dentition. The other New World monkeys have three premolars and three molars in each jaw quadrant and the old world monkeys have two premolars and three molars.

6. The marmosets used in this study were of the Callithrix jacchus jacchus variety.

7. Goss, White and Townsend (1983) and Towsend, White and Goss (1983) have researched the craniofacial growth of marmosets while Shaw and Auskaps (1954), Johnston, Dreizen and Levy (1970) and Goss (1984) reported on their dental development. Navia (1977) discussed the use of marmosets as animal models in dental research.

APPENDIX II

PREPARATION OF SPECIMENS

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

37-40% formalin	100 ml
distilled water	900 ml
sodium dihydrogen phosphate	4 gm
disodium hydrogen orthophosphate	6.5 gm

II.2 Decalcifying solution:

40% formic formate - 7 gm sodium formate
100 ml distilled water
40 ml formic acid

II.3 Tissue Processing:

The technique of Peterfi (Culling, 1974).

The specimens were subjected to the Double Embedding Technique. The following procedure was implemented at a constant temperature of 37°C.

1.	50% alcohol	.... 2 hours
2.	70% alcohol	.... 2 hours
3.	80% alcohol	.... 2 hours
4.	90% alcohol	.... 2 hours
5.	Absolute alcohol	.... 2 hours

6. Absolute alcohol .... 2 hours

7. Absolute alcohol .... overnight

8. One part absolute alcohol
   and one part methyl salicylate .... 2 hours

9. Methyl salicylate plus
   0.5% celloidin .... 2 days

10. Methyl salicylate plus
    1% celloidin .... 2 days

The tissues were then infused with paraffin wax at a constant temperature of 60°C.

1. 2/3 methyl salicylate
   1/3 paraffin wax .... 1 hour

2. 1/2 methyl salicylate
   1/2 paraffin wax .... 1 hour

3. 1/3 methyl salicylate
   2/3 paraffin wax .... 1 hour

4. Paraffin wax (first change) .... 2 hours

5. Paraffin wax (second change) .... 2 hours

6. Paraffin wax (third change) .... overnight

The specimens were then vacuumed in clean paraffin wax at a temperature of 60°C for 40 minutes prior to embedding in blocks.

APPENDIX III

PREPARATION OF GELATINIZED GLASS SLIDES

1. Clean slides were soaked in a dichromate cleaning solution which was prepared as follows:

   100 gm potassium dichromate was dissolved in 3/4 total volume of distilled water. 100 ml of concentrated sulphuric acid was added slowly to make up 1 litre of solution.

2. The slides were then washed in running tap water for at least 6 hours and then thoroughly rinsed in distilled water.

3. The slides were allowed to drain for 2 to 3 seconds.

4. The slides were dipped into the subbing solution which was prepared as follows:

   (i) 5.0 gm of U.S.P. gelatin was dissolved in 1 litre of warm distilled water.

   (ii) Then 0.5 gm of chrome alum (chromium potassium sulphate) was added.

   (iii) The solution was coded and then filtered through Whatman No. 1 filter paper.

5. After the slides had been dipped in the subbing sulution, they were allowed to dry vertically in a dust free atmosphere.

APPENDIX IV

STAINING TECHNIQUE

POLLACK'S TRICHROME STAIN (modified from Luna, 1968)

IV.1 Preparation of 0.5% Glacial Acetic Acid Solution

Glacial acetic acid	....	0.5 ml
Distilled water	....	100.0 ml

IV.2 Preparation of Pollack's Trichrome Solution

(i) Acid fuchsin

Acid fuchsin	....	0.5 gm
Ponceau 2R	....	1.0 gm
Light green, SF Yellowish	....	0.45 gm
Orange G	....	0.75 gm
Phosphotungstic acid, C.P.	....	1.5 gm
Phosphomolybdic acid, C.P.	....	1.5 gm
Glacial acetic acid	....	3.0 ml
Alcohol, 50% to make	....	300.0 ml

(ii) Mixing Procedure

1. Glacial acetic acid was added to the alcohol.
2. Four 50 ml portions of this acidified alcohol were placed in four beakers. In the first beaker, dissolve acid fuchsin and ponceau 2R; in the second, light green; in the third, orange G and phosphotungstic acid; and in the fourth, phosphomolybdic acid.

3. The remaining acidified alcohol was used to rinse out the beakers and to make up the volume.

4. The mixture was filtered and then it was ready for use.

IV.3 Staining Procedure

1. Deparaffinize and hydrate to distilled water

   i.e. xylol .... 10 minutes
   alcohol 100% .... 2 minutes
   alcohol 100% .... 2 minutes
   alcohol 70 % .... 2 minutes
   distilled water

2. Glacial acetic acid (0.5% solution) .... 10 seconds.

3. Rinse indistilled water.

4. Pollock's trichrome for 20 seconds.

5. Rinse in glacial acetic acid (0.5% solution) until no more stain washes off .... 3-5 seconds.

6. Rinse in distilled water.

7. Counter-stain in Light Green 0.05% for 70 seconds.

8. Rinse in 95% alcohol.

9. Dehydrate in 2 changes of 100% alcohol.

10. Clear in Xylol (2 changes).

11. Mount in D.P.X.

IV.4 Staining Reactions

Collagen	....	green
Bone	....	red
Erythrocytes	....	orange red to orange
Dentine	....	red

APPENDIX V

STAINING TECHNIQUE

VERHOEFF'S IODINE IRON HAEMATOXYLIN (modified from Culling, 1974)

V.1 Preparation of Verhoeff's iodine

Iodine	....	2 gm
Potassium iodide	....	4 gm
Distilled water	....	100 ml

V.2 Preparation of Special Staining Solution

Stock 5% alcoholic haematoxylin	....	20 ml
10% ferric chloride	....	8 ml
Verhoeff's iodine	....	8 ml

These were freshly prepared and the solutions were added to a flask in the order given.

V.3 Preparation of Van Gieson's Stain

Saturated aqueous solution of picric acid	....	100 ml
1% Acid fuchsin	....	10 ml

V.4 Staining Procedure

1. Deparaffinize and hydrate to water

i.e.	xylol	....	10 minutes
	alcohol 100%	....	2 minutes
	alcohol 100%	....	2 minutes
	alcohol 70%	....	2 minutes
	running tapwater		1 minute

2. Lugols iodine .... 3 minutes

3. Rinse in running tap water.

4. 5% sodium thiosulphate .... 2 minutes

5. Rinse in running tap water .... 1 minute

6. Special stain until jet black .... 30 minutes

7. Differentiate in 2% ferric chloride until elastic fibres are clearly seen, rinse in water and examine under the low power of a microscope.
   If over-differentiated, sections may be restained.

8. Rinse in distilled water.

9. Rinse in 95% alcohol.

10. Distilled water .... 5 minutes

11. Counter stain in Van Gieson's stain for 1 minute

12. Flick off stain, blot dry.

13. Quick dip in 100% alcohol and blot.

14. Clear in xylol.

15. Mount in D.P.X.

V.5 Staining Reactions

Elastic fibres and nuclei	....	Black to blue-black
Cytoplasm and muscle	....	Yellow
Collagen	....	Red

APPENDIX VI

STAINING TECHNIQUE

MILLER'S STAIN (from Miller, 1971)

VI.1 Preparation of Stain

(i) Victoria Blue 4R .... 1 gm

New fuschin .... 1 gm

Crystal violet .... 1 gm

Dissolve in 200 cm^3 of hot distilled water, then add in the following order:

Resorcin .... 4 gm

Dextrin .... 1 gm

30% ferric chloride (freshly prepared) .... 50cm^3

(ii) Mixing procedure

1. Boil for 5 min, then filter while hot.
2. Transfer precipitate plus filter paper to original beaker and re-dissolve in 200 cm^3 of 95% alcohol.
3. Boil on a hot plate or in a water bath for 15 to 20 minutes.
4. Filter and make up to 200 cm^3 with 95% alcohol.
5. Finally, add 2 cm^3 of concentrated hydrochloric acid.

VI.2 Staining Procedure

1. Deparaffinize and hydrate to water

   i.e. xylol .... 10 minutes
   alcohol 100% .... 2 minutes
   alcohol 100% .... 2 minutes
   alcohol 70% .... 2 minutes
   running tap water 1 minute

2. Lugols iodine .... 3 minutes

3. Rinse in running tap water.

4. 5% sodium thiosulphate .... 2 minutes

5. Pour on 0.5% potassium permanganate for 5 minutes.

6. Rinse in distilled water.

7. Recolorize in 1% oxatic acid for 3 minutes

8. Rinse in distilled water.

9. Rinse in 95% alcohol.

10. Transfer to the staining solution in a coplin jar for 1 to 3 hours.

11. Wash in 95% alcohol to remove excess stain.

12. Rinse in distilled water.

13. Counter stain with Van Gieson's stain for 1 minute.

14. Flick off stain and blot dry.

15. Quick dry in 100% alcohol and blot.

16. Clear in xylol.

17. Mount in D.P.X.

VI.3 Staining Reactions

Elastic fibres and mast cell granules	.... Jet Black
Cytoplasm and muscle	.... Yellow
Collagen	.... Red

APPENDIX VII

THE KEYBOARD OF MOP-1 (Carl Zeiss Inc., Oberkochen, West Germany)

VII.1 Diagram of the keyboard

PRIME FUNCTIONS

AREA	LENGTH PERIMET	NUMBER				X. Y. COORD
PROCESS FUNCTIONS			CHANNELS AND INPUT CODES			SPECIAL INPUTS
SET	CLEAR		7	8	9	INP
			4	5	6	Ex
SUMM	DISCR		1	2	3	.
PRINT	SEND		0		ENTER	-

VII.2 The Prime Functions

AREA = Area determination based on perimeter trace or by corner point sensing in combination with DISCR.

LENGTH PERIMET = Length from point a to point b, or perimeter length circumscribing the object. Either measurement can be by continuous trace or by point-to-point sensing in combination with DISCR.

NUMBER = Simple feature count by pin-pointing objects or in combination with SUMM and SET for simultaneous count of measured features.

X. Y. COORD = Series of measurements of X and Y coordinates.

VII.3 Process Functions

SET = Open program priorities and sequence for input of selected Prime and Process Functions (requires ENTER to finalize instructions).

PRINT = Print-out of selected functions, values and identifications. (PRINT must always be preceded by SET.)

SEND = Command for data transfer to interfaced calculator or computer.

CLEAR = 1 x CLEAR = Erase last measured value.
2 x CLEAR = Erace all measured values.
3 x CLEAR = Erase all values and selected functions.

SUMM = Summation of values.

DISCR = Measurement of areas, lengths, perimeter, angles and coordinates by discrete points.

VII.4 Channels and Input Codes

Data from up to 10 different sets of features can be stored, counted and summarized in 10 different channels. Once a channel is selected, all data will be entered into the

selected channel until another one is chosen. The channel keys are used as input codes in combination with INPUT instructions.

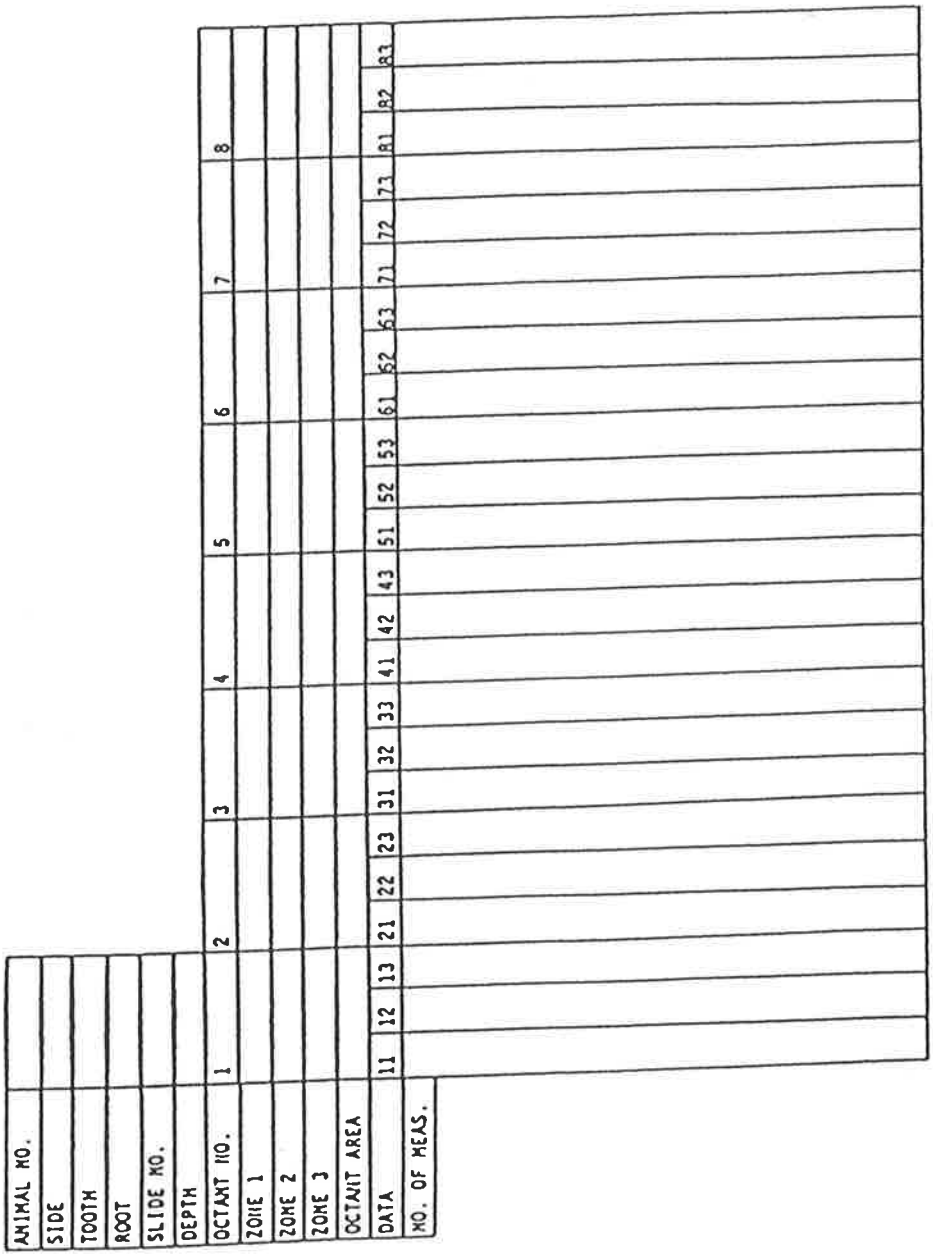
ENTER = Finalizes the input of Process Functions and Special Inputs (Sub-routines). ENTER must be pressed whenever SET with Process Functions or INPUT and codes are selected.

VII.5 Special Inputs

These are sub-routines.

APPENDIX VIII

THE DATA RECORDING SHEETS



APPENDIX IX

KEY TO DATA RECORDING SHEETS

KEY

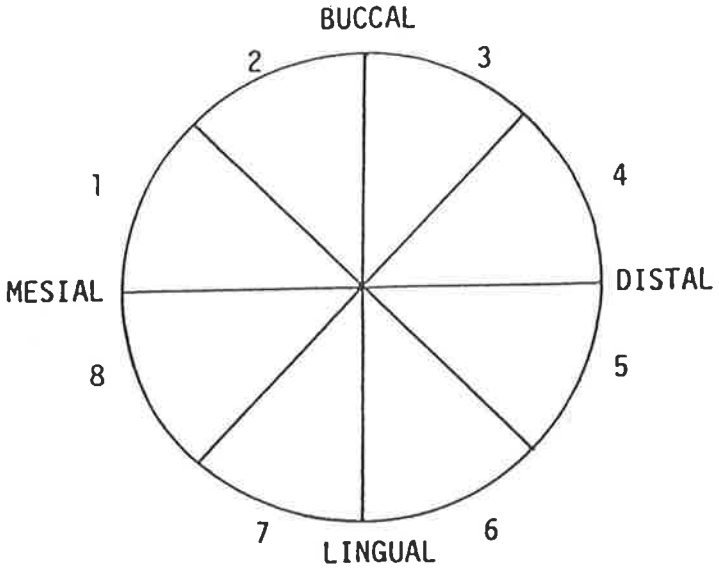
SIDE : RHS = 1
LHS = 2

TOOTH : central incisor = 1
lateral incisor = 2
canine = 3
1st premolar = 4
2nd premolar = 5
3rd premolar = 6
1st molar = 7
2nd molar = 8 = M_2

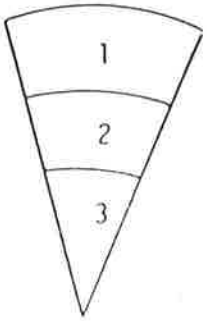
M_2 is to be referred to as 8 henceforth

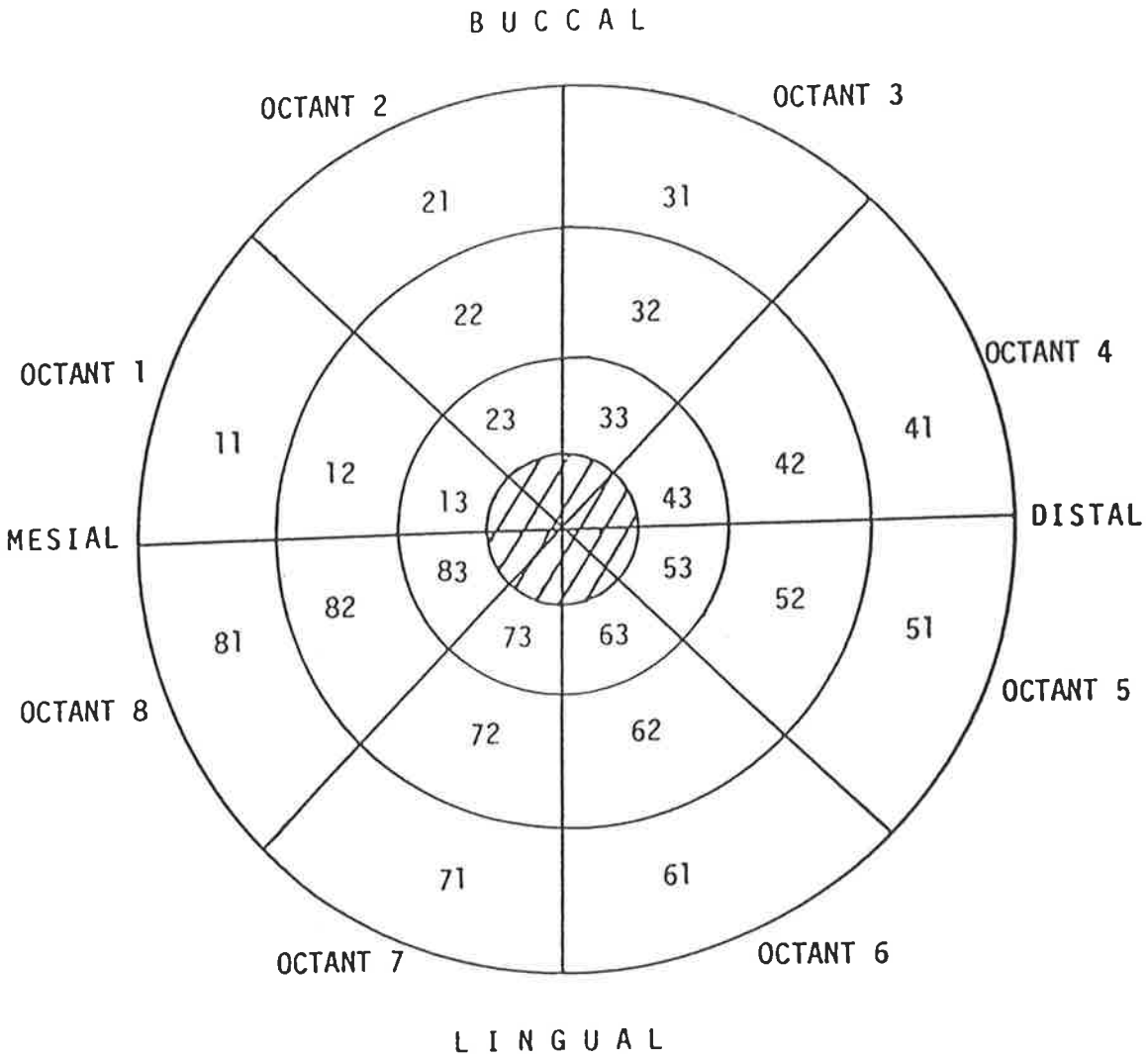
ROOT : Mesial Root = 1
Distal Root = 2
Single Root = 3

OCTANTS : There are 8 octants present on any one tooth or root ligament



ZONES : Each octant consists of 3 zones





Quadrant 1 = Octant 2 + Octant 3 = Buccal
Quadrant 2 = Octant 4 + Octant 5 = Distal
Quadrant 3 = Octant 6 + Octant 7 = Lingual
Quadrant 4 = Octant 8 + Octant 1 = Mesial
Quadrant 5 = Octant 3 + Octant 4 = Disto-buccal
Quadrant 6 = Octant 5 + Octant 6 = Disto-lingual
Quadrant 7 = Octant 7 + Octant 8 = Mesio-lingual
Quadrant 8 = Octant 1 + Octant 2 = Mesio-buccal

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