AN ULTRASTRUCTURAL STUDY OF VASCULAR ENDOTHELIAL JUNCTIONS IN NORMAL AND TENSIONED RAT PERIODONTAL LIGAMENT

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in partial fulfillment of the
degree of Master of Dental Surgery

by

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SUMMARY

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Ultrastructural changes in the microvascular bed of the periodontal ligament (PDL) incident to the applied forces have rarely been reported. Clark (1986) and Lew (1986) demonstrated changes in the fenestrae populations and Cooper (1988) established there was an increase of tissue channels when a tensile load was applied to the PDL. However, there are no published data on the effects on the endothelial junctions or their dimensions where there is an applied loading to the PDL.

The purpose of this investigation was to morphologically study and quantitatively compare the dimensions of endothelial junctions in the microvascular bed of the normal PDL with those of the tensioned PDL.

The rat maxillary molar specimen blocks were from Cooper's study (1988). Twelve week old male Porton rats were used as the experimental animal model. A 1.0 N. extrusive load was applied continuously, for a period of 30 minutes, to the right first molar. The contralateral left first molar served as the experimental control. The tissue was demineralized in a solution of 0.1 M. EDTA in 0.06 M. cacodylate buffer and 2.5% glutaraldehyde for 8 - 12 weeks. Then the tissue was post-fixed in 2% osmium tetroxide, dehydrated through a graded ethanol series, infiltrated with propylene oxide and Agar^{R} 100, and emdedded in Agar^{R} 100.

Four matching pairs of experimental and control blocks from four animals were selected for this investigation. The silver sections were prepared using a Reichert-Jung OM-U4 Ultramicrotome. The sections were stained with uranyl acetate and Reynolds' lead solution. The area to be examined was the PDL at the most mesial side of the first molar. The tissue was examined at levels 250 microns apart from the cervical to apical region. A minimal number of 150 complete junctions from the normal PDL and 150 complete junctions from tensioned PDL were selected randomly for the examination.

A transmission electron microscope, JEM -2000 FX, was used to examine and photograph the sections. The goniometer was used to explore the junctions so that the narrowest opposing membranes could be studied and measured. Measurements were made of the various widths and depths along the junctions.

Five types of blood vessels were encountered in the investigation, i.e., venous capillaries, arterial capillaries, postcapillary-sized venules, terminal arterioles and small collecting venules. The morphology of the junctions was found to be related to the type of blood vessel.

There were three types of junction found in the PDL microvasculature, i.e., close junctions, tight junctions and open junctions. No gap junctions were found. There were no significant differences in the numbers of each junction

type between normal and tensioned PDL. A Chi-square analysis also indicated that the proportion of tight junctions was not significantly different among different types of blood vessels. However, a significant change in the dimensions of junctions was found.

Close junctions were in the majority forming 80% of all the junctions investigated. The width of the close region was approximately 5-6 nm. The junction width of the close junctions at the non-close/tight region increased (9.4%) as a result of extrusive loads ($p<0.01$). Since the close junctions comprise the majority of junctions in the PDL, the increase in the junction width could imply that the fluid and substrate exchange through the junctions also increased incident to the extrusive load. It is therefore possible that close junctions are one of the major exchange pathways in the PDL microvasculature.

Tight junctions were found in approximately 16% of junction population. The tight regions where the opposing membranes fused were commonly found in the luminal third (87.5%). The tight regions appeared to be 'spot-fusion' rather than 'zone-fusion'. There was no significant change in the junction width of the tight junctions.

Open junctions were rarely found (4%). They were found only in the venous capillaries and postcapillary-sized venules where the walls were relatively thin. The pore sizes ranged from 136 nm. to 1231 nm.

The junction depth was found to have a strong positive correlation to the endothelial wall thickness across the middle of the junction ($p<0.001$).

The organization of the endothelial junctions in three dimensions could not be determined by thin section technique in this study. Since the three-dimensional reconstruction of tight junctions requires a series of ultra-thin sections. Further investigation of junctions by freeze-fracture replicas is suggested.

SIGNED STATEMENTS

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This project is submitted in partial fulfilment of the requirements for the degree of Master of Dental Surgery at The University of Adelaide.

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

Kanoknart Chintakanon

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==========

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CHAPTER 1 INTRODUCTION

Some investigators consider that the vascular system of the periodontal ligament (PDL) plays an important role in the tooth support system. Several functional hypotheses have been proposed, such as, hydraulic damping (Bien and Ayer, 1965), a squeeze film effect and cirsoid aneurysms (Bien, 1966), thixotropic concepts (Kardos and Simpson, 1980) and viscoelastic properties (Picton and Wills, 1978). Some evidence has shown that changes in vascular volume in the PDL influence the position of the tooth (Wills et al.,1976, 1978). Experimental tooth loading is also claimed to change the PDL vasculature (Bien and Ayers, 1965; Bien, 1966; Gianelly, 1969; Rygh, 1972a, 1972b, 1973, 1976; Gaengler and Merte, 1983; Lindskog and Lilja, 1984). However, the clear cut mechanism(s) of the role of the PDL vasculature and interstitial tissue in the tooth supporting system has not been demonstrated.

The transport pathways through the walls of blood vessels are :- direct cellular traverse through the membrane, close or open junctions, vesicles and fenestrae (Renkin, 1977; Casley-Smith, 1983). Clark (1986) and Lew (1986) demonstrated the changes of the fenestrae population in the PDL vasculature, incident to applied pressure and tension. Cooper (1988) and Cooper et al. (1990) investigated the transport of a tracer molecule, via the 'tissue channels', across the PDL vascular endothelium. It would seem to be

logical to examine the possible role of junctions in fluid flow incident to an applied force. However, apart from the mention of PDL endothelial junctions in an abstract report by Corpron et al. (1976), no studies of normal junctions or the effects of tooth loading on junctions have been reported in the dental literature.

Close junctions are assumed to be an important passage way for water and small molecules, and open junctions for macromolecules and cells (Casley-Smith, 1983; Simionescu et al., 1984). The passage through this pathway may become exacerbated during inflammatory reactions (Simionescu et al., 1984). The endothelial cells and their junctional barrier could be biologically manipulated to either increased or decreased permeability (reviewed by Shepro et al., 1988).

Junctional complexes in other microvascular beds have been studied qualitatively and quantitatively (reviewed by Casley-Smith, 1967, 1981, 1983; Renkin et al., 1977; Simionescu et al., 1972, 1974a, 1974b, 1975a, 1975b, 1976, 1978; Pitts and Simms, 1977; Schneeberger and Lynch, 1984). Some controversy exists, probably due to differences in the techniques used and the tissues investigated. The endothelium of each microvascular segment has its own junctional organization and significantly differs in thickness, vesicle density, occurence of channels, and other characteristics. Thus the search for the structural basis of permeability should be specially related to a given microvessel of a certain circulatory bed (Simionescu et al., 1984).

The purpose of this study is to morphologically study and quantitatively compare vascular endothelial junctions in normal PDL and in the PDL subjected to a short period of continuous tension.

CHAPTER 2 AIMS

1. To morphologically study the type, number and the dimensions of endothelial junctions in normal periodontal ligament (PDL) of rat maxillary first molars and in tensioned PDL incident to an extrusive force of 1.0 N. for a period of 30 minutes.

2. To quantitatively compare the type and dimensions of endothelial junctions in normal PDL of rat maxillary first molars with tensioned PDL.

3. To quantitatively compare the responses of different types of endothelial junction to the extrusive load related to different categories of blood vessels in the PDL of rat maxillary first molars. The analysis is to be based on a minimum of 150 random sections along the mesial side of the root length of normal PDL and 150 random sections of tensioned PDL incident to a continuous extrusive force.

4. To test the hypothesis that endothelial junctions in the PDL may undergo dimensional changes with tension loads and thus provide an enhanced route for fluid and particle flow between the blood vessels and interstitial tissue compartment.

CHAPTER 3 LITERATURE REVIEW

==========

1. THE VASCULATURE OF THE PERIODONTAL LIGAMENT

The periodontal ligament (PDL) is a highly vascular fibrous connective tissue which serves three main functions for the tooth and its surrounding structures. The vasculature must be adequate to provide the physiological exchange of material between the blood and the PDL tissue. The vascular system also acts as a cushion, assisting in the mechanisms of tooth support, and provides for the significant exchange between the blood and the PDL components in viscoelastic and/or thixotropic interstitial supporting systems (reviewed by Edwall, 1982).

1.1 GROSS ANATOMY :

The PDLs receive their arterial supply from several different sources. In the maxilla, the vessels supplying the PDL are derived mainly from the superior alveolar artery. Additional supplies come from the greater palatine artery via the lingual gingiva and from the labial branches of the facial and infraorbital arteries. In the mandible, the main vessels are derived from the inferior alveolar artery with additional branches from the sublingual branch of the lingual artery via the gingiva and also from branches of the buccal, inferior labial, masseteric and mental arteries. These vessels run into the marrow spaces of the alveolar bone and subsequently enter the PDL at various levels.

The veins in the PDL drain into the veins in the interalveolar and interradicular septi. The veins do not follow the arteries, but join to form larger veins in the interalveolar septi which are connected to a rich venous network surrounding the apex of each alveolus. In the maxilla, the veins accompany the superior arteries and drain either anteriorly into the facial vein or posteriorly into the pterygoid venous plexus. The main venous drainage of the mandible is via periosteal veins which themselves drain into the jugular veins. Several inferioralveolar veins accompany the inferioralveolar arteries and may drain anteriorly through the mental foramen into the facial vein, or posteriorly through the mandibular foramen into the pterygiod venous plexus. (Edwall, 1982)

1.2 MICROSCOPIC STRUCTURE AND VASCULAR ARRANGEMENT :

The vascular arrangements of the PDL of rat molars were observed by Kindlova and Matena (1962), using intra-arterial latex perfusion. The arrangements were found to be similar to those seen in primates. The main vessels of the ligament were arranged in palisades and ran parallel to the long axis of the tooth and branched to form a basket-like capillary network surrounding the root surface. These vessels were enclosed by loose connective tissue and passed between the principal fibre bundles of the ligament. There were no direct anastomoses between periodontal and gingival arteries, but communicating capillaries were seen between gingival arterioles and PDL veins.

Carranza et al. (1966) found that the vascular plexus was located closer to the bone than the cementum. However, the continuously growing incisors were the exception in that the vascular plexus of the periodontium around the enamel organ appeared closer to the enamel than the bone. This finding may be related to the need for a greater blood supply for enamel-forming tissue. Blood vessels supplying the alveolar bone provided branches to the PDL, particularly in the middle and apical thirds of the root. These vessels emerged perpendicular to the long axis of the root and immediately bent taking a direction parallel to the long axis of the tooth.

In rat and mouse molars, connections were seen between periodontal and pulpal blood vessels which were not found in other species. These connections occured particularly in the apical third of the root and in the bifurcation area. Vascular connections between gingival and periodontal blood vessels were rare.

Garfunkel and Sciaky (1971) studied the vascularization of the rat PDL using perfusion of an India ink suspension, composed of 4 to 5 micron granules, into the common carotid artery. The authors stated that each tooth with its surroundings had a complex vascular network that was directed to its particular functions. Each network was closely connected with others forming a complex blood supply source which could easily provide a compensatory blood supply when necessary.

The complex periodontal network was connected closely to the blood vessels of the pulp, the alveolar bone, the marrow space, and the supra-periosteal capillary network of the gingiva. The close relationship between the periodontal and gingival capillary network showed the existence of a plexus common to the terminal vessels of the inferioralveolar and facial arteries.

The maxillary and the mandibular molar vascular arrangements were similar. The main vessels in the apical region supplied the tooth pulp and the surrounding PDL. The "hammock-like" network was formed by larger vessels running parallel to the long axis of the tooth and the thinner vessels that crossed over and joined perpendicularly to the larger ones. The PDL vessels did not terminate at the apex of the alveolar crest, but were joined with the supra-periosteal blood vessels by a direct or an indirect link through the bone.

The maxillary incisor and the mandibular incisor showed similar vascular arrangements. The blood supply to the incisor was much richer than that to the molar. The blood vessels of the periodontium in the cementum region were wider and thicker than those of the enamel region, which indicated that there was a richer blood supply in the more active tissues.

2. MICROCIRCULATION OF THE PERIODONTAL LIGAMENT

The patterns of the microvasculature vary tremendously from one tissue to another representing a specialized functional unit within each organ. Different parts of the same organ system may have their own anatomical or physiological arrangement of the microvasculature (Majno, 1965). The microcirculatory bed of the PDL not only served functionally for the exchange of gases and nutritional demands but also served as a cushion assisting in the equalization of masticatory force. (Folke and Stallard, 1967).

At the histologic level, Folke and Stallard (1967) suggested that the PDL vasculature consisted of a greater number of either preferential channels or an overall increase in vessel diameter compared with those of the gingiva and periostium. Garfunkel and Sciaky (1971) also reported that the rat PDL vessels seemed to get smaller and thinner when they entered the alveolar bone.

2.1 CLASSIFICATION :

Microvasculature is a term used to describe a network of microscopic vessels. The arterioles, the capillaries, the venous capillaries, the postcapillary venules, the collecting venules and the muscular venules are referred to as the microvascular bed which is structurally and functionally part of the tissue they supply. An extensive general classification of microvessels, established by Rhodin (1967, 1968) is shown in Table 1.1 and Table 1.2

Table 1.1 Ultrastructural features of arterial blood vessels. (Rhodin, 1967)

Blood Vessel Type	Lumen Calibre	Wall Thickness	Endothelial Cell Morphology	Peri-endothelial Cells	Other
Arteriole	100-50μm	Greater than 6μm	Cell 0.15 to 2μm in width, few pinocytotic vesicles, upstream cell usually overlaps downstream cell.	2-3 layers of smooth muscle cells, some eosinophils, mast cells and macrophages.	Well developed elastica interna, non-myelinated nerves extending to smooth muscle layer.
Terminal Arterioles	Less than 50μm	Less than 6μm	Generally as above but with many filaments parallel to the long axis of the blood vessel and with more pinocytotic vesicles.	One layer of smooth muscle cells.	Little elastic interna, nerves closer to vessel wall with more frequent contacts with the smooth muscle layer, some myoendothelial junctions.
Precapillary	7-15μm	Less than 5μm	Cell protrudes towards vessel lumen, nucleus shorter, thicker and more lobulated than above, some cytoplasmic filaments, many pinocytotic vesicles.	One layer of smooth muscle cells.	An increased number of unmyelinated nerves associated with a decrease in lumen diameter, frequent neuromuscular and myo-endothelial junctions.

Table 1.2 Ultrastructural Features of Venous Blood Vessels. (Rhodin, 1968)

Blood Vessel Type	Lumen Calibre	Wall Thickness	Endothelial Cell Morphology	Peri-endothelial Cells	Other
Venous Capillary	4-7μm	0.3-1.3μm	Some rough endoplasmic reticulum, free ribosomes, mitochondria, vesicles, granules and filaments.	Occasional veil cells and pericytes. Some macrophages, leukocytes, lymphocytes and plasma cells.	Endothelium may be fenestrated.
Post-capillary venule	8-30μm	1-5μm	Cell rarely less than 0.2μm thick and generally larger than that of venous capillary. Slight over-lapping of adjoining cells.	More pericytes and veil cells than above. Some primitive smooth muscle cells around larger vessels.	Endothelium generally lacks fenestrae. Leukocytes may adhere to endothelial wall.
Collecting Venule	30-50μm	1.7μm	As above.	Continuous layer of pericytes and veil cells around vessel. More primitive smooth muscle than above. Smooth muscle cells around larger vessels.	Single layer of veil cells and some collagenous fibrils surround blood vessels.
Muscular Venule	50-100μm	2.0μm	As above.	1-2 layers of smooth muscle cells.	Veil cells form a complete layer around vessel wall. Myoendothelial junctions present.
Small collecting vein	100-300μm	2-3μm	As above but with specific endothelial granules.	2 or more layers of smooth muscle cells.	Unmyelinated nerves situated 5-10 microns from muscular layer.

In mouse PDL, Freezer (1984) classified the microvessels into 4 types, based on the vessel diameter and the appearance of pericytes as follows :

1.Capillary-sized vessels with an internal diameter of four to seven microns, with a partial or complete pericytic cellular investment.

2.Capillary-sized vessels without a pericytic cellular investment.

3.Postcapillary-sized vessels with an internal diameter of 8 to 30 microns, with associated pericytes being few in number or absent.

4.Postcapillary-sized vessels with a complete pericytic cellular investment.

Clark (1986) modified Rhodin's classification to describe the microvascular bed in the PDL of the rats into arterial and venous types as shown in Table 1.3

TABLE 1.3 Classification of blood vessels in the PDL of the rat maxillary first molar. (Clark, 1986)

Blood vessel type	Lumen	Endothelial Wall
Terminal Arteriole	10-30 um	No fenestrae. 0.5-1.5 um. smooth muscle layer. Wall thickness 0.4-2.0 um
Arterial Capillary	5-10 um	Fenestrae present. Single pericyte covering. Nuclei bulges within endothelial wall.
Venous Capillary	8-10 um	Fenestrae and perikaryon layer. Wall thickness 0.04-0.3 um
Postcapillary Venule	10-30 um	Incomplete pericytic covering. Some fenestrae. Wall thickness 0.05-0.5 um

2.2 TYPE OF BLOOD VESSELS :

The types of vessel in the microvascular bed of the PDL, according to vessel luminal diameters and ultra-structural criteria, range from terminal arterioles through precapillary sphincters, arterial capillaries, venous capillaries to postcapillary-sized venules. The vessel luminal diameters vary from 4 microns to approximately 35 microns. Vessels are predominantly postcapillary-sized venules with a lesser number of capillaries (Sims 1983, Weekes 1983, Freezer 1984, Weekes and Sims 1986, Freezer and Sims 1987).

Frank, Fellinger and Steuer (1976) examined the rat molar PDL and found that most of the blood capillaries are of the continous type but a few fenestrated capillaries may be seen. Shore, Berkovitz and Moxham (1983) investigated the fenestrated capillaries in rat mandibular incisor PDL and found that the PDL vessels exhibited significant numbers of fenestrations, although such capillaries are not usually found in fibrous connective tissues. Fenestrated capillaries in the PDL were also reported by Corpron et al. 1976, Rygh 1976, Sims 1983, Shore et al. 1983, Moxham et al. 1985, Clark 1986, Lew 1986. Sims (1983) has suggested the term 'apericytic' venules for the venules that did not have the complete layers of pericytes and veil cells. Freezer and Sims (1987) reported that 70% of postcapillary-sized venules were devoid of a pericytic cellular investment in the plane of section.

2.3 DISTRIBUTION OF BLOOD VESSELS :

Anisotropic distribution of microvascular volume in the PDL of the mouse mandibular molar was described by Sims (1987), Freezer and Sims (1987). Maximum vascular volume occured in the lingual quadrants and minimum volume characterised the buccal quadrants. The vascular volumes in the mesial and the distal quadrants were slightly larger than those in the buccal quadrants. Vertically, the vascular volume increased from the cervical to apical region. Across the ligament, the middle third contained the greatest volume, and the tooth third contained the smallest volume.

Corpron et al. (1976) described the distribution of different types of blood vessels in the PDL of the mouse molar. Continuous capillaries were found in the central part of the PDL, whereas the fenestrated capillaries were found peripherally and close to the osteoblastic layer. Small arterioles with incomplete muscular coatings were found near alveolar bone or near the cementum. Shore et al. (1983) described the distribution of fenestrated capillaries along the rat mandibular incisor root. In the apical region, the periodontal vessels contained more fenestrations (3.5×10^6 fenestrations per $mm.^3$ of tissue) than those in occlusal region (1×10^6 fenestrations per $mm.^3$ of tissue).

The microvascular bed of the mouse mandibular molar PDL was investigated by Sims (1983). Postcapillary-sized venules were the predominant structures in the venous pool in both the cervical and apical regions. In the apical region, these vessels were noticeably larger and more numerous.

Morphologically, the vessels in the apical region appeared to be devoid of pericytes and therefore conformed to 'apericytic' post-capillary venules.

Stereological study of the blood vessels of the mouse molar PDL, using a transmission electron microscope (Freezer and Sims, 1987), showed that 88% of the vessels were venous type and 12% of the vessels were capillary type. The alveolar third contained the greatest number of both types of vessels. None of the venous vessels were found in the tooth third. Of the venous vessels, 70% were devoid of a pericytic cellular invesment in the plane of section.

2.4 ULTRASTRUCTURE :

Corpron et al. (1976) described the ultrastructure of the terminal vessels in the PDL of mouse molars. They stated that capillaries showed finger-like projections that protruded into the lumen. The cytoplasm of the endothelial cells contained numerous microvesicles, scattered ribosomes, and small oval mitochondria. Occasional tight junctions appeared between endothelial cells lined completely by a distinct basement lamina but incompletely encircled by pericytes. In fenestrated capillaries, pores appeared along flattened areas of the capillary wall as 30 to 50 nm. openings bridged by thin membranes continuous with either side of the endothelial cell. They also suggested that these fenestrations may represent rapid pathways for metabolite transport across the endothelium to the extravascular compartment, where high metabolic requirements of growth or repair occur.

Three-dimensional microvascular morphology of the rat molar PDL, using the SEM vascular casting technique, was demonstrated by Weekes and Sims (1986). The vessels around the buccal and lingual walls and over the interdental septum were arranged in a similar pattern, but at the interdental septum the vessels were more closely packed. Tracts of four to six vessels which coursed occluso-apically, had two sizes of blood vessels. Vessels with a diameter of six to ten microns were classified as capillaries and those with an average diameter of 20 microns as venules. Capillaries branched and intertwined around the venules, but anastomoses were infrequent. In the interradicular septum region, the postcapillary venules coursed occluso-apically for a distance of between 100-400 microns before re-entering the bone.

The direction of vascular flow at the buccal and lingual walls, and adjacent to the interdental septum, was postulated to be from the occlusal down to the apical region. However, in the interradicular area the flow was from the apical aspect up toward the crest of the septum.

2.5 MICROVASCULAR WALL :

The ultrastructure of the blood vessel walls in mouse molar PDL, using the transmission electron microscope, was reported by Sims (1983). The results of the investigation differed significantly from Rhodin's classification (1968) in their scarcity of pericytes, the presence of fenestrae, and the existence of unmyelinated nerves and nerve endings alongside the endothelium. The

endothelium of postcapillary-sized venules was relatively thin with occasional fenestrae being present.

The frequency of fenestrae accorded with the observations of Casley-Smith (1970,1971) who demonstrated that fenestrae were several times more common in the venous limbs of capillaries than arterial limb capillaries. Postcapillary-sized vessels appeared to have a continuous basement membrane and many showed an incomplete layer of pericytes. Large areas of the endothelial wall in postcapillary-sized vessels, as well as in venous capillaries, were exposed to the enveloping collagen bundles with only the basement membrane intervening.

Unmyelinated nerves and nerve endings are frequently found alongside the endothelium of venous capillaries and postcapillary-sized venules. In the apical third, the venous vessels become significantly larger. They appear to be devoid of pericytes and comprise endothelial-like tubes with only the basement membrane and occasionally veil cells intervening between the endothelium and the enveloping collagen fibre bundles. The ratios of luminal diameter to vascular wall thickness were also reported. Venous capillaries in the cervical region averaged 20:1. Cervical zone postcapillary venules approximated 30:1 whereas many apical postcapillary venules had ratios which exceeded 60:1 (Sims, 1983).

3. THE EFFECT OF FORCES ON THE PERIODONTAL LIGAMENT VASCULATURE

The complex hydrodynamic structure of the PDL consists of three major fluid systems which are :-

1. The circulatory systems of blood which course within vessel walls.

2. The viscoelastic system made up of the cells and fibres of the periodontium.

3. The extra-circulatory system of interstitial fluids which fill the space between the cells, fibres, blood vessels, tooth and bone.

Each system has its characteristic response to pressure of various application rates and intensities. (Bien and Ayers, 1965; Bien, 1966a, 1966b; Picton, 1963; Picton and Wills, 1978).

3.1 MICROSCOPIC CHANGES :

Bien and Ayers (1965) described the characteristics of a hydraulic damper provided by the blood flow that is 'soft' when there is a slow application rate of force, but becomes 'hard' when the application rate of force is increased.

Bien (1966a) stated that the extracellular fluid system dissipated part of the force acting on a tooth and acted to restore the tooth to the equilibrium position. The drag effect of the PDL on teeth was exerted by the intracellular fluids confined within the cells and fibres. He also proposed that there were two damping effects in the tooth support mechanism. The first damping effect was a

squeeze film effect involving the interstitial fluid. The second damping effect was provided by the passage through the walls of smaller blood vessels of fluid, entrapped because of constriction of the vessels by the stretched periodontal fibres. The flow of blood in the vessels was occluded by the entwining periodontal fibres. Minute gas bubbles were formed below the stenosis due to the drop of pressure. Above the stenosis, fluid diffused through the walls of the 'cirsoid aneurysms' formed by the build-up of pressure. To date, no morphologic evidence has been published to support this hypothetical structure.

Gianelly (1969) indicated the close relationship between vascular integrity of the PDL and the type of resorption resulting from an applied force. He suggested that the vascular supply of the PDL played a strategic role in determining the type of bone resorption that follows force application. Light force stimulated frontal resorption while heavy force, which occluded the vascular channels, obliterated the nutritional supply for cellular activity and hydraulic transmission of force in the ligament.

Walker et al. (1978) and Ng et al. (1981) suggested that the major contribution of tooth support is provided by the 'solid' collagen fibers and semi-solid ground substance compartments of the ligament acting in conjunction with the periodontal vasculature.

A microscopic study in rats, investigated by Gaengler and Merte (1983), revealed that both compression and tension in the PDL led to ischemic areas beginning in the

venules and capillaries spreading in the small veins and arteries, and ending with the main arteries. However,the compression zones were more affected. This investigation also demonstrated the independence in structure and function of the PDL and gingival blood circulation.

3.2 ULTRASTRUCTURAL CHANGES :

Rygh (1972a, 1972b, 1973, 1976) has reported on the ultrastructural vascular changes in both pressure and tension zones of PDL incident to orthodontic tooth movement in humans and rats. The findings indicated that advanced cellular and vascular changes precede the fibrillar alterations.

In pressure zones, the ultrastructural vascular changes were distinguished into three stages :-

The initial stage showed the slowing of blood circulation which was characterized by the dilatation of blood vessels, packing of erythrocytes, and the presence of platelets and floccular material interspersed between the cellular elements.

The second stage was the partial disintegration of blood vessels, degeneration of endothelial and perivascular cells and basal lamina, thus allowing communication between the lumen of the blood vessels and the perivascular tissue space. Degeneration of endothelial cells was characterized by intercellular swelling, dilatation of the endoplasmic reticulum, separation of the nucleus from the cytoplasm and the formation of clear vacuoles. Fragmentation of red blood cells also occured in this stage.

The third stage showed the complete disintegration of blood vessels which was characterized by close packing of the erythrocytes and a tortuous appearance of basement membrane. Morphological alterations increased in severity with increasing time. Despite the time factor, the findings in rats were morphologically similar to those in humans, except the crystallization of erythrocytes which was found only in rats. However, rat and human tissue cannot be compared directly because of the experimental conditions and the differences in vascularities (Götze, 1980; Wills et al., 1976).

In tension zones, marked changes occurred in the cells, fibre systems and ground substance on the cementum and alveolar bone surfaces of the PDL. Vascular changes did not seem to be dramatic, but a slight dilatation seemed to occur. Fenestrated vessel walls were occasionally observed.

Orthodontically induced vascular injuries to the PDL were studied by Lindskog and Lilja (1984), using a scanning electron microscope. Extravasated erythrocytes, eichinocytes and platelets in various stages of degeneration were found in the pressure zones. This study indicated that the tissue reactions leading to remodeling of the PDL are associated with local vascular injuries and subsequent formation of a coagulum. Macrophages seemed to be the cells which play the most important role during removal of the hyaline zone.

3.3 EXTRUSIVE LOADS :

Much less is known about the source and nature of extrusive loads and their effects upon tooth position and the PDL than is known about intrusive loads. (reviewed by Moxham and Berkovitz, 1982b) Oral soft tissues, gravity, sticky foods, certain habits and orthodontic procedures can produce extrusive movements. On applying the extrusive load, a biphasic response is seen. In the first phase, instantaneous extrusion occured rapidly. The second phase involved a more gradual extrusion. Bien and Ayers (1965), Bien (1966a), Moxham and Berkovitz (1982b) have suggested that intrusive and extrusive loads may be resisted by different mechanisms. However, Picton (1986) showed the similar character of the load displacement and recovery response to both intrusive and extrusive loads applied to the PDL of adult monkeys. The report suggested that the mechanism of tooth support may be similar for either type of load.

Lew (1986) investigated the effects of a 1.0 N tension load, for a period of 30 minutes on the fenestral number and dimensions of the small blood vessels. The extrusive load resulted in an increase in both number and diameter of fenestrae in arterial capillaries, venous capillaries and postcapillary-sized venules. The increase of PDL tissue channels in the apical region of rat maxillary first molars incident to a continuous extrusive load was reported by Cooper (1988) and Cooper et al.(1990).

4. JUNCTIONAL SPECIALIZATIONS

Farquhar and Palade (1963), in describing the morphology of junctional complexes in a variety of epithelia, distinguished three components :

1. *Zonular occludens* (tight junction) which was closest to the lumen, characterized by fusion of opposing cell membranes over a variable distance and resulting in obliteration of the intercellular space. Within this zone, the membranes converged one or more times and their outer leaflets fused, forming short pentalaminar segments.

2. *Zonular adhaerens* (intermediate junction) was on the cell boundary below the tight junction. The membranes in this area coursed parallel for 0.2 to 0.5 microns at a distance apart of approximately 20 nm., with a dark band of dense fibrillar material associated with the cytoplasmic surfaces of the membranes. It was primarily involved in maintenance of cell cohesion.

3. *Macula adhaerens* (desmosome) characterized by dense plaques which were believed to be spaced at more or less regular intervals in a circumferential row parallel to the zonular adhaerens. Elsewhere on the adjoining cell surfaces, similar macula were distributed at random.

Fawcett (1981) stated four principal types of junctions could be recognized in vertebrates, i.e., zonula occludens, zonula adherens, macula adherens (desmosome) and gap junctions (Figure 3.1). These types of junction were classified into three groups according to their permeability and functions.

The *occluding junctions (zonula occludens, tight junction)* prevented small molecules from passing through the intercellular spaces of epithelia and thus enabled the organism to maintain an internal environment that was chemically distinct from its surroundings.

The *adhering junctions* which included both zonula adhaerens and macula adherens (or desmosomes), provided sites for attachment of fibrillar cytoskeletal and contractile elements onto the cell membrane and maintained cell cohesion.

The *gap junctions (communicating junction)* permitted passage of ions and small molecules from cell to cell and thus functioned in coordinating the activities of groups of cells.

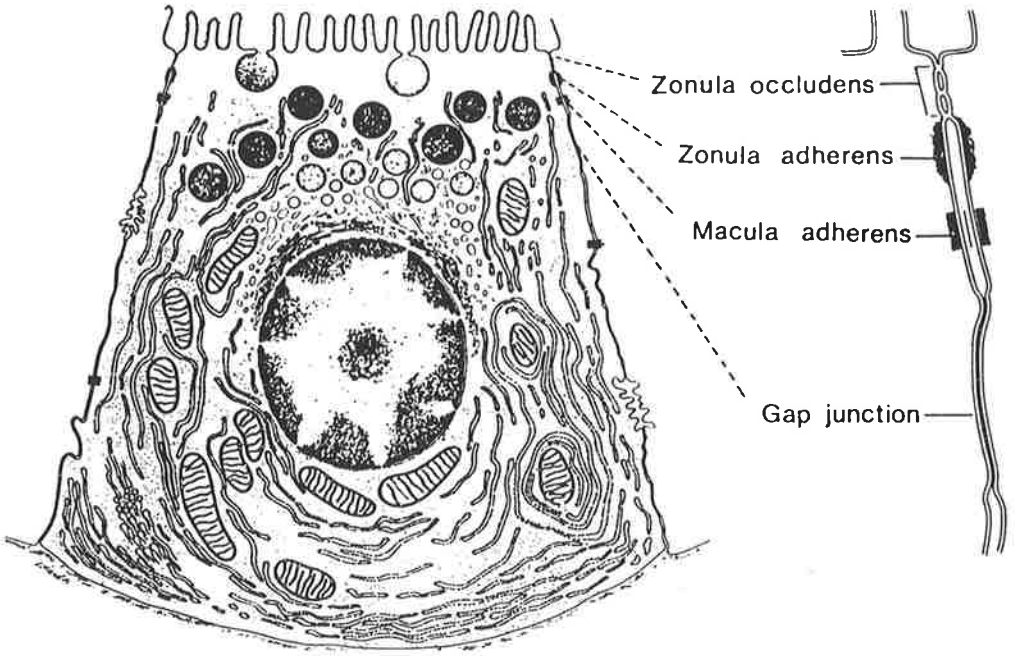

FIGURE 3.1 *Illustration of the location and components of and membrane relationships at the junctional complex between epithelial cells. A gap junction is depicted near the junctional complex but these may be located elsewhere on the lateral surfaces of the cells.* (Fawcett, 1981 in *'The Cell'*)

4.1 ENDOTHELIAL JUNCTIONS IN THE MICROVASCULATURE :

In the microvascular endothelium, the cells are linked to one another by intercellular junctions that consist of two basic types ; occluding (tight) junctions and communicating (gap) junctions (Simionescu et al.,1975a, 1976, 1984). Each microvascular segment has characteristically organized endothelial junctions that reflect various degrees of tightness and intercellular coupling. The authors described each segment as follows.

ARTERIOLES : In these vessels, which have a diameter of 30 - 100 microns and a continuous layer of smooth muscle cells in their media, the cells of the endothelium are joined together by a combination of tight and gap junctions. This combination insures strong cell to cell adhesion. Their functions are; 1) to seal off the intercellular spaces with the occluding junctions, and 2) to establish cell to cell communication through the gap junctions. In freeze-cleaved preparations, the tight junctions appear as a system of two to six strands which form a continuous network. Many of the meshes of the tight junction's network are fully or partly occupied by gap junctions. There are no interruptions affecting all tight junction strands and there are few strands with free ends. It is probable that these junctions are true occluding zonules.

CAPILLARIES : In these vessels, which measure 5 - 10 microns in diameter, the cellular elements of the wall are reduced to the endothelium and a few pericytes. The endothelium is characterized by continuous or quasi-

continuous occluding junctions. The junctions consist of two to three successive rows either branched and continuous, or staggered and discontinuous. Gap junctions are absent. It can be assumed that the type of intercellular communication they mediate is missing in the endothelium of blood capillaries.

PERICYTIC (POSTCAPILLARY) VENULES : These vessels are the immediate postcapillary vessels, approximately 10 to 50 microns in diameter, in which the endothelium is covered by an extensive but not continuous layer of pericytes. This segment corresponds to the postcapillary venule and collecting venule in the perimuscular connective tissue. These vessels show loosely organized endothelial junctions with discontinuous low-profile ridges and grooves devoid of particles. Gap junctions are absent. Approximately 30% of the junctions appear to have a space of up to 6 nm.

MUSCULAR VENULES : This type of vessel has a larger diameter of approximately 50 to 200 microns and a media which consists of a continuous layer of smooth muscle cells. The organization of the tight junctions in these vessels is very similar to that already described in pericytic venules, except that small and irregular gap junctions occur associated with (but not surrounded by) the system of low-profile ridges and grooves of the tight junctions.

TRANSITION OR INTERMEDIARY FORMS : Such forms have not been recognized so far at the arterial end of capillaries

but seem to occur at the venous end. The low frequency with which they are encountered suggests that they are of rather limited distribution.

The authors also proposed the term '*communicating junction*' for gap junction which has the following advantages; it relates to the main function established for this type of junction. Hence it is preferable to use the strictly morphological term 'nexus'; it describes appropiately the macular geometry of the structure; and its nomenclature is in line with that already used for other junctional elements.

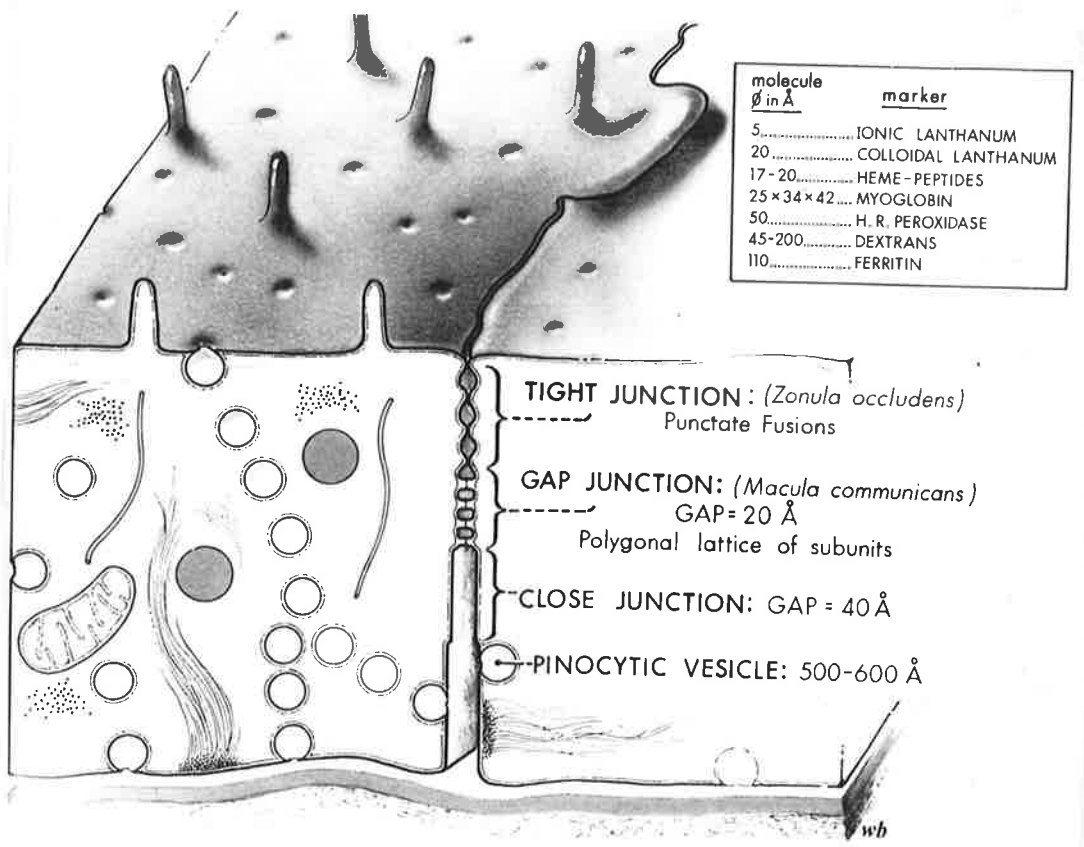

FIGURE 3.2 *Summary of the general appearance of the several types of cell junctions between endothelial cells as seen in sectioned material. The most commonly used markers for testing transendothelial transport in electron microscopy are indicated in the box together with approximate molecular diameter. Tight junctions appear as punctate fusions of outer leaflets of opposed cell membranes, and is believed by most investigators to allow penetration of only ionic lanthanum. Gap junctions in sections also appear to consist of cell membrane fusions, but have been shown to allow penetration of colloidal lanthanum and heme peptides with an average diameter of about 20 A°, therefore qualifying this junction as a gap junction, 20 A° wide. The close junction does not show a membrane fusion and allows penetration of myoglobin with a molecular diameter up to 40 A°. The pinocytis vesicles have been demonstrated to transport all tracer substances listed, particularly horseradish peroxidase, dextrans, and ferritin particles. Note that the size of the junctions has been slightly exaggerated in the drawing to explain more readily their substructure.*
(From Handbook of Physiology - The Cardiovascular system II; chapter 1; - Rhodin, 1984)

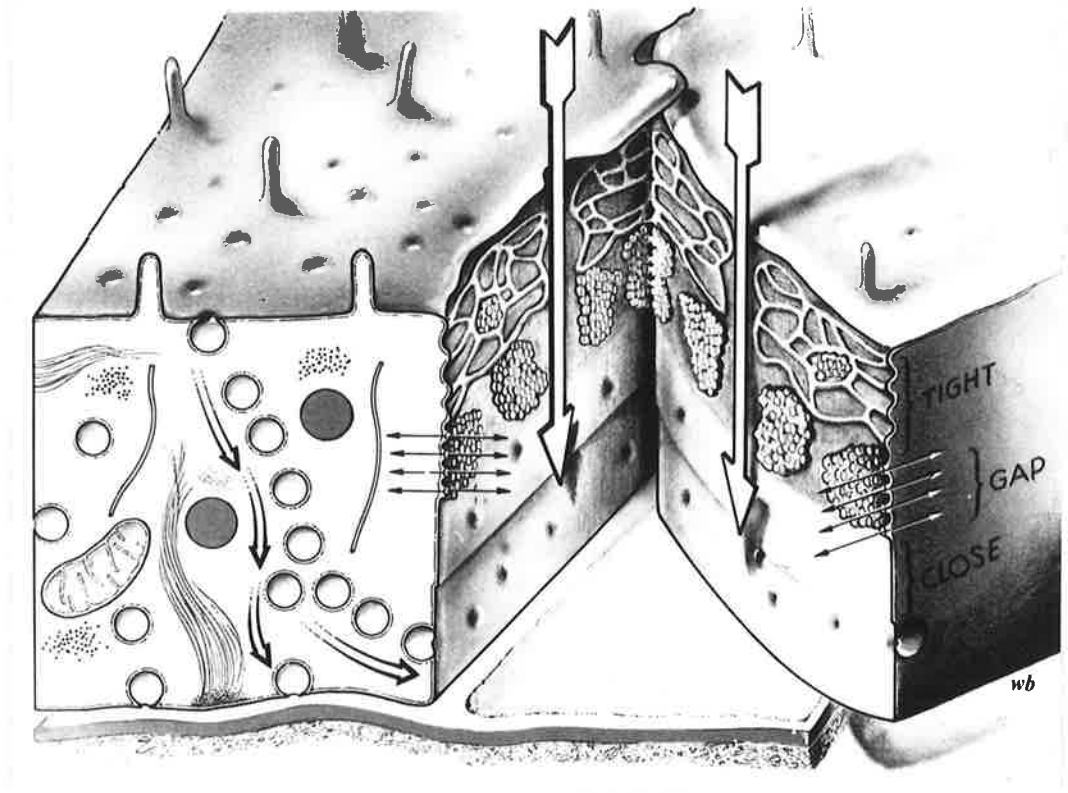

FIGURE 3.3 *Summary of the general appearance of the several types of cell junctions between endothelial cells as seen in freeze-cleaved material. It is assumed that by using this preparation technique, one has been successful in separating the two adjoining cells shown in fig 3.2 , so that it is possible to examine the apposing cell surfaces. In reality, the freeze-cleavage technique splits along the central plane of cell membranes and other membranes. It now appears that the punctate fusions of the tight junction are in fact derived from a system of ridges formed by minute particles within the cell membranes. Large arrows indicate that some investigators have demonstrated penetration of colloidal lanthanum across these junctions in some segments of the cardiovascular system, which is assumed to prove that these ridges are interupted at some points along the tight junctions, either permanently or momentarily. Gap junctions are, in fact, patch-like areas (maculae) with minute membrane particles forming small linkages, which serve as areas for transfer of ions and metabolites from one cell to the next, indicated by small double headed arrows. Double arrows indicate movement of pinocytic vesicles. This movement can also be assumed to occur in the opposite direction.* (From Handbook of Physiology - The cardiovascular system II; chapter 1; - Rhodin, 1984)

4.2 TIGHT JUNCTIONS :

The tight junction was first described in epithelia by Farquhar and Palade (1963). It is also called a punctate junction, occluding junction or zonular occludens. Two important biological functions have been ascribed to the tight junction : (1) it acts as a seletive permeability barrier in epithelia and endothelia, and (2) it forms and maintains structural, compositional, and functional polarity of cells.

4.2.1 STRUCTURE OF TIGHT JUNCTIONS :

The ultrastructural appearance of tight junctions in thin-sectioned, plastic-embedded tissue consists of a series of punctate contacts in which the outer leaflets of the adjacent membranes appear to fuse, thereby eliminating the intercellular space (Staehelin, 1974). Freeze fracture studies show that tight junctions consist of a belt-like network of varying complexity, of linearly arranged particles and/or strands (Simionescu et al., 1975a, 1975b, 1976). It has been claimed that, in leaky epithelia, such as the choroid plexus, a fragmented appearence of tight junction strands could provide a structural explanation for the relatively high permeability of these junctions (van Deurs and Koehler, 1979).

4.2.2 PERMEABILITY BARRIER :

The tight junction acts as a selective permeability barrier in epithelia and endothelia. The number of tight junction strands is generally taken to express the tightness of the junction. The greater the

number of strands the higher the transepithelial resistance. In leaky epithelia, such as the renal proximal tubule or the gall bladder , the average number of tight junction strands is two to four. Whereas, in tight epithelia, such as those of the stomach and urinary bladder, the average number is eight or more strands (Schneeberger and Lynch, 1984). However, Von BÜlow et. al. (1984) studied tight junction structure in the frog choroid plexus and found that the correlation between the number of tight junction strands and the transepithelial resistance did not exist in the frog choroid plexus.

Other factors beside the number of strands may be important in determining junctional resistance. Cereijido et al. (1983) reported that changes in temperature resulted in a reversible increase in junctional resistance. The length of the junction as related to the unit area of absorptive surface is of considerable importance in determining the paracellular resistance.

The properties of tight junctions can be altered by physiological requirements, fetal maturation, pathological conditions and experimental challenge. Therefore, the resistance of the tight junctions can be modulated so as to regulate transepithelial fluxes through the paracellular route. (Schneeberger and Lynch, 1984)

4.2.3 TIGHT JUNCTIONS IN ENDOTHELIUM :

There are certain important differences in the morphology and behavior between epithelial and endothelial

tight junctions. Those between endothelial cells are often located at the midpoint rather than the apex of the intercellular space. Unlike epithelial tight junctions, which form smooth, interconnected strands, those between endothelial cells remain particulate regardless of the length of time of fixation. Furthermore, the structure of the endothelial tight junction varies with the segment of the vascular bed examined (Simionescu et al., 1975a, 1975b, 1976).

4.2.4 THREE-DIMENSIONAL ORGANIZATION :

The three-dimensional organization of tight junctions in the capillary endothelium of a rat heart revealed by serial-section electron microscopy was investigated by Bundgaard (1984). The endothelial junctions were organized as irregular networks of lines of contact between neighboring cells, with paracellular pathways circumventing these contact lines. A quantitative comparison of the reconstructed cleft segments from the venules and the capillaries gave the impression that the general pattern of the lines of contact was similar. Occasionally, however, the venular junctions showed a complexity greater than observed in capillaries.

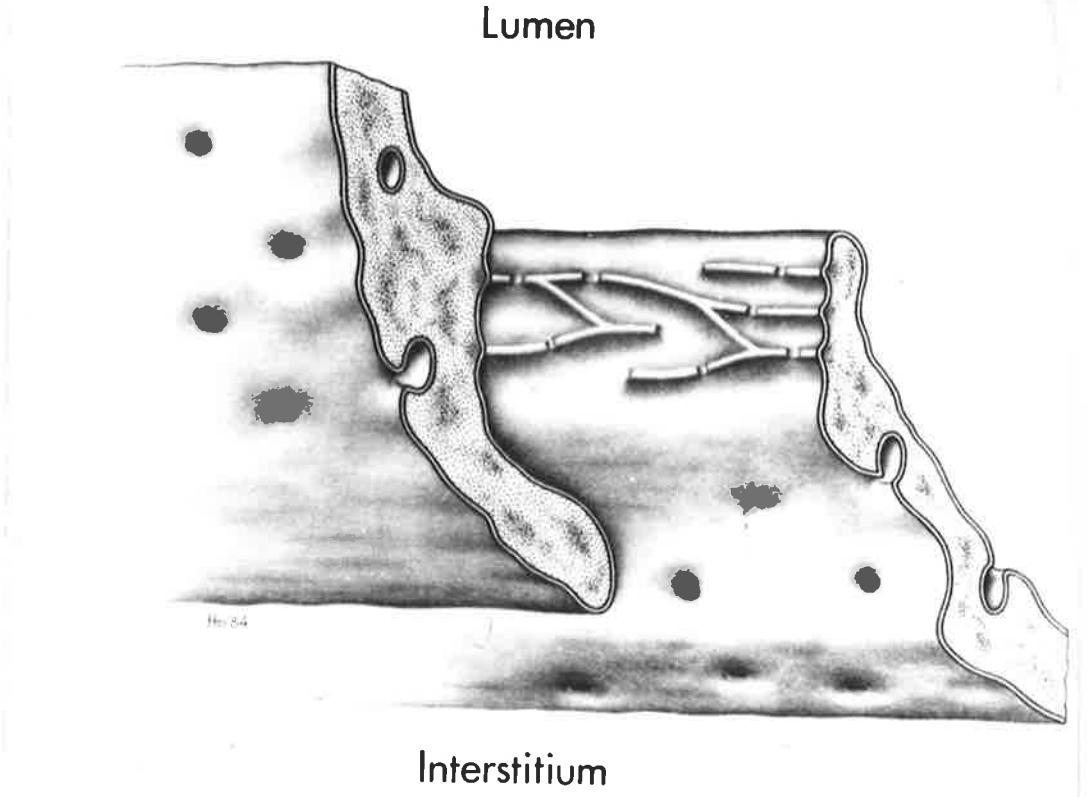

FIGURE 3.4 *Schematic drawing of the organization of capillary endothelium. A small segment of a capillary wall is viewed from the pericapillary space. The junctional region on the lateral surface of an endothelial cell is exposed. Three-dimensional reconstructions based on consecutive thin sections have shown that the punctate junctions observed in random individual thin sections represent an irregular network of lines of contact. Passage of hydrophilic solutes through the junctional region can occur by circumvention of lines of contact and via discrete interruptions in the lines. The pathways are accessible to hydrophilic solutes of dimensions at least up to the size of small proteins.* (Bundgaard, 1984)

These findings led to the conclusion that intercellular clefts are the main pathways for exchange of hydrophilic solutes across the wall of continuous capillaries. The junctions of capillary endothelium are focally permeable to hydrophilic solutes of dimension up to the size of small proteins, the upper size limit being somewhat ill-defined.

4.3 CLOSE JUNCTIONS :

Close junctions or narrow junctions are shown to have spaces of ~6 nm. between the two lamina membranes. Freeze substitution techniques reveal essentially similar dimensions to those found by chemical fixation (Casley-Smith, et al., 1975; Casley-Smith, 1981). The proportion of close junctions to total close and tight junctions is approximately 5% for the skeletal muscle capillaries of dog (Casley-Smith et al., 1975) and 7% for the mouse diaphragm capillaries (Casley-Smith, et al., 1979, Casley-Smith, 1981). The author suggested the proportion of close junctions varies greatly with different types of cells.

It has been said by some workers (Simionescu et al., 1978) that the close junctions are much more common in post-capillary venules than they are in the capillaries. However, this has been denied (Bundit ans Wissig, 1978; Casley-Smith et al., 1979) and the difference ascribed rather to the difficulties of observing close regions in the more convoluted junctions of capillaries without using a goniometer stage. The variations in the increase in the width of the close junctions of cardiac capillaries in the

inner portion of myocardium, and perhaps in the testis were reviewed by Casley-Smith (1983).

4.3.1 PERMEABILITY OF CLOSE JUNCTIONS :

The close junctions have been shown to be permeable to small tracers such as horseradish peroxidase, cytochrome C and saccharated iron oxide. The differences of the permeability of close junctions in arteries, capillaries and venules to other tracers were demonstrated by several investigators. However, there are some contradictions among the studies (reviewed by Casley-Smith, 1983).

The close junctions seem well fitted to represent the small pores of Pappenheimer (1953). It has been suggested that certain conditions such as inflammation or raised capillary pressure may stretch the pores, or shorten them. However, neither the width nor the depth of close and tight junctions were significantly altered by burning or histamine (Casley-Smith and Window, 1976).

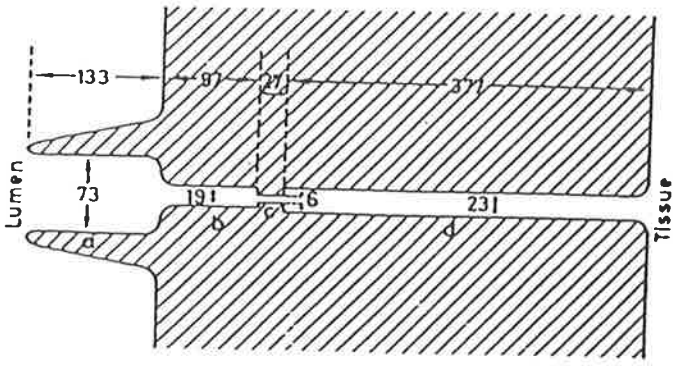

FIGURE 3.5 *A diagram of the close junction of blood capillaries. The mean dimensions are indicated in nm.* (From Casley-Smith et al., 1975).

4.4 GAP JUNCTIONS :

This type of junction is also called a nexus, macular communicans or macula occludens. The term 'communicating junction' was proposed by Simionescu et al. (1975a, 1978). Gap junctions are found in most animal tissues except skeletal muscle, many nerve cells and blood cells (Pitts, 1980). These junctions presumably represent the structural substrate of the direct two-way communication between cells. They are considered instrumental in the electron coupling and intercellular exchange of ions and small metabolites. Recent evidence has shown, however, that even in endothelia known to lack morphologically distinct communicating junctions such as capillaries and pericytic venules, an interendothelial transfer of dye (Lucifer yellow) can occur. This transfer may take place either via very small gap junctions that are commonly missed during investigation or via the tight junctions provided by cell-to-cell channels (Simionescu et al., 1984).

4.4.1 STRUCTURE OF GAP JUNCTIONS :

It appears that there is a 'spot weld' joining of one cell with the next: the interior of the 'weld' is 'drilled out' so that the cytoplasmic matrices of the two cells communicate with each other. Their precise significance is uncertain in endothelial cells (Casley-Smith, 1983).

In isolated rat liver gap junctions, electron microscopy shows that the junctions are composed of two closely apposed plasma membranes, separated by a narrow extracellular gap. The plane of the junctional membrane contains annular particles, called connexons, arranged in a regular hexagonal lattice. The connexons transverse the entire membrane, protrude into the extracellular gap, and are coaxially aligned with those in the other half of the junction (Zampighi, 1980). Each of the connexons may move with respect to each other to control channel permeability (Revel et al., 1980).

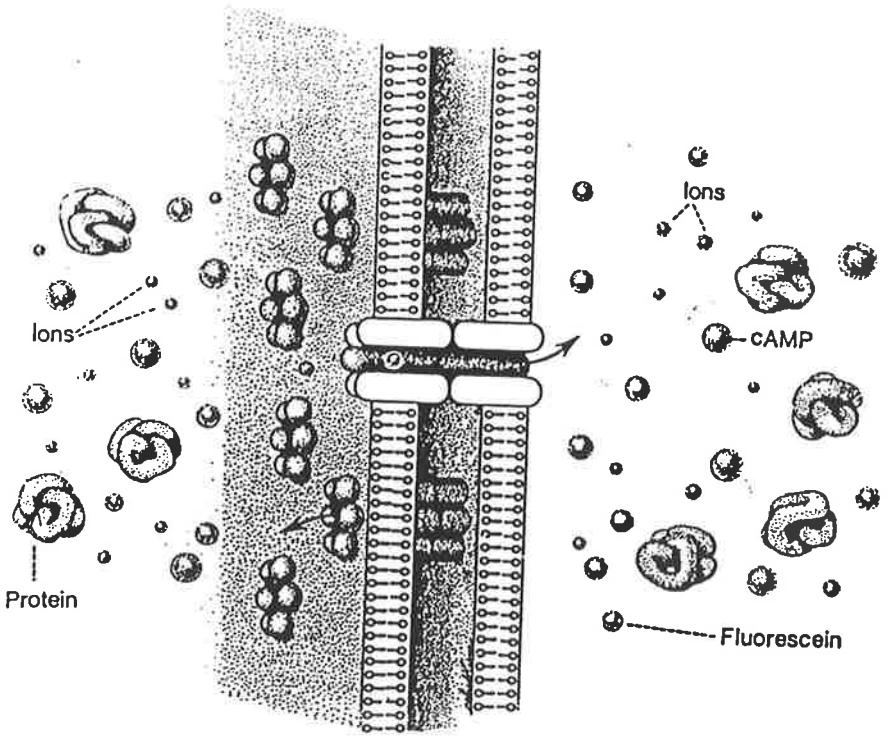

FIGURE 3.6 *Schematic depiction of the connexons and their subunits in a gap junction. The hydrophilic pore permits passage of ions and small molecules such as cyclic AMP or fluorescein but excludes macromolecules.*

(From 'The Cell - Junctional spacialization', Fawcett 1981)

4.4.2 THREE-DIMENSIONAL ORGANIZATION :

The three-dimensional structure was obtained by tilting the negatively stained communicating junctions at angles of between 0° and 79° to the incident electron beam. The three-dimensional maps show the membrane surfaces as well as the hydrophilic surfaces of the connexons. The clusters are hexamers arising from the exposed portions of six connexon subunits protruding from the cytoplasmic and external surfaces of each membrane. These subunits protrude less from the cytoplasmic face than from the extracellular face (Zampighi, 1980).

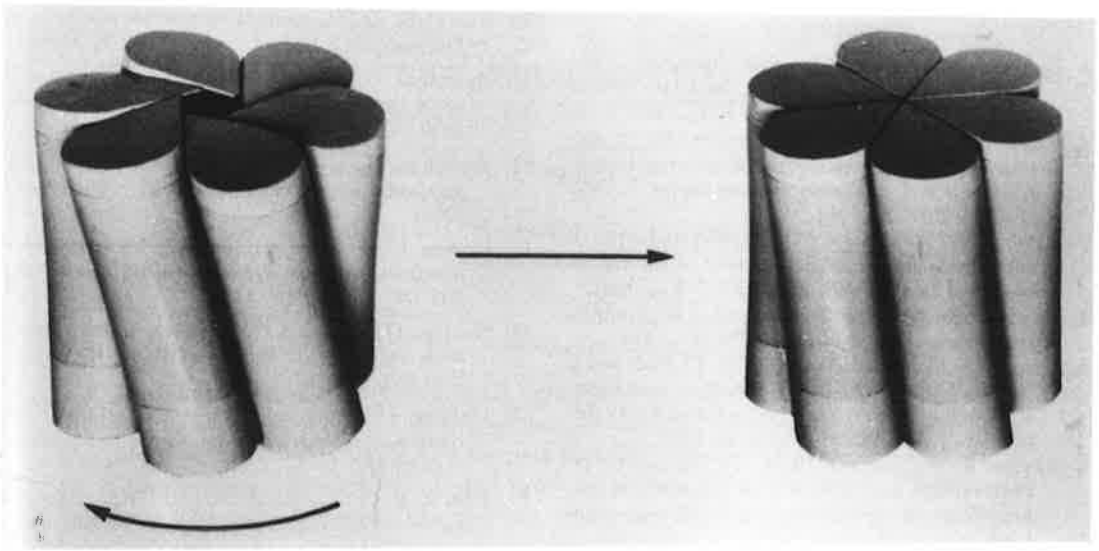

FIGURE 3.7 *Model of the connexon showing the transition from the 'open' to the 'closed' configuration. The closure of the channel on the cytoplasmic face (upper most) is achieved by the subunits sliding against each other, decreasing their inclination, and hence rotating, in a clockwise sense, at the base.*(Zampighi, 1980)

Gap junctions have the capacity to change from a low resistance to a high resistance state. Oxygen deprivation and aldehyde fixation result in a change from the low to the high resistance state. Therefore the majority of ultrastructural studies of gap junctions describe their appearance in the high resistance state. A rise in intracellular calcium or a lowering of intracellular pH is associated with uncoupling of the cell. It is believed that the intercellular channels may open and close in physiological circumstances in vivo (Fawcett, 1981). Gap junctions are found to reduce in size and numbers, while those of tight junctions are relatively unchanged, in regenerating cells (Revel et al.,1980). Conditions affecting the concentration of intracellular calcium ions: elevation of the external Ca load, treatment of cell cultures with a Ca-transporting ionophore, treatment with a combination of cyanide and iodoacetate, or with high levels of carbon dioxide, all cause depression of channel permeability. Treatment of cell culture with cyclic AMP or its more permeable derivative, produces an increase in permeability.

4.5 OPEN JUNCTIONS :

The open junctions are found in injured blood vessels, normal sinusoidal endothelium, and in normal and injured initial lymphatics. It is impossible to give any precise figure for when the gap between two cells becomes so large that it should be called an open junction (Casley-Smith, 1983). Casley-Smith and Window (1976) used 30 nm. as

an arbitrary lower limit. The opening of junctions in injured blood vessels occurs usually in the postcapillary venules.

4.5.1 OPEN JUNCTIONS AND ACUTE INFLAMMATION :

It appears that all inflammatory stimulae may produce an immediate (mediated) opening of the endothelial intercellular junctions of the post-capillary venules, which close again after some 30 minutes, and delayed (possibly direct, non-mediated) opening of the junctions of both capillaries and venules, which lasts for varying periods (reviewed by Casley-Smith, 1979).

It appears that these open junctions allow material to pass out of the blood vessels. On the other hand, it is suggested that more macromolecules may pass in via these openings than pass out, especially since they alomst always occur on the venous side of the circulation where the hydrostatic pressure is low. The open venular junctions perhaps function like venous-limb fenestrae, and actually remove a net amount of macromolecules from the tissues (Casley-Smith, 1979; 1983).

4.6. ENDOTHELIAL JUNCTIONS IN PULPAL BLOOD VASCULATURE :

4.6.1 CAPILLARIES :

The TEM examination of blood capillaries in rat incisor pulp was studied by Tabata and Semba (1987). Tight junctions of continuous capillaries in the subodontoblastic region are not perfect zonula occludens. These discontinuous zonula occludens (or macula occludens) are formed by 2 to 3

parallel strands and have a gap of approximately 10 nm. Gap junctions are not found.

4.6.2 ARTERIES AND VEINS:

Tabata and Semba (1988) did a TEM study of freeze-fractured replicas of the arteries and veins in rat incisor pulp. The junction of central arteries is composed of tight junctions with intercalated gap junctions. The tight junctions appear as 2 to 6 strands. There are many particles both on the ridges of the P-face and in the grooves of the E-face. These junctions continuously enclose the endothelial cells.

In the small arterioles, the junction is composed of 7 to 10 strands of tight junctions. No gap junctions are found.

The endothelium junctions of the central veins are composed of 2 to 4 strands of discontinuous low-profile ridges and grooves. There are no particles in the grooves on the E-face.

The endothelial junctions of the small venules are composed of 1 to 2 strands of tight junctions and linear creases. There are several particles in the grooves of this tight junction on the E-face. This junction is interpreted as being intermediate between that of the capillaries and that of the central veins.

CHAPTER 4 MATERIALS AND METHODS

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1. REVIEW OF COOPER'S LABORATORY PROCEDURE AND TISSUE PREPARATION.

1.1 EXPERIMENTAL DESIGN :

In order to eliminate the variable of cyclic hormonal changes on the vasculature and provide the mature periodontal ligament (PDL), Cooper selected 12 week old male Porton rats, approximately 250-350 g. in weight, as the experimental animal model. Sodium ferrocyanide (M.W. 212) contained in experimental primary perfusate (Appendix 1), which precipitated with a cobalt salt, tris (ethylenediamine) cobalt III Chloride (M.W.399.64) (Appendix 2) contained in experimental primary fixative (Appendix 3), was the ultrastructural tracer. The right maxillary first molars were chosen to receive the experimental extrusive force (1.0 N. tensile load applied continuously for a period of 30 minutes) with the appliance designed by Clark and Lew (1986). The contralateral left first molars served as the experimental control.

1.2 LABORATORY PROCEDURE :

The animals were anesthesised with I.P. injection of Nembutal^{R} (Appendix 5). Heparin B.P. (Appendix 6) was injected I.V. to avoid undesirable blood coagulation. Subsequently, the appliance designed by Clark and Lew (1986) was used to provide a 1.0 N. extrusive force on the right

maxillary first molars and maintained for a period of 30 minutes. The neck dissection was commenced 10 minutes after application of the experimental tension. The experimental tracer ion, sodium ferrocyanide contained in experimental primary perfusate (Appendix 1), was perfused for a period of 5 minutes. Then, the experimental primary fixative (Appendix 3) containing the cobalt salt (Browning 1980) was perfused for a period of 5 minutes while the experimental molar was maintained under tension. The animals were decapitated after the extrusion appliance was removed. The maxillae were carefully dissected out and trimmed coronally mesial to the first molars and distal to the third molars.

1.3 TISSUE PROCESSING :

The maxillae were demineralized at 4°C in a solution of 0.1 M. EDTA in 0.06 M. cacodylate buffer and 2.5% glutaraldehyde (Appendix 8) at pH 6.0. The solution was constantly agitated and changed daily. The completion of decalcification was determined by radiographic clearance and confirmed by probing a fine needle in an unwanted area of the tissue.

Following decalcification, the tissue was embedded in alginate impression material contained in a small metal mould. It was proposed to cut the maxillary first molars through the mesial root mid-sagittally, as in figure 4.1, using a sharp razor blade under a stereo-dissecting microscope.

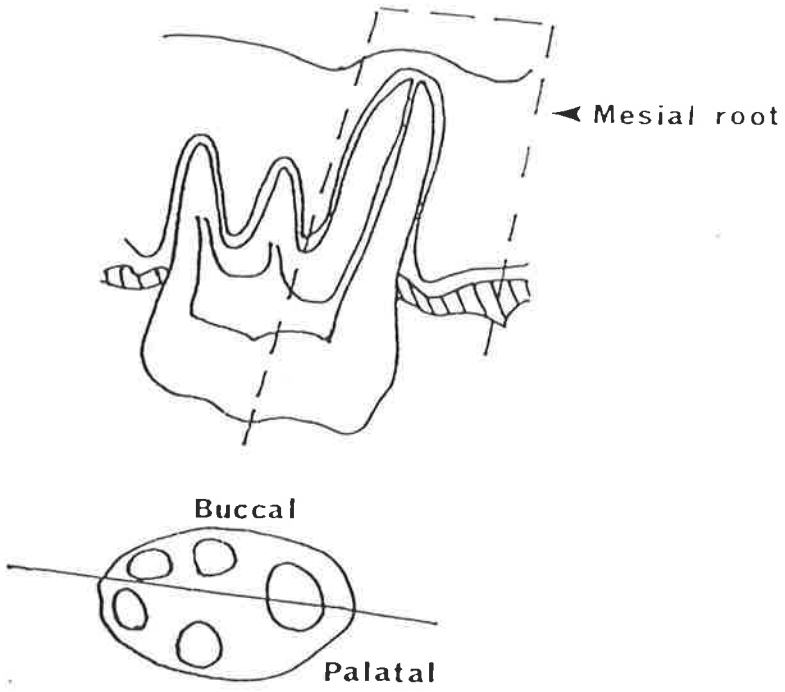


FIGURE 4.1 *The mid-sagittal cut of the rat's right maxillary first molar.*

Four pairs of matching right and left first molar sections containing bisected apical foramina were selected for ultrastructural investigation of the ionic tracer in control and experimental PDL.

The tissue processing sequence continued for 3 days (Appendix 10) until the specimens were embedded, then followed by 4 days of curing. The tissues were post-fixed in 2% OsO_4 in d.d. H_2O (Appendix 11) and dehydrated through a graded series of ethanol and propylene oxide solutions. The tissue was infiltrated with propylene oxide and Agar 100^R, and embedded in fresh Agar 100^R (Appendix 12).

2. ULTRAMICROTOME SECTIONING

2.1 ROUGH TRIMMIMG :

A Reichert-Jung Om-U4 Ultramicrotome was used for tissue sectioning in the experiment. The specimen block was mounted in a Reichert specimen holder chuck. The block face trimming was done by hand with a de-greased single edge razor blade. The block face was cut in a mesa shape, with the sloping sides having an angle of approximately 50° to the vertical to give adequate rigidity during the sectioning without the section size increasing too rapidly as cutting progressed into the block.

2.2 THICK SECTIONING :

Orientation cutting at 1 micron thickness was done with glass knives using a Reichert ultracut. The clearance angle of the glass knives was 6°, the same clearance angle as the diamond knife used for silver sectioning. The water trough on the glass knife was filled with double distilled millipored water (m.d.d. H_2O). The 1 micron sectioning proceded until the desired number of sections had been cut. Then, the sections were transfered from the trough and placed on a glass slide for staining.

2.3 THICK SECTION STAINING :

After having been dried on a hot plate at 90°C for 1-2 minutes, the sections were stained with millipored solutions of 0.05% toluidine blue and 1% borax at a 1:1 ratio by volume (Appendix 13) for 1 minute. The excess stain was rinsed off and the sections were dried again on the hot

plate. The stained sections were then viewed with a light microscope to determine the level depth.

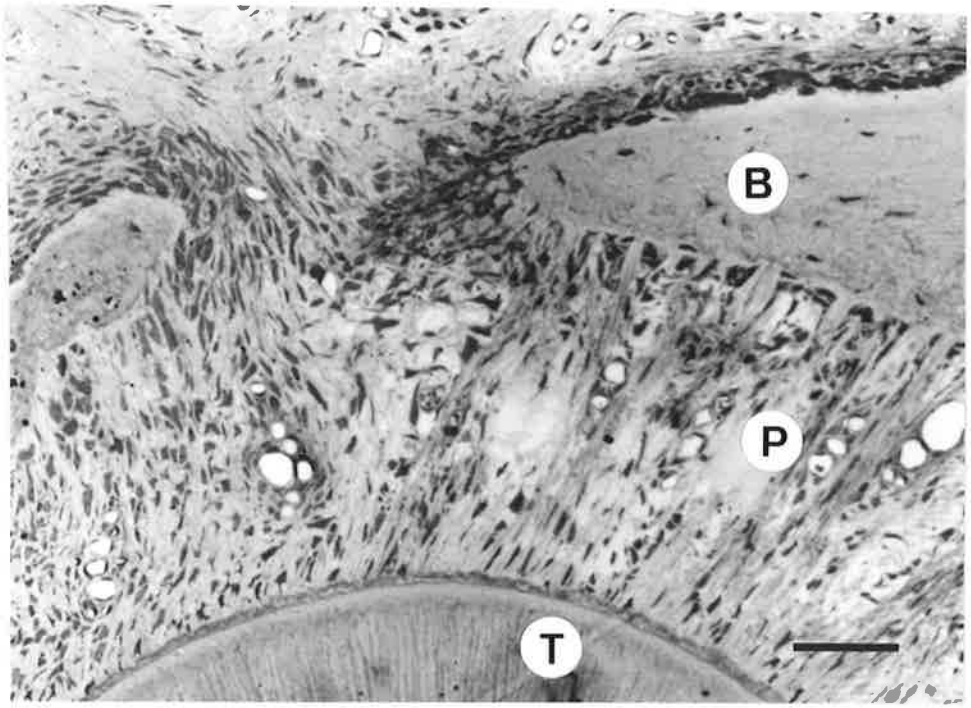
2.4 DETERMINATION OF ZERO LEVEL :

The sections were examined approximately 50 microns apart until the alveolar bone was noted at the mesial side. The first section reaching the mesial alveolar bone was regarded as the zero level. Silver sectioning for electron microscopy was obtained from this level.

2.5 FINAL MESA :

After reaching zero level, a polaroid photograph was taken from a 1 micron thick, toluidine blue stained section, at 40 times magnification. The area for the final mesa was determined from this photograph and a trapezium shape was outlined to cover the PDL at the most mesial part of the first molar. Bone and tooth substance was included at the base and the top of the trapezium, respectively, to give better support for the section.

The mesa was cut and the excluding part was removed with a degreased scapel blade number 11. Then a 1 micron thick section was cut using a glass knife with a water trough and stained with toluidine blue. A polaroid photograph was taken at 100 times magnification which helped locate the site of the blood vessels during electron microscopy.



FIUGRE 4.2 *A photomicrograph of the final mesa at zero level. T, tooth; P, periodontal ligament; B, bone; Bar, 100 um. Toluidine blue stain X 138*

2.6 SILVER SECTIONING :

The silver sections were cut with a diamond knife. The knife angle was 6° and the cutting speed was 1 mm. per second. The water trough was filled with double distilled millipored water. The automatic advancing knob was used during sectioning. This knob was varied until the right thickness of the section was obtained as determined by the silver interferance colour of the sections as they floated in the collecting trough. The thickness of each section was approximately 70 nm.

2.7 SECTION COLLECTING :

Three silver sections were collected at a time on a 150 square mesh copper grid with a diameter of 3 mm. Any sections lost during collecting were recorded. Twelve copper grids were collected at each level. All the grids were dried at room temperature with the tissue side up.

2.8 SILVER SECTION STAINING :

A 150 square mesh grid with good sections was selected and stained with uranyl acetate solution at 37°C for 12 minutes, then with Reynolds' lead at room temperature for 4 minutes (Appendix 14). The grid was dried at room temperature and stored in a clean and dry dustproof grid storage box.

2.9 OBTAINING THE NEXT LEVEL :

On the basis of previous investigations (Freezer, 1984), it was decided to collect the sections of the PDL at 250 micron intervals along the root length. The tissue

block was trimmed roughly again with a razor blade, then the block face was cut down to a distance of 250 microns with glass knives. The orientation section, one micron thick, was cut and stained with toluidine blue. A polaroid photograph was taken at a magnification of 40 times to help design the area of the final mesa. This photograph of the orientation section was compared to the one from the previous level to help locate the area for the final mesa so that it covered the same mesial area as used previously. A thick section of the final mesa was then cut and stained with toluidine blue. A polaroid photograph was taken at a magnification of 100 times. Then the silver sectioning and section collecting commenced.

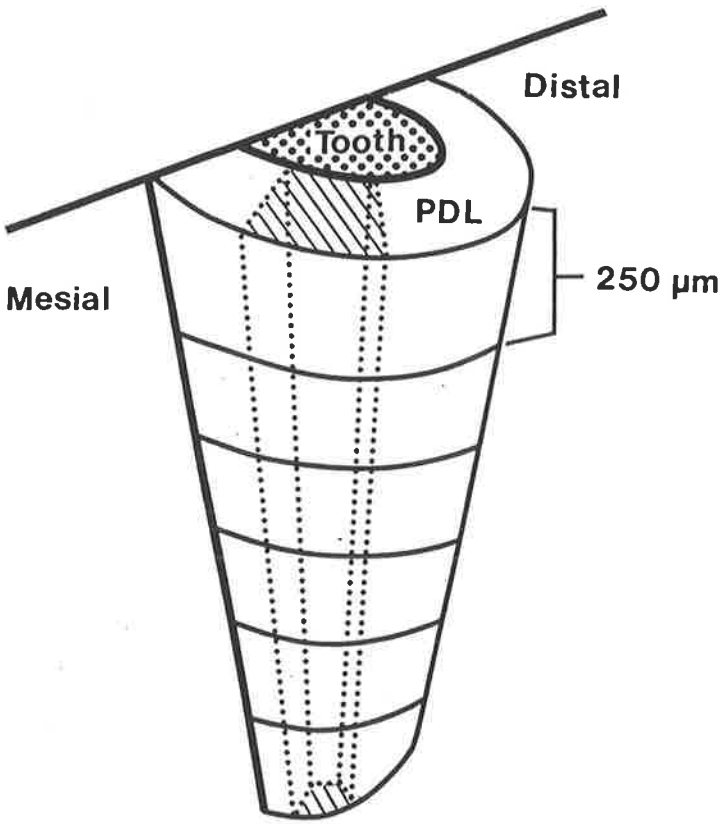


FIGURE 4.3 *Diagram illustrating the region of tissue collected along the root length. The dotted lines indicate the margin of the final mesa collected.*

2.10 DETERMINATION OF THE END LEVEL :

The tissue block was cut down apically and the sections were collected at 250 micron intervals for approximately 7 to 8 levels (1500 - 1750 microns). Then the block was sectioned slowly and the sections were frequently viewed. The appearance of a small area of cellular cementum indicated the termination of the root length, i.e. the end level.

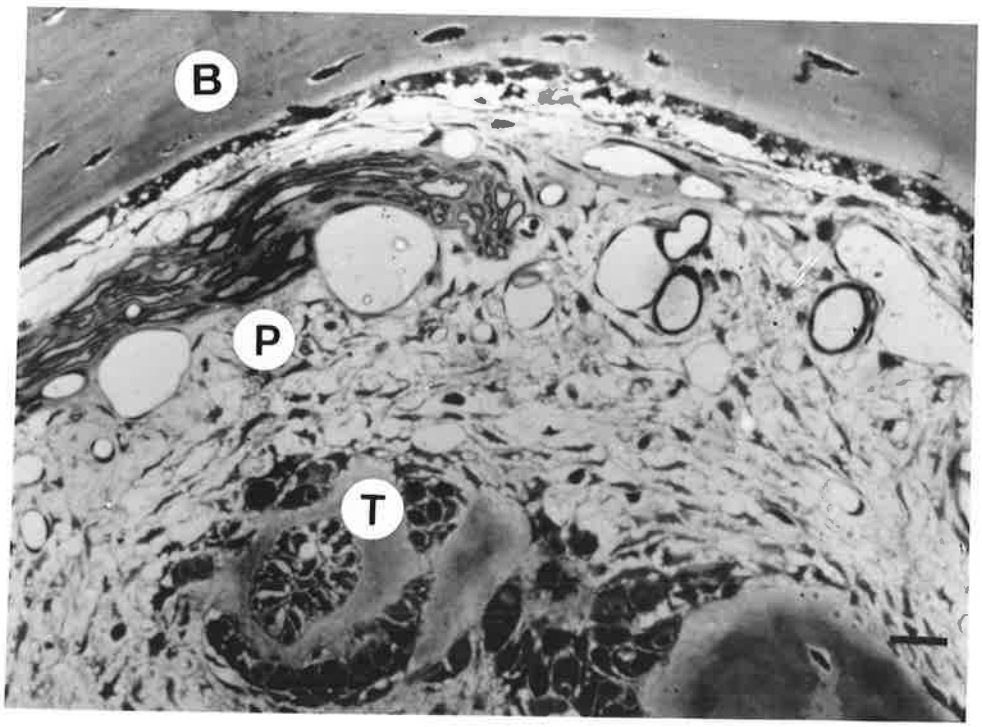


FIGURE 4.4 *A photomicrograph of the final mesa of the end level. T, tooth; P, periodontal ligament; B, bone; Bar, 10 um. Toluidine blue stain. X 750*

3. TISSUE SAMPLING

The grid square containing a group of blood vessels in the PDL, which was located at the most mesial area of the tooth, was selected. The polaroid photograph of the final mesa (X100) was used to help locate the group of blood vessels. An electron micrograph was taken at a magnification of X500 to cover all the area in that square grid. All the blood vessels were identified as in the circumferential tooth third, middle third or bone third of the PDL.

Each blood vessel was devided into quadrants (Figure 4.5). The first qudrant was examined for the presence of a complete endothelial junction which could be seen clearly along the luminal side to the abluminal side. If there was no complete endothelial junction in the first quadrant, then the second, the third or the forth quadrant was examined, respectively, until an endothelial junction that matched the criteria was found.

One junction was required for each blood vessel. Five junctions were needed as a constant number of junctions per level. Therefore, if there were less than five blood vessels in the first grid square, then the adjacent grid square was examined as well. On the other hand, if there were more than five blood vessels in the grid square, all the blood vessels were examined at this stage.

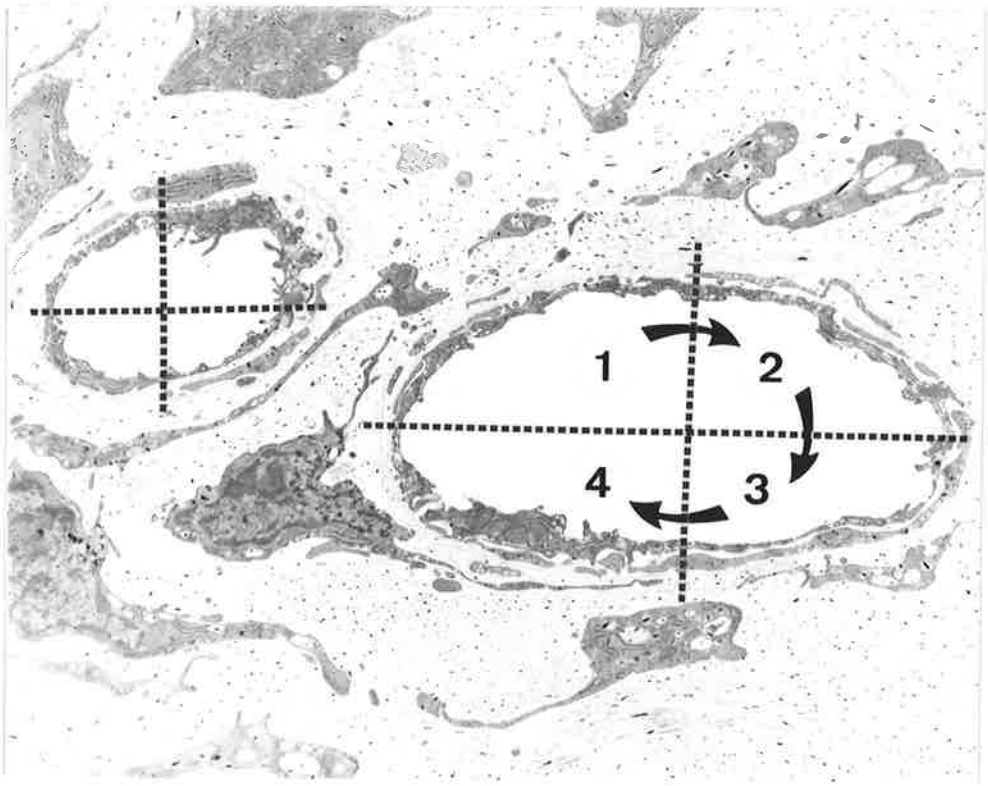


FIGURE 4.5 *Each blood vessel was divided into 4 quadrants. The first quadrant to have a 'complete' junction, examining clockwise from the first to the forth, respectively, was selected. The number of each quadrant is shown in the larger blood vessel.*

4. TRANSMISSION ELECTRON MICROSCOPY

A transmission electron microscope, JEM - 2000 FX, was used to examine the sections at the accelerating voltage of 120 kV.

4.1 CARBON COATING :

The stained grid was coated with a thin film of carbon from ultra-'F' purity carbon rods. The carbon coating was carried out in a Dentron vacuum evaporator. The thickness of the carbon layer was approximately 25 A°. The carbon layer was needed to help absorb scattered rays during specimen illumination in the transmission electron microscope.

4.2 TRANSMISSION ELECTRON MICROSCOPE :

A single tilt, double specimen holder was used to hold both a trial carbon grid and a carbon coated, 150 square mesh, copper grid at the same time. The electron beam aligning, focusing and specimen height adjustment were carried out on the trial carbon grid to avoid damaging the sections. The carbon coated, 150 square mesh, copper grid was then examined.

The sections were examined under low magnification to identify geometrical structures. The grid square which contained the group of blood vessels in the PDL at the most mesial area was selected. An electron micrograph was taken at a X500 magnification to cover all the area of the grid square.

Each blood vessel was then examined at the higher magnification, approximately X80,000. Each vessel was scanned along the endothelial layer until a complete endothelial junction was obtained in the first quadrant, as described in the tissue sampling technique. An electron micrograph was taken at the untilted angle. The magnification used was selected to fit the whole junction in the micrograph. The exposure time was approximately 1.5 - 2.0 seconds.

4.3 GONIOMETER :

The goniometer was used to tilt the specimen either clockwise or anti-clockwise around the horizontal axis of the specimen holder. All 'tight regions' of the endothelial junction were examined using the goniometer. A section was tilted, either clockwise or anti-clockwise, so that the area could be seen more clearly as 'open' or confirmed as a 'tight region'. An electron micrograph was taken at this angle. The magnification used was the same as that used at the zero angle.

Then the rest of the blood vessels were examined until the data for a minimum of five junctions per level was obtained. Electron micrographs were taken from each junction selected both at the zero angle and the tilted angles. If there was more than one tight region in the same junction, extra electron micrographs were taken for each tight region.

4.4 MAGNIFICATION CALIBRATION :

The instrumental magnification was calibrated with a carbon replica of an optical grating (E.F. Fullam Inc. Schendectady, N.Y., 2160 lines/mm.). Electron micrographs of the replicating graticule were taken at the magnification of X500 and at the highest magnification possible when examining the endothelial junctions.

5. DEVELOPING AND PRINTING

TEM plate negatives were developed in Kodak^{R} D19 developer at 20°C for 4 minutes. The negatives were rinsed in deionised water for 1 minute and fixed in Ilford Hypam^{R} Rapid Fixer for at least 5 minutes. They were rinsed again for 15 minutes and dried in an air-dryer for 2 hours.

The negatives were printed on multigrade Ilfospeed^{R} photographic paper using a Durst^{R} laboratory "54" enlarger. A grade 5 filter was used in order to achieve a good contrast. The prints were developed in Ilfospeed^{R} paper developer for 1 minute, dried in Ilford Hypam^{R} Rapid Fixer for 5 minutes, then rinsed in water for 15 minutes.

In order to clarify the type of blood vessel, the micrograph of the grid square was enlarged to approximately X1500 to X2000. The measurement of the luminal diameter and the recognition of the blood vessel wall structure were obtained from this enlarged print.

The measurement of the dimensions of the endothelial junction were made on a print enlarged to a magnification of X200,000. However, the measurement of 4

long junctions were made on the prints enlarged to only X100,000 due to the limitations of the enlarger.

The dimensions of the open junctions were also measured on prints enlarged to a magnification of X100,000.

6. DATA COLLECTION

As described previously in the tissue sampling method, five junctions for each level were selected. For any grid square that had more than five blood vessels, some of the blood vessels were excluded under the following conditions;-

1. There was no junction in that vessel.
2. The vessel intersected the grid bar and showed less than 3/4 of the vessel which made indicating the correct luminal diameter difficult.
3. The electron micrograph had poor quality which caused ambiguity in classification of the junction type.

Five blood vessels were then selected randomly as respresentative of each level.

Due to the small number, every open junction encountered was included in the sample.

6.1 RECORDING SHEET :

The data from each level was recorded in the recording sheet as follows. Vessel type, region of vessels, type of junction and the location of the tight region were assigned digital values which were more convenient for statistical management.

RECORDING SHEET

Block Number Level					
BLOOD VESSEL					
Number					
Type (1,2,3,4,5)					
Luminal diameter (u)					
Location (1,2,3)					
JUNCTION					
Type (1,2,3,4)					
Depth (nm.)					
Width W1 (nm.)					
W2 (nm.)					
W3 (nm.)					
W4 (nm.)					
Tight region depth (nm.)					
Tight region location (1,2,3)					
Endothelium thickness (T)(nm.)					

6.2 DESIGNATION AND IDENTIFICATION :

6.2.1 BLOCK NUMBER

Each block was identified by three sets of letters/numbers.

The first two letters were;-

either RC (for Rat-Control)

or RE (for Rat-Experiment).

The numbers in the middle represented the animal number.

The final combined number and letter represented the block side of the mesial root ;-

1B = right-buccal 2L = right-lingual

3L = left-lingual 4B = left-buccal

6.2.2 CLASSIFICATION OF BLOOD VESSELS

The classification of the blood vessels was based on the luminal diameter as well as the structure of the blood vessel walls. The narrowest diameters were used for classification. The vessel type nomenclature was modified from the criteria suggested by Rhodin (1967, 1969) and Clark (1986). The blood vessels were classified as follows;-

1.Venous capillary: 1.5 to 10 microns in diameter; the endothelial lining was relatively thin and composed of 1 to 3 endothelial cells, a continuous basement membrane, pericyte and fenestrae occasionally present.

2.Arterial capillary: 2 to 7 microns in diameter; in comparison to the venous capillary the ratio of endothelial lining to the luminal diameter was greater, the vessel wall was usually composed of 1 to 3 endothelial cells with nuclei often bulged within the endothelial wall, pericytes and fenestrae were sometimes present.

3.Postcapillary-sized venules: 10 to 30 microns in diameter, thin endothelial lining, partial or complete pericytic investment was occasionally seen.

4.Terminal arteriole: 7 to 15 microns in diameter; endothelial nucleus was thicker and more lobulated, one layer of smooth muscle cell was present.

5.Collecting venules: luminal diameter was larger than 30 microns, pericytes were usually seen.

6.2.3 LOCATION OF BLOOD VESSELS

The location where the blood vessel came from was classified into 3 regions across the PDL by using the polaroid photograph of the final mesa at the magnification of 100 times. The PDL area from tooth to bone was devided into 3 parts as tooth 1/3, middle 1/3 and bone 1/3. If any blood vessel was present in both regions, its classification was for the region where the major part of it appeared.

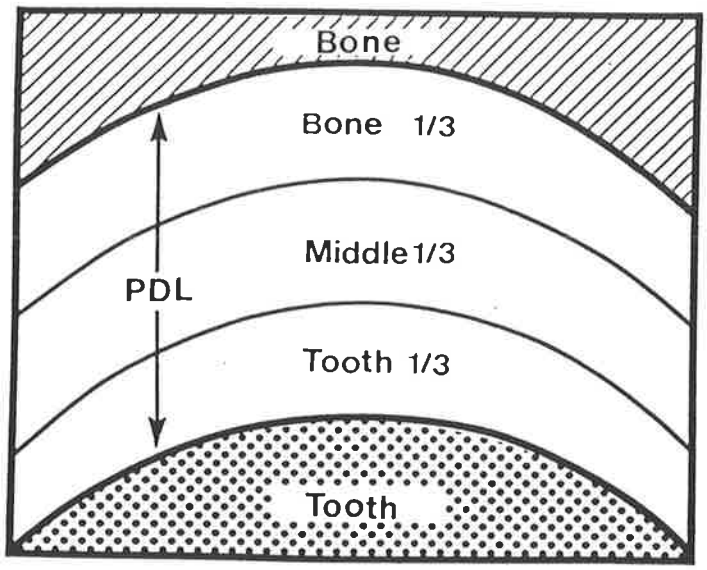


FIGURE 4.6 *Diagram illustrating the division of the PDL area into three regions.*

6.2.4 TYPES OF ENDOTHELIAL JUNCTION

Endothelial junctions were classified into 4 types as follows (Figure 4.7);-

1.CLOSE JUNCTION : The junction where the "close region" was present and the outer two lamellae were separated all along the depth of the junction. (See Figure 5.8)

CLOSE REGION : The region where the width of the intercellular cleft was reduced but the outer two lamellae were still seperated by a small gap.

2.TIGHT JUNCTION : The junction where any "tight region" was present even if there was also a "close region". (See Figure 5.9)

TIGHT REGION : Where the two outer lamellae of the opposing cells were fused.

3.OPEN JUNCTION : The junction where the intercellular distance increased more than 100 nm. all along the depth of the junction. (See Figure 5.12).

4.GAP JUNCTION : The junction where two plasma membranes were closely opposed and appeared intercalated between the two opposing membranes.

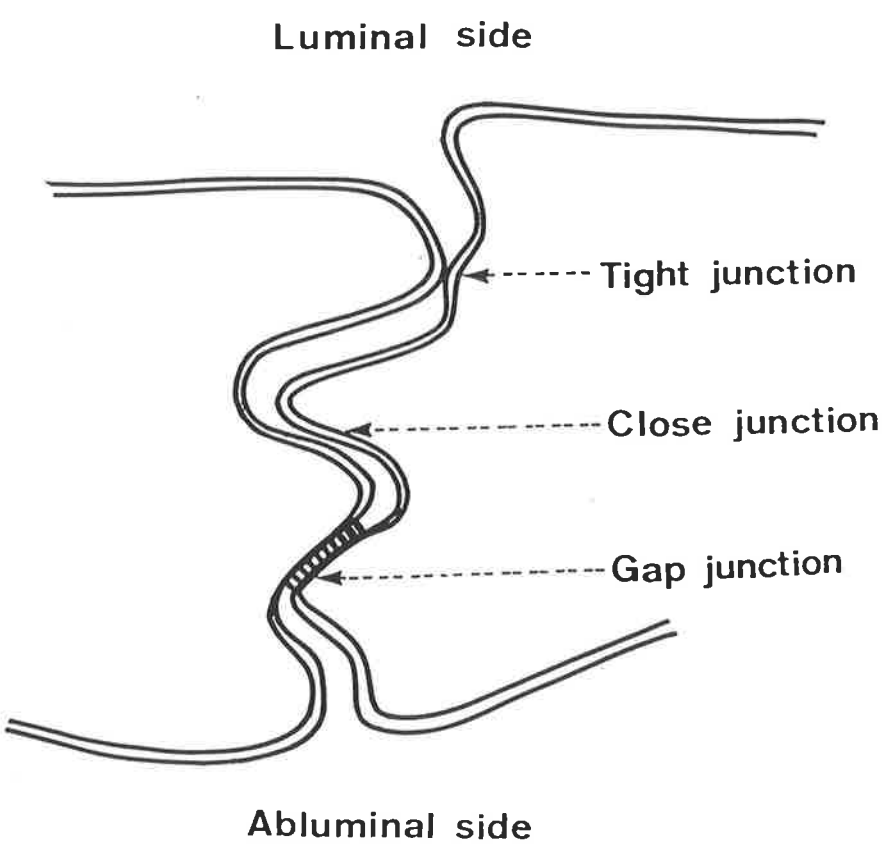


FIGURE 4.7 *Diagram illustrating different types of endothelial junction.*

6.2.5 DIMENSIONS OF ENDOTHELIAL JUNCTION

Terms used to describe the endothelial junctions in this investigation were based on terms used by Casley-Smith (1975, 1978, 1981, 1983).

LENGTH : The length of the junction around the cell in the plane of the luminal surface.

DEPTH : The distance between the luminal and abluminal surfaces.

WIDTH : The distance between one endothelial cell and the next, i.e. between the two external laminae of the two plasma membranes.

6.3 METHOD OF MEASUREMENT :

6.3.1 TIGHT AND CLOSE JUNCTIONS

The dimensions of junctions were measured with a digital planimeter with a digitizing tablet (MOP-3, Carl Zeiss Inc.). The error of this method was found to be less than 0.5% (Casley-Smith, 1981).

The depth of each junction was measured from the midpoint between the two opposing membranes at the luminal end, through the junction, to the midpoint between the two opposing membranes at the abluminal end.

To measure the width of the junctions, each junction was devided into four parts ;-

W1 - the width at the luminal side

W2 - the width at 1/3 depth of the junction

W3 - the width at 2/3 depth of the junction

and W4 - the width at the abluminal side.

Due to the difficulty in accurately locating the outer lamellae of the two unit membranes, the width was measured between the darkest lines. The measured distance was then subtracted from the width of a unit membrane (75 Å). The result was therefore the absolute distance between the two opposing membranes.

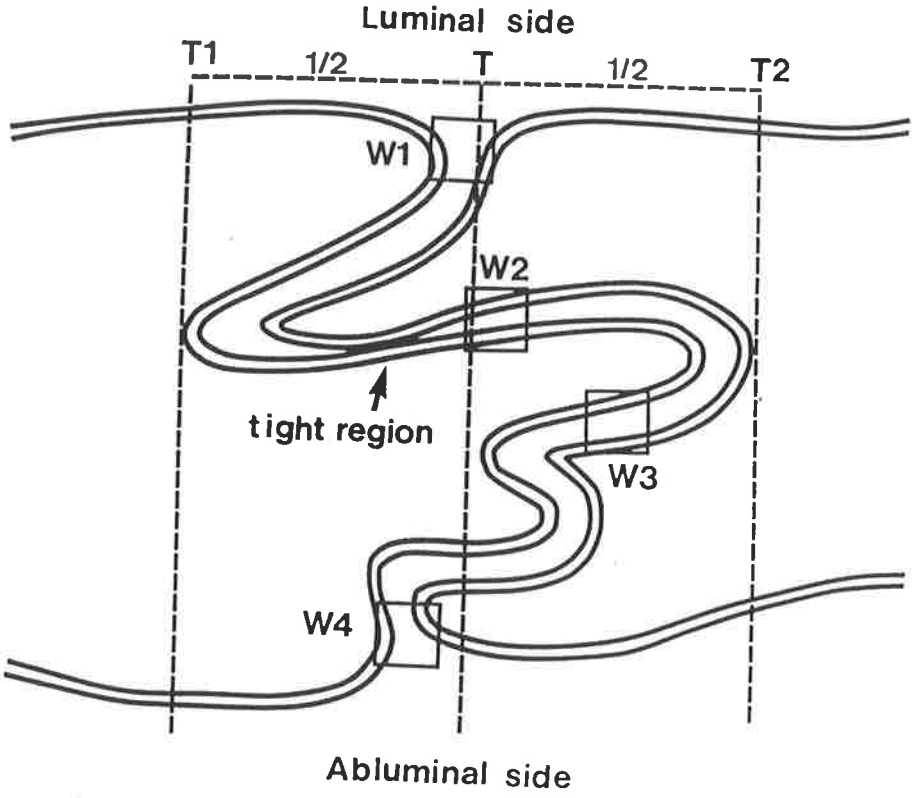


FIGURE 4.8 *Diagram illustrating the method of measuring the width of the junction in four regions and the thickness of the endothelium across the junction.*

The width and the length of the junctions were measured from the enlarged electron micrograph prints that showed the widest distance between the two opposing membranes. At this angle, the electron micrographs were assumed to represent the morphology of the junctions at a right angle to the plane of the two opposing membranes.

For the tight junctions, the data were recorded for the type and dimensions as described above, including the depth and the location of the tight region. The location of the tight region was recorded as in the luminal 1/3, middle 1/3 or abluminal 1/3.

To measure the thickness of the endothelium across the junction, the margins of the junction on both sides of the two endothelial cells were located as T1 and T2. The endothelial cell thickness (T) at the midline between T1 and T2 was recorded.

Each junction dimension was measured twice. The mean values from the two measurements were used in the statistical analysis.

6.3.2 OPEN JUNCTIONS

The dimensions recorded from the open junction were, the distance between the two unit membranes, and the thickness of the endothelial lining of both cells on both sides of the junction.

7. STATISTICAL ANALYSIS

Statistical analysis of 16 variables was carried out using a general mixed model analysis of variance performed by the BMDP3V statistical package (BMDP statistical Software Inc., Los Angeles, Calofornia, USA.) at the Department of Statistics, University of Adelaide.

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The mesial root length of rat molars ranged from 1500 to 2000 microns. As a sample was collected every 250 microns there were therefore between 7 and 9 levels per mesial root. The length of the root of the extruded teeth and the control teeth was not significantly different except in animal number 11. Due to the root length of the control tooth of the animal number 11 being longer than the root length of the experimental tooth, the number of blood vessels investigated was higher in the control samples. The number of blood vessels investigated was 160 in the control samples and 150 in the experimental samples.

1. BLOOD VESSEL

1.1 BLOOD VESSEL TYPES :

The blood vessels investigated consisted of five types, i.e., venous capillaries, arterial capillaries, post capillary-sized venules, terminal arterioles and collecting venules. Both continuous and fenestrated capillaries were found. Approximately 3/4 of the total blood vessels were venous type. Collecting venules were found only in animal number 14 and at the end (apical) levels. Both the observed and predicted mean diameters of each vessel type are shown in Table 5.1. The predicted means were calculated when the animal variability was taken into account. The average size of the luminal diameter of blood vessels in the experimental samples was smaller than those in the control samples. This

was highly significant for venous capillaries ($p<0.001$) but not for other types of vessels.

TABLE 5.1 The mean diameter of each blood vessel type in nanometers.

Vessel Type	Observed mean	Predicted mean	S.D.dev. Pred.	
Venous capillary				
Control	7.15	7.14	0.44	
Experiment	5.32	5.21	0.43	***
Arterial capillary				
Control	4.80	4.84	0.39	
Experiment	3.88	4.27	0.35	(N.S.)
Postcapillary-sized venules				
Control	15.26	14.53	0.67	
Experiment	14.87	13.98	1.03	(N.S.)
Terminal arteriole				
Control	9.65	9.65	0.60	
Experiment	9.86	9.25	1.18	(N.S.)

*** ($p<0.001$)
N.S. (Non-significant)

The majority of the blood vessels in the experimental samples were small arterial and venous capillaries. The percentage of each blood vessel type is shown in Table 5.2 and illustrated in Figure 5.1.

TABLE 5.2 The percentage of blood vessel types.

Vessel type	Control	Experiment
Venous capillary	38.75%	56.67%
Arterial capillary	11.25%	22.66%
Postcapillary-sized venule	34.38%	14.67%
Terminal arteriole	14.37%	5.33%
Collecting venule	1.25%	0.67%

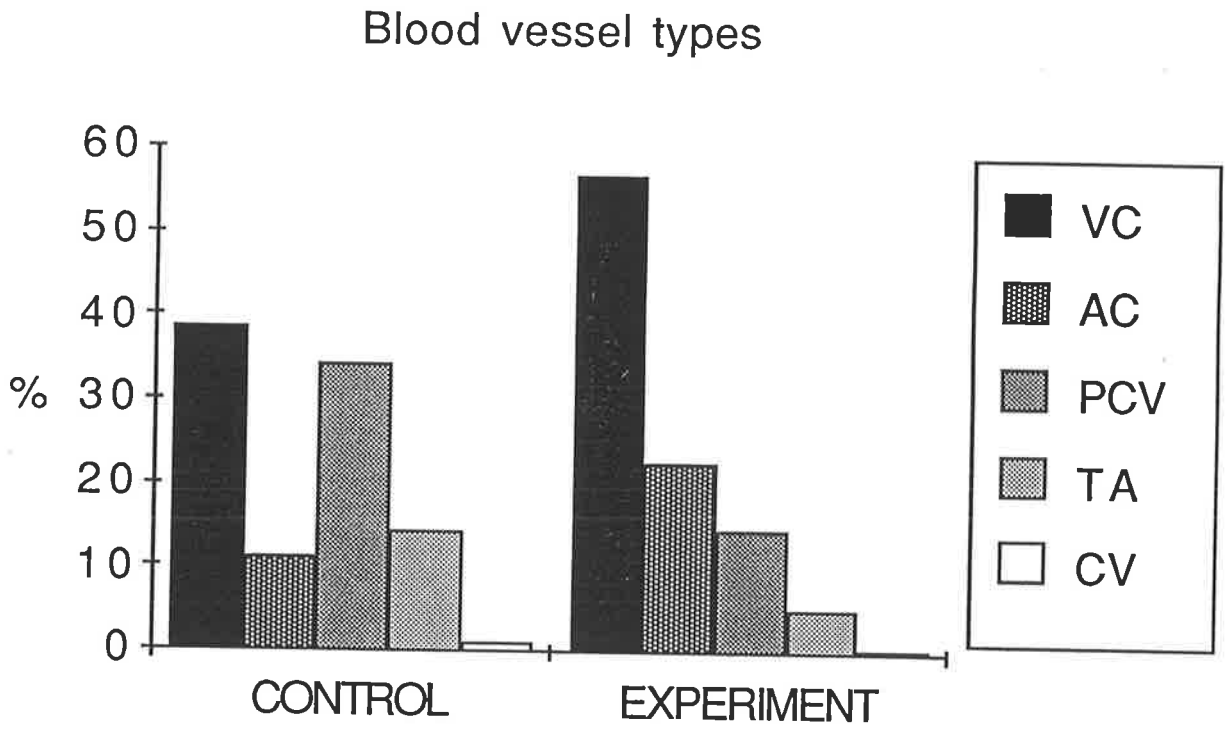

FIGURE 5.1 *Histogram illustrating the percentage of each blood vessel type.*

1.2 BLOOD VESSEL MORPHOLOGY :

There were some differences in the general appearance of the blood vessel morphology between the control and the experimental PDL. Generally, the blood vessels selected from the control samples had a more rounded shape and a smoother endothelial wall. The blood vessels from the experimental samples were usually more irregular in shape and the endothelial lining was corrugated. Furthermore, there were more finger-like projections of the endothelial cells into the vessel lumen in the experimental blood vessels than in the control. (Figure 5.2)

The appearance of blood vessels in the experimental samples was not the same as the appearance of tissue with acute inflammation or degenerative tissue. There was no sign of a swollen endothelium nor the presence of inflammatory cells. The mitochondria were intact. The basement membrane completely surrounded the endothelial wall. The differences in the general appearance of the blood vessel morphology were most marked in animal number 13. However, these differences were less likely to be seen at the end (apical) level for all animals.

1.3 BLOOD VESSEL LOCATION :

The majority of the blood vessels investigated in the control were from the middle 1/3, while in the experimental PDL they were from the bone 1/3. The blood vessels from the tooth 1/3 were the minority both in the control and in the experimental samples. The distribution of the blood vessels in different locations is shown in Table 5.3 and Figure 5.3.

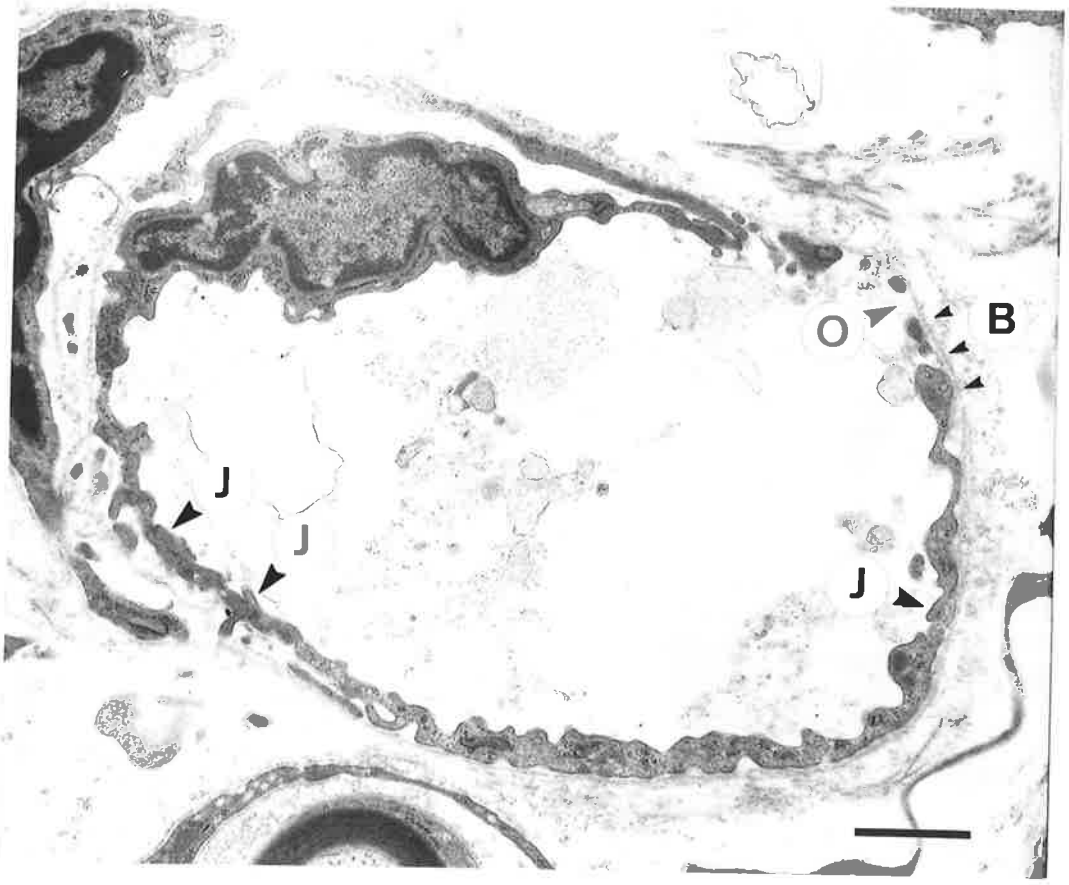

FIGURE 5.2 *A venous capillary with a diameter of 4.8 microns from an experimental sample. The endothelial lining was corrugated. Inflammatory cells were not present.*
B, basement membrane; O, open junction; J, junction; Bar, 1um. Uranyl acetate and Reynolds' lead stain. X 15K

TABLE 5.3 The distribution of blood vessel location across the PDL.

Location	Control	Experiment
Tooth 1/3	5.6%	1.3%
Middle 1/3	52.5%	40.0%
Bone 1/3	41.9%	58.7%

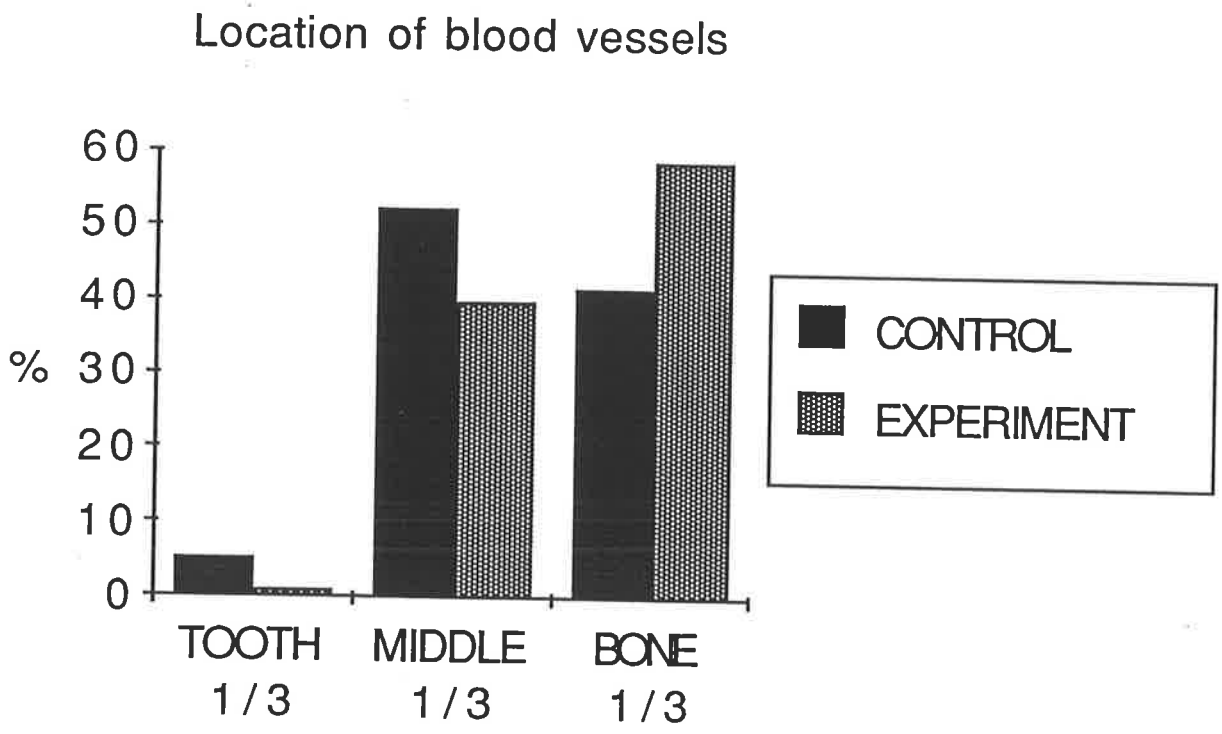

FIGURE 5.3 *Histogram illustrating the distribution of the blood vessels in the different locations across the PDL.*

2. JUNCTION MORPHOLOGY & BLOOD VESSEL TYPE

In the morphological study, a different type of blood vessel was found to be associated with the complexity in the appearance of the junctions. Generally, the blood vessels with relatively thin walls displayed a 'simple type' of junction. The junction between the overlaping endothelial wall was relatively straight. This type of junction was commonly found in venous capillaries, postcapillary-sized venules and collecting venules. An example of a 'simple junction' found in a venous capillary is shown in Figure 5.4.

Arterial capillaries had relatively thicker walls compared to venous capillaries. The junctions found in this type of blood vessel often appeared as 'curvy types'. This type of junction had two or more curves along the depth of the junction. However, the 'simple type' junctions were occasionally found in this type of blood vessel as well. An example of a 'curvy junction' is shown in Figure 5.5.

Terminal arterioles appeared to have the thickest walls among the vessels investigated in this study. The junction found in this type of blood vessel was relatively long and displayed a 'convoluted type' of junction. The morphology of the junction was more complicated where the wall was thicker. In small terminal arterioles, which were the intermediate form between the arterial capillaries and the true terminal arterioles, the 'curvy junctions' were also found. Figure 5.6 illustrates a very 'convoluted junction' from a large terminal arteriole.

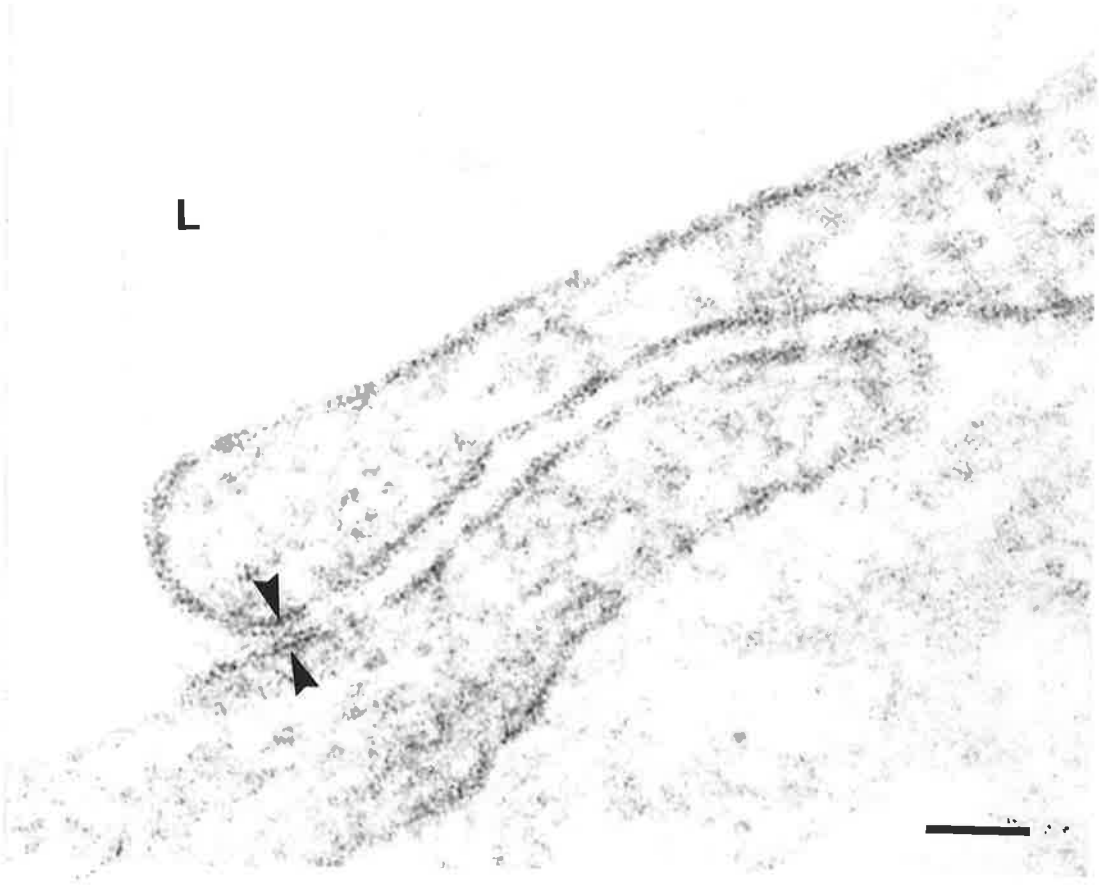

FIGURE 5.4 *A 'simple junction' from a venous capillary with a diameter of 6 microns. The arrow heads indicate the tight region. L, luminal side; Bar, 50 nm.*
Uranyl acetate and Reynolds' lead stain. X 266K

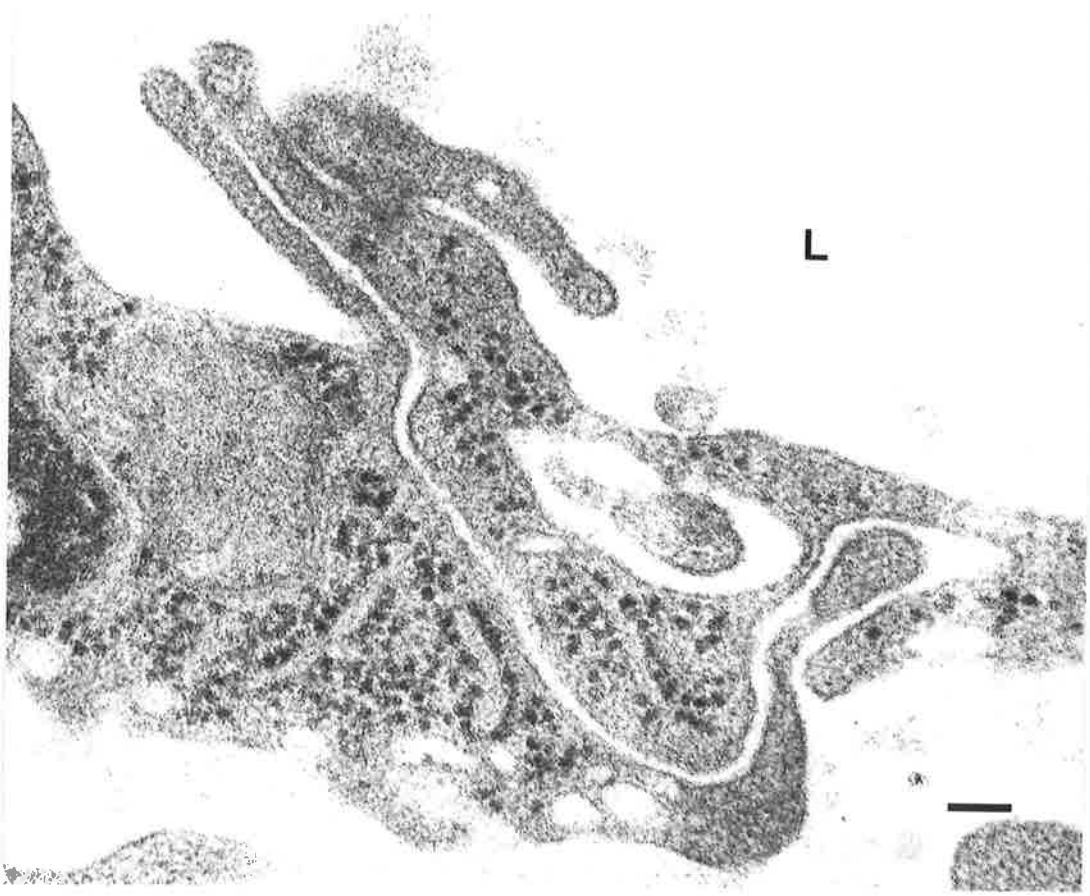

FIGURE 5.5 *A 'curvy junction' from an arterial capillary with a diameter of 6.7 microns. L, luminal side; Bar, 100 nm. Uranyl acetate and Reynolds' lead stain. X 82K*

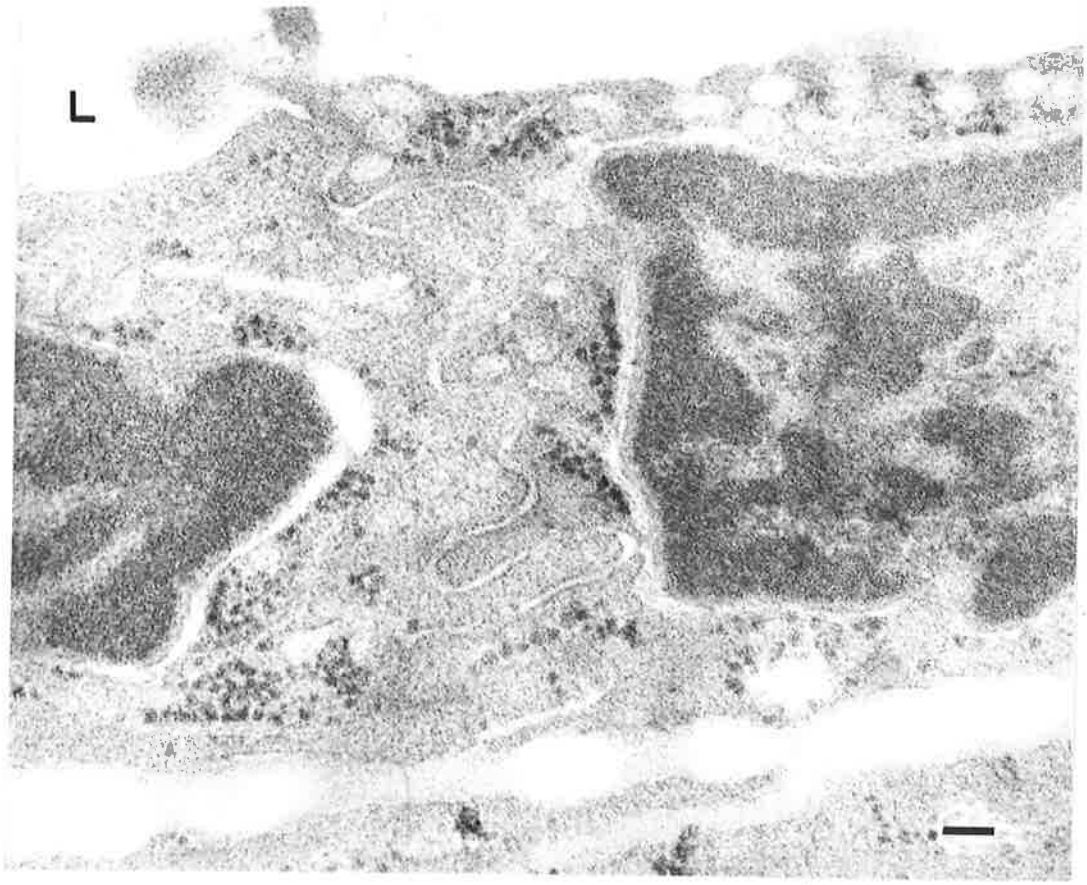

FIGURE 5.6 *A 'long-convoluted junction' from a terminal arteriole with a luminal diameter of 15 microns. The endothelial layer is 1.2 microns thick. L, luminal side; Bar, 100 nm.*

Uranyl acetate and Reynolds' lead stain. X 70K

3. JUNCTION TYPES

There were three types of junction found in the microvascular bed of the PDL in this study. They were close junctions, tight junctions and open junctions. No gap junctions were found. Close junctions formed the majority of the junctions found both in the control and the experimental groups. Open junctions were rarely found. The analysis of the open junctions was done separately due to the very small number, their distinctive characteristics and the different methods of sampling and measuring.

3.1 CLOSE JUNCTIONS AND TIGHT JUNCTIONS :

As reported above, close junctions formed the majority of the junctions found in this study. After the extrusive load was applied, the percentage of the close junctions reduced. As a result, there was an overall increase in the percentage of the tight junctions. However, these changes were not statistically significant and not consistant in every animal. The percentage of the tight junctions and the close junctions is shown in Table 5.4 and Figure 5.7. Examples of a tight junction and a close junction are shown in Figure 5.8 and Figure 5.9, respectively.

TABLE 5.4 The percentage of tight junctions and close junctions.

Junction type	Control	Experiment
Tight junction	15.6%	21.3%
Close junction	84.4%	78.4%

FIGURE 5.7 *Histogram illustrating the percentage of tight junctions and close junctions.*

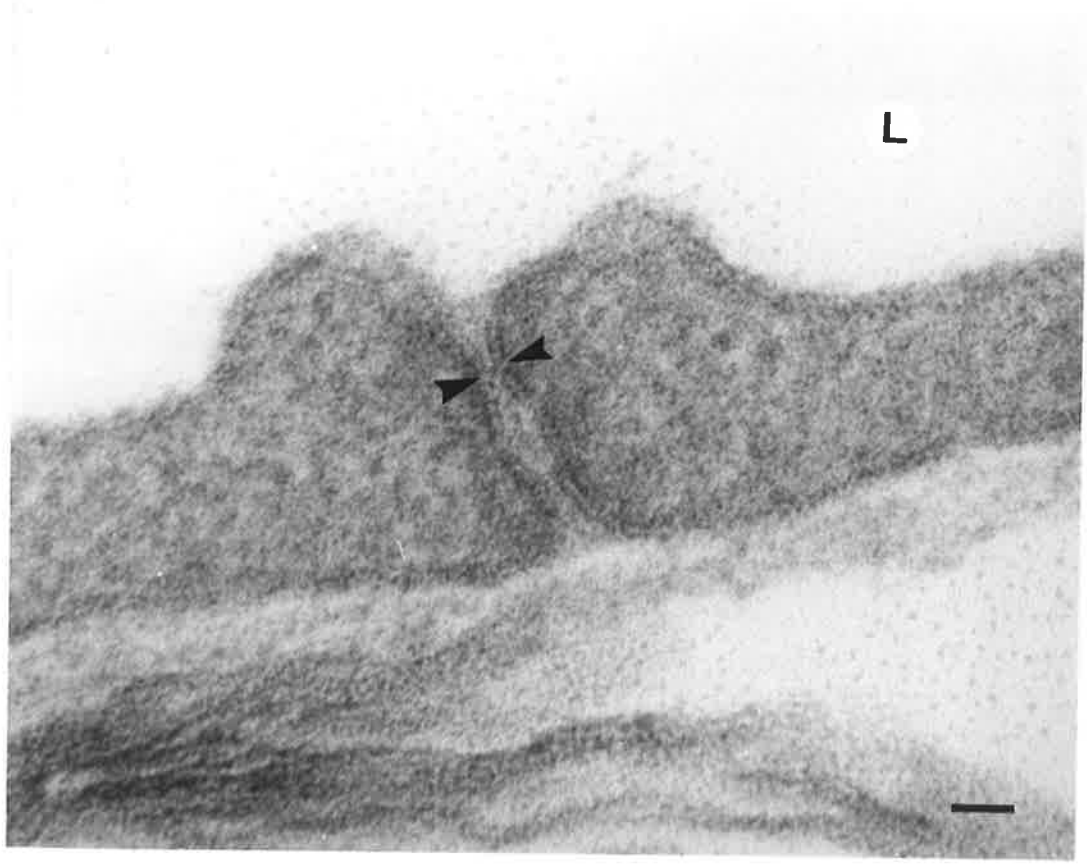

FIGURE 5.8 *A tight junction from a postcapillary-sized venule with the diameter of 11 microns. The arrow heads indicate where the outer lamellae of the two opposing membranes fused. L, luminal side; Bar, 25 nm.*
Uranyl acetate and Reynolds' lead stain. X 323K

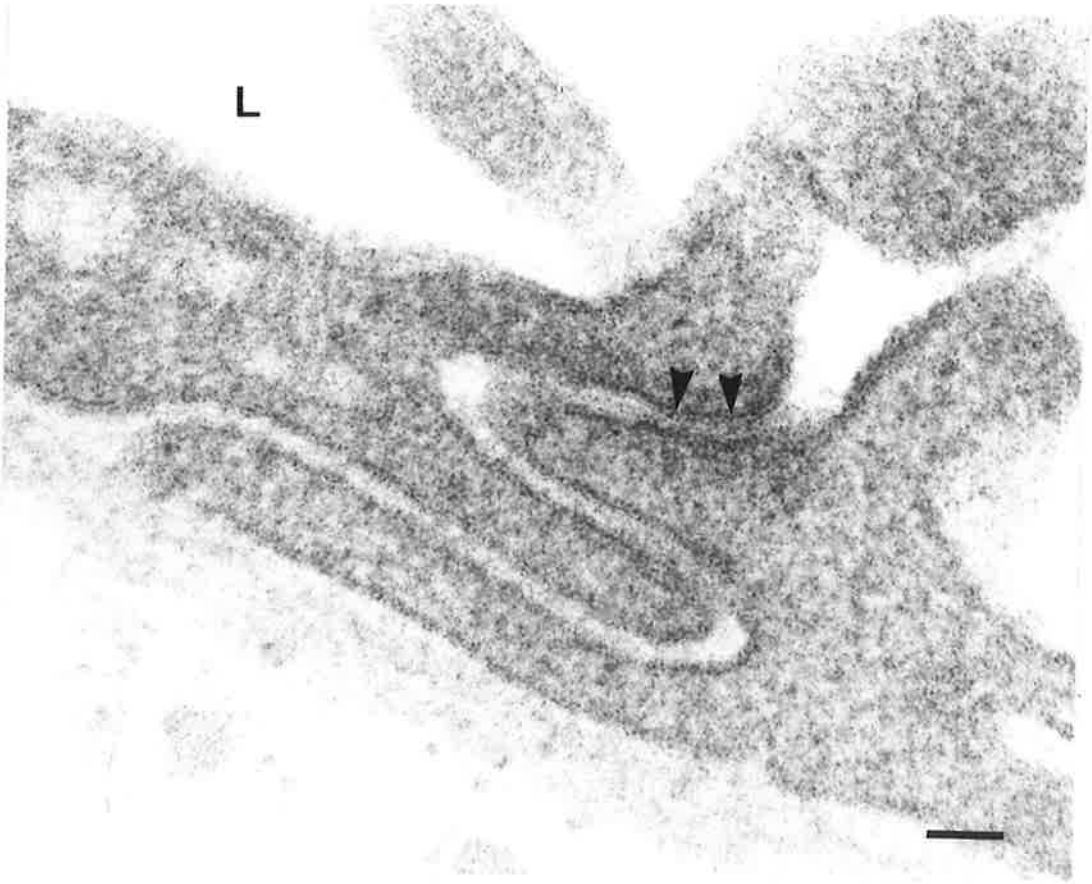

FIGURE 5.9 *A close junction from a venous capillary with a diameter of 6.7 microns. The arrow heads indicate the close region with a gap of approximately 5 nm. between the two opposing membranes. L, luminal side; Bar, 100 nm. Uranyl acetate and Reynolds' lead stain. x 200K*

A statistical analysis of the percentage of tight junctions was carried out for different types of blood vessels. The collecting venules were excluded from the analysis because of a very small sample size. The percentage of tight junctions in every type of blood vessel between the control and the experiment was not statistically significant. Table 5.5 shows both observed and predicted mean percentages of tight junctions. The predicted means were calculated when the animal variability was taken into account.

TABLE 5.5 The percentage of tight junctions in each type of blood vessel.

Vessel Type	Observed mean	Predicted mean	SD.dev. Pred.
Venous capillary			
Control	9.8%	12.2%	7.2%
Experiment	23.5%	22.1%	6.3%
Arterial capillary			
Control	22.2%	31.5%	12.0%
Experiment	23.5%	34.5%	10.0%
Postcapillary-sized venule			
Control	14.2%	12.9%	5.7%
Experiment	13.6%	12.5%	8.4%
Terminal arteriole			
Control	26.0%	22.0%	8.6%
Experiment	12.5%	19.4%	16.5%

A Chi-square analysis was carried out to test whether there were any significant differences in the proportion of tight junctions for different types of blood vessels. The analysis was tested separately for the experiment and control as shown in Table 5.6 and Table 5.7. The results from this analysis indicated that the difference in proportion of tight junctions in both the control and experiment samples was not statistically significant for different types of blood vessels.

TABLE 5.6 The Chi-square analysis of the proportion of tight junctions in different types of blood vessel in control samples.

CONTROL	V1	V2	V3	V4	
Tight junction	6	4	8	6	24
Close junction	56	14	47	17	134
Total	62	18	55	23	158
	.0968	.2222	.1455	.2609	.1519

$X^2 = 4.296$ $(0.10 < p < 0.20)$

TABLE 5.7 The Chi-square analysis of the proportion of tight junctions in different types of blood vessel in experimental samples.

EXPERIMENT	V1	V2	V3	V4	
Tight	20	8	3	0	31
Close	65	26	19	8	118
Total	85	34	22	8	149
	.2353	.2353	.1579	.0000	.2080

$X^2 = 3.72$ $(0.10 < p < 0.20)$

V1 = Venous capillary V2 = Arterial capillary
V3 = Postcapillary-sized venule V4 = Terminal arteriole

The distribution of tight junctions varied greatly among different animals. The difference in the percentage of tight junctions between experimental and control samples was highest in animal number 13. The distribution of tight junctions in different animals is shown in Figure 5.10.

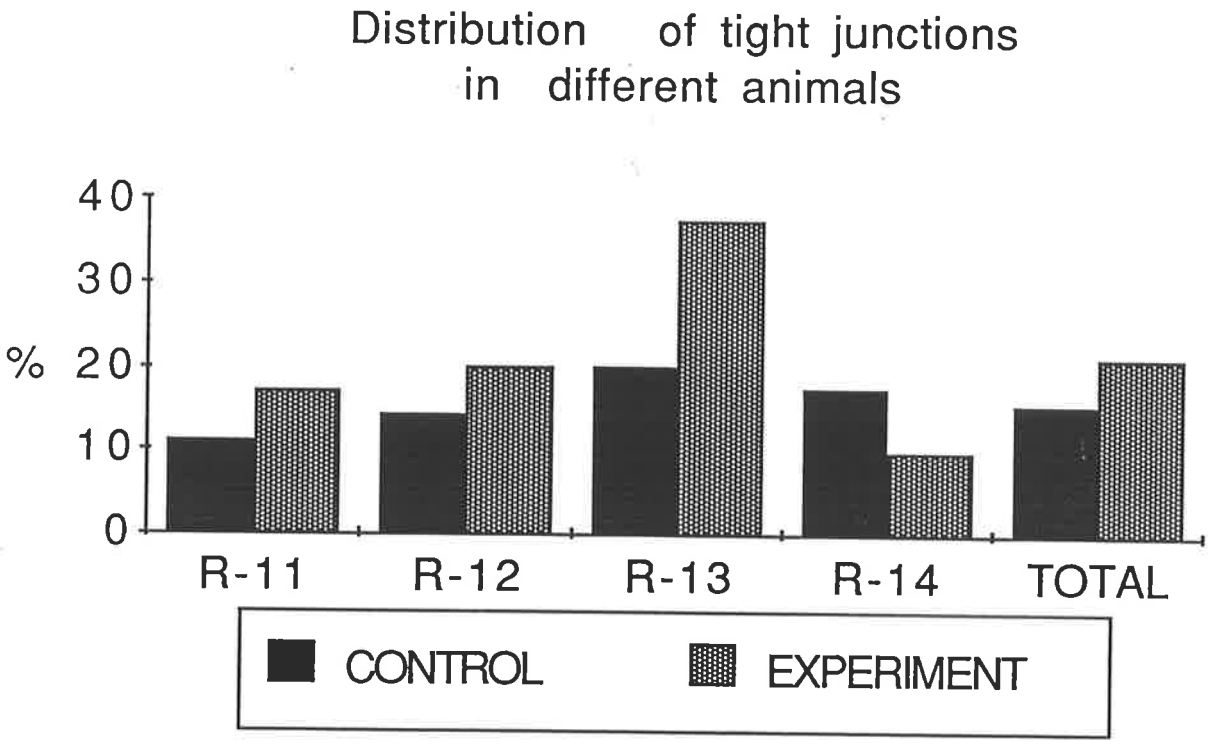

FIGURE 5.10 *The histogram illustrates the distribution of tight junctions in different animals.*

3.2 TIGHT REGION OF TIGHT JUNCTIONS

The location of the tight region of tight junctions along the junction depth was investigated. The tight regions were predominantly found in the luminal third (87% of the control samples and 88% of the experimental samples). The tight regions that were in the middle third or abluminal third were found only in the apical levels. There was no difference in the location of the tight regions between the experimental and control samples.

3.3 OPEN JUNCTIONS :

A small number of open junctions were found in three out of four animals in this study. The open junctions were found only in the venous capillaries and the post-capillary-sized venules where the vessel walls were relatively thin. The basement membrane was intact and completely surrounded the vessels. The percentage of open junctions found was 4.8% of the total number of junctions investigated (comprising 1.6% from the control samples and 3.2% from the experimental samples).

In the control samples, open junctions were found in 2 animals (animals number 12 and 13). The percentage of open junctions formed 3.1% of the total for the control samples. In the experimental samples, open junctions were also found only in 2 animals but in a different combination (animals number 13 and 14). The percentage of open junctions formed 6.6% of the total for the experimental samples.

In animal number 13, the number of open junctions was significantly higher than in other animals which was 60% of the total number of open junctions found. If the animal number 13 was excluded, the percentage of open junctions in the control samples (2.5%) was close to the percentage of open junctions in the experimental samples (2.7%). However, due to the very small number of open junctions, a statistical analysis of the significant differences between the experimental and control samples could not be done. The distribution of the open junctions in the experimental and control samples is shown in Figure 5.11. The pore size of the open junctions is shown in Table 5.8.

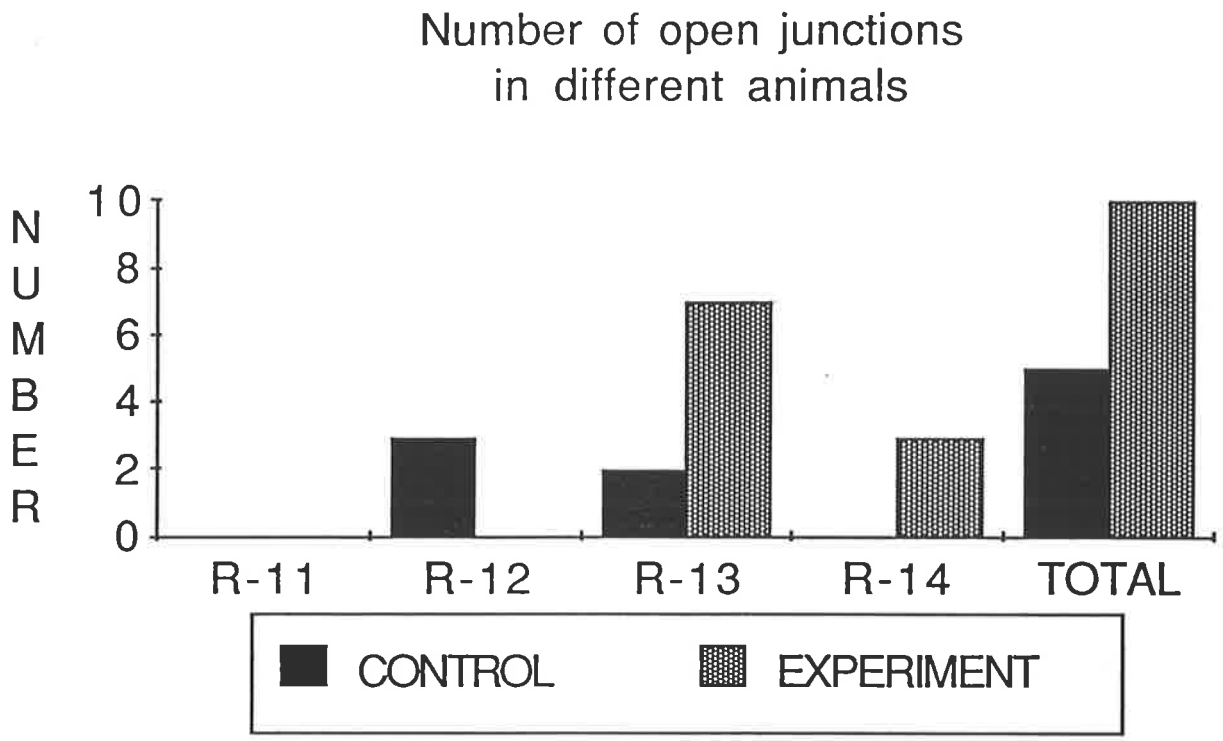

FIGURE 5.11 *Histogram illustrating the distribution of the open junctions in the four animals.*

TABLE 5.8 The width of the open junctions in nanometers.

	Number	Mean	S.D.	minimum	maximum
Control	5	767.8	414.9	136.2	1231.2
Experiment	10	640.2	357.2	227.8	1180.2
Total	15	682.8	367.6	136.2	1231.2

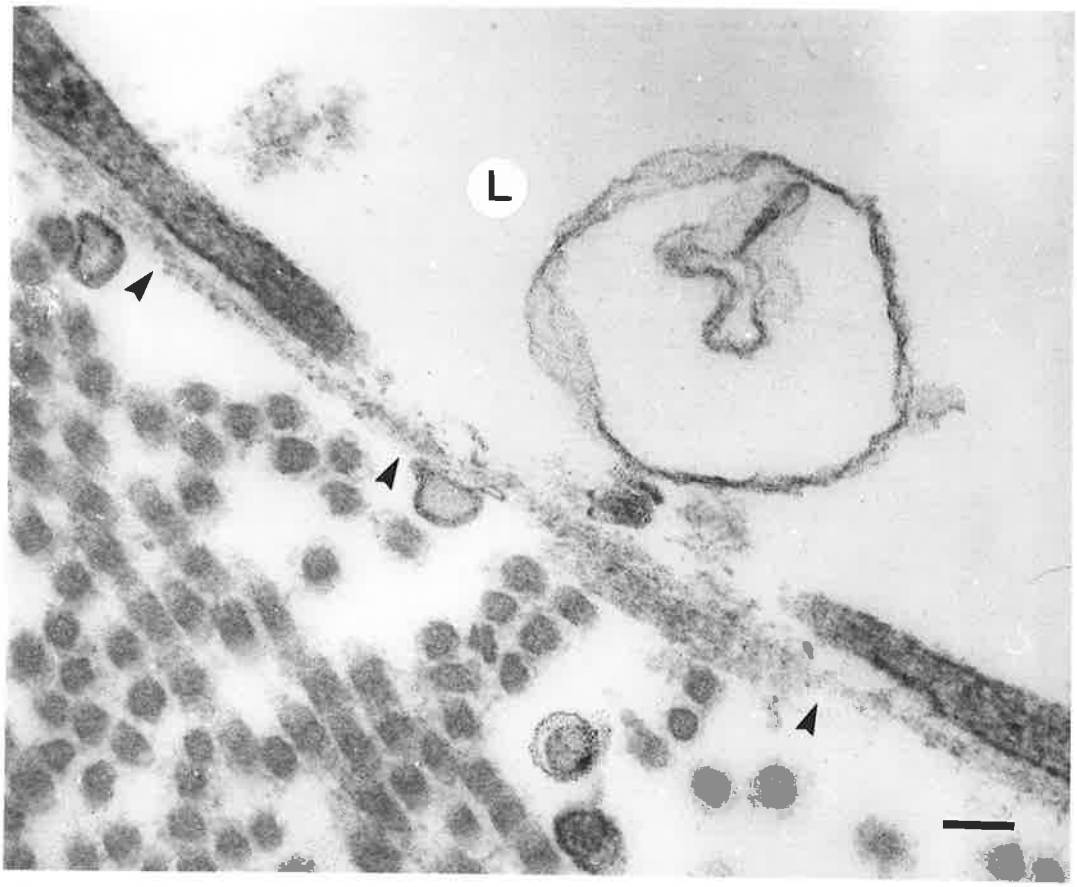

FIGURE 5.12 *An open junction from a venous capillary with a diameter of 6.7 microns. The size of the pore is 378 nm. The surrounding basement membrane is indicated by arrow heads. L, luminal side; Bar, 50 nm.*

Uranyl acetate and Reynolds' lead stain. X 185K

4. JUNCTION DIMENSIONS

4.1 JUNCTION DEPTH :

The depth of the junction measured from the luminal end to the abluminal end had a strong correlation to the endothelial wall thickness measured across the middle of the junction. Because there was no statistically significant difference of junction depth and endothelial wall thickness, the results were combined for a test of significance of the regression between the junction depth and wall thickness. The equation of the regression line is:-

$$Q2 = 208.9 + 0.048\ (\pm 0.011)Q1$$

$$F^{1}_{292} = 17.22\ ,\quad (p<0.001)$$

Q1 = Junction depth, Q2 = endothelial wall thickness

From the equation of the regression line, it means that for every unit of increase in the junction depth, the endothelial wall thickness increases 0.048 unit.

The depth of junctions in the control and the experimental PDL is shown in Table 5.9. Collecting venules were excluded due to the small sample size.

TABLE 5.9 The depth of junctions in nanometers.

Vessel Type	Observed mean	Predicted mean	SD.dev. pred.
Venous capillary			
Control	778.9	697.1	146.7
Experiment	851.0	810.5	127.5
Arterial capillary			
Control	719.7	761.1	242.5
Experiment	1022.7	984.1	222.0
Postcapillary-sized venules			
Control	951.1	909.0	165.5
Experiment	675.3	625.7	221.9
Terminal arteriole			
Control	994.1	994.3	144.3
Experiment	768.0	619.5	223.3

4.2 JUNCTION WIDTH :

The width of the junction was measured in four different regions. The width at the luminal and the abluminal ends showed a greater variability than the width measured at the luminal 1/3 and the abluminal 1/3. However, there were no statistical differences among the four regions. Therefore, the width of the junction in different regions (W1, W2, W3, W4) was combined together in the statistical analysis.

The width of the junctions was measured in specific regions classified as ;-

- tight regions,
- close regions,
- non-close/tight regions with a uniform width,

and - non-close/tight regions with a non-uniform width.

4.2.1 Tight region: Because there was a fusion between the two opposing membranes at the tight region, there was no space between the two endothelial walls (width = 0).

4.2.2 Close region: The width at the close region was approximately 5-6 nm. There was no difference in the width of the close region between the control and the experimental samples.

4.2.3 Non-close/tight region: The junctions investigated were divided into two types according to the width at the non-close/tight region ;-

I. Uniform type - This type of junction displayed a relatively uniform width between the two opposing membranes (Figure 5.13). The width at the non-close/tight region was approximately 20 nm.

II. Non-uniform type - The enlarged width between the two opposing membranes (lacunae) was seen along the depth in this type of junction (Figure 5.14). The width of the 'lacuna' varied greatly. The width of this region varied from 25 nm. up to 85 nm. There was a tendency for this type of junction to be found more often in the experimental than in the control vessels.

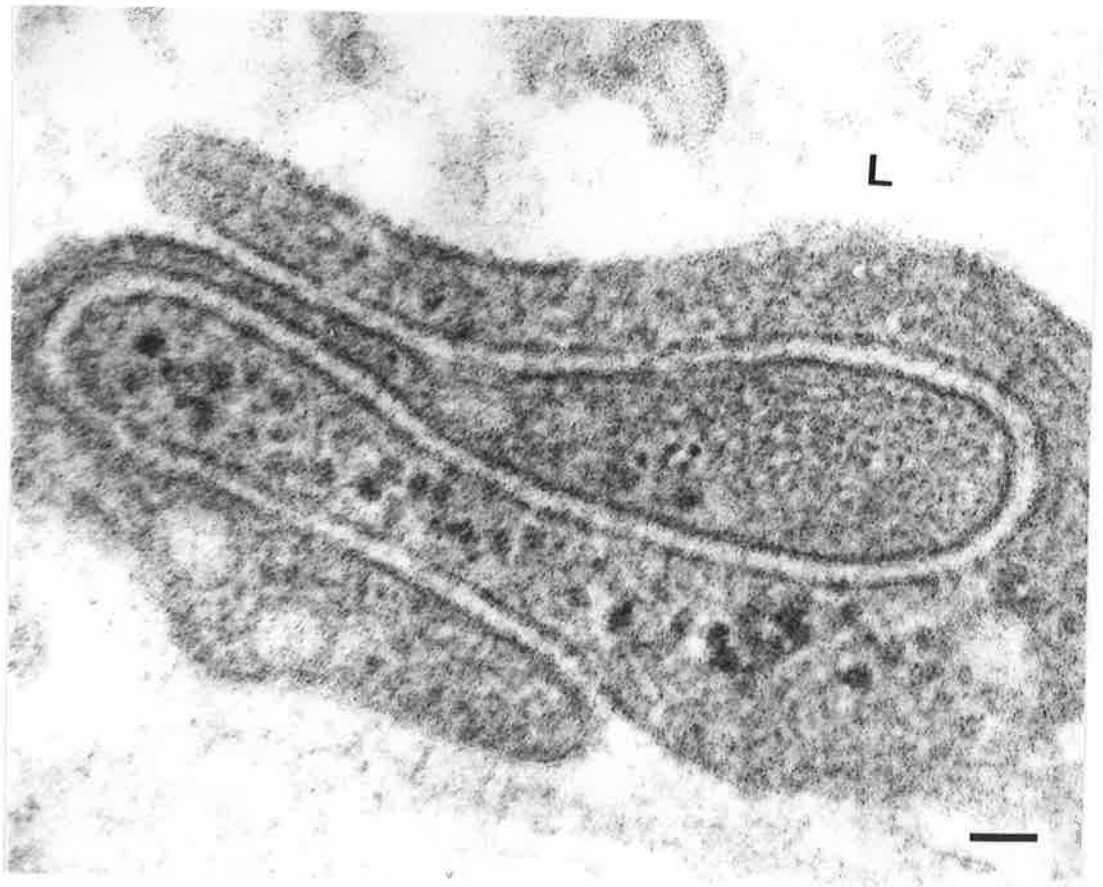

FIGURE 5.13 *A junction with a uniform width from a venous capillary with a diameter of 4.4 microns. The width of the* non-close/tight *region is approximately 18 nm. L, luminal side; Bar, 50 nm.*

Uranyl acetate and Reynolds' lead stain. X 170K.

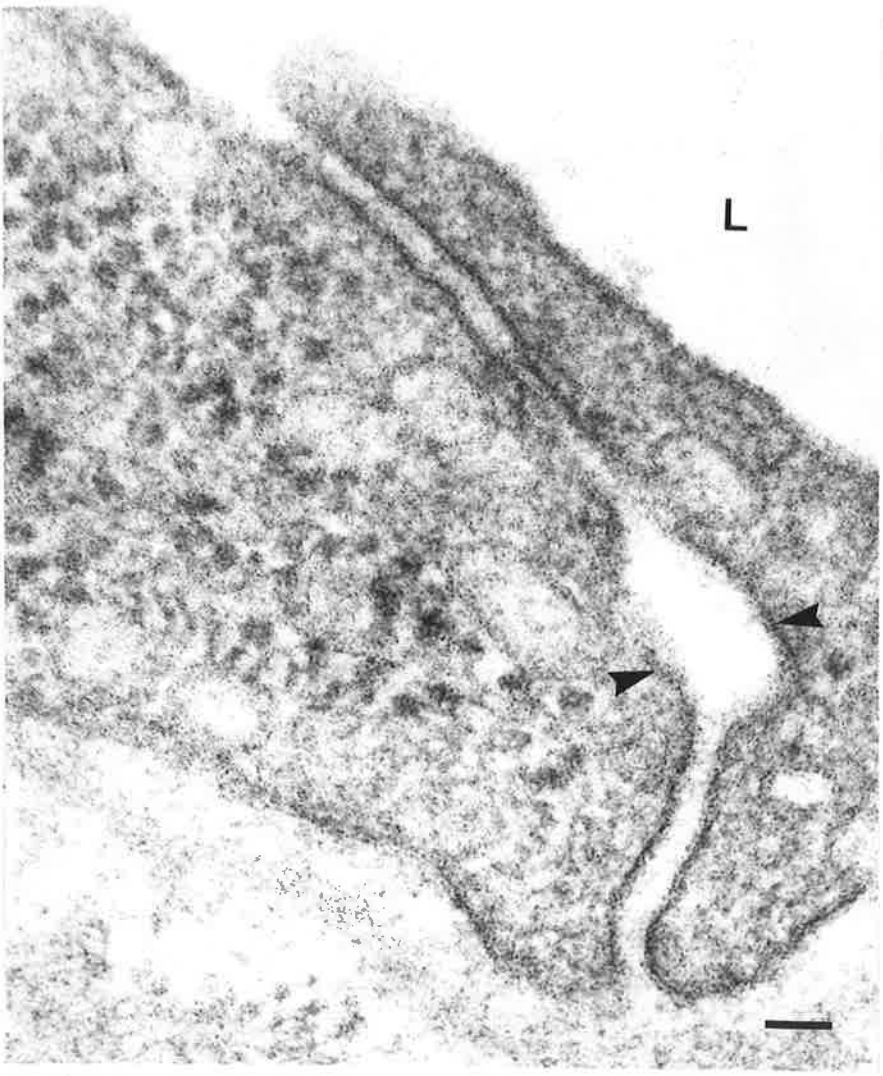

FIGURE 5.14 *A junction with non-uniform width from a terminal arteriole with a diameter of 8 microns. The arrow heads indicate the 'lacuna' where the width between the two membranes increased. The width across the lacuna is 67 nm. L, luminal side; Bar, 50 nm.*

Uranyl acetate and Reynolds' lead stain. X 170K.

4.3 A COMPARISON OF JUNCTION WIDTH IN TIGHT JUNCTIONS AND CLOSE JUNCTIONS

In the separate analysis of junction width between tight junctions and close junctions, it was found that tight junctions and close junctions behaved differently in changing width as the result of extrusive loads. The junction width of the close junctions increased significantly after the extrusive load was applied ($p<0.01$), while the change in the junction width of tight junctions was not significant. The increase in the junction width was mainly from the non-close/tight regions, while the width at the close regions was fairly constant. The increase in the junction widths was 9.4%. Table 5.10 shows the average junction width in close junctions.

TABLE 5.10 The average width of close junctions in nanometers.

	Observed mean	Predicted mean	SD.dev. pred.
Control	20.69	20.80	1.99
Experiment	22.35	22.76	2.22

($p<0.01$)

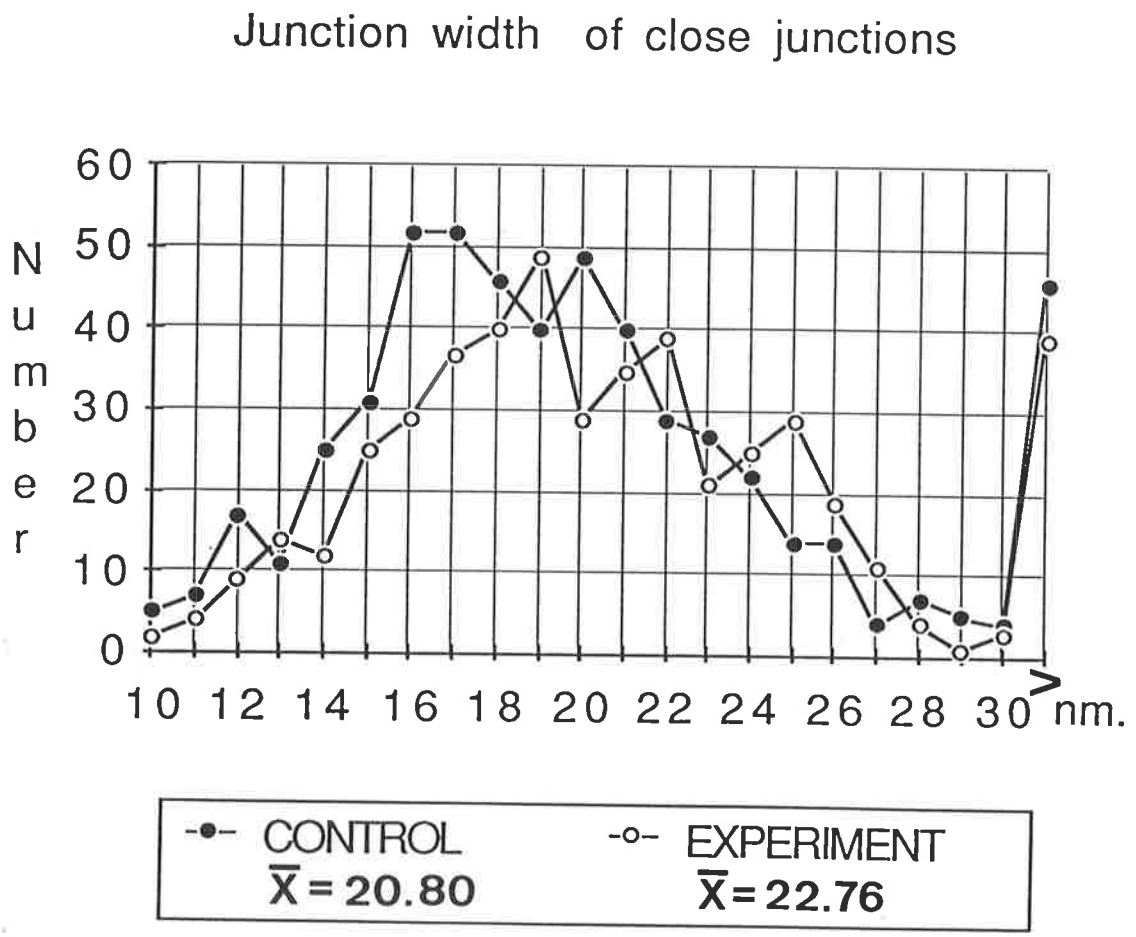

FIGURE 5.15 *The distribution of close junction width in control and experimental samples.*

5. GONIOMETER

In order to classify the type of junctions correctly, the use of a goniometer was necessary. In several junctions what appeared to be a membrane fusion when viewed at the plane of section was shown not to be so with the use of the goniometer. When viewed at a tilted angle there was a clearly visible gap between the two opposing membranes (Figure 5.16).

While using the goniometer to tilt the section to different angles relative to the electron beam, the dimensions of the junction changed. If the section was tilted around the axis that was parallel to the endothelial wall, the change in the dimension was not significant (Figure 5.16). However, if the section was tilted around the axis that was at right angles to the plane of the endothelial wall, the dimensions of the junction were dramatically changed (Figure 5.17). Although the dimensions of the junction were to be measured from the micrographs that were presumably taken at a right angle to the plane of the opposing membranes, the selection of the micrograph was based on visual judgement. The variability in the dimensions of the junction was therefore very high. The variability in the depth of the junction was also greater than that of the width.

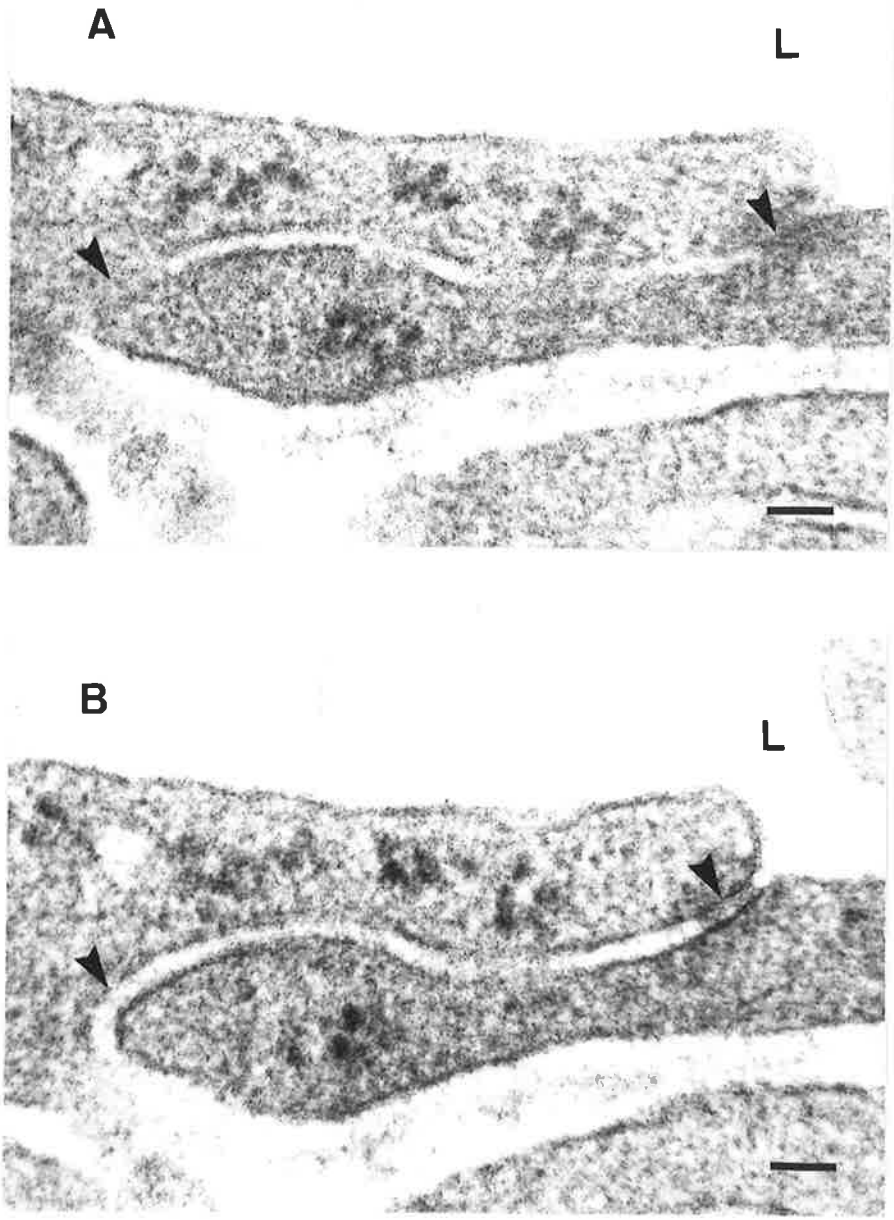

FIGURE 5.16 *The junction in the figure (A) appears to have two areas of membrane fusion (arrow heads) at the luminal and the abluminal ends. After the section was tilted by a goniometer clockwise 25° around the X-axis, as in figure (B), the two fusion areas appear to have a gap of approximately 12 nm. between them. This junction was then classified as a 'close junction'. L, luminal side; Bar, 50 nm. Uranyl acetate and Reynolds' lead stain. X 170K*

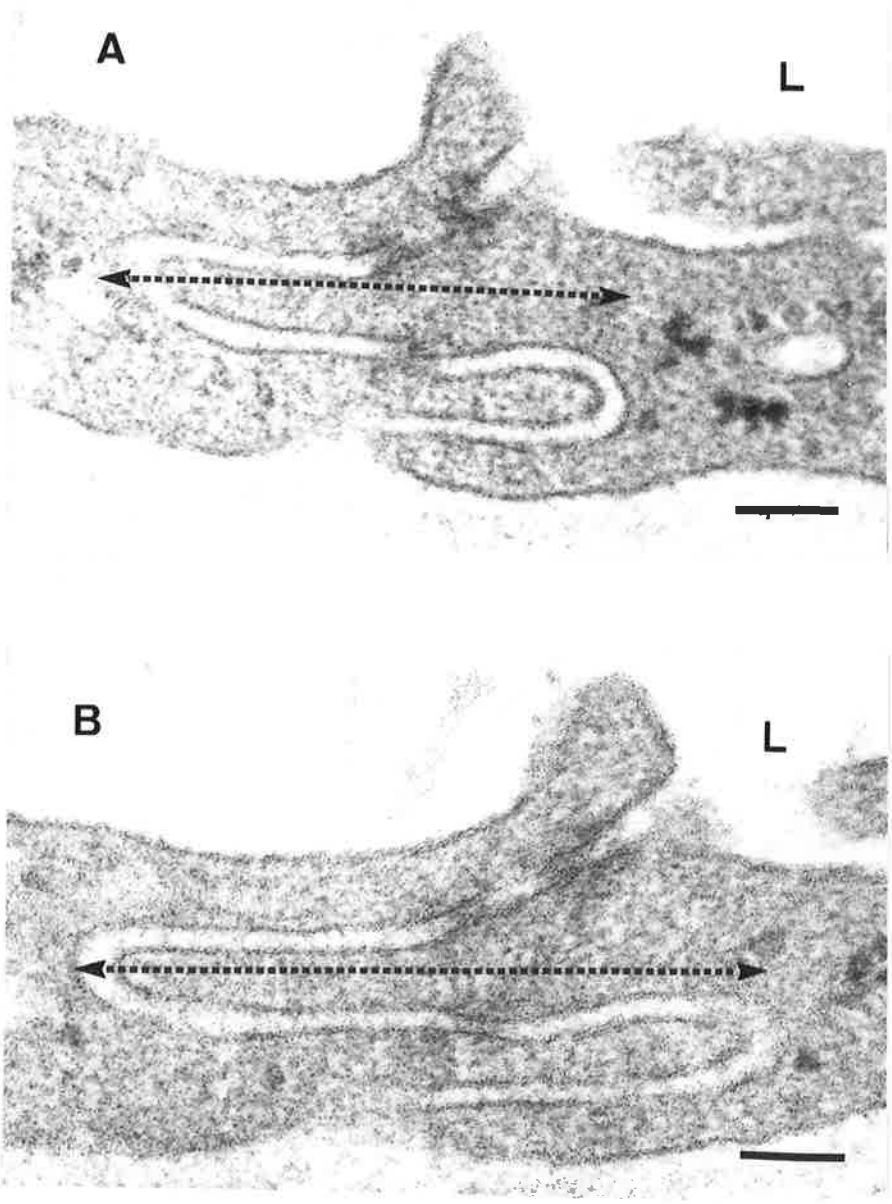

FIGURE 5.17 *The micrograph in figure (a) was taken at 0°. After the section was tilted clockwise 34° around the Y-axis the dimension of the junction was dramatically changed as seen in figure (B). L. luminal side; Bar, 100 nm. Uranyl acetate and Reynolds' lead stain. X 133K*

CHAPTER 6 DISCUSSION

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1. GENERAL DISCUSSION

1.1 RAT AS AN EXPERIMENTAL MODEL :

Several investigators have used rats as an experimental model in studies of the periodontal ligament (PDL), including Macapanpan, 1954; Kindlova and Matena, 1959, 1962; Bernick, 1962; Kindlova, 1968; Garfunkel and Sciaky, 1971; Edwall, 1982; Weekes, 1983; Weekes and Sims, 1986. The effects of applied forces, tension or pressure, to the periodontal ligament of rats have also been reported (Piekarski and Cohen, 1962; Carraza et al., 1966; Baumrind, 1969; Rygh, 1972a, 1972b, 1976; Bondevik, 1980; Cho et al., 1982; Gaengler and Merte, 1983; Shore et al., 1984; Michaeli et al., 1985; Clark, 1986; Lew, 1986; Berkovitz et al., 1981; Engstrom et al., 1988).

Cooper (1988) chose the rat as the experimental animal in her study because of easy access, low cost and ease of standardization. In addition, more is known collectively regarding the rat PDL than for any other animal species, so further investigations can only lead to the development of more complete hypotheses related to the structure and function of the PDL.

1.2 AREA OF INVESTIGATION :

The PDL at the mesial root region was chosen as the area of the investigation. The mesial root is the largest and least curved compared to the other roots. The process of bilateral sectioning is therefore easier to perform. Furthermore, this investigation was designed to study the tensioned PDL incident to extrusive loads. If the root is curved, both tensioned and pressured areas would occur depending on the morphology of the root.

The effect of an extrusive load at the apical region of the mesial root has been investigated by Clark (1986), Lew (1986) and Cooper (1988). Cooper (1988) stated that according to the morphology of the mesial root which has a complicated mesio-palatal deviation from the vertical axis, a purly axial extrusion without incorporating a tipping vector was almost impossible. Relative to the "pressure-tension" hypothesis (Baumrind, 1969), the apical zone of the mesial root of the maxillary first molar should be representative of a tension zone after application of an extrusive force.

In this investigation, the area of study was the PDL at the most mesial area from the alveolar bone crest level down to the end of the root. According to Cooper (1988), it was likely that these areas represented both tensioned and pressured PDL depending on the direction of the tipping vector present during the application of extrusive load. It was impossible to identify the pure tensioned or pressured areas of the PDL in this study because of the tipping vector. This study was therefore a demonstration of

vascular reaction to an external force which was assumed to cause principally tension in the PDL. In addition, the vascular distribution in the PDL from the cervical to apical regions is different (Sims, 1983). Therefore, the direct comparison of the effect of extrusive loads between the present study and the previous studies (Clark, 1986; Lew, 1986; Cooper, 1988) would be inappropriate.

1.3 EXTRUSIVE LOAD :

The 1.0 N force was selected to be used in this investigation to be comparable with the loads used by other authors in their studies on tooth movement of rat molars ;

Gianelly (1969) - used tipping forces of 0.5 N - 1.5 N.
Azuma(1970) - used tipping forces of 0.8 N - 2 N.
Lew (1986) - used an extrusive force of 1.0 N.
Cooper (1988) - used an extrusive force of 1.0 N.
Moxham and Berkovitz (1989)
- used extrusive forces of 0.01 - 0.2 N.

According to the small size of the rat, 1.0 N of this extrusive load was considered to be very high compared to orthodontic forces used in humans.

1.4 FIXING AGENTS AND ENDOTHELIAL JUNCTIONS :

The preservation of membranes is important in the study of intercellular junctions. The appearance of the trilaminar structure, refered to as a unit membrane, should be clearly visible. The measurement between the two opposing membranes will then be accurate. This appearance was also

important in the identification of junction type. A pentalaminar structure should also be visible where there is membrane fusion.

In the present study, the tissue was fixed by the 'double fixation' method, i.e. primary fixation in aldehyde (glutaraldehyde and paraformaldehyde) followed by 'post-fixation' in osmium tetroxide. This method combines the advantages of both fixatives. Phospholipid membranes are well preserved by osmium tetroxide while the protein components such as cytoplasm and mitochondria are well preserved by aldehyde (Weakley, 1972).

It is likely that the space between neighboring cell membranes could have been influenced by the fixation procedures (Palade et.al., 1979). Aldehyde fixation results in a change of the endothelial junctions from the low to the high resistance state (Fawcett, 1981). Therefore, the rare leaks found in certain investigations are a response to the experimental fixation (Bundgaard, 1984). On the other hand, mechanical manipulation and exposure of the tissue to light and air, preceding fixation was found to induce leak formation (Simionescu et al., 1978). Casley-Smith (1981) reported that the width and depth of the various regions of the junctions in freeze-substitution observations are similar to those observed in chemical fixation.

Although the effect of fixing agents can influence the size of the junction, this investigation would still be valid as it is the comparison between normal and tensioned PDL under the same conditions.

1.5 ANIMAL VARIABILITY :

In the statistical analysis of animal variability in the present study, it was found that the differences amongst these four animals were significant ($p<0.05$). The results from animal number 13 had the highest variation compared to other animals. The results of the whole experiment may then be effected by the result from animal number 13. Although the animal variability was taken into account in the analysis, the standard error of the experiment was still effected by the results from this animal. There was no definite reason why these differences occured. Therefore, there was no reason why animal number 13 should be excluded even though the results were significantly different from the other animals. In order to overcome the problem of animal variability, a larger number of animal samples should be considered in future research.

1.6 SAMPLE SIZE :

The total number of vessels in this study was 310 (control = 160, experiment = 150). When all the vessels were included in the analysis, the sample size was considered to be adequate. However, after the vessels were divided into different types, the sample size of each vessel type was then reduced significantly. Collecting venules were excluded from the study due to inadequate sample size. The sample size for terminal arterioles was also considered to be very small (control = 21, experiment = 8).

In the analysis of the junction widths, the result from each vessel type was not statistically significant

except in terminal arterioles. After the the vessels were combined as venous type (venous capillaries, post-capillary sized venules, collecting venules) and arterial type (arterial capillaries, terminal arterioles), the results became marginally significant (venous type $p=0.055$, arterial type $p=0.057$). When all the vessels were combined together in the analysis, the change in the size of the junction width was significant ($p<0.01$).

In order to improve accuracy in the experiment, an adequate sample size for each type of blood vessel should be considered. The sample size of each vessel type should be equal, the direct comparison among different vessel types would then be valid.

2. ACCURACY IN MEASURING METHOD

The factors that effected the accuracy in the measurement of the junction dimensions were as follows;-

2.1 THE INTERPRETATION OF TWO DIMENSIONAL IMAGES FROM THREE DIMENSIONAL STRUCTURES :

In measuring the junction dimensions, the identification of the opposing membranes is important. The image of a sheet-like object varies depending on the plane of section relative to the electron beam. Figure 6.1 shows the different images of the same object viewed at the different angles.

To correctly define the margin of the cell membrane, the membrane needs to be viewed exactly at a right-angle. If the membrane goes through obliquely the margin of

the membrane will appear less definite. What appears to be black dense lines when viewed at a right-angle will become a collection of granules of varying densities when viewed obliquely.

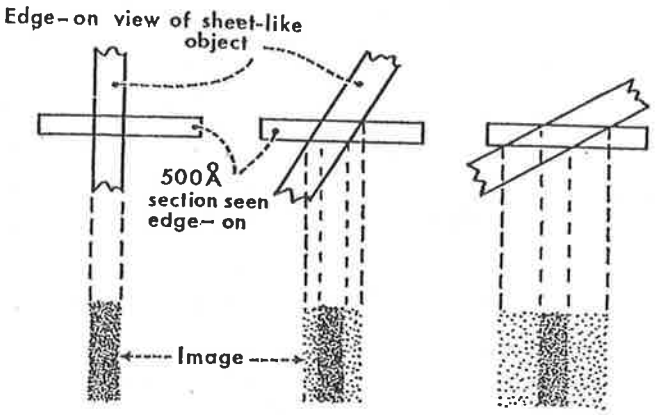

FIGURE 6.1 *Variation in apparent image of a sheet-like object passing at different angles through a section.* (Weakley, 1972)

By using a goniometer, the section can be tilted at various angles relative to the electron beam. However, the endothelial junction is a 3-dimensional structure, and what appears to be a right-angle at the luminal end may not appear to be a right-angle at the abluminal end.

2.2 SECTION THICKNESS :

Ultra-thin sections are necessary for this study. With increases in section thickness, fewer and fewer gaps between the two opposing membranes can be identified. Figure

6.2 shows the effect of the section thickness and the identification of a gap between the two cells.

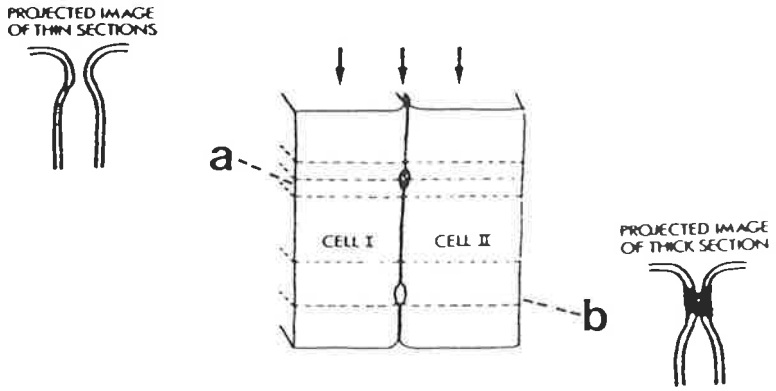

FIGURE 6.2 *The effect of section thickness in the identification of a gap between cells.*(Bundgaard, 1984)

Bundgaard (1984) stated the basic assumption for the analysis; that a gap can be identified only if it extends throughout more than 50% of the section thickness (Figure 6.2 a). If the membranes corresponding to the closed part of the cleft extend through 50% of the section or more, the electron density of these membranes implies that the gap becomes blurred in the projected image (Figure 6.2 b).

The appearances of punctate junctions studied by Bundgaard (1984) have three subdivisions (Figure 6.3). However, it is possible that these differences in appearance are the result of section thickness and the angle at which the sections intersect the perpendiculars to the membrane.

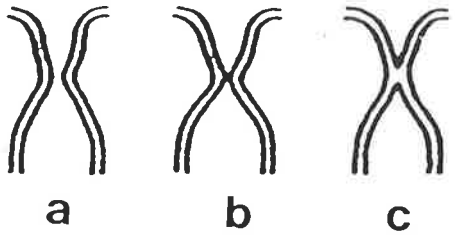

FIGURE 6.3 *Subdivision of the appearances of the punctate junctions. (a) close apposition; (b) contact between membranes; (c) apparent fusion of membranes.* (Bundgaard, 1984).

2.3 STAINED SECTIONS :

When the sections are stained, it is highly likely that the staining, or its effect on contrast, is non-uniform through the section. In section-stained material, much of the stain lies in the section itself, rather than actually on the surface. This stained depth is shown to be approximately 4 nm. deep, but this depth may include surface aggregates (approximately 1 nm.) as well (Casley-Smith et al.,1978). The estimation of the junction dimension has to take this factor into account. Since the staining is effectively confined to the first 4 nm., then the sections are often effectively only 4 nm. thick (Casley-Smith, 1981).

2.4 GONIOMETER :

A goniometer is very necessary for exploring the tight regions of the junctions. Because of the irregularity

of the PDL vessels in the constant plane of sectioning, it is unlikely that the majority of junctions will be perfectly cross-sectioned. Therefore, it is more likely that the opposed plasma membranes appear to fuse with each other. With the goniometer, the narrowest portions can be rotated so that fusion areas can be more readily clarified. The classification of each junction is therefore more accurate.

Casley-Smith (1981,1983) stated that because Simionescu et al.,(1978) did not use a goniometer to study the junctions, the results of their studies are different. Simionescue et al., (1978) found that close junctions are much more common in post-capillary venules than they are in the other capillaries, while Casley-Smith (1981,1983) found them equally common in all types of capillaries.

In the present investigation, with routine TEM examination most of the junctions were found to have at least one tight region which would be classified as a tight junction. However, after using a goniometer, most of the tight regions appeared as close regions. Consequently, the junctions were re-classified as close junctions.

A difference in the dimensions of junctions was found during examination at different angles. The difference in the depth was greater than the difference in the width. The measurements were taken from the prints that were assumed to be taken at the right angles to the plane of junction. However, this assumption was justified by the visual judgement. The errors of the measurement will be less if the measurement technique is consistent.

2.5 RESOLUTION AND ACCURACY OF THE ELECTRON MICROSCOPE :

The electron microscope used in the present study (JEM-2000 FX) has a resolution of 30 Å. This means that if the gap between the two membranes is smaller than 30 Å (3 nm.) it would be invisible.

The accuracy of the electron microscope was tested at the Electron Optical Center, The University of Adelaide. The series of micrographs taken at different magnification was tested against a replica of a diffraction grating. The error was found to be less than 5%. This was also confirmed by the test in this study.

2.6 POSITION OF THE SPECIMEN :

Magnification will vary with the position of the specimen on the electron microscope axis. If the grid that holds the specimens is not perfectly flat, a variation in the magnification will occur. The micrographs that are taken from the area away from the center of the field also have higher distortion.

2.7 PRINTING :

Because the width of the junctions was very small, the direct measurements taken from the micrographs were inappropriate. The micrographs were photographically enlarged. The reference lines were drawn on the prints which made for more consistency in the measurement. However, errors could have occured during processing. The error in determining the exact enlargement of the print would effect

the accuracy of the final results. Moreover, some shrinkage of the print may occur while processing.

Although there were several factors affecting the measuring method, these factors would be similar to both experimental and control samples. Therefore, these errors would cancel out in the analysis of the junctions.

3. BLOOD VESSELS

3.1 CLASSIFICATION OF BLOOD VESSELS :

The classification of blood vessels in this study was based on the criteria established by Rhodin (1967,1968), Freezer (1984), Clark (1986) and Lew (1986). However, the diameters of venous capillaries and arterial capillaries were adjusted to conform with the morphology of the present material. The diameters of both venous and arterial capillaries in this study were smaller compared to the previous studies. Due to the low magnification used in the identification of blood vessels, a distinction between pericytic and non-pericytic venules could not be conducted accurately. Therefore, pericytic and non-pericytic venules were classified as the same type of blood vessel.

The vessels classified as 'terminal arterioles' in this study were classified as 'precapillary sphincters' in Cooper's study (1988). Classifications of blood vessels are slightly different according to different authors (Rhodin, 1967,1968; Wolff, 1977; Simionescu and Simionescu, 1984; Freezer, 1984; Clark, 1986). This is partly because of the different tissues studied. Caution should be exercised when a comparison is attempted.

3.2 BLOOD VESSEL MORPHOLOGY :

There were some differences in blood vessel morphology between the control and experiment samples. The walls of the blood vessels in the experimental samples were corrugated and had more finger-like projections into the lumen (Figure 5.2). These differences were seen more often in the venous capillaries. According to Casley-Smith (1979), the immediate vascular reaction in acute inflammation is characterised by the appearance of inflammatory cells which pass through the open junctions. This generally occurs within 30 minutes. It is therefore unlikely that these phenomena represented the inflammatory stage of the tissue due to the absence of inflammatory cells.

It is postulated that these phenomena were caused by the reduction of internal vascular pressure. After the extrusive force was applied, tension in the PDL occured. The vascular flow of the blood vessels that were trapped between the stretched PDL was then reduced. As a consequence of internal pressure reduction, an elastic recoil of the vessel walls occurred which gave the appearance of corrugated walls. This hypothesis was also supported by the statistical analysis that the diameter of the venous capillaries was reduced in the experimental samples ($p<0.001$, Table 5.1). However, for the other types of vessels, the change in the vessel diameter was not significant.

3.3 DISTRIBUTION OF BLOOD VESSELS :

The distribution of the PDL vasculature has been reported by Freezer and Sims (1987). This study supports

their study that the tooth 1/3 contains the smallest vascular volume. However, the vascular volume found in the middle 1/3 and bone 1/3 in this study was equal while in the previous study, the middle 1/3 contained the greatest volume.

The distribution of the vessels from the cervical region down to the apical region was not consistent. A significant conclusion could not be drawn from this study.

3.4 BLOOD VESSEL TYPE AND JUNCTION MORPHOLOGY :

In this study the venous type vessels (venous capillaries, postcapillary-sized venules, collecting venules) showed the simplest appearance of the junctions. The appearance of the arterial capillary was more curvy and the junctions in terminal arterioles had the most complexity.

Simionescu et al. (1975a) described the segmental differentiations of cell junctions in the microvasculature. Their report that the small venules (pericytic venules) have the simplest orientation of the junction was confirmed by this study.

The organization of endothelial junctions studied by Bundgaard (1984) showed a similar pattern of the lines of contact (tight junctions) reconstructed from the venules and the capillaries. Occasionally, however, the venular junctions showed a complexity greater than that observed in capillaries. Direct comparision with the present study was not valid since the reconstruction of junctions from thin serial sections is required.

4. ENDOTHELIAL JUNCTIONS

There were three types of junctions found in this study, i.e., tight junctions, close junctions and open junctions. No gap junctions were found. This study supports the study of Simionescu et.al. (1975a) that gap juctions were not present in the capillaries and pericytic venules. The presence of gap junctions is more common in the larger vessels such as arterioles, arteries and veins.

In the present study, the vessels in the PDL consisted of venous capillaries, arterial capillaries, post-capillary-sized venules, terminal arterioles and small collecting venules. The vessel walls were very thin and consist of 1 to 2 layers of cells. The exchange of fluid and substrates is the major function of these vessels. The communication between cells may not be very important. This might be the reason why the communicating junctions or gap junctions were absent.

4.1 TIGHT JUNCTIONS :

The percentage of tight junctions compared to the total number of junctions in this study was approximately 20%. There was no significant difference in the number of tight junctions between the control and the experimental samples. Furthermore, from the Chi-square analysis there were no differences in the proportion of tight junctions among different typs of blood vessels.

Approximately 87% - 88% of the tight regions were in the luminal third. This result was consistant with the

result found in epithelium (Fawcett 1981). However, Schneeberger and Lynch (1984) made the generalised statement that the tight regions are often found in the midpoint rather than at the apex of the intercellular space.

The appearance of tight junctions in the PDL in this study was similar to the appearance of tight junctions in the dental pulp described by Tabata and Semba (1987). The area of membrane fusion was a 'spot-fusion' rather than a 'zone-fusion'. This appearance should be confirmed by the examination of freeze-fracture replicas.

The extrusive load did not have any effect on the number or dimensions of the tight junctions. Neither the width nor the depth of the tight junctions changed significantly. The results of this study suggest that the tight regions of the tight junctions were permanent structures which would not change from the effect of short-term mechanical stimuli. Inoue et.al. (1987) showed the evidence of the morphological changes of intercellular junctions in the rat submandibular gland treated by long-term (10 days) repeated administration of isoproterenal. Muhleisen et.al. (1989) demonstrated the structural alterations in vascular endothelial tight junctions *in vitro* during the period of 1 - 6 days. Whether long-term mechanical stimuli can have an effect in changing the structure of tight junctions, needs to be studied in future research.

4.2 CLOSE JUNCTIONS :

Close junctions comprised the majority of junction types found in the PDL microvascular bed. The percentage of close junctions in the present study was approximately 80%. This was considered to be very high compared to the 5% of close junctions found in dog muscular capillaries (Casley-Smith, 1975) and 7% found in mouse diaphragm capillaries (Casley-Smith 1981). This difference may imply that the PDL microvasculature is highly permeable.

According to the responses to the extrusive loads, close junctions were found to behave differently from tight junctions. Although the number of close junctions did not change significantly, the change in the junction width was significant ($p<0.01$). Thus, it can be concluded that an extrusive load has the effect of increasing the width of the close junctions. As close junctions comprised the majority of junctions, it is then possible that the permeability of the PDL vasculature is increased as a result of an extrusive load.

4.3 OPEN JUNCTIONS :

Open junctions were rarely found in the PDL microvascular bed. Although open junctions are commonly found in inflammatory tissue, they can also be found occasionally in normal tissue. No inflammatory cells were found in this study. Therefore, the presence of the open junctions did not indicate that the tissue was in the inflammatory stage.

Simionescue et.al. (1975a) stated that there was no agreement about the relative frequency in the appearance of either tight or open junctions. The authors suggested that this may be because of the difference in the tissues and areas studied.

Open junctions were found only in venous capillaries and post-capillary sized venules in this study. These types of vessels are more 'leaky' and more sensitive to stimulation than other types of vessels (Casley-Smith 1979, 1983).

Because of a very small sample size, a statistical analysis could not be done. Although the number of the open junctions increased in the experimental samples, it could not be concluded that this was a result of the extrusive load.

5. SUGGESTIONS FOR FUTURE RESEARCH

In the study of endothelial junctions, there are three major methods for preparing membranes for examination in the electron microscope. They are thin-sectioning, negative staining and freeze-cleaving (freeze-etching).

Freeze-cleaving and freeze-etching techniques have recently contributed important new information on membrane ultrastructure. The combination of the two techniques allows a three-dimensional reconstruction of membrane structure at the supramolecular level (reviewed by Staehelin, 1974). The information on the inner sides of the two opposing membranes (E-face and P-face) can be obtained by these methods.

It is not only the type of junction that is important in cell permeability but also its orientation. The number of lines of the tight junctions was found to be associated with the permeability of the tissue (Schneeberger and Lynch, 1984). Fragmentation of the line of tight junctions was also found in leaky epithelia (van Deurs and Koheler, 1979). This information cannot be obtained by the thin-sectioning technique in the present study. Currently, there is no information on the orientation of junctions in the PDL microvascular bed. Further research in this area should be considered.

1. There were five types of blood vessels found in the PDL microvascular bed of the rat maxillary first molar, i.e., venous capillaries, arterial capillaries, post-capillary-sized venules, terminal arterioles and small collecting venules.

2. As a result of the extrusive load, the diameter of venous capillaries reduced significantly ($p<0.001$). A change in the morphology of this type of vessel was found, such as, corrugated endothelial walls and numerous finger-like projections into the lumen.

3. The endothelial junctions of the vessels with relatively thin walls such as venous capillaries, post-capillary-sized venules and collecting venules displayed 'simple type' morphology. Arterial capillaries tended to have a 'curvy type' junction whereas terminal arterioles had the most complicated 'convoluted type' junctions.

4. There were three types of endothelial junction found in this study, i.e., tight junctions, close junctions and open junctions. No gap junctions were found.

5. Close junctions were the most common type of junction found and comprised approximately 80% of all PDL junctions. Open junctions were rarely found (4%).

6. Open junctions were found only in the venous capillaries and postcapillary-sized venules.

7. There was no significant difference between normal and tensioned PDL in the number of close junctions, tight junctions and open junctions.

8. According to the Chi-square analysis, the proportion of tight junctions was not statistically significant for different types of blood vessels.

9. The junction width was classified in four categories;-
   1) Tight region width was 0 nm. (fusion of membranes)
   2) Close region width was approximately 5-6 nm.
   3) Non-close/tight region width of uniform type junctions was approximately 20 nm.
   4) Non-close/tight region width of non-uniform type junctions ranged from 20 nm. up to 85 nm.

10. The width of the close junctions increased (9.4%) as a result of the extrusive force ($p<0.01$) while the width of the tight junctions did not change significantly. Because close junctions comprised approximately 80% of all the junctions, it is hypothesized that the close junctions were one of the major pathways of fluid and substrate exchange incident to the extrusive load.

11. The change in the junction width was significant at the non-close/tight regions while the width at the close regions was fairly constant.

12. The endothelial junctions of the tensioned PDL are often found to have more non-uniform width morphology than normal PDL.

13. Tight regions of tight junctions were commonly found in the luminal third (87.5%). Those tight junctions which had tight regions in the middle third or the abluminal third of the endothelial junction, were found only in the apical third levels.

14. The tight regions appeared as 'spot-fusion' between the two opposing membranes rather than 'zone-fusion'. However, the ultrastructural organization of the tight junctions needs to be confirmed with a freeze-fracture replica investigation.

15. There was a strong correlation between the junction depth and the thickness of the endothelial walls across the middle of the junction.

16. The animal variability in this study was significantly high ($p<0.05$) especially in animal number 13 which resulted in the high standard deviations. However, for statistical purposes, all the animals were included in the statistical analysis.

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1. Experiment Primary Perfusate (E.P.P.)

1.0 g. $Na_4Fe(CN)_6.10H_2O$

0.475 g. NaCl

3.0 g. Dextran (M.W.~70,000)

Test ion is Sodium ferrocyanide. (M.W.212)
(B.D.H.,England)

Dissolve the powders in 100 ml. of millipored d.d. water. Prepare the perfusate on the day required. To fill the perfusion pressure bottle for perfusion of 3 rats, 200 ml. of solution is sufficient.

2. Tris (Ethylenediamine) Cobalt III Chloride

Referred to as cobalt salt, this salt is unavailable commercially, but is not difficult to prepare (Work, 1946) and stores indefinately.

The salt used in this investigation was prepared at the Department of Physical and Inorganic Chemistry, The University of Adelaide.

Chemical formula: $[Co(en)_3]Cl_3$ (M.W. 399.64)

To prepare cobalt salt, cool a solution of 25 ml. 98% ethylenediamine in 53 ml. of water on ice. Then add 11.7 ml. HCl (conc.). To this, add a solution of 25.7 g. $CoCl_2.6H_2O$ in 53 ml. of water.

Add 33 ml. H_2O_2 and rotate in a vacuum condenser until a precipitate begins to form. At this point, add 17 ml. HCl (conc.) and 33 ml. ethanol. After this, cool the solution on ice, filter and wash with 90% ethanol until the washing solution is colourless. Dry it at 110°C in an oven to constant weight. The yield is approximately 20 g.

3. Experimental Primary Fixative (E.P.F.)

The fixative contains 2% paraformaldehyde, and 2% glutaraldehyde in 0.1 cacodylate buffer. The osmolarity is adjusted with the cobalt salt and NaCl. Dextran is added to avoid possible crystalloid and osmotic pressure alterations at the moment of fixation. (Bohman, 1970)

Per 100 ml. solution:

2.14 g. Sodium cacodylate

0.475 g. NaCl

1.0 g. Cobalt salt (Appendix 2)

3.0 g. Dextran (M.W. ~70,000)

Make fresh as required. Dissolve the powder in 72 ml. millipored d.d. water. When the animal is ready to be cannulated add :-

8 ml. 25 % glutaraldehyde (2% in solution)

20 ml. 10% paraformaldehyde (Appendix 4)

Adjust the pH to 7.0 and refrigerate until ready to perfuse with fixative.

4. 10% Paraformaldehyde

Dissolve 10 g. paraformaldehyde powder (TAAB Lab, Reading, Berkshire) in 100 ml. millipored d.d. water at 65°C. Add a few drops of NaOH to clear the solution. Cool and store at 4°C.

5. Nembutal^{R}

Pentobarbitone sodium (Ceva chemicals) was injected I.P. at room temperature, using a single-use short 26 guage needle attached to a 1 ml. syringe.

Dosage: 0.4 ml. of 20 mg./ml. per 100 g. body weight. A dosage of approximately 0.4 ml. of 60 mg./ml. was required for a 300 g. rat.

Presentation: Provided in a bulk 100 ml. dispenser, for use with animal only.

6. Anticoagulant

Heparin injection B.P. (containing no antiseptic) was supplied in a 5 ml. glass ampoule (C.S.L., Melbourne) containing 5000 units (IU) per 5 ml.

Dosage: 0.02 ml. of heparin sodium per 100 g. body weight.

Route: I.V. injection femoral vein.

Shelf life: Discard unused heparin once glass vial seal is broken.

Storage: Below 25°C

7. Millipore Filtering

Double distilled (d.d.) water was millipore filtered through a 0.22 micrometre filter (Millipore Corporation, Bedford, Mass.) using a vacuum filtration technique (Gellman, Ann Arber, Mich.). Filtration through a millipore filter will remove any particulate matter that has the potential to obstruct fine microcirculatory beds.

8. Decalcifying Solution

74.45 g. EDTA

1800 ml. 0.06 M cacodylate buffer (Appendix 9)

200 ml. 25% glutaraldehyde

Dissolve EDTA in a cacodylate buffer by gentry heating. When cooled, add glutaraldehyde (pH to 6.0 at 4°C).

Shelf life: 7 days at 4°C

9. 0.06 M Cacodylate Buffer

25.68 g. Sodium cacodylate

2000 ml. d.d. water

Dissolve the powder in water , pH to 7.4 at 20°C

Shelf life: 7 days at 4°C

10. Tissue Processing

Transfer the trimmed tissues to separate 2 ml. soda glass vials, half-filled with 0.06 M

cacodylate buffer, and press in a rotator overnight. The following day, discontinue pressing but leave the tissues in the rotator until final embedding occures. Pipette the solution in and out of the individually labelled glass vials, being careful not to touch the tissues.

Day 1

1. Post-fixation:	1 hour
2% OsO_4 in d.d. H_2O	
2. Wash: d.d. H_2O	15 mins.
3. Wash: 70% alcohol	15 mins.
4. Dehydrate:	
70% alcohol	15 mins.
70% alcohol	15 mins.
80% alcohol	15 mins.
80% alcohol	15 mins.
90% alcohol	15 mins.
90% alcohol	15 mins.
100% alcohol	15 mins.
100% alcohol	15 mins.
propylene oxide	15 mins.
propylene oxide	15 mins.
propylene oxide	30 mins.
propylene oxide	30 mins.
5. Infiltrate:	
1:1 propylene oxide : Agar 100^R	overnight.

Day 2

1:3 propylene oxide : Agar 100^R	4 hours.
100% Agar 100^R I	4 hours.

100% Agar 100^{R} II overnight.

Make up final embedding 100% Agar 100^{R}

Day 3

Embed: Remove tissue samples from their vials, and using a stereo-dissecting microscope, place with known orientation into silicone rubber moulds. Incubate resin filled moulds at 37°C for 48 hours, then at 60°C for a further 48 hours before coding and storing at room temperature.

11. 2% Osmium Tetroxide in Double-distilled Water

1 g. OsO_4 in 50 ml. d.d. H_2O

Method: Beakers A - hot tap water

B - cold tap water

C - ethanol 100%

Prepare the OsO_4 solution in a fume cupboard with the operator wearing two pairs of gloves, and a respiratory mask.

Place ampoule into Beaker A to dissolve the crystals. Remove the label from the ampoule. Rotate the ampoule to get an even film of OsO_4 around the ampoule. Thoroughly clean the ampoule with a tissue saturated in ethanol. Place the ampoule into a thick walled screw top jar containing 50 ml. d.d. H_2O. Ensure the lid is firmly secured, and shake the jar until the ampoule brakes. Wrap the jar in aluminium foil to exclude the light. If the solution is exposed to the light, it will oxidise rendering it useless.

Leave in fume cupboard at room temperature to dissolve overnight.

Osmium tetroxide solution should not be kept in the refrigerator as the osmium oxidises more rapidly at 4°C. The solution can only be used when it is clear.

12. Agar 100^{R} Embedding Resin

Agar 100^{R} (Agar Aids, Essex) is used for embedding the tissue for transmission electron microscope investigations. The weight per oxide equivalent for the agar is 145. The volumetric ratio of the agar included is 1 solution A : 2 solution B.

Calculate the volume required as a multiple of the basic WPE 145, 1:2 formula as follows:

Solution A:	Agar 100 (Agar Aids, Essex)	1.00 ml.
	DDSA (Dodecenyl succinic anhydride, Agar Aids,Essex)	
	$\frac{1.00\text{ml}}{145} \times 266 \times 0.7$	1.28 ml.
	Total	2.28 ml.
Solution B:	Agar 100	2.00 ml.
	MNA (Methyl nadic anhydride, Agar Aids, Essex)	
	$\frac{2.00\text{ ml.}}{145} \times 178 \times 0.7$	1.12 ml.
	Total	3.12 ml.

Add B to A and mix together for a further 10 minutes. Total volume of 5.04 ml. Add 0.07 ml. of BMDA (Benzyldimethylamine, Agar Aids, Essex), calculated as 0.014 x 5.40 ml. (fraction of the total volume), and mix the resultant resin for another 10 minutes.

Allow to stand for at least 30 minutes for tissue infiltration , or overnight for final embedding.

13. Staining for 1 micron Orientation Sections

1:1 ratio of 1% Borax and 0.05% Toluidine Blue

a) 1% Borax Stain

1 g. Sodium thiosulphate (Borax)

100 ml. d.d. H_2O

Dissolve by stirring.

Avoid contact with skin.

Shelf life: 6 months at room temperature.

b) 0.05% Toluidine Blue Stain

0.05 g. Toluidine blue

100 ml. d.d. H_2O

Dissolve by stirring.

Avoid contact with skin.

Shelf life: 6 months at room temperature.

14. Staining for Silver Sections

a) Uranyl Acetate

0.5% uranyl acetate in 70% alcohol

0.125 g. uranyl acetate

7.5 ml. alcohol made up to 25 ml. with millipored water.

Dissolve by stirring.

Exclude light by wrapping in aluminium foil.

Shelf life : 3 months at room temperature.

b) Reynolds' lead

Modified Reynolds' lead.

(i) 1.33 g. lead nitrate

1.76 g. sodium citrate

30 ml. d.d. water

(ii) 8 ml. 1 N. sodium hydroxide

Vigorously shake (i) and allow to stand for 30 minutes, add (ii), then dilute to 50 ml. with d.d. water, mixing by inversion.

Shelf life : 30 days at 4°C.

Discard if pH drops below 11.

CHAPTER 9

BIBLIOGRAPHY

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ANDERSON,D.J., HANNAM,A.G. and MATTHEWS,B. 1970
Sensory mechanisms in mammalian teeth and their supporting structures.
Physiol.Rev. 50:171-195

AZUMA,M. 1970
Study on histologic changes of periodontal membrane incident to tooth movement.
Bull.Tokyo Med.Dental Univ. 17:149-178

BAEZ,S. 1977
Microvascular terminology.
In "Microcirculation"
(KALEY,G. and ALTURA,B.M.-editors),
Volume 1, Chapter 1
University Park Press,Baltimore. pp.23

BAUMRIND,S. 1969
A reconsideration of the propriety of the "pressure-tension" hypothesis.
Am.J.Orhtod. 55:12-22

BENNETT,H.S., LUFT,J.H. and HAMPTON,J.C. 1959
Morphological classifications of vertebrate blood capillaries.
Am.J.Physiol. 196:381-390

BERKOVITZ,B.K.B. 1990
The structure of the periodontal ligament : an update.
Eur.J.Orthod 12:51-76

BERKOVITZ,B.K.B. and MOXHAM,B.J. 1982
The ground substance of the periodontal ligament.
In "The periodontal ligament in health and disease"
(BERKOVITZ,B.K.B., MOXHAM,B.J., NEWMAN,H.N. -editors),
pp.119-149, Pergamon Press, Oxford

BERKOVITZ,B.K.B., WEAVER,M.E., SHORE,R.C. and MOXHAM,B.J. 1981
Fibril diameters in the extracellular matrix of the periodontal connective tissues of the rat.
Conn.Tiss.Res. 8:127-132

BERNICK,S. 1962
Age changes in the blood supply to molar teeth of rats.
Anat.Rec. 144:265-274

BEVELANDER,G. and NAKAHARA,H. 1968
The fine structure of the human periodontal ligament.
Anat.Rec. 162:313-326

BIEN,S.M. 1966(a)
Fluid dynamic mechanisms which regulate tooth movement.
In "Advances in Oral biology", Vol. 2
(STAPLE,P.H. -editor), pp.173-201,
Academic Press, New York

BIEN,S.M. 1966(b)
Hydrodynamic damping of tooth movement.
J.Dent.Res. 45:907-914

BIEN,S.M. and AYERS,H.D. 1965
Responses of rat maxillary incisors to loads.
J.Dent.Res. 44:517-520

BISHOP,M.A. 1985
Evidence for tight junctions between odontoblasts in the rat incisors.
Cell Tissue Res. 239:137-140

BISHOP,M.A. 1987
An investigation of pulp capillaries and tight junctions between odontoblasts in cats.
Anat.Embryol. 177:131-138

BONDEVIK,O. 1980
Tissue changes in the rat molar periodontium following application of intrusive forces.
Eur.J.Orthod. 2:41-49

BONDAREFF,W. 1957
Submicroscopic morphology of connective tissue ground substance with particular regard to fibrillogenesis and aging.
Gerodontologia 1:222-223

BRUNS,R.R. and PALADE,G.E. 1968
Studies on blood capillaries II., Transport of ferritin molecules across the wall of muscle capillaries.
J.Cell Biol. 37:277-299

BRYER,L.W. 1957
An experimental evaluation of the physiology of tooth eruption.
Int.Dent.J. 7:432-478

BULLIVANT,S. 1981
Possible relationships between tight junction structure and function.
In "Epithelial ion and water transport"
(MACKNIGHT,A.D.C., LEADER,J.P. -editors),
pp.265-275, Raven Press, New York

BUNDGAARD,M. 1984
The three-dimensional organization of tight junctions in a capillary endothelium revealed by serial section electron microscopy.
J.Ultrastruc.Res. 88:1-17

BUNDIT,V. and WISSIG,S.L. 1978
Comparison of the permeability of capillaries, venous capillaries and small venules to protein tracers.
J.Cell Biol. 79:384a (abstract)

CARRANZA,F.A., ITIOZ,M.E., CABRINI,R.L. and DOTTO,C.A. 1966
A study of the periodontal vascularization in different laboratory animals.
J.Perio.Res. 1:120-128

CASLEY-SMITH,J.R. 1967
An electron microscopical study of the passage of ions through the endothelium of lymphatic and blood capillaries, and through the mesothelium.
Quart.J.Exp.Physiol. 52:105-113

CASLEY-SMITH,J.R. 1970
The functioning of endothelial fenestrae on the arterial and venous limbs of capillaries, as indicated by the differing passage of protiens.
Experimentia 26:852-853

CASLEY-SMITH,J.R. 1971
Endothelial fenestrae in intestinal villi: differences between the arterial and venous ends of capillaries.
Microvasc.Res. 3:49-68

CASLEY-SMITH,J.R. 1977
Lymph and lymphatics.
In "Microcirculation",
(KALEY,G. and ALTURA,B.M. -editors),
Univeristy Park Press, Baltimore.

CASLEY-SMITH,J.R. 1979
The fine structure of the microvasculature in inflammation.
Biblthca Anat. 17:36-53.

CASLEY-SMITH,J.R. 1981
Freeze-substitution observations on the endothelium, and the passage of ions.
Microcirculation 1(1):79-109

CASLEY-SMITH,J.R. 1983
The structure and functioning of the blood vessels, interstitial tissues and lymphatics.
In "Lymphangiology", Chapter 2,
(FOLDI,M., CASLEY-SMITH,J.R. -editors),
pp.27-164, F.K.Schattauer Verlag, Stuttgart - New York

CASLEY-SMITH,J.R. and CARTER,D.B. 1979
The passage of macromolecules across inflamed capillary endothelium vis large vacuoles.
Microvasc.Res. 18:319-327

CASLEY-SMITH,J.R. and DAVY,P. 1978
The estimation of distances between parallel membranes on thick sections.
J.Microscopy 114:1-11

CASLEY-SMITH,J.R., GREEN,H.S. and WADEY,P.J. 1975
The quantitative morphology of skeletal muscle capillaries in relation to permeability.
Microvasc.Res. 10:43-64

CASLEY-SMITH,J.R. and WINDOW,J. 1976
Quantitative morphological correlations of alterations in capillary permeability, following histamine and moderate burning in the mouse diaphragm; and the effects of benzopyrones.
Microvasc.Res. 11:279-305

CASPER,D.L.D., GOODENOUGH,D.A., MAKOWSKI,L. and PHILLIPS,W.C. 1977
Gap junction structures I. Correlated electron microscopy and x-ray diffraction.
J.Cell Biol. 74:605-628

CASTELLI,W.A. and DEMPSTER,W.T. 1965
The periodontal vasculature and its response to experimental pressures.
J.Am.Dent.Assoc. 70:890-905

CATCHPOLE,H.R. 1973
Capillary permeability III Connective tissue.
In "The Inflammatory Process", 2nd ed., Vol. 2,
(ZWEIFACH,B.W., GRANT,L., McCLUSKEY,R.C. -editors),
pp.121-147, Academic Press, New York

CEREIJIDO,M. GONZALES-MARISCAL,L. and BORBOA,L. 1983
Occluding junctions and paracellular pathways studied
in monolayer MDCK cells.
J.Exp.Biol. 106:205-215

CHAMBERS,R. and ZWEIFACH,B.W. 1947
Intercellular cement and capillary permeability.
Physiol.Rev. 27:436

CHO,M.I. and GARANT,P.R. 1982
A freeze-fracture study of the periodontal ligament of
the rat.
J.Periodont.Res. 18:249-261

CLARK,A.B. 1986
A quantitative analysis of the effect of intrusion on
apical periodontal ligament fenestrae of the rat
maxillary molar.
M.D.S. Thesis, University of Adelaide, South Australia

CLAUDE,P. and GOODENOUGH,D.A. 1973
Fracture faces of zonular occludens from "tight" and
"leaky" epithelia.
J.Cell Biol.58:390

CLIFF,W. 1976
Blood Vessels
Cambridge University Press, Cambridge

COOPER,S.M. 1988
An ultrastructural study of tracer molecule passage
across periodontal ligament vascular endothelium.
M.D.S. Thesis, University of Adelaide, South Australia

COOPER,S.M., SIMS,M.R., SAMPSON,W.J. and DREYER,C.W. 1990
A morphometric, electron microscopic analysis of
tissue channels shown by ionic tracer in normal and
tensioned rat molar apical periodontal ligament.
Archs.Oral Biol. 35(7):499-507

CORPRON,K.E., AVERY,J.K., MORAVA,A.A. and LEE,S.D. 1976
Ultrastructure of capillaries in mouse periodontium.
J.Dent.Res. 55(3):551

COTRAN,R.S. 1967
The fine structure of the microvasculature in relation to normal and altered permeability.
In "Physical basis of circulatory transport" (REEVE,E.B. and GUYTON,A.C. -editors), pp.249, W.B. Saunders Co., Philadelphia

CRONE,C. and LEVITT,D.G. 1984
Capillary permeability to small solutes.
In "Handbook of physiology", section 2 "The cardiovascular system", Vol. IV, Part I, "Microcirculation" (RENKIN,E.M., MICHEL,C.C. -editors), pp.411-466, American Physiological Society, Bethesda, Maryland

DE ALMEIDA,O.P. and BOHM,G.M. 1970
Vascular permeability in the rat gingiva.
A model of vessel response in chronic inflammation.
J.Path. 127:27-34

DECKER,R.S. 1976
Hormonal regulation of gap junction differentiation.
J.Cell Biol. 69:669-685

DOUVARTZIDIS,I. 1984
A morphometric examination of the periodontal vasculature of the marmoset molar.
M.D.S. Thesis, University of Adelaide, South Australia

EDWALL,L.G.A. 1982
The vasculature of the periodontal ligament.
In "The periodontal ligament in health and disease" (BERKOVITZ,B.K.B., MOXHAM,B.J., NEWMAN,H.N. -editors), Chapter 7, pp.151-171, Pergamon Press, Oxford

EISENSTEIN,R. 1978
Vascular extracellular tissue and atherosclerosis.
Artery 5:207-221

EGELBERG,J. 1966
Permeability of the dento-gingival blood vessels. I
Application of the vascular labelling method and fluid measurements.
J.Periodont.Res. 1:180-191

EMBERY,G., PICTON,D.C.A. and STANBURY,J.B. 1987
Biochemical changes in periodontal ligament ground substance associated with short-term intrusive loadings in adult monkeys. (Macaca fascicularis)
Archs.Oral.Biol. 32(8):545-549

ENGSTROM,C., GRANSTROM,G. and THILANDER,B. 1988
Effect of orthodontic force on periodontal tissue metabolism : A histologic and biomechanical study in normal and hypocalcemic young rat.
Am.J.Orthod.Dentofac.Orthop. 93:486-495

FARQUHAR,M.G. and PALADE,G.E. 1963
Junctional complexes in various epithelia.
J.Cell Biol. 17:375-412

FAWCETT,D.W. 1963
Comparative observations on the fine structure of blood capilllaries.
In "The Peripheral Blood Vessels"
(ORBISON,J.L. and SMITH,D. -editors),
pp.17 -44, MD:Williams and Wilkins, Baltimore

FAWCETT,D.W. 1981
The cell, its organelles and inclusions.
W.B.Saunders Co., Philadelphia

FERRIER,J.M. and DILLON,E.M. 1983
The water binding capacity of the periodontal ligament and its role in mechanical function.
J.Periodont.Res. 18:469-473

FIRTH,J.A., BAUMAN,K.F. and SIBLEY,C.P. 1983
The intercellular junctions of guinea pig placental capillaries : A possible structural basis for endothelium solute permeability.
J.Ultrastruct.Res. 85:45-57

FLAGG-NEWTON,J.L. 1980
The permeability of the cell-to-cell membrane channel and its regulation in mammalian cell junctions.
In Vitro 16(12):1043-1048

FLAGG-NEWTON,J.L. and LOEWENSTEIN,W.R. 1979
Experimental depression of junctional membrane permeability in mammalian cell culture : A study of tracer molecules in the 300 to 800 Dalton range.
J.Membrane Biol. 50:65-100

FLAGG-NEWTON,J.L., SIMPSON,I. and LOEWENSTIEN,W.R. 1979
Permeability of cell to cell membrane channels in mammallian cell junction.
Science 205:404-407

FOLKE,L.E.A. AND STALLARD,R.E. 1967
Periodontal circulation as revealed by plastic microspheres.
J.Periodont.Res. 2:53-63

FOLKOW,B. and NEIL,E. 1971
Circulation
Oxford University Press, New York

FRANK,R.M., FELLINGER,E. and STEUER,P 1976
Ultrastructure du ligament alveolo-dentaire du rat.
J.Biol.Buccale 4:295-313

FREEMAN,E. 1980
Periodontium
In "Oral histology. Development,structure and function."
(TEN CATE,A.R. -editor),
pp.234-269, The C.V. Mosby Co., St.Louise

FREEMAN,E. and TEN CATE,A.R. 1978
Early ultrastructural changes in the periodontal ligament during orthodontic movement.
J.Dent.Res. 57:1138, Special Issue A

FREEZER,S.R. 1984
A study of periodontal ligament mesial to the mouse mandibular first molar.
M.D.S. Thesis, University of Adelaide, South Australia

FREEZER,S.R. and SIMS,M.R. 1987
A transmission electron-microscope stereological study of the blood vessels, oxytalan fibres and nerves of mouse-molar periodontal ligament.
Arch.Oral Biol. 32(6):407-412

FRIEND,D.S. and GILULA,N.B. 1972
Variations in tight and gap junctions in mammalian tissues.
J.Cell Biol. 53:758-778

FURSTMAN,L. and BERNICK,S. 1972
Clinical considerations of the periodontium.
Am.J.Orthod. 61(2):138-155

GABBIANI,G. and MAJNO,G. 1977
Fine structure of endothelium.
In "Microcirculation", Chapter 5,
(KALEY,G. and ALTURA,B.M. -editors),
pp.133-144, University Park Press, Baltimore.

GAENGLER,P. and MERTE,K. 1983
Effects of force application on periodontal blood circulation. A vital microscopic study in rats.
J.Periodont.Res. 18:86-92

GARFUNKEL,A. and SCIAKY,I. 1971
Vascularization of the periodontal tissues in the adult laboratory rat.
J.Dent.Res. 50:880-887

GARLICK,D.G. and RENKIN,E.M. 1970
Transport of large molecules from plasma to interstitial fluid and lymph in dogs.
Am.J.Physiol. 219(6):1595-1605

GERSH,I. and CATCHPOLE,H.R. 1960
The nature of ground substance of connective tissue.
Perspect.Biol.Med. 3:282-319

GIANELLY,A.A. 1969
Force induced changes in the vascularity of the periodontal ligament.
Am.J.Orthod. 55:5-11

GILCHRIST,D.R. 1978
Ultrastructure of periodontal blood vessels.
M.D.S. Thesis, University of Adelaide, South Australia

GILULA,N.B. 1974
Junctions between cells.
In "Cell Communication", (COX,R.P. -editor), pp.1-29, John Weiley & Sons, New York

GÖTZE,V.W. 1980
Uber den Volumenanteil von faserbundelabschnitten und blutgefaBen im desmodont menschlicher frontzahne.
Dtsch.zahnarztl. 35:1103-1104

GOULD,T.R.L., BRUNETTE,D.M. and DOREY,J. 1982
Cell turnover in the periodontal ligament determined by continuous infusion of H_3-thymidine using osmotic minipumps.
J.Periodont.Res. 17:662-668

GREENE,E.C. 1971
Gross Anatomy
In "The rat in laboratory investigation" (FERRIS,E.J. and GRIFFITH,J.Q. -editors), pp.24-51, J.Q. Hafner Publishing Co., New York

GRIFFIN,C.J. and SPAIN,H. 1972
Organization and vasculature of human periodontal mechanoreceptors.
Arch.Oral.Biol 17:913-921

GUYTON,A.C. 1972
Compliance of the interstitial space and the measurement of tissue pressure.
Pflugers Arch.Suppl. 336:S1-S20

GUYTON,A.C., SCHEEL,K. and MURPHREE,D. 1966
Interstitial fluid pressure. III : Its effect on resistance to tissue fluid mobility.
Circ.Res. 19:412-419

HAYAT,M.A. 1970
Principles and technique of electron microscopy. Biological application., 2 nd edition, Vol. I, University Park Press, Baltimore, USA

HENERS,M. 1974
Syndesmotic limiting movement of the periodontal ligament.
Int.Dent.J. 24:319-327

HEULKE,D.F. and CASTELLI,W.A. 1965
The blood supply of the rat mandible.
Anat.Rec. 153:335-342

HRUZA,Z. 1977
Connective tissue
In "Microcirculation"
(KALEY,G. and ALTURA,B.M. -editors),
pp.167-184, University Park Press, Baltimore

IIDA,J., KURIHARA,S., TAGUSHI,M. and MIURA,F. 1974
A study of vascular permeability increase by mechanical stimulation.
Craniofac.Biol. 21:248 (Abstract)

INOUE,T., YAMANE,H., YAMAMURA,T., and SHIMONO,M. 1987
Morphological changes of intercellular junctions in the rat submandibular gland treated by long-term repeated administration of isoproterenol.
J.Dent.Res. 66(8):1303-1309

INTAGLIETTA,M. 1977
Transcapillary exchange of fluid in single microvessels.
In "Microcirculation"
(KALEY,G. and ALTURA,B.M. -editors),
pp.197-212, University Park Press, Baltimore

ITO,S., SATO,E. and LOEWENSTEIN,W.R. 1974
Studies on the formation of a permeable cell membrane junction. II : Evolving junctional conductance and junctional insulation.
J.Membrane Biol. 19:339-355

KARDOS,T.B. and SIMPSON,L.O. 1979
A theoretical consideration of the periodontal membrane as a collagenous thixotropic system and its relationship to tooth eruption.
J.Periodont.Res. 14:444-451

KARDOS,T.B. and SIMPSON,L.O. 1980
A new periodontal membrane biology based upon thixotropic concepts.
Am.J.Orthod. 77(5):508-515

KARNOVSKY,M.J. 1967
The ultrastructural basis of capillry permeability studied with peroxidase as a tracer.
J.Cell Biol. 35:213-236

KARNOVSKY,M.J. 1968
The ultrastructural basis of transcapillary exchanges.
J.Gen.Physiol. 52:64s-93s

KELLER,G.J. and COHEN,D.W. 1955
India ink perfusions of the vascular plexus of oral tissues.
Oral Surg.,Oral Med.,and Oral Path. 539-542

KHOUW,F.E. and GOLDHABER,P. 1970
Changes in vasculature of the periodontium associated with tooth movement in the rhesus monkey and dog.
Arch.Oral.Biol. 15:1125-1132

KINDLOVA,M. 1968
Development of vessels in the marginal periodontium in rat.
J.Dent.Res. 47:507

KINDLOVA,M. and MATENA,V. 1959
Blood circulation in rodent teeth of the rat.
Acta anat. 37:163-192

KINDLAVO,M. and MATENA,V. 1962
Blood vessels of the rat molar.
J.dent.Res. 61:775-779

KING,G.J. and FISCHLSCHWEIGER,W. 1982
The effect of force magnitude on extractable bone resorption.
J.dent.Res. 81:126-130

KRAUS,B.S., JORDAN,R.E. and ABRAMS,L. 1969
Dental anatomy and occlusion.
Waverley Press Inc., Baltimore, USA

KVAM,E. 1972
Scanning electron microscopy of tissue changes on the pressure surfaces of human premolars following tooth movement.
Scand.J.Dent.Res. 80:357-368

KVAM,E. 1973
Organic tissue chacteristics on the pressure side of human premolars followimg tooth movement.
Angle Orthod. 43:18-23

LANDIS,E.M. and PAPPENHEIMER,J.R. 1963
Exchange of substances through the capillary walls.
In "Handbook of Physiology", Section 2, Circilation Vol.III, (HAMILTON,W.F. and DOW,P. -editors), pp.961-1034, Waverley Press Inc., Baltimore

LAURENT,T.C. 1970
The structure and function of the intercellular polysaccharides in connective tissue.
In "Capillary permeability"
(CRONE,C. and LASSEN,N.A. -editors), pp.647-653, Academic Press, New York

LAURENT,T.C. 1972
The ultrastructure and physico-chemical properties of interstitial connective tissue.
Pflugers Arch.Suppl. 336:S21-S42

LEW,K. 1986
Microvascular changes in the rat molar PDL incident to extrusion with special reference to fenestrae.
M.D.S. Thesis, University of Adelaide, South Australia

LINDSKOG,S. and LILJA,E. 1984
Scanning electron microscopic study of orthodontically induced injuries to the periodontal membrane.
Scand.J.Dent.Res. 92:334-343

LUFT,J.H. 1965
The ultrastructural basis of capillary permeability.
In "The Inflamatory Process", 1st edition, Vol.I,
(ZWEIFACH,B.W. and GRANT,L. -editors),
pp.47-93, Academic Press, New York, London

LUFT,J.H. 1973
Capillary permeability I, structural considerations.
In "The Inflamatory Process", 2nd edition, Vol. I,
(ZWEIFACH,B.W., GRANT,L. and McCLUSKEY,R.T. -editors),
pp.47-93, Academic Press, New York, London

MACAPANPAN,L.C., WEINMANN,J.P. and BRODIE,A.G. 1954
Early tissue changes following tooth movements in rats.
Angle orthod. 24:79-95

MAJNO,G. 1965
The ultrastructure of the vascular membrane.
In "Handbook of Physiology", Section 2, Circulation
Vol.III, Chapter 64,
(HAMILTON,W.F. and DOW,P. -editors),
pp.2293-2375, Waverley Press, Baltimore

MAJNO,G. and PALADE,G.E. 1961
Studies on inflammation.I : The effect of histamine and serotonin on vascular permeability : An electron microscopic study.
J.Biophys.Biochem.Cyto. 11:571-606

MAJNO,G. and PALADE,G.E. 1961
Studies on inflammation.II : The site of action of histamine and serotonin along the vascular tree : A topographical study.
J.Biophys.Biochem.Cyto. 11:607-626

MATSUO,M., KISHI,Y. and TAKAHASHI,K. 1980
Morphological changes of the vascular network of the periodontal membrane and alvaolar bone incident to orthondontic tooth movement.
J.Periodont.Res. 15:254 (Abstract)

MARTINEZ-PALOMA,A. and ERLIJ,D. 1975
Structure of tight junctions in epithelia with different permeability.
Proc.Natl.Acad.Sci.USA 72:4487-4491

MAYERSON,H.S. 1963
The physiological importance of lymph.
In "Handbook of Physiology", Section 2, Circulation,
Vol. II, (HAMILTON,W.F. and DOW,P. -editors),
American Physiological Society, Washington D.C.

MAYERSON,H.S., WOLFRAM,C.G., SHIRLEY,H.H. and
WASSERWAN,K. 1960
Regional differences in capillary permeability.
Am.J.Physiol. 198:155-160

McCULLOCH,C.A.G. and MELCHER,A.H. 1983
Cellular density and cell generation in the periodontal
ligament of mice.
Am.J.Anat. 167:43-58

MEEK,G.A. 1976
Practical electron microscopy for the biologist,
2nd edition.
J.Wiley and Sons, New York-Sydney-Toranto.

MELCHER,A.H. 1976
Periodontal ligament
In "Orban's oral histology and embryology",
8th edition, (BHASKER,S.N. -editor),
pp.288, C.V.Mosby Co., St.Louis

MELCHER,A.H. and WALKER ,T.W. 1976
The periodontal ligament in attachment as a shock
absorber.
In "The Eruption and Occlusion of teeth"
(POOLE,D.F. and STACK,M.V. -editors),
pp.183-192, Butterworths, London-Boston

MERKER,H.J. and GUNTHER,T. 1972
Ultrastructure of interstitial tissue.
Pfluger Arch.Suppl. 336:S33-S35

MICHAELI,Y., STEIGMAN,S. and HARARI,D. 1985
Recovery of the dental and periodontal tissues of the
rat incisor following application of continuous
intrusive loads : A long term study.
Am.J.Orthod. 87:135-143

MOHAMED,A.H., WATERHOUSE,J.P. and FRIEDERICI,H.H.R. 1973
The fine structure of gingival terminal vascular bed.
Microvasc.Res. 6:137

MOXHAM,B.J. and BERKOVITZ,B.K.B. 1979
The effect of axially directed extrusive loads on movements of the mandibular incisors of the rabbit.
Arch.Oral Biol. 24:759-763

MOXHAM,B.J. and BERKOVITZ,B.K.B. 1981
A quantitative assessment of the effects of axially directed extrusive loads on displacement of the impeded and unimpeded rabbit mandibular incisor.
Arch.Oral Biol. 26:209-215

MOXHAM,B.J. and BERKIVITZ,B.K.B. 1982(a)
The periodontal ligament and physiological tooth movements.
In "The periodontal ligament in health and disease" (BERKOVITZ,B.K.B., MOXHAN,B.J., and NEWMAN,H.N.-editors), pp.215-247, Pergamon Press, Oxford

MOXHAM,B.J. and BERKOVITZ,B.K.B. 1982(b)
The effect of external forces on the periodontal ligament - the response to axial loads.
In "The periodontal ligament in health and disease" (BERKOVITZ,B.K.B., MOXHAM,B.J. and NEWMAN,H.N.-editors), pp.249-268, Pergamon Press, Oxford

MOXHAM,B.J. and BERKOVITZ,B.K.B. 1989
A comparison of the biomechanical properties of the periodontal ligaments of erupting and erupted teeth of non-continuous growth (ferret mandibular canines).
Archs.Oral biol. 34(10):763-766

MOXHAM,B.J., SHORE,R.C. and BERKOVITZ,B.K.B. 1985
Fenestrated capillaries in the periodontal ligaments of the erupting and erupted rat molar.
Arch.Oral Biol. 32(7):477-481

MUHLEISEN,H., FINGIRLE,J., WOLBURG,H. and BETZ,E. 1989
Structural alterations in vascular endothelial tight junctions in the course of their gradual degradation in vitro.
Tissue & Cell 21(6):803-811

NEWMAN,H.N. and STRAHAN,J.D. 1982
Trauma and the periodontal ligament.
In "The periodontal ligament in health and disease" (BERKOVITZ,B.K.B., MOXHAM,B.J. and NEWMAN,H.N.-editors), pp.307-313, Pergamon Press, Oxford

NG,G.C., WALKER,T.W., ZIGG,W. and BURKE,P.S. 1981
Effect of tooth loading on the periodontal vasculature of the mandibular forth premolar in dogs.
Arch.Oral Biol. 26:189-195

OLIVEIRA-CASTRO,G.M. and LOEWENSTEIN,W.R. 1971
Junctional membrane permeability. Effect of divalent cations.
J.Membr.Biol. 5:51-77

ORLOWSKI,W.A. 1978
Biochemical study of collagen turnover in rat incisor periodontal ligament.
Arch.Oral Biol. 23:1163

PACKMAN,H., SHOHER,I. and STEIN,R.S. 1977
Vascular responses in the periodintal ligament and alveolar bone detected by photoelectric plethysmography : the effect of force application to the tooth.
J.periodont. 48:194-200

PALADE,G.E., SIMIONESCU,M. and SIMIONESCU,N. 1979
Structural aspects of the permeability of the microvascular endothelium.
Acta Physiol.Scand. (Suppl.) 463:11-33

PAPPENHIEMER,J.R. 1953
Passage of molecules through capillary walls.
Physiol.Rev. 33:387-423

PAPPENHIEMER,J.R. 1970
Osmotic reflection coefficients in capillary membranes.
In "Capilary Permeability"
(DRONE,C. and LASSEN,N.A. -editors),
pp.278-286,288,293-295, Munksgaard, Copenhegen

PERSSON,C.G.A. and SVENSJO,E. 1985
Vascular responses and their suppression : Drugs interfering with venular permeability.
In "Handbook of inflammation"
(BONTA,J.L., BRAY,M.A. and PARNHAM,M.J. -editors),
pp.61-82, Elsevier, Amsterdam, New York

PICTON,D.C.A. 1962
Tilting movement of teeth during biting.
Arch.Oral Biol. 7:151-159

PICTON,D.C.A. 1963
The effect on normal vertical tooth mobility of the rate of thrust and time interval between thrusts.
Arch.Oral Biol. 8:291-299

PICTON,D.C.A. 1986
Extrusive mobility of teeth in adult monkeys (Macaca fascicularis).
Archs.Oral Biol. 31:369-372

PICTON,D.C.A. 1989
The periodontal enigma : eruption versus tooth support.
Eur.J.Orthod. 11:430-439

PICTON,D.C.A. and WILLS,D.J. 1978
Viscoelastic properties of the periodontal ligament and mucous membrane.
J.Prosth.Dent. 40:263-272

PIEKARSKI,J. and COHEN,W. 1962
Vascular changes in the periodontal ligament in experimental movement of the rat molar.
Int.Ass.Dent.Res.(Meeting Abstract No.252),pp.67

PINTO DA SILVA,P. and KACHER,B. 1982
On tight junction structure.
Cell 28:441-450

PITTS,J.D. 1980
The role of junctional communication in animal tissues.
In Vitro 16(12):1049-1056

PITTS,J.D. and SIMMS,J.W. 1977
Permeability of junctions between animal cells.
Exp.Cell Res. 104:153-163

REITAN,K. 1951
The initial tissue reaction of orthodontic tooth movement as related to the influence of function.
Acta.Odont.Scand.Suppl.6

REITAN,K. 1971
Comparative behavior of human and animal tissue during experimental tooth movement.
Angle Orthod. 41:1-14

REITAN,K. 1974
Tissue changes in orthodontic tooth movement.
In "Orthodontics in dialy practice"
(SALZMANN,J.A. -editor),
J.B. Lippincott Co., Philadelphia

REITAN,K. 1985
Biomechanical principles and reactions.
In "Orthodontics, Current principles and techniques"
(GRABER,T.M. AND SWAIN,B.F. -editors),
pp.101-192, The C.V. Mosby Co., St.Louis

RENKIN,E.M. 1977
Multiple pathways of capillary permeability.
Circ.Res. 41:735-743

RENKIN,E.M., WATSON,P.D., SLOOP,C.H., JOYNER,W.M.
and CURRY,F.E. 1977
Transport pathways for fluid and large molecules in
microvascular endothelium of the dog paw.
Microvasc.Res. 14:205-214

REVEL,J.P. and KARNOVSKY,M.J. 1967
Hexagonal array of subunits in intercellular junctions
of the mouse heart and liver.
J.Cell Biol. 33:C7

REVEL,J.P., YANCEY,S.B., MEYER,D.J. and NICHOLSON,B. 1980
Cell junctions and intercellular communication.
In Vitro 16(12):1010-1017

RHODIN,J.A.G. 1967
The ultrastructure of mammalian arterioes and
precapillary sphincters.
J.Ultrastruct.Res. 25:452-500

RHODIN,J.A.G. 1968
The ultrastructure of mammalian venous capillaries,
venules, and small collecting veins.
J.Ultrastruct.Res. 25:452-500

RHODIN,J.A.G. 1973
Fixation of microvasculature for electron microscopy.
Microvascular Res. 5:285-291

RHODIN,J.A.G. 1973
Ultrastructure of the microvascular bed.
In "The microcirculation in clinical medicine"
Chapter 2, (WELLS,R. -editor),
pp.13-31, Academic Press, New York

RHODIN,J.A.G. 1984
Architecture of the vessel wall.
In "Handbook of physiology", Section 2, Vol.IV,
Chapter 1, (RENKIN,E.M. and MICHEL,C.C. -editors)
pp.1-31, American Physiological Society,
Baltimore, Maryland

RHODIN,J.A.G. 1984
Anatomy of microcirculation.
In "Blood vessels and lymphatics in organ systems"
(ABRAMSON,D.I., and DOBRIN,P.B. -editors)
Chapter 3, Microcirculation.
Academic Press, Inc.

RIPPE,B., KAMIYA,A. and FOLKOW,B. 1978
Simultaneous measurement of capillary diffusion and
filtration exchange during shifts in filtration-
absorption and at graded alterations in the capillary
permeability surface area product (PS).
Acta Physiol.Scand. 104:318-3336

RYGH,P. 1972a
Ultrastructural vascular changes in pressure zones of
rat molar periodontium incident to orthodontic tooth
movement.
Scand.J.Dent.Res. 80:307-321

RYGH,P. 1972b
Ultrastructural cellular reactions in pressure zones of
rat molar periodontium incident to orthodontic tooth
movement.
Acta Odontol.Scand. 30:575-593

RYGH,P. 1973
Ultrastructural changes in pressure zones of human
periodontium incident to orthodontic tooth movement.
Acta Odont.Scand. 31:109-122

RYGH,P. 1976
Ultrastructural changes in tension zones of rat molar
periodontium incident to orthodontic tooth movement.
Am.J.Orthod. 70:269-281

RYGH,P., BOWLING,K., HOVLANDSDAL,L. and WILLIAMS,S. 1986
Activation of the vascular system : A main mediator of
periodontal fibre remodeling in orthodontic tooth
movement.
Am.J.Orthod. 89(6):453-468

SAGATIES,M.J., RAVIOLA,G., SCHAEFFER,S., and MILLER,C. 1987
The structural Basis of the inner blood retina barrier in the eye of macaca mulatta.
Invest.Ophthalmol.Vis.Sci. 28(2):2000-2014

SCHARER,P. and HAYASHI,V. 1969
A quantitative study on intra-periodontal pressure.
Paradentolgie 23:3-10

SCHNEEBERGER,E.E. and LYNCH,R.D. 1984
Tight junctions : Their structure, composition and function.
Circ.Res. 55(6):723-733

SCHOUR,I. and MASSLER,M. 1971
The teeth
In "The rat in laboratory investigation"
(FARRIS,E.J. and GRIFFITH,J.Q. -editors), Chapter6, pp.104-160, J.Q.Hafner Publishing Co., New York

SCHRODER,H. and HAUCK,G. 1977
Fluid and substance pathway through the extravascular space.
Biol.Anat. 15:231-233

SHEPRO,D. ET AL. 1988
Endothelial cells, inflammatory edema, and the microvascular barrier : Comment by a "free radical".
Microvasc.Res. 35:247-264

SHORE,R.C., BERKOVITZ,B.K.B. and MOXHAM,B.J. 1984
Histological study, including ultrastructural quantification, of the periodontal ligament in the lathyritic rat mandibular dentition.
Arch.Oral Biol. 29(4):263-273

SHORE,R.C., MOXHAM,B.J. and BERKOVITZ,B.K.B. 1983
Fenestrated capillaries in the periodontal ligament.
J.Dent.Res. 63:513a

SIMIONESCU,M. and SIMIONESCU,N. 1984
Ultrastructure of the microvascular wall - functional corrections.
In "Handbook of physiology", Section 2, Vol.IV, (RENKIN,E.M. and MICHEL,C.C. - editors), pp.41-101, American physiological Society, Baltimore, Maryland

SIMIONESCU,M., SIMIONESCU,N. and PALADE,G.E. 1974a
Morphometric data on the endothelium of blood capillaries.
J.Cell Biol. 60:128-152

SIMIONESCU,M., SIMIONESCU,N. and PALADE,G.E. 1974b
Characteristics endothelial junctions in sequential segments of the microvasculature.
J.Cell Biol. 63:632a (Abstract)

SIMIONESCU,M., SIMIONESCU,N. and PALADE,G.E. 1975a
Segmental differentiations of cell junctions in the vascular endothelium : The Microvasculature.
J.Cell Biol. 67:863-885

SIMIOMESCU,M., SIMIONESCU,N. and PALADE,G.E. 1976
Segmental differentiations of cell junctions in the vascular endothelium : Arteries and veins.
J.Cell Biol. 68:705-723

SIMIONESCU,M., SIMIONESCU,N. and PALADE,G.E. 1984
Partial chemical characterization of the anionic sites in the basal lamina of fenestrated capillaries.
Microvascular Res. 28: 352-367

SIMIONESCU,N.,SIMIONESCU,M. and PALADE,G.E. 1972
Permeability of intestinal capillaries. Pathways followed by dextrans and glycogens.
J.Cell Biol. 53:365-392

SIMIONESCU,N., SIMIONESCU,M. and PALADE,G.E. 1975b
Permeability of muscle capillaries to small heme-peptides. Evidence for the existance of trans-endothelial channels.
J.Cell Biol. 64:586-607

SIMIONESCU,N., SIMIONESCU,M. and PALADE,G.E. 1978
Structural basis of the permeability in sequential segments of the microvasculature of the diaphragm. I. Bipolar microvascular fields. II.
Pathways followed by microperoxidase across the endothelium.
Microvasc.Res. 15:1-36

SIMS,M.R. 1975
Oxytalan-vascular relationships observed in histologic examination of the periodontal ligaments of man and mouse.
Arch.Oral Biol. 20:713-716

SIMS,M.R. 1980
Angular changes in collagen cemental attachment during tooth movement.
J.Periodont.Res. 15:638-645

SIMS,M.R. 1983
The microvascular venous pool and its ultrastructural associations in mouse molar periodontal ligament - periodontal microvasculature and nerves.
Aust.Orthodont.J. 8:21-27

SIMS,M.R. 1984
Ultrastructural analysis of the microfibrillar component of mouse and human periodontal oxytalan fibres.
Con.Tis.Res. 13:59-67

SIMS,M.R. 1987
A model of the anisotropic distribution of microvascular volume in the periodontal ligament of the mouse mandibular molar.
Aust.Orthodont.J. 10:21-25

SOBIN,S.S. and TREMER,H.M. 1977
Three-dimensional organization of microvascular beds as related to function.
In "Microcirculation"
(KALEY,G. and ALTURA,B.M. -editors),
pp.43-67, University Park Press, Baltimore

SOUTHARD,K.A. and FORBES,D.P. 1982
The cellular response to tension across the inter-premaxillary suture.
Craniofac.Biol. 27:298 (Abstract)

STAEHELIN,L.A. 1974
Structure and function of intercellular junctions.
Int.Rev.Cytol. 39:191

STEIGMAN,S., HADARI,D. and MICHAELI,Y. 1983
Long term effect of intrusive loads of varying magnitude upon the eruptive potential of rat incisors.
Am.J.Orthod. 84:254-259

STEIGMAN,S., MICHAELI,Y. and WEINREB,M. 1987
Structural changes in the dental and periodontal tissue of the rat incisor following application of orthodontic loads.
Am.J.Orthod.Dentofac.Orthop. 91:49-56

TABATA,S. and SEMBA,T. 1987
Examination of blood capillaries in rat incisor pulp by TEM of thin sections and freeze fracture replicas.
J.Electron Microsc. 36(5):283-293

TABATA,S. and SEMBA,T. 1988
Examination of arteries and veins in rat incisor pulp by TEM of freeze-fracture replicas.
J.Electron Microsc. 37(6):323-328

TANG,M.P.F. 1990
A TEM study of tissue channels in normal and tensioned rat molar periodontal ligament.
M.D.S.Thesis, The University of Adelaide,South Australia

TAO-CHENG,J., NAGY,Z. and BRIGHTMAN,M.W. 1987
Tight junctions of brain endothelium in vitro are enhanced by astroglia.
J.Neuroscience 7(10):3293-3299

TAYLOR,A.E. and GRANGER,D.N. 1984
Exchange of macromolecules across the microcirculation.
In "Handbook of physiology" Cardiovascular system IV, (RENKIN,E.M. and MICHEL,C.C. -editors), pp.467-520, American Physiological Society, Baltimore, Maryland

THEILADE,J., EGELBURG,J. and ATTSTROM,R. 1971
Vascular permeability to colloidal carbon in chronically inflamed gingiva.
J.Periodont.Res. 6:100-109

THORBALL,N. and TRANUM-JENSEN,J. 1983
Vascular reaction to perfusion fixation.
J.Microc. 129:23-139

TONER,P.G. and Carr,K.E. 1972
Cell structure : An introduction to biological electron microscopy.
Second edition, Chuechill Livingstone Northumberland Press, Edinburgh and London.

TURNER,D.F., MARFURT,C.F. and SATTELBERG,C. 1989
Demonstration of physiological barrier between pulpal odontoblast and its perturbation following routine restorative procedures : A horseradish peroxidase tracing study in the rat.
J.Dent.Res. 68(8):1262-1268

VAN DEURS,B. and KOEHLER,J.K. 1979
Tight junctions in the choroid plexus epithelium. A freeze fracture study including complementary replica.
J.Cell Biol. 80:662-673

VON BULOW,F., MOLLGARD,K. and VAN DEURS,B. 1984
Tight junction structure in relation to transepithelium resistance in the frog choroid plexus.
Euro.J.cell Biol. 33:90-94

WAGNER,R.C., FROEHLICH,R., HOSSLER,F.E. and ANDREWS,S.B. 1987
Ultrastructure of capillaries in the red body (rete mirabile) of the eel swim bladder.
Microvasc.Res. 34:349-362

WALKER,T.W. 1980
A model of periodontal vasculature in tooth support.
J.Biomech. 13:149-157

WALKER,T.W., NG,G.C. and BURKE,P.S. 1978
Fluid pressures in the periodontal ligament of the mandibular canine tooth in dogs.
Arch.Oral Biol. 23:753-765

WATSON,P.D. and GRODINS,F.S. 1978
An analysis of the effects of the interstitial matrix on plasma lymph transport.
Microvasc.Res. 16:19-41

WEAKLEY,B.S. 1972
A beginner's handbook in biological electron microscopy.
Cherchill Livingstone, Northumberland Press, Edinburgh and London.

WEEKES,W.T. 1983
Vascular morphology of rat molar periodontium.
M.D.S. Thesis, Univeristy of Adelaide, South Australia

WEEKES,W.T. and SIMS,M.R. 1986
The vasculature of the rat molar periodontal ligament.
J.Periodont.Res. 21:186-194

WEIDERHIELM,C.A. 1966
Transcapillary and interstitial transport phenomena in the mesentery.
Fed.Proc. 25:1789-1798

WIEDEMAN,M.P., TUMA,R.F. and MAYROVITZ,H.N. 1981
"An introduction to microcirculation"
pp.57-96, Academic Press, New York

WILLIAMS,M.A. 1977
Practical methods in electron microscopy.
Quantitative methods in biology.
(GLLAUERT,A.M. -editor),
North Holland Publishing Co.,Amsterdam-New York-Toronto

WILLS,D.J., PICTON,D.C.A. and DAVIES,W.I.R. 1976
A study of the fluid systems of the periodontium in Macaque monkeys.
Arch.Oral Biol. 21:175-185

WILLS,D.J., PICTON,D.C.A. and DAVIES,W.I.R. 1978
The intrusion of the tooth for different loading rates.
J.Biomechanics. 11:429-434

WILLS,D.J. and PICTON,D.C.A. 1981
Changes in the force intrusion relationship of the tooth with its resting position in Macaque monkeys.
Arch.Oral Biol. 21:175-185

WOLFF,J.R. 1977
Ultrastructure of the terminal vascular bed as related to function.
In "Microcirculation ", Vol.I, Chapter 4,
(KALEY,G. and ALTURA,B.M. -editors),
pp.96-130, University Park Press, Baltimore

WONG,R.S.T. 1983
Vascular morphology of the mouse periodontium.
M.D.S. Thesis, University of Adelaide, South Australia

WONG,R.S.T. and SIMS,M.R. 1987
A scanning electron-microscopic, stereo-pair study of methacrylate corrosion casts of the mouse palatal and molar periodontal microvasculature.
Arch.Oral Biol. 32(8):557-566

YANCEY,S.B., EASTER,D. and REVEL,J. 1979
Cytological changes in gap junctions during liver regeneration.
J.Ultrastruc.Res. 67:229-242

YEE,A.G. and REVEL,J.P. 1975
Endothelial cell junctions.
J.Cell Biol. 66:200-236

ZAMPIGHI,G. 1980
On the structure of isolated junctions between communicating cells.
In Vitro 16(12):1018-1028

ZAMPIGHI,G., CORLESS,J.M. and ROBERTSON,J.D. 1980
On the gap junction structure.
J.Cell Biol. 86:190-198

ZWEIFACH,B.W. 1961
The structural basis of the microcirculation.
In "Development and structure of the cardiovascular system", Chapter 13,
(LUISADA,A.A. -editor),
pp.198-205, McGraw-Hill, New York

ZWEIFACH,B.W. 1973
Microvascular aspects of tissue injury.
In "The inflammatory process", 2nd edition, Vol.II,
(ZWEIFACH,B.W., GRANT,L. and McCLUSKEY,R.T. -editors),
pp.3-46, Academic Press, New York-London

ZWEIFACH,B.W. 1977
Perspectives in microcirculation.
In "Microcirculation", Vol.I,
(KALEY,G. and ALTURA,B.M. -editors),
pp.1-19, University Park Press, Baltimore

ZWEIFACH,B.W. and LIPOWSKY,H.H. 1984
Pressure-flow relations in blood and lymph microcirculation.
In "Handbook of Physiology", Section 2, Vol.IV,
(RENKIN,E.M. and MICHEL,C.C. - editors),
pp.251-307, American Physiological Society,
Baltimore, Maryland