

VASCULAR CHANGES IN THE PERIODONTAL
LIGAMENT FOLLOWING THE REMOVAL OF
ORTHODONTIC FORCES

by

ELBERT FREDERICK MURRELL

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Preventive Dental Science
Faculty of Dentistry
University of Manitoba
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ABSTRACT

The objective of this study is to document periodontal ligament (PDL) vascularity coincident to orthodontic tooth movement and following release of orthodontic force. The maxillary right first molar tooth of 28 rats were tipped in a mesial direction by a fixed appliance using a 30 gm force (experimental animals, E). 28 age-matched rats served as untreated controls (C). After 2 weeks, the appliance was deactivated (Time 0) and 4E and 4C animals were fixed by intracardiac perfusion of Karnovsky's fixative; an equivalent number were perfused 3, 5, 7, 10, 14, and 21 days (d) later. The right hemimaxilla was prepared for light microscopy. The number of patent blood vessels per 10,000 μm^2 (#BV) and the % blood vessels (proportional area, %BV) were determined by computerized image analysis of buccal (B), mesial (M), palatal (P) and distal (D) quadrants; alveolar bone (AB) and cemental (Ce) regions, and middle (Mi) and apical (A) levels of the PDL of the mesiobuccal root. Means were compared by ANOVA and a multiple comparison test and differences were considered significant when $p < 0.05$. At Time 0, #BV in the AB region at both Mi and A levels were greater in E than C animals, suggesting the cumulative tissue effects of orthodontic force. From 0-3 d., in E, %BV was greatest in M quadrant, suggesting that PDL vascularity had increased due to tissue compression. From 3-7 d., no differences were evident between E and C, suggesting vascular equilibration coincident to the resumption of normal distal physiologic drift. From 7-14 d., %BV were greatest in the D quadrant of the AB region of the Mi PDL level of E animals, which is likely a compression region. From 14-21 d., there were no significant regional differences between E and C in PDL vascularity.

This data suggests increased vascularity in PDL regions experiencing compression coincident to either therapeutic tooth movement or physiologic drift following release of orthodontic force. Since these vessels contain blood, they likely contribute to regional variations in PDL viscoelasticity which may either dampen therapeutic tooth movement or contribute to early relapse of teeth subsequent to release of orthodontic force.

DEDICATION

This thesis would not have been possible without the love and support of my parents Yvonne and Nigel; brothers Rohan, Troy, Sean, Alan; sister Nigela; and girlfriend Liza. I love each of you dearly, and I look forward to getting home.

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CHAPTER I
LITERATURE REVIEW

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Following orthodontic tooth movement teeth have a tendency to return to their original position prior to treatment. This is an undesirable phenomenon which has plagued orthodontists for decades. It is believed that the cause for this undesirable consequence may be found within the periodontal ligament of the tooth.

A critical review of the literature dealing with the periodontal ligament, in particular the vascular system and tooth movement has been done. The major areas reviewed include:

- (a) relapse;
- (b) the periodontal ligament under normal circumstances;
- (c) the periodontal ligament changes in response to orthodontic tooth movement;
- (d) osteoblasts;
- (e) and angiogenesis.

The connection between the major areas and the study conducted will attempt to derive a possible cause of undesirable tooth movement following orthodontic therapy .

Relapse:

"Not infrequently cases are presented that require more skill in retaining the teeth than in regulating them."(Kaplan, 1988) As a result orthodontists have been in search

of the etiologic factors governing relapse, much the same as knights of past once searched for the "holy grail."

Orthodontic relapse may be defined as the tendency for a relocated tooth to move back toward its original position. Relapse may be of various etiologies which include:

- (a) failure of gingival and periodontal fibres to remodel or slow remodelling of these fibres;
- (b) unstable tooth position (a lack of parallelism in tooth roots at the completion of orthodontic therapy) (Proffit, 1986);
- (c) musculature;
- (d) apical base (that arbitrary junction of the alveolar bone at the apices of the teeth with the basal bone of the maxilla and the mandible) (Lundstrom, 1929; Kaplan, 1988; Koole, 1990);
- (e) tissue fluids;
- (f) occlusion;
- (g) intercanine width;
- (h) habits;
- (i) third molars;
- (j) incisor shape;
- (k) direction of growth;
- (l) eruptive patterns;
- (m) tooth size discrepancies;

- (n) excessive mandibular dental arch width increase so as to accommodate teeth without extractions (Parker, 1972; Boese, 1980a, b; Little, 1981, 1988, 1989; Punekey *et al.*, 1984; Shields *et al.*, 1985; Glen *et al.*, 1987; Pangrazio-Kulbersh, 1990; Row *et al.*, 1990).

The pressure created by the muscles of the cheeks, lips, and/or tongue are great enough to cause orthodontic relapse of the teeth as well as changes in the occlusal relationship if teeth are not placed into a position of equilibrium with these forces (Reitan, 1969; Proffit, 1986). Therefore, the best means of dealing with these potential causes of relapse is to be sure that the dentition is placed in balance with muscular forces.

The presence of third molars has been implicated as a cause of relapse of anterior incisor teeth for over 115 years (Kaplan, 1974). Broadbent (1943) cephalometrically disputed third molars as a cause of anterior lower incisor relapse. Dewey (1917) contended that "in some cases the third molar became impacted because of lack of space behind the second molar, while in others it provided room for its eruption by causing the anterior teeth to crowd" (Kaplan, 1974). Many investigators of scientific evidence and anecdotal experiences have indicted the third molar as a culprit in orthodontic relapse (Nance, 1947; Vego, 1962) while others have acquitted the third molar as causing orthodontic relapse (Moore, 1960; Hixon, 1972). Hence it is clear that the involvement of the third molar in relapse requires more investigation.

In simple terms it has been stated with regard to the apical base that " In cases of arch length deficiency, expansion of the dental arches will fail.", "...molar width and

canine width are of...an uncompromising nature..." (Reitan, 1969; Little *et al.*, 1981).

Little explanation is needed here, save to say that if orthodontic therapy involves expansion then beware of the relation to the apical bases, since expansion of the teeth from over the apical bases is more prone to relapse.

Peck *et al.*, (1972) advocated that an index representative of the dimensions of the lower incisors is important for the correction and prevention of relapse. Tied to this index is the shape of the lower incisors. The index is the result of the mesio-distal length divided by the facio-lingual length multiplied by 100. For the lower central incisor the value of this assessment should be between 88 and 92 percent; while the figure for the lateral incisor was 90 to 95 percent (Fastlicht, 1970; Peck *et al.*, 1972; Gilmore *et al.*, 1984; Puneky, *et al.*, 1984). The conclusion to be drawn is that violation of this index is an invitation to unwanted relapse potential. However, the parameters of the sample selection may be somewhat suspect and hence not represent the population at large (Peck *et al.*, 1972).

Post-retention growth in the mandible and not the maxilla will possibly lead to anterior mandibular relapse of the teeth. This occurs since the lower incisors are brought in contact with the upper incisors causing the lower to be moved back into less space and hence crowd or relapse, depending on whether or not treatment has ever been instituted (Little *et al.*, 1981).

Oral habits, such as tongue thrusting, thumb sucking, too will result in relapse post orthodontically. If stability from this relapse factor is to be accomplished then the

habit must be broken before or during the course of orthodontic treatment (Reidel, 1976; Profitt, 1986).

From an occlusal perspective it appears that the stability of the case will be enhanced when ideal cusp-to-fossa interdigitation is achieved, providing for cuspid guided excursions and anterior guided protrusion. This is based on the premise that the teeth with their cuspal arrangement will act to lock the upper and lower teeth in a given arrangement with each other (Little *et al.*, 1981).

The direction in which a tooth is moved in correcting its position may also play a role in relapse. Labial and lingual tipping tooth movements to corrected positions may be more prone to relapse than are mesial and distal bodily movements (Reidel, 1960).

Relapse may be a normal, predictable, physiologic response to abnormal forces (Parker, 1972), including orthodontic ones. Since relapse is a phenomenon which occurs after the given force has been removed; it becomes necessary to institute a retentive phase of orthodontic treatment aimed at preventing relapse which should be considered as "secondary orthodontic treatment" (McCoy, 1935). As a result, most orthodontists consider a retentive phase for relapse control, to be a necessity (Reitan, 1969; Watson, 1988; McReynolds *et al.*, 1990). In orthodontics the control of relapse is a valuable component of successful therapy (Peck *et al.*, 1972). The significant reasons for controlling relapse include:

- (a) preservation of post-treatment aesthetics following relocation of rotated and crowded teeth (Skillen *et al.*, 1940; Edwards, 1968, 1970; Bowling *et al.*, 1988);

- (b) increased cost of treatment resulting from retreating a completed case (Watson, 1988);
- (c) increased number of treatment procedures including fixed or removable retainers, or surgery; (Thompson, 1959; Brian, 1969);
- (d) increased time for the completion of treatment.

There is an abundance of literature dealing with the role played by the fibrous components of the periodontium in the etiology of tooth relapse following removal of orthodontic forces. Rygh *et al.*, (1986) and others strongly advocated that orthodontic stability is dependant on the remodelling of the periodontal fibres (Oppenheim, 1911, 1933; Thompson *et al.*, 1958). These fibres include the periodontal ligament, transseptal, and dentogingival fibres. Reitan (1969) through his work on dog and human specimens concluded that contraction of stretched fibrous structures is most important when looking at the causes of relapse. Transseptal fibres connect the mesial and distal root surfaces of adjacent teeth above the crest of the alveolar bone and maintain the mesiodistal relations between adjacent teeth stabilizing them against any separating forces (Orban, 1936; Waldrom, 1942; Chase *et al.*, 1944; Erikson *et al.*, 1945; Huckaba, 1952; Edwards, 1988). The transseptal fibres bridge the shortest distance between two adjacent teeth, irrespective of any rotations. Orthodontic therapy increases the stress within the transseptal fibres as they attempt to maintain the original tooth position (Orban, 1936; Waldron, 1942; Chase *et al.*, 1944; Erikson *et al.*, 1945; Huckaba, 1952). At one time, although functioning to maintain tooth position, transseptal fibres were regarded as being slow to remodel (Reitan, 1960, 1969). Clinically this is of importance: If the retentive

period is too short then all of the fibres of the periodontal ligament tend to contract and rearrange themselves (Boese, 1969; Brian, 1969; Edwards 1970). But if the teeth are retained for at least 2 to 3 months, there is less tendency toward relapse (Fullmer *et al.*, 1958; Thompson, 1959; Reitan, 1967, 1969). In looking at cross-sections of the stretched transseptal fibres of the tooth during rotation Wiser (1966) was unable to prove that these fibres caused relapse. However he did report that their removal resulted in minimal relapse tendency. The method by which soft tissues such as the transseptal fibres and the principal fibres of the periodontal ligament might apply a force capable of moving teeth is not clear. Edwards (1988) reported that transseptal fibres are composed primarily of nonelastic collagenous fibres, unlike the blood vessel walls of the periodontal ligament which have elastic tissue. Contrary to the idea of transseptal fibres remodelling slowly, it has been reported that transseptal fibres tend to remodel very quickly and efficiently within 2 to 3 months after orthodontic rotation of teeth (Reitan, 1959; Ten Cate, 1972; Ten Cate *et al.*, 1976; Sodek, 1977; Beertsen, 1979; Deporter *et al.*, 1984; Edwards, 1988); as a result these investigators reject any hypotheses attributing slow transseptal fibre remodelling as being responsible for orthodontic relapse. However, rotational relapse can be reduced by prolonged retention periods or by surgical sectioning of the transseptal fibres. Those who have reported that the transseptal fibre remodels quickly have done so on the basis of collagen turnover measurements. Since surgical sectioning of these fibres or increased periods of retention seem to reduce relapse; perhaps the physiological collagen turnover does not result in transseptal fibre re-orientation (Edward, 1988). Hence in cases of stretched transseptal fibres, relapse may

still be possible regardless of collagen turnover, since turnover does not necessarily imply change in orientation.

Oxytalan fibres which are concentrated in the supracrestal tissues during rotational movement of teeth (Edwards, 1968; Sims, 1976) have also been implicated as a cause of relapse. However, there is no direct evidence that they are physiologically similar to elastic fibres. It has been reported that oxytalan fibres attach from the cementum of the tooth to blood vessels within the periodontal ligament (Sims, 1975, 1979). The purpose of this attachment is unclear, however, it may be related to maintain tooth vascularity.

Dentogingival fibres which originate in the cementum and terminate in the connective tissue of the gingiva have an unalterable cemental attachment, hence orthodontically rotated teeth will tend to relapse at the completion of fixed therapy if not retained, at least partly due to the nature of these fibres (Grant *et al.*, 1963; Crum *et al.*, 1974).

In summary there is no substantial evidence at the present time to explain the mechanism by which the gingival soft tissues may apply a force capable of moving teeth (Edwards, 1988).

In response to some of the causes of orthodontic tooth relapse a number of varied strategies have been compiled in an attempt to minimize relapse. The strategies include:

- (a) circumferential fiberotomies;
- (b) fixed retainers for long durations of time;
- (c) extraction of third molars;
- (d) overcorrection of malposed teeth;

- (e) removable retainers;
- (f) using activators and/or functional appliances alone or with fixed appliances;
- (g) interproximal stripping of the teeth thereby changing the anatomy of given teeth and the establishment of an ideal occlusion with corresponding proper tooth alignment;
- (h) proper tooth positioning over basal bone with parallelism of roots so as to better withstand functional loads;
- (i) serial extraction in selective cases, that is "It seems logical that if a tooth completes its formation in a site where it will remain when treatment is completed it will be more stable (Dale, 1985). Conversely, if a tooth is left in a crowded, tipped, and rotated position for several years and then is moved to a new position relatively rapidly, it will be less stable for a time and will require a longer retention period;
- (j) correction of rotated or mass-aligned teeth as soon as possible after tooth eruption into the arch (Reitan, 1969; Edwards, 1970; Andrews, 1972; Peck *et al.*, 1972; Crum *et al.*, 1974; Reidel, 1976; Beertsen, 1979; Ahrens *et al.*, 1981; Gilmore *et al.*, 1984; Dale, 1985; Proffit, 1986; Kryshalskyj *et al.*, 1987; Kaplan, 1988; Watson, 1988; Edwards, 1988; Koole, 1990).

Studies have been proposed in opposition and in favour of the hypothesis that the vascularity is in some way responsible for tooth eruption and/or maintained tooth position

following eruption. Those in opposition would include reports by Taylor (1951) who suggested that changes in blood flow to the periodontal tissues did not alter the eruption rate. Main (1966) used hypotensive drugs and showed no effects on the rate of eruption.

Studies in favour of the vascular etiology of tooth eruption/tooth position theory include the following who reported or suggested:

- (a) Pressure derived from the cardiovascular system might contribute to eruption (Davidovitch, 1988).
- (b) Increasing rates of tooth eruption were associated with an increased vascularity (Bryer, 1957).
- (c) Small pulsating movements of teeth were found to be synchronous with the arterial pulse. It was reported that this was evidence that vascular pressure has the ability to produce a force which is sufficient to move a tooth under physiologic conditions (Korber, 1970; Ng *et al.*, 1981).
- (d) The fluid (vascular) at the apex of an erupting tooth attained a pressure sufficient enough to facilitate tooth eruption (Berkovitz, 1971).
- (e) After experimentation on the rabbit mandibular incisor, significant changes in eruption occurred following spontaneous drops in arterial blood pressure which were not induced experimentally. Furthermore, following death of the rabbit, the incisor ceased to erupt with the sudden drop in arterial pressure and cessation of the heart beat, also the tooth gradually intruded once the arterial pressure dropped to zero (Moxham, 1979; Myhre *et al.*, 1979).

It is evident from the preceding studies (vascular) that the vascularity may play some role in the position of each tooth within the dentition, during and following eruption into its final functional position within the jaws.

It is the author's contention that the literature is deficient, and thus in need of reports that deal with the vascularity (blood vessels) of the periodontal membrane as a direct potential source contributing to tooth position following orthodontic tooth movement. This is based on the fact that many papers which have discussed tooth movement sometimes discussed vascular changes but have gone no further in associating relapse to the vascular changes (Schuback *et al.*, 1957; Boyer *et al.*, 1962; Zaki *et al.*, 1963; Castelli *et al.*, 1965; Folke *et al.*, 1967; Weekes *et al.*, 1986; Rygh, 1976; Lew, 1989). I am suggesting that the association is compelling and should be examined further.

The Normal Vascular Arrangement of the Periodontal Ligament:

There are numerous morphological studies of the periodontal ligament under normal circumstances (Edwall, 1982; Rygh *et al.*, 1986; and Melsen, 1986). Methods to investigate the microvasculature of the periodontal ligament include: injection of

- (a) india ink,
- (b) colored latex or
- (c) colloidal or silicone rubber (Keller *et al.*, 1955; Schuback *et al.*, 1957; Kindlova *et al.*, 1962; Kindlova, 1963; Cohen, 1960; Adams, 1962; Boyer *et al.*, 1962; Castelli *et al.*, 1965; Egelberg, 1966; Cutwright *et al.*, 1967; Garfunkel *et al.*, 1970) into blood vessels.

Folke *et al.*, (1967) used plastic microspheres to study the

- (a) vasculature beneath the oral and 'col' epidermis and
- (b) microcirculation within the periodontal ligament (Folke *et al.*, 1967).

Additional methods for study of the periodontal microcirculation, include lead chrome precipitation (Boyer *et al.*, 1962), and radiology following dye injection (Schuback *et al.*, 1957; and Saunders, 1966).

In spite of the various methods, few studies of the ultrastructure of micro-vessels of the periodontal ligament (Johnson and Highison, 1985; Weekes *et al.*, 1986; and Nakamura *et al.*, 1986) are available. Furthermore, most investigations of the vasculature of periodontal ligament are histological descriptions of either the arterial supply (ignore venous drainage) or "blood vessels" with no further categorization (Johnson and Highison, 1985).

In the literature both human and animal studies have been conducted to study the vascularity of the periodontal ligament, under normal circumstances.

Human studies:

As early as 1909 there was much speculation as to the origins of the periodontal ligament blood vessels. Schweiter (1909) reported that the vessels of the periodontal ligament passed

- (a) from alveolar bone through perforations in the alveolar wall (Birn, 1966)
and
- (b) from the gingiva (Birn, 1966).

Hayashi (1932) studied serial sections of human cadavers following injections of Berlin Blue to determine the distribution of the blood supply to the different parts of the periodontal ligament and alveolar bone (Birn, 1966). At that time, his study was considered to be the most complete. He concluded that the main blood supply to the periodontal ligament was from the dental artery which is a branch of the inferior alveolar artery in the mandible and the superior alveolar arteries in the maxilla.

Branches of the dental artery are:

- (a) the interalveolar artery;
- (b) two interalveolar branches; and
- (c) two longitudinal periodontal arteries given off within the periodontal ligament.

Finally, the dental artery passes through the apical foramen to enter the pulp. From the interalveolar branches, 4 to 5 perforating alveolar branches pass through foramina in the alveolar wall to the periodontal ligament. Hayashi (1932) reported that the best supply to each periodontal ligament was found on the buccal margin of the alveolar wall, then in the middle of the mesial surface and apically on the distal surface with the blood supply being greater to the molars than the incisors (Birn, 1966). The distal surface of the apical third of the periodontal ligament had the least number of blood vessels. Steinhardt (1935) reported that the number of blood vessels was greater in the gingival and apical regions of the periodontal ligament than in the middle (Birn, 1966).

Birn (1966) used a system whereby the fenestrations in the alveolar wall of the human dentition were used to assess number, size, area and distribution of blood vessels within the periodontal ligament. He reported that

- (a) all fenestrations enclose blood vessels;
- (b) the mesial and distal surfaces of the periodontal ligament were more vascular than the buccal and lingual surfaces; and
- (c) the larger blood vessels were within the gingival and apical thirds of the periodontal ligament.

The correlation of fenestrations to blood vessels has also been shown in the periodontal ligament of the rat (Birn, 1966). This correlation is reassuring since the distribution of blood vessels in the rat periodontal ligament has been reported to be similar to that of dog, cat and human (Cohen, 1954; Cohen, 1960; Castelli, 1963).

The rationale for the greater number of blood vessels within the gingival and apical regions of the periodontal ligament may be related to tooth movement. Physiologic drift in a mesial direction has been well established in human dentition (Birn, 1966). As the fulcrum of movement lies approximately in the middle of the root, the magnitude of movement is greatest in the gingival and apical thirds of the periodontal ligament. If this movement promotes tissue remodelling, there may be greater demands on the vasculature. Hence, greater blood vessel diameter on mesial and distal surfaces, would imply that apposition/resorption, may be occurring there (Birn, 1966; Ten Cate *et al.*, 1980). Furthermore Edwall (1982) reported that the blood vessels of the

periodontal ligament are oriented vertically and anastomose with vessels from the alveolar bone via fenestrations in the alveolar wall.

Animal studies:

In the rodent a variable pattern was reported by (Boyer *et al.*, 1962). The variation may be attributed to vessels from the surrounding soft tissues, especially muscles, which also supply the rodent periodontal ligament (Boyer *et al.*, 1962).

Frolich (1964) reported a "diagonal symmetry" in the vascularity of the periodontal ligament of the rodent; that is, the number of vessels in the apical region of the lingual surface is matched on the facial surface of the gingival region (Boyer *et al.*, 1962). Extrapolation of Frolich's (1964) theory would indicate that the number of vessels in the apical region of the mesial surface would be matched at the gingival surface of the distal region. The significance of Frolich's (1964) theory will be discussed with regard to the fulcrum of the tooth.

Castelli *et al.*, (1965) reported from their studies on monkeys, that arteries pass through foramina in the alveolar wall into the apical two-thirds of the periodontal ligament and that there are no arteries which pass from the gingiva into the periodontal ligament. Veins, drain the interradicular and interalveolar septa through the apical perforations (Castelli *et al.*, 1965).

Using an India ink infusion technique, Garfunkel *et al.*, (1970) reported that the periodontal vessels of the rat molars do not terminate at the apex of the alveolar crest as advocated by Kindlova *et al.*, (1962), but join with the supraperiosteal blood vessels by a direct or an indirect link through the bone itself. In the 'col' region the blood supply

to the periodontium comes from the adjacent periodontium and the buccal and lingual sides of the periosteum (Kindlova *et al.*, 1962). Folke *et al.*, (1967) and Boyer *et al.*, (1962) suggested that communicating blood vessels from the crestal bone also supply the periodontium. It was further reported by Garfunkel *et al.*, (1970), that in the molar apical region the blood vessels form a basket-like network which supplies both the tooth pulp and the adjacent periodontal ligament and anastomoses between the two networks occurs via lateral canals in the root. Anastomoses between adjacent bone and periodontal ligament blood vessels had also been shown by Garfunkel *et al.*, (1970). The basket-like vascular network was also demonstrated near the root apex by Kindlova *et al.*, (1962) using the latex injection method and also by Nakamura *et al.*, (1983) who perfused with Mercox.

By means of the corrosion casting technique Murakami, (1971); Gannon, (1981) and Weekes *et al.*, (1986) were able to provide a detailed three dimensional, complex view of the microvascular distribution within various regions of the periodontal ligament of the rat molar. They reported that each region had a distinct distribution of blood vessels as follows. On the buccal and lingual wall, continuous vessels were traceable from the gingival plexus to the apex. Three to six vessels were grouped in tracts. Two distinct sizes of vessels were seen in each tract. The smaller vessels were approximately 6 to 10 μm in diameter (capillaries) while larger vessels were about 20 μm in diameter (postcapillary venule). The smaller vessels intertwined with the larger vessels; however, they seldom anastomosed with them, but rather penetrated the socket wall to anastomose with the medullary plexus. Within the ligament the vessels were randomly

arranged within an annulus approximately 50 to 100 μm in diameter adjacent to the alveolar bone. Generally, both the pre-capillary venule and capillaries arose directly from the gingival vessels. Coronally, some vessels entered the ligament from the alveolar bone to form a hairpin loop, pointing occlusally prior to joining the vertical tract of vessels. In the periapical region loops were also seen.

In the interdental septum the vessels around the buccal and lingual walls and over the interdental septum were arranged in similar patterns to each other, except that all the vessels in the interdental region were close together and were approximately 20 μm in diameter. Vessels of adjacent teeth arose from the interproximal col and were initially together; above the interdental alveolar crest these vessels then separated to course into the alveolar socket of each tooth.

In the interradicular septum region the microvascular bed exhibited a unique morphology. The postcapillary venules were not continuous from the apex to the crest of the septum. They passed in an occluso-apical direction for 100 to 400 μm in a series of repeating segments within the ligament. The vessels were sinusoidal. The coronal extremity of each segment penetrated the socket wall at an acute angle, and drained into a large postcapillary venule or small collecting venules. The apical extremities of these short venular segments also re-entered the bone and emptied into small collecting veins, enlarging in diameter as they went.

The arteriolar blood supply arose from the alveolar bone and usually joined the middle of a venular segment. However, venular branches from the middle of the segment were more frequent than arteriolar communications. A segment was often

supplied by more than one arteriole. The metarterioles either joined with, or enlarged into, postcapillary vessels of 15 to 20 μm in diameter that coursed occluso-apically within the ligament (Weekes *et al.*, 1986). A capillary bed did not exist between the arteriolar supply and the venous side of the circulation.

At the crest of the interradicular septum, the vessels were arranged differently. In the ligament the vessels were mainly venules showing a random venule-venule anastomosing pattern. The venular drainage from the crest into the bone was via vessels with a greater diameter than those draining the sides of the septum. Additionally, larger venules (up to 60 μm in diameter) were present in the ligament area itself. The crestal vessels had a random arrangement. It was concluded by Weekes *et al.*, (1986) that the predominant vessel type in the periodontal ligament was the postcapillary venule, ranging in diameter from 15- 20 μm . Capillaries were present but in smaller numbers.

The report by Weekes *et al.*, (1986) differed from that of Kindlova *et al.*, (1962) as they (Weekes *et al.*, 1986) were not able to show demarcation of rat molar ligament vessels into paired arterial and venous systems as advocated by Kindlova *et al.*, (1962). In the study by Weekes *et al.*, (1986), the vessels were either capillaries or postcapillary venules with few horizontal connecting branches. Arterioles were not found coursing in the ligament itself.

From scanning electron microscopic studies on the periodontal ligament of the rat Nakamura *et al.*, (1986) reported that the periodontal ligament had an abundant number of blood vessels. The distribution was closer to the alveolar bone, arranged vertically from the apex of the root to the alveolar crest (Nakamura *et al.*, 1986). This vertical

arrangement parallel to the long axis of the tooth has been confirmed by Kindlova (1965) in *Macacus rhesus* monkeys, and by Carranza *et al.*, (1966) in the rat, cat, mouse, hamster, and dog.

It is generally agreed that blood vessel density is greatest in the apical region of the periodontal ligament (Moxham *et al.*, 1985; Lew, 1987). Lew (1987) reported that three main types of blood vessels can be found in the apical region of the rat molar:

- (a) metarterioles
- (b) capillaries, and
- (c) postcapillary venules.

Metarterioles ranged in diameter from 18-24 μm and had 1-2 continuous layers of smooth muscle cells, each layer averaging 0.5-1.0 μm thickness.

Capillaries had a single continuous layer of simple squamous endothelial cells (0.05-0.5 μm thick) in the apical region of the periodontal ligament. The luminal size of the capillaries ranged from 6.8 to 7.8 μm .

Postcapillary venules ranged in luminal diameter from 21-26 μm . It was further reported by Lew (1987) that the average vascular volume in the apical periodontal ligament of the rat was almost 20%. This seemed somewhat excessive to Casley-Smith (1971). If one considers the ligament to be a connective tissue, then it should only require 5% vascular volume in order to provide the necessary nutrition. This excessive vascular volume in the apical region may be regarded as being required for other metabolic needs such as occlusal force dissipation as well as bone and cemental resorption/repair.

Lew (1987) further reported that the postcapillary venule is the most predominant vessel in the apical region of the periodontal ligament, similar to reports by Weekes *et al.*, (1986), comprising 82.4 % of the total vascular volume. Furthermore, it was reported that the blood vessels occupy only 1 to 2 % of the periodontal ligament space (Ng *et al.*, 1981).

"Collateral" blood supply of the periodontal ligament vessels has been reported and should not be overlooked. This supply accounts for the rather uniform size of the lumens throughout the length of the tooth's root. The orthodontic significance of "collateral" blood supply of the capillary bed is such that, if one of the sources of blood supply is cut off during orthodontic tooth movements, the remaining vessels can still bring about repair and regeneration.

Of additional significance is the fenestrated character of the periodontal ligament microvasculature of the rat (Moxham *et al.*, 1985). Fenestrated capillaries are found in organs engaged in the production/absorption of fluids, or where there is a need for a more direct exchange with the blood stream (Majno, 1965). This has been suggested to be related to the high metabolic activity of the ligament (particularly in the proliferative basal region). It should be noted that fenestrated capillaries have permeability characteristics that differ markedly from those of continuous capillaries (Dressel *et al.*, 1966; Eliassen *et al.*, 1973; Haraldsson, 1982). Therefore, the presence of fenestrated capillaries might have important implications for the function of the periodontal ligament.

Additionally, it has been suggested that the vasculature of the periodontal ligament, among other things, plays a role in tooth support (Parfitt, 1960; Bien, 1966a; Moxham *et al.*, 1982b) and eruption (Moxham *et al.*, 1982a).

Review of both human and animal literature has provided a general picture of the periodontal ligament under normal (non-orthodontic force) conditions. Studies have indicated that the periodontal vascularity of both human and animal models are very similar (Cohen, 1954; Castelli, 1963; Carranza *et al.*, 1966). Given the similarity, certain conclusions may be drawn from the studies cited, animal and human, regarding the periodontal ligament vascularity:

- (a) fenestrations in the alveolar wall of the periodontal ligament provide blood vessels which penetrate the ligament along its vertical length;
- (b) the blood vessels are arranged vertically along the length of the tooth from the apex to the coronal region;
- (c) the vascular supply seems to be greatest on the mesial and distal surfaces of the ligament;
- (d) the number of vessels are greatest in the apical region and to a lesser extent in the coronal and middle regions;
- (e) post-capillary venules predominate in the ligament;
- (f) a 'basket-like' network appears to be present in the apical region of the ligament;
- (g) venous drainage is apical and via the interradicular and interalveolar septum;

- (h) the gingival vessels seem to provide some vascularity to the periodontal ligament;
- (i) the ligament has a higher vascular content than would be expected;
- (j) anastomoses of blood vessels occurs in the ligament as well as collateral blood supplies;
- (k) the capillaries of the ligament are highly fenestrated;
- (l) the periodontal ligament vascularity of the animal models cited appears to be similar to human; and
- (m) the vertically arranged blood vessels give off branches which supply the ligament.

Further studies of the rat buccal and lingual vasculature are required to determine if the arteriolar supply to these regions matches the pattern found in the interradicular septum where arterioles anastomose directly with postcapillary venules. Also under normal circumstances

- (a) the mesio-distal, bucco-lingual location of the blood vessels within the periodontal ligament needs to be more readily defined;
- (b) the relative proportion of the vessels within the ligament at these locations also needs to be determined.

Changes in the Periodontal Ligament in Response to Tooth Movement:

The changes in tooth position and their supporting tissues coincident to orthodontic tooth movement have been studied in dogs (Schwartz, 1932; Follin *et al.*, 1986); monkeys (Johnson *et al.*, 1926; Oppenheim, 1944; Picton *et al.*, 1967; Melsen,

1986); rats (Bien, 1966b; Rygh, 1976; Walker, 1980; Steigman *et al.*, 1983; Sims 1987; Row *et al.*, 1990) and humans (Reitan, 1951; Zachrisson *et al.*, 1973; Sadowsky *et al.*, 1981; Hamp *et al.*, 1982; Sims, 1983). The rat has often been used as a model to study the periodontal ligament because its molar teeth and periodontium are similar to human, and because it is relatively easy to house and manage.

The periodontal ligament like the suture; defined as a complex of cellular and fibrous tissues intervening between, and surrounding the bony edges of the tooth socket, regenerates after orthopaedic therapy instead of forming scar or repair tissue (Ten Cate *et al.*, 1977; Wagemans *et al.*, 1988).

Movement of the crown of the tooth by an orthodontic appliance produces forces within the periodontium (Moyer, 1950; Ten Cate *et al.*, 1976; Davidovitch, 1981; Rygh *et al.*, 1986; and Michaeli *et al.*, 1985). However, the mechanism by which this mechanical stimulus is converted into cellular activity is still largely unknown (Goldman *et al.*, 1972; Wagemans *et al.*, 1988; and Southard *et al.*, 1988), although Binderman *et al.*, (1988) has reported it to be initiated by a membrane-linked processes. During orthodontic therapy the cellular reactions are thought to be initiated indirectly. One theory suggests that with tooth movement the fibres and blood vessels of the periodontal ligament are distorted which in turn affects cell activity. Additional hypotheses as to the mechanism of converting mechanical stimulus to cellular activity include:

- (a) alterations of available circulating hormones or alterations in the surface properties of the cells (Rygh, 1982);
- (b) calcium ions and cyclic AMP may influence mitosis (Melcher, 1976);

- (c) changes in oxygen tension and pH in the microenvironment may affect resorption and deposition of bone (Rygh, 1982);
- (d) there may be secretion by cells of the periodontal ligament of substances capable of stimulating differentiation of osteoclasts and osteoblasts in response to appropriate stimuli (Melcher, 1976).

There is evidence suggesting that osteoclasts are derived from haemopoietic cells and transported via the circulation, hence differentiation of osteoclasts/osteoblasts as suggested by Melcher (1976), may not be the case.

Dating back to studies by Sandstedt (1904), orthodontists have believed that a compressive force to the periodontal ligament results in adjacent bone resorption, while tensile forces to the periodontal ligament initiate bone and fibre formation (Macapanpan *et al.*, 1954; Reitan, 1967; and Kuitert *et al.*, 1988). The shape or the rate of flow of the blood within periodontal vessels may be altered, if the force is great enough (Rygh *et al.*, 1986). Application of either light or heavy force to teeth produces compression of the periodontal ligament between the tooth root and adjacent alveolar bone. For example, if the tooth is moved in a mesial direction, the mesial periodontal ligament will be compressed. This results in tension within collagen fibres on the opposite side of the tooth (Azuma, 1970; Zaki *et al.*, 1963; Kuftinec, 1968).

On the tension side of the ligament, the periodontal space becomes widened as the tooth is drawn away from the alveolar wall. Bundles of fibres are stretched and the alveolar crest is stretched in the same direction. The blood vessels between these stretched fibres cause them to assume an ellipsoid appearance, since they are usually

oval. If the force exceeds 25 gm/cm^2 (Goldman *et al.*, 1972; Storey, 1973) and occurs over a sufficient time period, the result will be compression of the periodontal ligament between the tooth and alveolus. Ischemia will lead to hyalinization due to insufficient nutrition (Moyers, 1950; Reitan, 1960, 1967; Kvam, 1972; Rygh, 1974; Diaz, 1978; and Kuitert *et al.*, 1988). Another factor necessary to bring about hyalinization is the amount of initial periodontal ligament compression (Moyers, 1950). Since the periodontal ligament is in the range of thickness from 0.15 to 0.38 mm, the tooth must be moved at least 0.15 to 0.38 mm to ensure alveolar bone resorption and an adequate degree of periodontal ligament compression. With light forces that are insufficient to occlude the blood vessels on the pressure side the bone resorption is mediated by osteoclasts which migrate from other regions of the ligament. This type of resorption is termed "frontal resorption" (Reitan, 1951; 1964; Goldman *et al.*, 1972; Ericsson *et al.*, 1977; and Kuitert *et al.*, 1988).

Conversely, if the force is great enough to occlude the blood vessels on the pressure side, cellular migration is unable to occur in the ligament because of a lack of nutrient supply or anoxia. In this case cells migrate from the adjacent marrow space on the endosteal surface of the alveolus above and below the point of vascular occlusion, since these areas still have patent blood vessels. Bone resorption hence occurs on periosteal surfaces superior and inferior to endosteal surfaces adjacent to the zone of compression. This resorptive process has been termed "undermining resorption" (Reitan, 1951; Goldman *et al.*, 1972; Ericsson *et al.*, 1977; and Kuitert *et al.*, 1988).

Injury, inflammation, degeneration and repair to the supporting tissues of the tooth are factors regulating tooth movement (Stuteville, 1937; Rygh, 1974, 1976). The rate of tooth movement is related to the rate of recovery of periodontal ligament; from hyalinization, which must occur before tooth movement can occur (Moyers, 1950; Michaeli *et al.*, 1985). The hyalinized area inevitably disappears and is repopulated with blood vessels and cells (Rygh, 1974).

There are a number of degenerative changes evident during hyalinization (Rygh 1974). These changes include: degraded blood vessels, remnants of cell nuclei, and compressed fibrils. In the rat, vasodilation can be seen on the tension side while vasoconstriction with haemorrhage can be seen on the compression side in response to force (Kuitert *et al.*, 1988; Lew *et al.*, 1989). With increased levels of force, the pressure zones occur over a greater area, when examined in the rat (Rygh, 1974). After two days, tissue repair begins in the smaller foci of hyalinization and these zones are repopulated by cells within a few days. In experiments involving rat molars, subjected to greater forces (10-25g) or with a tipping force which concentrates force in the pressure region, hyalinized areas can persist for up to five days (Rygh, 1974). These large hyalinized areas have a relatively larger amount of vascular elements than the localized compressed foci. Resorption of alveolar bone is frequently both frontal and undermining types (Rygh, 1974). In the elimination of the hyalinized tissues there is an initial invasion of pioneer cells and blood vessels penetrating the hyalinized tissue followed by cells and vessels (Rygh, 1974). It has been suggested that inflammatory cells are capable of initiating angiogenesis, within the compression area. The new capillaries remove the

necrotic material and transfer nutrients to the ligament (Rygh, 1974; and Follin *et al.*, 1986). Macrophages are derived from these pioneer blood vessels and are found adjacent to the hyalinized areas (Kvam, 1970; and Rygh, 1974) and participate in removal of hyalinized tissue.

Subsequent to the initial angiogenesis is an area of repair located about 30-50 μm , from the zone of angiogenesis. In this region, removal of degradation products left by the pioneer cells and vessels, and some collagen breakdown, occurs near the remnants of the hyalinized zone, while synthesis of new tissue components occurs further away (Rygh, 1974).

The exact pathway whereby collagen is removed from the hyalinized zone has yet to be determined. However, Freeman (1989) has suggested that the periodontal fibroblasts were responsible, due to the observation of intracellular collagen within these cells. Conversely, Rygh, (1974) suggested that removal is accomplished by macrophages. Perhaps both fibroblasts and macrophages play a role in the breakdown of the collagen, to differing or similar degrees.

The more extensive amorphous masses containing coalesced blood vessel components of degraded blood cells are eliminated by the development of foreign body giant cells (Rygh, 1974).

Hyalinized tissues are removed and repaired by invasion of cells and blood vessels from the adjacent periodontal ligament and alveolar bone. The mechanism of tissue removal is influenced by the form and composition of the degraded material being

removed. Irrespective of this, the total elimination of the hyalinized structures seems to be the rule (Rygh, 1974).

Continuous fibrous attachments are provided between bone and the periodontal ligament on both the "depository (tension) and "resorptive" (pressure) surfaces of each alveolar socket, during both physiologic and orthodontic tooth movement (Kurihara *et al.*, 1980a). On the tension side, the fibres of the ligament must undergo change as bone is formed over the existing fibres, or else they would be totally covered after 0.5 mm of bone deposition.

The mechanism of periodontal fibre attachment and successive reattachments to pressure (resorption) and tension (deposition) surfaces has been proposed by several authors (Kraw *et al.*, 1967; Garant *et al.*, 1979; Johnson, 1987; and Kurihara *et al.*, 1980a,b). Kurihara *et al.*, (1980a) have postulated three types of periodontal fibre attachments to resorbing alveolar bone: "continuous" (Type I), "intermediate" (Type II), and "adhesive" (Type III). On depository surfaces only the Type I or the "continuous" mechanism is seen, while on resorptive surfaces all three types may be seen. Of the three types of periodontal ligament to bone attachment on the resorptive surface the "adhesive" (Type III) mechanism is the most abundant while the continuous mechanism is the least observed (Kurihara *et al.*, 1980b).

On the tension (depository) surface, an uninterrupted continuity of collagenous periodontal ligament fibre bundles can be seen from the ligament to the alveolar bone. These fibres become embedded in the bone as a result of progressive surface bone deposition occurring around them to form Sharpey's fibres (Kraw *et al.*, 1967). This

mechanism occurs under physiologic conditions and has been regarded by Kurihara *et al.*, (1980b), as continuous attachment capable of occurring on both tension or pressure surfaces. However an additional mechanism seems to occur on the tension surface when subjected to orthodontic load. The additional mechanism suggests that Sharpey's fibres are secreted simultaneously with new bone deposition. With the migration of fibroblasts from the bone, they deposit either entirely new Sharpey's fibres or new fibrils which are incorporated into existing fibres (Garant *et al.*, 1979). On the tension side there is both an incorporation of pre-existing collagen fibres into new osteoid and a considerable production of new collagen fibres near the advancing bone front. Also, lengthening of fibres seems to occur by incorporation of new fibrils into existing fibres (even at some distance from the alveolar wall) (Rygh, 1976). It should be noted that, continuous periodontal attachments are identical for both depository and resorption surfaces (Johnson, 1987).

Adhesive periodontal attachments are of a proteoglycan nature and provide adhesion of unit collagen fibrils of periodontal ligament fibres onto the resorbed surface of alveolar bone. Intermediate periodontal attachments are composites of continuous and adhesive mechanisms. In the intermediate attachment, unit collagen fibrils of the periodontal fibre bundle pass through the reversal line to join fibrils of the Sharpey's fibre bundle. Reversal of bone resorption involves synthesis of bone matrix and entrapment of the periodontal ligament fibres by the appositional front (Kurihara *et al.*, 1980a,b). It has been suggested by Kurihara *et al.*, (1980a,b) that all three types of

attachments persists after reversal. Johnson (1987) would argue that the evidence supporting the intermediate attachment was not very good.

A number of cellular processes are activated within the periodontal ligament, if the tooth is stabilized in the new position by a continuous force. The number of connective tissue cells increases due to cell division. On the tension side, osteoblasts are mobilized from the ligament within three to five days. Following this, osteoid tissue will be deposited on the alveolar wall. In areas where the fibrous bundles are thick, new bone appears to be deposited along them. In the presence of thin bundles, a more uniform layer is deposited along the alveolar wall. Once the osteoid layer is laid down, calcification in its deeper layers starts, while the superficial layers of osteoid remain uncalcified. Hence within three months a mature layer of new bone may exist after the final tooth movement (Crumley, 1964; Kraw *et al.*, 1967; Reitan, 1969; Rygh, 1982).

The periodontal ligament vasculature also undergoes detailed changes on both the resorptive (pressure) and deposition (tension) surfaces in response to tooth movement. As late as 1986, there were still few published studies of the relationship between tooth movement and the adjacent blood vascular system (Nakamura *et al.*, 1986).

The immediate response of the periodontal ligament vasculature to applied forces has been viewed as a response to fluid changes as opposed to altered changes in the cellular activity. The blood acts as a "shock absorber," to dissipate applied forces. However, shortly after force application, the blood flow adapts to rearranged tissue spaces within the periodontal ligament creating favourable conditions for cellular activity (Kuftinec, 1968).

Numerous studies of both short and long experimental periods have been conducted so as to ascertain the pathological vascular changes incident to tooth movement.

Tensile forces within the periodontal ligament have been reported to cause blood vessel dilation and distension, within hours of force application (Macapanpan *et al.*, 1954; Kufinec, 1968; Rygh, 1976). Brooke (1965) reported that the vessels of the periodontal ligament adjacent to areas of bone deposition (tension areas) had significantly larger diameter than those found within the periodontal ligament of control teeth. There was no evidence of new vessel formation. The increased diameter of the vessels was probably due to mechanical stretching by the periodontal ligament, since the duration of the study was short. Khouw *et al.*, 1970) reported histomorphometric data, supporting Brooke (1965), suggesting that the only vascular changes coincident to tension within the periodontal ligament showed increased vessel diameters. Increased number of blood vessels was not reported.

Gaengler *et al.*, (1983) examined the effects on the rat periodontium under both continuous and intermittent loads of short duration (10 minutes to 180 minutes). He reported ischemia/stasis and dilation in the microvascular vessels, arteries and veins, in the tension zones.

Lew *et al.*, (1989), suggested that a short term extrusive load of 30 minutes, simulating clinical orthodontic tensile movement, increased the apical vascular volume by 42.7%. Since the force was short term then it is fair to assume that the increase in vascularity was a result of blood vessel diameter increase as opposed to an increase in

number of vessels. Lew *et al.*, (1989) further suggested that activation of the vascular system may precede activation of both the cellular and fibrillar elements within tension zones of the periodontal ligament, thus the vasculature plays a key role in periodontal ligament remodelling coincident to tooth loading.

Lew (1989) simulated changes in the microvasculature of the tension zone, of the periodontal ligament of the rat molar, by means of an extrusive force for 30 minutes. A force of one Newton was chosen since it had been used by other authors to study orthodontic tooth movements in the rat (Azuma, 1970; Giannelly, 1969; and Bondevik, 1980). From his experiment of short duration Lew (1989) reported degenerative changes in the endothelial cell membrane and nucleus in 20-30 % of venules. However the arteries generally were unaffected. The affected venules had endothelial cells that were swollen with the formation of abnormally large, thin walled vacuoles. In some endothelial cells, the cell membrane adjacent to the blood vessel lumen disintegrated, releasing their cytoplasmic contents into the lumen. Pyknotic nuclei were also noted. These endothelial cell changes were not observed by Rygh (1976), who studied the changes in the tension zones of the rat molar periodontal ligament upon application of a 0.05 to 0.2 Newton force over periods of 30 minutes to 28 days. The discrepancies in the data may be due to the lighter force and/or the increased duration of the study by Rygh (1976). It is conceivable that the heavier force used by Lew (1989) caused some damage to the periodontal ligament, resulting in inflammation. The vulnerability of the venules to the force coincident to inflammation may be due to their relative inability to be distended, larger number of fenestrae or a defective basement membrane (Zweifach,

1973). Although such degenerative changes have been demonstrated in pressure zones of rats (Rygh, 1974), vascular degeneration had not been described previously in tension zones. The early vascular changes reported by Lew (1989) support the findings of others (Rygh *et al.*, 1986), who implicated the vascular system as the "main mediator" in periodontal ligament remodelling associated with tooth movement.

In the presence of heavy forces, the tension side of an orthodontically moved tooth demonstrates haemorrhage and increased numbers of osteoclasts (Oppenheim, 1944). Ten Cate *et al.*, (1977) have reported on initial mild injury that included cell death in rat parietal sutures during rapid expansion. This is significant since Ten Cate *et al.*, (1977) considered the suture to be very similar to the periodontal ligament when subjected to tensile forces. Experimental tooth movement produced increased vascular activity in both areas of tension and in areas of frontal bone resorption within the periodontal ligament (Rygh *et al.*, 1986). Furthermore, the report by Rygh *et al.*, (1986) supports previous observations showing

- (a) the occluding effects on the blood vessels in areas of pressure in the periodontal ligament (Khouw *et al.*, 1970; Gianelly, 1969);
- (b) an increased number of blood vessels adjacent to and angiogenesis into compressed hyalinized areas (Khouw *et al.*, 1970; Gianelly, 1969),
- (c) an increased blood supply to areas of osteoclastic activity (Orban, 1958),
and
- (d) an increased vascularization in areas of tension (Rygh, 1974; Khouw *et al.*, 1970).

In contrast to the tension zone, subsequent to tooth movement, the vascular changes in the pressure zone of the periodontal ligament coincident to tooth movement are well documented. Early changes include vascular stasis and tissue ischemia (Piekarsky *et al.*, 1962; Castelli, 1965; Gaengler *et al.*, 1983), followed by a gradual decrease in the number of patent capillaries (Macapanpan *et al.*, 1954; Azuma, 1970). Complete obliteration of the blood vessels (Buck *et al.*, 1972) and eventual vascular degeneration (Rygh, 1974) occurs within days. Regeneration and proliferation of the blood vessels into the pressure zone within the pressure zone usually occurs after 7 days (Khouw *et al.*, 1970; Rygh *et al.*, 1986).

Nakamura *et al.*, (1986) report that following both one and two days of rat molar tooth movement in a buccal direction, the vessels in the periodontal ligament were displaced toward the buccal side of the pressure zone. They were able to show their results three-dimensionally by means of the vascular casting/SEM method. Extrapolation of these results suggests that blood vessels would be displaced toward the mesial alveolar bone when a mesial force was applied to the maxillary first molar. It was also found that near the apex of the molar, the blood vessels formed a basket-like vascular arrangement similar to that seen under control conditions. The vessels in areas of extreme compression had various diameters; some were partially dilated while others showed tapering structures, after one day of experimental tooth movement in response to a 10-20 gram force (Nakamura *et al.*, 1986). After three days vessels showed a great deal of change. That is, the vessels were highly compressed and arranged in rows at the alveolar margin while collapsed vessels and "bell shaped" ones were frequently observed.

However, there was no apparent basket-like vascular arrangement observed near the apex of the root, as seen after one day of force.

In studying the periodontium of the cat, Nakaruma (1967) reported on ischemia occurring at the alveolar crest (zone of compression) after a continuous light force was applied for more than one day.

Rygh, (1974), reported on blood vessel dilation, packing of erythrocytes and broken vessel walls in response to tooth movement 2 hours and 2-3 days following force application.

Khouw *et al.*, (1970) reported a significant difference in the vascular pattern of the periodontal ligament of resorption and apposition sides of the rhesus monkey and dog. Despite the similar width of the ligament space on both sides, the blood vessels on the resorptive side were found to be more numerous but smaller in diameter than on the appositional side. The observation of an increase in the number of blood vessels in areas of resorption has also been reported by (Zaki *et al.*, 1963; Piekarsky *et al.*, 1962). On both the tension and compression sides, the blood flow is reduced; however, this reduction is greatest on the compressive side. Khouw *et al.*, (1970) further reported that the periodontal ligament next to the bone has a richer supply than that next to the cementum in the dog and monkey. This might account for the greater reactivity of bone with respect to remodelling as compared to cementum.

Using fluorescent microspheres injected into the rat to measure blood flow, the maxillary first molar on one side was moved with an orthodontic spring delivering a 30 to 50 gram force to move the tooth for five days, at which time the animal was sacrificed

(Kvinnsland *et al.*, 1989). Kvinnsland *et al.*, (1989) reported a substantial increase in the rate of blood flow in the periodontal ligament of all the molar teeth on the experimental side as compared to the contralateral control side of the animal. It should be noted that, increased blood flow rate is dependent on vasodilation, absence of gross leakage and absence of stasis. The report by Kvinnsland *et al.*, (1989) appeared to contradict reports of other authors (Gaengler *et al.*, 1983; Rygh *et al.*, 1986) who suggested stasis and reduced blood flow in areas of compression. In the report by Kvinnsland *et al.*, (1989) a generalized stasis of blood flow in the tissue would have lead to a decrease in blood flow and not an increase as was reported. The basic vascular responses to inflammation are vasodilation, increased vascular permeability and eventual stasis. Had inflammation been the case with the study by Kvinnsland *et al.*, (1989), there would have been an initial period of increased blood flow followed by eventual stasis. Inflammation does not appear to have been the case with the Kvinnsland *et al.*, (1989) study. An alternative hypothesis for the Kvinnsland *et al.*, (1989) report may be as follows: local areas of compression and/or stasis will be found in periodontal ligament coincident to orthodontic tooth movement in the rat. There will be some regions of vasodilation to allow for an increased blood flow in certain areas subsequent to the orthodontic stimulus (Kvinnsland *et al.*, 1989), to maintain the increased cellular activity (Baumrind *et al.*, 1970; Kvam, 1972). Since the Kvinnsland *et al.*, (1989) report looks at a specific instance in the orthodontic tooth movement, that is at 5 days, it is plausible that this in fact may be the situation at this period in time. A further explanation for the contradictory report by Kvinnsland *et al.*, (1989) is that localized vasculature may show

compression or stasis as seen under transmission and scanning electron microscopic observations using semi-thin and thin sections (Kvinnslund *et al.*, 1989).

Summarizing the literature regarding orthodontic tooth movement; on the compression surface there is bony resorption while on the tension surface there is apposition of bone. On the tension side the periodontal ligament space becomes widened thereby stretching the fibres between the alveolar bone and cementum of the tooth. Conversely on the compression side the ligament and fibres within tend to become compressed. On both the compression and tension surfaces there is a maintenance of fibre attachment between the tooth and alveolar bone. The vascular response on the compression surface is one of a reduced blood flow, eventual stasis, and hyalinization, due to blood vessel occlusion and eventual obliteration. Within days the compressed zone is repopulated by new blood vessels and cells which results in repair to the hyalinized area. On the tension surface there is an increase or at the minimum a maintenance in the blood flow. The blood vessels on the tension side are reported to be dilated.

It is evident that pathological changes accompany tooth movement, some of which are not fully understood, however, repair of the periodontal ligament in response to tooth movement seems to occur. Repair is evident by virtue that following orthodontic tooth movement the tooth still remains attached to a vital periodontal membrane. There have been some qualitative descriptions of the effects of experimental tooth movement on blood vessels but quantitative data on changes in the tension zone of the periodontal ligament are lacking (Lew, 1989). To date there seems to be little data on the

morphometric changes of blood vessels of the periodontal ligament. Questions that still need to be addressed regarding the periodontal ligament in response to orthodontic tooth movement include: is there an increase in the number of capillaries; does one area exhibit a greater number of vessels over another; does the size of the vessels change; does the location of vessels relative to the alveolar wall and cementum change; does the number of vessels change with time following tooth movement; and does total area of blood vessel occupation of the ligament change with time in response to force. Without bone resorption, tooth movement in a given direction is highly unlikely, and thus the osteoclast becomes quite important.

Osteoclast:

Morphologically, the osteoclast is a large multinucleated cell, responsible for the physiological removal of calcified matrix in the skeleton and dentition (Chambers, 1985; Mundy *et al.*, 1987; Vaes, 1988). Osteoclasts are not a cell of fixed morphology and location in the tissue, but instead may be described as a temporary state of fusion of a number of (mononuclear) precursors. Makris *et al.*, (1982) report that fusion occurs in correlation with resorbing activity and the number of nuclei per osteoclast may vary from one to twenty.

In vitro (Walker, 1975; Loutit *et al.*, 1976) and *in vivo* (Ko *et al.*, 1981; Burger, 1982; Schneider *et al.*, 1988) reports suggest that osteoclasts are derived from the haematopoietic stem cell. It has also been reported that in most cases the circulating osteoclast precursor is a rare cell among blood leukocytes. However, the level of

circulating osteoclast precursors is increased in certain physiological and pathological situations (Chambers, 1991).

During the resorptive process osteoclast will be found attached to the calcified matrix that it is resorbing. The osteoclast accomplishes the resorptive process by forming a sealing zone of close adhesion between itself and the bone, which extends around the circumference of the bone-apposed surface of an osteoclast (Miller, 1984). Marchisio, (1989) suggest that special adhesive structures are involved in creating this sealed zone and subjacent cytoplasm contains concentrations of cytoskeletal elements that exclude other organelles (clear zone). Within this circumferential sealing zone the osteoclastic plasma membrane is thrown into complex folds (ruffled border), the elaboration and size of which is proportional to bone resorbing activity (Doman *et al.*, 1986). It is likely that the ruffled border represents a means whereby the membrane-associated activities required for bone resorption are concentrated. Contemporary evidence suggest that the osteoclast resorbs bone through secretion of proteins and enzymes into the confined space between bone and the ruffled border within the circumferential sealing zone. Within the specialized extracellular environment, bone is degraded by acid hydrolase activity (Chambers, 1991).

During the physiological and/or orthodontic movement of teeth through alveolar bone, one side of the socket mostly shows bone formation, while the opposite side, towards which the displacement occurs, is subjected to an overall bone resorption process (Rygh, 1982). The osteoclast has been reported as being the centrepiece of the

remodelling sequence, allowing orthodontic tooth movement to occur through resorption (Vaes, 1988; Marks *et al.*, 1988).

Various hypotheses have been suggested that might account for the conversion of the mechanical force to the cellular signal responsible for initiating bone resorption (Kahn *et al.*, 1991); however, only two appear intuitively likely to be involved in the process. The first hypothesis, advocates that changes in force are detected at the cellular level due to presence of mechano- or stretch receptors on osteoblast, bone lining cells and/or osteocytes. The force deforms these receptors leading to transmembrane ion fluxes (an effect that would be transmitted to electrically-coupled bone cells) and ion induced alterations in cell function (ie: the activation of remodelling). Hasegawa *et al.*, (1985) has reported data which substantiate the idea that osteoblast and osteoblast-like cells are directly responsive to mechanical stress; however documentation of stretch receptors has been limited and requires further study. The second hypothesis has received more attention. It is suggested that bone cells respond to either endogenous potential (Brighton *et al.*, 1986) or alterations in electrical potential produced by the piezoelectric properties of bone matrix (Pollack, 1984) and/or streaming potential resulting from an ionic exchange between the bone matrix and bone fluid/blood vascular compartment (Eriksson, 1974). These potentials generated by stress would hence represent the signal event in initiating resorption/remodelling (Kahn *et al.*, 1991). Any cell type associated with the bone surface might be the target of the initiating signal in resorption/remodelling. The bone lining cell/osteoblast seems best suited as the cell most likely receiving the signal. This tentative conclusion is based on the fact that

- (a) osteoblasts are in proximity to both blood borne and matrix derived regulatory factors such as ionic changes resulting from streaming potential;
- (b) the osteoblasts mediate ionic exchange between the blood vascular component and the mineral component of the bone matrix (Talmage, 1967; Parfitt, 1979) and
- (c) their probable role in controlling the recruitment and activity of osteoclasts (Kahn *et al.*, 1991), the cells ultimately responsible for bone resorption.

The postulation that the osteoblasts is an intermediate between the signal and the osteoclasts stems from the observation that osteoblast and not osteoclast have receptors for and/or exhibit responses to resorption stimulating factors such as parathyroid hormone (Kahn *et al.*, 1991). Thus the idea is that a mechanical force is placed on the periodontium which affects receptors on the osteoblasts/osteocytes who then activate the osteoclast thereby starting the actual resorptive process (Kahn *et al.*, 1991).

The osteoclasts are extremely rich in lysosomal enzymes. It has been reported that the cells of the "clasts" family contain high concentrations of specific lysosomal enzymes of particular interest is, tartrate resistant acid phosphatase (TRAP). The TRAP enzyme is one of many isozymes in a broad spectrum of acid phosphatase enzymes (Hammerstrom *et al.*, 1971). The presence of TRAP within a cell membrane is considered to be a cytochemical marker for osteoclasts (Minkin, 1982; Cole and Walters, 1987). In the present study the TRAP stain was used to locate and quantify the osteoclast-like cells as well as their immediate precursors.

It is possible that a correlation exists between the periodontal ligament vascularity and TRAP cells found within the ligament. An increase in TRAP positive cells may suggest an increase in bone resorption and a possible correlation with more tooth movement. Hence, the relationship between TRAP-positive cells and blood vessel characteristics should be examined.

MICROCIRCULATION

All body functions are dependent on the steady stream of blood flowing through millions of microscopic vessels collectively known as the microcirculation (Renkin *et al.*, 1984; Granger, 1988). The microcirculation connects the arterial and venous systems allowing the most important function of circulation, that is transport of nutrients to the tissues and removal of cellular excreta, to occur (Zweifach, 1961; Guyton, 1991). The arteries and veins are the primary channels for blood flow, while lymphatics divert tissue fluid back into the venous system via a succession of lymph channels. The microcirculation maintains cellular and intercellular hydration in addition to transferring nutritive substances into the intercellular space for subsequent cellular uptake. The arterioles, minute arteries which control the blood flow to each tissue area, and local conditions in the tissues themselves control the diameters of the arterioles in turn. Consequently, each tissue in most instances controls its own blood flow in relation to its needs.

The vascular system is classified based on the structure of the vessel wall. The ratio of the thickness of the arterial wall to the lumen is greater than for veins; arteries

can also be distinguished from veins by the greater amount of smooth muscle and elastin in their media. Arterioles, small arteries, regulate blood flow between arteries and the capillary bed (Zweifach, 1961; Sims, 1983).

The special needs of each organ of the body are met by a specific organization of the microcirculation. Generally each nutrient artery entering an organ branches six to eight times before the arteries become small enough to be considered "arterioles," which have internal diameters less than 20 micrometers. In turn, the arterioles branch two to five times, reaching diameters of 5 to 9 micrometers at their ends where they supply blood to the capillaries which are the principal sites of the exchange between blood and tissue of nutrients, waste products, fluid and other materials. From the arteriole the blood passes into a series of metarterioles, called terminal arterioles by some physiologists. Metarterioles have a structure which is midway between that of arterioles and capillaries. The blood enters the capillaries once it leaves the metarteriole. Some of the capillaries are large and are called preferential channels while the smaller ones are called true capillaries. Once the blood passes through the capillaries it then enters the venule and returns to the general circulation (Zweifach, 1961; Guyton, 1991).

The diameter of the arterioles may change manyfold, since it is a highly muscular endothelial tube wrapped with one to three layers of smooth muscle cells. The metarteriole, unlike the arteriole, lacks a continuous muscular coat, however, smooth muscle fibres encircle the vessel at intermediate points. A smooth muscle fibre usually encircles the capillary at the point where the true capillaries originate from the metarterioles. This point is called the precapillary sphincter, having the capacity to open

and close the entrance to the capillary. Capillaries are the major component of the microcirculation and they compose more than 90% of the vascular system. The capillary has a diameter ranging from 4 to 9 micrometers. Interestingly, this diameter is just large enough to allow passage of the erythrocyte (about 8 micron) through the circulatory system (Zweifach, 1961; Guyton, 1991). Capillaries, composed of a single layer of endothelial cells surrounded by a basal lamina, permeate the tissues thoroughly and are not usually greater than 25 to 50 microns from any cell (Zweifach, 1961; Guyton, 1991).

Lining the entire circulatory system are endothelial cells which are joined by intercellular junctional complexes. These cells are less than one micron thick except in areas where the nucleus causes bulging of the cytoplasm (Zweifach, 1961).

Venules are generally larger and more numerous than arterioles and have a weaker muscular coat. As a result of this a larger fraction of tissue blood volume is resident in these microvessels (Granger, 1988). The pressure in the venules is much less than that in the arterioles, however the venules can still contract considerably (Guyton, 1991).

This typical arrangement of the microcirculation is not found in all parts of the body; however, some similar arrangement serves the same purpose. Of extreme importance, the metarterioles (and the precapillary sphincters when they also exist) are approximated to the tissues that they serve. Hence, the local conditions of the tissues such as the concentrations of nutrients, end products of metabolism, hydrogen ions, and so forth can directly effect the rate of blood flow in each minute tissue area (Guyton, 1991).

Microvascular function reflects the behaviour of two primary tissue types, endothelium and vascular smooth muscle; however deeper insight into the cellular basis of microcirculation function still needs to be examined (Granger, 1988).

Blood flow through capillaries is not usually continuous but rather intermittent, being turned off and on every few seconds or minutes. This on/off phenomenon is called vasomotion, meaning intermittent contraction of the metarterioles and precapillary sphincter (Granger, 1988; Guyton, 1991).

Currently, the concentration of oxygen in the tissues, appears to be the most important factor affecting the degree of opening and closing of the metarterioles and precapillary sphincter. When the oxygen demand is high as in tissue injury, the intermittent periods of blood flow occur more often and the duration of each period of flow lasts longer thus allowing the blood to carry increased quantities of oxygen (and other required nutrients) to the tissues. Conversely, during times of low oxygen demand the intermittent periods of blood flow are reduced. While capillary blood flow is intermittent, there are so many capillaries present in the tissues that there is a constant rate of blood flow through each tissue capillary bed, constant capillary pressure, and a constant rate of transfer of substances between the blood of the capillaries and the surrounding interstitial fluid. This constant function is in reality the function of literally billions of individual capillaries, each operating intermittently in response to the local conditions in the tissues (Guyton, 1991).

The periodontal ligament is like other organs in that it too has a microcirculatory system which reacts in accordance to its needs for viability; such as during tooth movement. However, the mechanism for capillary formation is angiogenesis.

Angiogenesis:

Angiogenesis is an important event during several processes, including wound healing, inflammation, recanalization of thrombi, tumour growth and metastasis, normal growth and development, and is also important to immunological reactions (Polverini *et al.*, 1977a; Wagner, 1980; Nicosia *et al.*, 1982; Knighton *et al.*, 1990). After wounding, the formation of a rich vascular bed is essential for optimal reconstitution of parenchyma and repair. It is also evident that this process is dynamic and usually transient in nature during the repair process. The stimulation and inhibition of the endothelial cell during wound repair has been the subject of past studies. In addition, the fact that the metastatic spread of a variety of neoplasms is dependent on angiogenesis has stimulated work in the area of tumour-induced angiogenesis and its control (Langer *et al.*, 1980; Crum *et al.*, 1986; Ingber *et al.*, 1986b).

Angiogenesis is the development of new blood vessels within a tissue enabling the circulatory system to extend into wounded/avascular regions (Nicosia *et al.*, 1982; Folkman, 1985). We know little about the basic mechanisms of angiogenesis, in particular, how endothelial cells form buds, establish patency and anastomose with adjacent vessels. Most studies of angiogenesis utilize *in vivo* models of the rabbit ear chamber, the rat cornea, the dorsal sac of the rat, and the chorioallantoic membrane of

the chick embryo (Clark *et al.*, 1932; Gimbrone *et al.*, 1974; Nicosia *et al.*, 1982). Some of the earliest *in vivo* studies of angiogenesis were by Lewis (1931) where he described the growth of capillaries in cultures of chick embryo skin.

Stimulation:

Angiogenesis is regulated by local acting growth factors. Direct angiogenesis factors stimulate capillary formation by acting directly on endothelial cells. Indirect angiogenesis factors stimulate inflammation which then elicits angiogenesis (Folkman, 1985; Knighton *et al.*, 1990).

Inflammation and coincident migration of monocytes and macrophages is often necessary for successful wound healing. Monocytes/macrophages produce many locally active growth factors (for new capillary formation) which could influence angiogenesis (Wissler, 1982; Polverini *et al.*, 1977b; Knighton *et al.*, 1990).

"Angiogenic factors" may produce stimulation, migration, or proliferation of endothelial cells (Leibovich *et al.*, 1988). Vascularized tissue response to injury is dependant upon complex and diverse endothelial cell activities. Angiogenesis is required for the development of granulation tissue during wound healing (Leibovich *et al.*, 1988). The angiogenic response of soft tissue injury, allows division of angiogenesis into the following main events: stimulation, migration, proliferation, tube formation, stabilization/differentiation and regression/downregulation. Regardless of the type of angiogenic stimulus, capillary growth occurs by this series of sequential steps (Madri *et al.*, 1988).

In order for angiogenesis to be initiated there must be a stimulus. Normally microvascular endothelial cells have complex intercellular junctions and are surrounded by a basement membrane (Rhodin, 1974). When soft tissues are injured, a variety of cellular and tissue factors are released into the damaged tissue. Two of these compounds, histamine and serotonin, are potent vasoactive agents that bind to specific membrane-bound receptor molecules on the luminal surface of postcapillary venule endothelial cells (Simionescu *et al.*, 1982). These vasoactive compounds once bound to the cell surface, cause both the separation of the cell-cell junctions and retraction of the endothelial cells, thereby allowing increased flow of fluids and cells from the vessel lumen into the surrounding interstitium. Stimulated microvascular endothelial cells also permit leukocyte adhesions and diapedesis (Robbins *et al.*, 1984).

In order for angiogenesis to occur, the endothelial cells must be freed from the constraints of their investing basement membranes. It has been reported that migrating leukocytes produce and secrete collagenases which degrade vascular basement membrane, freeing the endothelial cells from their basement membranes (Horowitz *et al.*, 1977; Sholley *et al.*, 1978; Mainardi *et al.*, 1980; Russo *et al.*, 1981). Evidence for this phenomenon is unclear since extensive fragmentation of the microvascular basement membrane is not detected during leukocyte emigration. An alternative hypothesis, is that microvascular basement membrane may be breached by enzymes produced by endothelial cells. Gross *et al.*, (1982, 1983); and Kalebic *et al.*, (1983) have reported that when induced to migrate, cultured endothelial cells produce several enzymes, specifically plasminogen activator, plasmin, and collagenases, all capable of digesting basal lamina

components. Thus endothelial cell proteases provide an attractive explanation for the loss investing basement membrane that precedes endothelial cell migration and proliferation during angiogenesis.

In addition to stimulation factors within the inflammatory exudate, degradation products of matrix and autocrine stimulation by endothelial cell-derived factors, the microvascular endothelial cells may also be stimulated by the drastic changes in the surrounding matrix, from basement membrane to interstitial connective tissue. There is a degree of complexity in the endothelial cell stimulation that occurs during the early phases of inflammation and neovascularization derived in part from resident mast cells and macrophages, recently emigrated leukocytes, endothelial cells themselves, and the matrix components in the immediate extracellular environment (Madri *et al.*, 1988).

Migration:

The migration of microvascular endothelial cells into the injured area is paramount to the formation of granulation tissue (Bryant, 1977; Robbins *et al.*, 1984). This migration may be the result of one or more environmental factors, such as (1) chemotactic factors secreted by leukocytes; (2) degradation products of various matrix components; (3) release from investing basement membrane constraints; (4) change in matrix composition from a basement membrane to interstitial connective tissue; (5) change from a polarized two-dimensional to a relatively nonpolarized three-dimensional extracellular environment. However, microvascular endothelial cells are metabolically active, mitotically quiescent cells invested by an identifiable basement membrane. The basement membrane provides the overlying endothelial cells with an organized

substratum for attachment and expression of a differentiated phenotype (Folkman *et al.*, 1979; Denekamp, 1982) but does not allow any significant migration.

Leukocytes precede vascular ingrowth into an injured area during the inflammatory process. It is scientifically accepted that leukocytes contain chemotactic factors that are released when they are stimulated by inflammation (Madri *et al.*, 1988). The release of these factors may then initiate a migratory response by the surrounding microvascular endothelial cells. However, in the absence of leukocytes, angiogenesis seems to occur; thus it is believed that release of chemotactic factors by leukocytes is not essential for initiation of angiogenesis and endothelial migration (Sholley *et al.*, 1978). Therefore, during angiogenesis many factors are available that can act as stimulator of the migratory response.

Significant connective tissue destruction occurs during an angiogenic response in inflammation. Matrix component fragments are known to be chemotactic to a number of mesenchymal cells (Postlethwaite *et al.*, 1976, 1978; Chaing *et al.*, 1978; McCarthy *et al.*, 1984) however there are no indications that microvascular endothelial cells should be different. Matrix components dramatically affect endothelial cell attachment and migration (Folkman *et al.*, 1980; Madri *et al.*, 1982). The matrix components and their breakdown fragments appear to provide a stimulus for endothelial cell migration during the angiogenic response.

Physical constraints imposed on cells by surrounding supporting structures (matrices) can be powerful modulators of cell function, and release from this organization/orientation by partial destruction of the basement membrane may be

sufficient to stimulate and initiate endothelial cell migration (Ingber *et al.*, 1985). In the resting state, microvascular endothelial cells exist in a polarized environment, having a luminal surface in contact with a fluid phase and an abluminal surface in intimate contact with the basement membrane (Yannariello-Brown *et al.*, 1985a,b; Madri *et al.*, 1987). Thus it is possible that the asymmetric, polarized cell contact with the basement membrane might indicate a polarized distribution of cell-surface matrix-binding proteins. Asymmetric localization of cell-surface matrix-binding proteins may be important to the organization of the cell cytoskeleton, which may permit the expression of a particular cell phenotype (nonmigrating, mitotically quiescent, metabolically active and differentiated). This change of microenvironment, and the probable subsequent changes in type and distribution of cell-surface matrix-binding proteins and cytoskeleton may permit the expression of an alternative phenotype, namely a migrating, mitotically active cell type (Terranova *et al.*, 1984; Madri *et al.*, 1987). *In vitro* studies of vascular endothelial and glandular epithelial cells demonstrated dramatic changes in cell morphology, cell function, and multicellular organization when the cells were placed in or migrated into a three-dimensional environment. This supported the concept of extracellular matrix-mediated change in cellular phenotype (Hall *et al.*, 1982; Bissell *et al.*, 1982; Madri *et al.*, 1986; Lwebuga-Mukasa *et al.*, 1984; Ingber *et al.*, 1986a).

Organization of the cell substratum is an important determinant of cell function; however, the surrounding extracellular matrix is also important. Following release from their investing basement membranes (containing type IV collagen), endothelial cells come into contact with the interstitial matrix components, consisting mainly of types I and III

collagen (Stenn *et al.*, 1979). This change in adjacent matrix composition has been implicated in changes in subsequent cell behaviour. Microvascular endothelial cells grown on native or denatured interstitial collagen (type I or III collagen), form a confluent monolayer; growth on selected basement membrane components (type IV collagen or laminin) causes them to form multicellular tube-like structures (Madri *et al.*, 1986). Pratt *et al.*, (1984); Madri *et al.*, (1986); Leto *et al.*, (1986); and Form *et al.*, (1986) have all demonstrated this apparent relationship between matrix composition and cell behaviour in other vascular endothelial cells. Local changes in matrix composition may be caused by endogenous endothelial cell-matrix biosynthesis during this period. Madri *et al.*, (1986), reported that cultured microvascular endothelial cells alter their profiles of collagen biosynthesis according to the substratum on which they are grown. For example, microvascular endothelial cells grown on plastic, produced less type IV and V collagen compared to cells cultured on collagenous substrate. Furthermore, cells cultured on interstitial collagen substrate synthesize types IV and V collagen in a 1:2 ratio, while cells cultured on basement membrane collagen substrate have a 1:1 ratio between types IV and V. Thus, as microvascular endothelial cells migrate through an interstitial matrix, they probably continue to alter biosynthesis of matrix proteins. Previous experiments with large vessel and microvascular endothelial cells and epithelial cells have demonstrated a definite staggered appearance of denovo synthesized matrix components during migration. At the migratory front, type V collagen and laminin are the first matrix molecules to appear in close apposition to the migrating cells. Later at some distance from the migratory front edge, laminin, followed by type IV collagen, are

deposited beneath the cells and become part of the structurally identifiable basement membrane (Stenn *et al.*, 1979; Hintner *et al.*, 1980; Stanley *et al.*, 1981; Madri *et al.*, 1982; Form *et al.*, 1986).

Proliferation:

Proliferation of neovascular endothelial cells occurs early in the angiogenic phase. It has been suggested by Ausprunk *et al.*, (1977); and Burger *et al.*, (1981), that the cells immediately distal to the migratory front make up the actively proliferating cell region. Proliferative stimuli are diverse and complex, including (1) proteases acting cell surfaces, (2) mitogenic factors secreted by leukocytes and degradation products of matrix components, (3) release from the constraints of basement membrane, (4) changes in matrix composition, (5) changes from a two-dimensional to a three dimensional cell environment, and (6) migration. Let us now examine each of these factors in greater detail.

Following injury there is release and activation of a number of proteases (Madri *et al.*, 1988). Thrombin is an enzyme known to interact with endothelial cells enhancing the mitogenic potential of fibroblast growth factor (Isaacs *et al.*, 1981). Thus, following injury and activation of the clotting cascade, activated thrombin may trigger endothelial cell proliferation by 1) direct interaction with the endothelial cell surface and 2) enhancing the formation of a fibrin network which provides a scaffold for the neovasculature (Nicosia *et al.*, 1987).

In recent years a multitude of endothelial cell growth factors have been identified (Lobb *et al.*, 1986). From these studies, it is evident that a diverse number of molecules

exhibit this activity, however they may have similar features, in particular an affinity for heparin. Increased levels of endothelial cell growth factors enhance the suppressive effect on angiogenesis by certain steroids (Crum *et al.*, 1986). A great deal of work still needs to be done in order to more clearly define the roles of growth factors in angiogenesis.

Sholley *et al.*, (1977) successfully demonstrated the relationship between cell migration and proliferation. During angiogenesis, migration always precedes proliferation by approximately 24 hours (Ausprunk *et al.*, 1977; Burger *et al.*, 1981; and Sholley *et al.*, 1984). Hence, the loss of cell-cell and cell-substratum contact and subsequent cell migration (triggered in part by loss of basement membrane structural integrity) may stimulate endothelial cell proliferation. The stimulus may be the result of complex changes in the way the cells interact with the matrix. It is generally agreed that matrices contain information resident within their structure/composition. Thus, it is not unreasonable to presume that microvascular endothelial cells perceive this information via cell-surface receptors (Madri *et al.*, 1982; Lesot *et al.*, 1983; Madri *et al.*, 1983, 1984; Brown *et al.*, 1983; Yannariello-Brown *et al.*, 1985a,b). Information may then be transferred from cellular membrane proteins, (such as spectrin and protein 4.1), to the filamentous intracellular cytoskeletal network to the cell nucleus, eliciting changes in cell behaviour and proliferative rate (Folkman *et al.*, 1978; Pratt *et al.*, 1984; Leto *et al.*, 1986; Madri *et al.*, 1987). Cell shape may be the end result of complex, incompletely understood behaviour. Folkman *et al.*, (1979) and Schor *et al.*, (1979) reported that a gelatin substratum was required for continual culture of capillary endothelial cells, consistent with a permissive effect on matrix growth suggested by Gospodarowicz *et al.*,

(1980, 1983). Later studies showed that microvascular endothelial cells cultured on different matrix components exhibited different rates of proliferation, which correlated with differences in cytoskeletal organization, interactions with matrix, and cell shape (Nicosia *et al.*, 1982; Madri *et al.*, 1984; Kramer *et al.*, 1984). Thus, it may be deduced that release of endothelial cells from the constraints of the basement membrane, exposes them to a microenvironment that allows for an increased proliferation rate. Madri *et al.*, (1984) and Kramer *et al.*, (1984) proposed that endothelial cells respond to their microenvironment by altering their matrix biosynthetic profiles in a variety of ways. Synthesis of matrix components during angiogenesis has been demonstrated to be similar to that for migrating aortic endothelial cells and epithelial cells (Stenn *et al.*, 1979; Madri *et al.*, 1982). From these studies it was concluded that migrating aortic endothelial cells synthesize and secrete laminin early in the migratory phase, while type IV collagen is detected later. Also, during angiogenesis, particular matrix components appear to have a regulatory effect on proliferation rate *in vitro* (Form *et al.*, 1985, 1986). More specifically, endothelial cells grown on a laminin substratum (a matrix component evident soon after injury) exhibit a high proliferation rate, while cells grown on type IV collagen (a component observed later in the response) exhibit a much lower rate. Furthermore, mixing type IV collagen with laminin elicited a lower proliferation rate, further suggesting specific matrix modulation of cell proliferation (Form *et al.*, 1986).

The change in dimensionality experienced by the endothelial cells, should also be considered as a potential modulator of proliferation during angiogenesis. For example, the change from a two-dimensional environment (luminal and abluminal) to a three-

dimensional one where the cells are completely surrounded by matrix (with resultant changes in types of and organization of cell surface matrix-binding molecules and cytoskeleton) may greatly affect cell proliferation. While invested by a basement membrane, microvascular endothelial cells are known to exhibit a very low mitotic index, however, Burger *et al.*, (1981) demonstrated that cells in the interstitium near the migratory front in angiogenesis are very mitotically active. As angiogenesis progresses, tube formation occurs concurrent to deposition of a basal lamina-like material. Mitotic index is reduced in these areas, suggesting that changing from a three-dimensional to a polarized two-dimensional environment influences cell proliferation rate.

Tube formation:

Tube formation is observed in areas proximal to the migrating and proliferating cells, in the later stages of angiogenesis. At the present time this complex phenomenon is not completely understood. Cell and tissue factors, matrix composition, organization and dimensionality, and cell-cell interactions are important factors in tube formation (Bryant, 1977; Folkman *et al.*, 1980; Wagner, 1980; Madri *et al.*, 1986; Montesano *et al.*, 1983). The cell-matrix and cell-cell interactions in the development and maintenance of tubes during angiogenesis is very important. Following migration and proliferation, endothelial cells form highly organized, three-dimensional structures with lumina. Lumen formation is thought to occur by means of both intercellular and intracellular mechanisms (Wagner, 1980). One theory of lumen formation suggests that they result from either the development of spaces between the plasma membranes of adjacent cells or the joining of plasma membrane processes of individual cells and/or adjacent cells.

Montesano *et al.*, (1983) demonstrated lumen formation by two and more contiguous endothelial cells in collagen gels. Madri *et al.*, (1988), have demonstrated the presence of tube-like structures composed of endothelial cell plasma membrane processes joined by junctional complexes (Williams *et al.*, 1980; Maciag *et al.*, 1982; Pratt *et al.*, 1985). An alternative mechanism for lumen formation involves development of extensive intracellular vacuolization followed by intracellular vacuolar fusion, and ultimately cell-cell plasma membrane fusion, forming a seamless endothelium. Support for this hypothesis comes from laboratory studies; in particular, the discovery of ring cells, which have no intercellular junctions and form a continuous cytoplasmic ring (Folkman *et al.*, 1980). Extensively vacuolated cells have amorphous material in the lumen and typical luminal plasmalemmal membrane specializations, including plasmalemmal vesicles with and without diaphragms, coated pits, and fenestra-like structures, consistent with the second mechanism of tube formation (Folkman *et al.*, 1980). Since there is good evidence in support of both of these hypothetical mechanisms they should not be considered as being mutually exclusive (Madri *et al.*, 1988).

Stimuli for tube formation are also not fully understood. Various conditioned media and endothelial cell growth factor preparations are necessary for the development of tubes *in vitro*, suggesting that a variety of cell- and tissue-derived factors may be involved in the *in vivo* process (Folkman *et al.*, 1979, 1980; Zetter, 1980; Fenselau *et al.*, 1981; Maciag *et al.*, 1982; Alessandri *et al.*, 1983). The surrounding substratum also appears to be important to tube formation. Schor *et al.*, (1979) and Montesano *et al.*, (1983) suggested that matrix composition, organization, and integrity are controlling

factors of various stages of the angiogenesis process. For example, Folkman *et al.*, (1980) noted that tube formation by cultured capillary endothelial cells required a gelatin substrate. Madri *et al.*, (1983) noted more specific effects of cell substrate. Madri *et al.*, (1983) further showed that capillary endothelial cells formed tubes rapidly when exposed to types IV and V collagen, as compared to the interstitial collagen (types I and III). In other experiments, the basement membrane aspect of the amnion supported rapid tube formation relative to its stromal aspect (Madri *et al.*, 1983, 1986). An amorphous electron-dense basal lamina-like material surrounding the abluminal surfaces of the endothelial cells following tube formation was further demonstrated (Montesano *et al.*, 1983). The concept that specific matrix components have significant influences in directing endothelial cell behaviour during angiogenesis, is consistent with the above observations.

The role played by the surrounding interstitial tissue should not be reduced, in the wake of the evidence suggesting that the appearance, accumulation and organization of basement membrane components promotes rapid tube formation. The potential roles of the surrounding interstitial tissue are: (1) trapping and stabilizing secreted basal lamina components in areas immediately adjacent to the endothelial cells, providing the desired cell-matrix interactions for tube formation; (2) creating a change in endothelial cell connective tissue biosynthesis, resulting in an increased basement membrane component synthesis. Cultured microvascular endothelial cells change their collagen synthetic profiles in response to changes in underlying substratum (Madri *et al.*, 1983, 1986). Furthermore, endothelial cells exposed to interstitial or basement membrane components

synthesize less interstitial and more basement membrane collagenous components than the same cells grown on a plastic substratum (Madri *et al.*, 1988).

New capillaries must be stabilized to attain functional integrity. Their stability can be achieved by envelopment in a continuous basal lamina synthesized by the capillary endothelial cells themselves. The basal lamina structure itself being highly organized may provide a stable attachment surface for the two-dimensional polarized configuration. Additionally the basal lamina may afford the capillary a reasonable degree of strength against the forces of distention that may otherwise destroy the cell-cell junctional complexes that exist in the capillary bed. An additional means of stabilization may be in the information resident in the matrix and its organization/composition. This matrix may specifically structure the biosynthetic machinery of the endothelial cells via cytoskeletal organization, thereby directing luminal and abluminal secretion of specific functional and structural cell products that allow for the maintenance and proper physiologic function of the capillary network (Bissell *et al.*, 1982). *In vitro* models give support to this hypothesis of stabilization as follows (1) MDCK cells form tube-like structures with apical microvilli and cultured mammary gland epithelial cells form acinar-like structures and secrete milk-specific proteins into luminal spaces when cultured in collagen gels (Sholley *et al.*, 1977; Lesot *et al.*, 1983; Lee *et al.*, 1984); (2) type II pneumocytes grown on amnion basement membrane maintain their polarized cuboidal morphology and apical microvilli and secrete lamellar bodies into the medium; these same cells cultured on the interstitial surface of the amnion take on a flattened morphology and become devoid of microvilli and lamellar bodies (Lweubuga-Mukasa *et*

al., 1984); (3) rat pancreatic adenocarcinoma cells cultured on amnion basement membrane exhibit polarized distribution of zymogen granules, a golgi complex, nucleus and lipid droplets (from apex to base). However, when cultured on the interstitial aspect of the amnion, these cells attach poorly and remain rounded and unpolarized (Ingber *et al.*, 1986a).

Tube formation is an inherent property of the endothelial cell, but the microvascular vessel also has the pericyte which is closely associated (Joyce *et al.*, 1985). The possibility that the pericyte may play a role in newly formed capillary maturation, stability, and function, exists. The function of the pericyte is unknown. However, (1) it may regulate the tone of the microvasculature due to its contractile properties or (2) it may assist in the maintenance of the vascular basement membrane. In order to ascertain the precise function of this cell, additional *in vitro* and *in vivo* experiments are required (Madri *et al.*, 1988).

With injury, there is subsequent capillary ingrowth, thereby increasing blood flow to the injured area. This repair process continues with the reconstitution of parenchyma or replacement with new connective tissue (Bryant, 1977; Robbins *et al.*, 1984). Upon completion of repair to the injured site, the recently formed capillary network and associated pericytes and basal laminae are removed, a process termed "regression". The regression phase has been studied by Ausprunk *et al.*, (1978) and Folkman (1985) using a corneal pocket assay. Removal of the angiogenic stimuli produces mitochondrial swelling (within endothelial cells) at the migratory front. Vascular stasis occurred as a result of platelet adhesion to the endothelial cell laminal surfaces, followed by endothelial

cell death and degradation of cell debris by local macrophages. Withdrawal of a specific angiogenic signal may be adequate to initiate regression, thus continuous positive stimuli are probably required for sustained angiogenesis. However, this does not necessarily exclude a requirement for negative or suppressive stimuli such as those observed in inhibition of tumour induced angiogenesis by heparin, particular steroids, and selected tissue factors, and the downregulation of smooth muscle proliferation by endothelial cell products (Langer *et al.*, 1980; Castellot *et al.*, 1981, 1982, 1984; Karnovsky, 1981; Azami *et al.*, 1984; Crum *et al.*, 1986; Ingber *et al.*, 1986b).

Whether the angiogenic signal originates from tumour, inflammatory agents, or immunological sources, the angiogenic sequence and events are similar (Folkman, 1985). A difference between tumour and normal blood vessel angiogenesis is the rate of endothelial proliferation. The rate of tumour endothelial proliferation can be 30-40 times that of normal vessels (Denekamp, 1982; Denekamp *et al.*, 1982; Hobson *et al.*, 1984). As angiogenesis takes place, vasoproliferation is accompanied by other components of inflammation such as white blood cell migration, edema, and fibroblast proliferation (Ausprunk, *et al.*, 1977). Conversely, there is a mild inflammatory reaction coincident to tumour-induced angiogenesis, hence an endothelial cell response predominates (Cavallo *et al.*, 1972). Angiogenesis coincident to wound healing is self limiting, whereas tumour angiogenesis is not. Once tumour-induced angiogenesis begins it continues indefinitely until all of the tumour is either eradicated or until the host dies (Folkman, 1985).

Regression:

The signals required for the initiation of the capillary regression phase in the wound-repair process are not understood. Potential signals could include (1) containment of the injury and subsequent downregulation of the acute inflammatory response, leading to a marked decrease in the production of various inflammatory cell and tissue derived stimulatory factors; (2) changes in the tone of the affected vasculature, resulting in stasis and thrombosis, followed by endothelial cell death and dissolution of the capillary network structure; (3) specific immune cell-mediated modulation of the neovascular endothelial cells and pericytes, leading to down regulation and death of the vascular cells and dissolution of their connective tissue scaffolding; and (4) epithelial and/or mesenchymal cell factors or products that downregulate neovascular cells such as transforming growth factor-beta (TGF-B) (Ignotz *et al.*, 1986; Frater-Schroder *et al.*, 1986).

Conclusion:

The organization of extracellular and interstitial tissue matrix and of cell membrane-cytoskeleton interactions as they relate to the endothelial cell repair response have been recently investigated (Wong *et al.*, 1983; Pratt *et al.*, 1984; Yurchenco *et al.*, 1984; Leto *et al.*, 1985). Several investigators have reported that matrix composition and organization influences cell behaviour (Hay, 1981; Madri, 1982; Bissell *et al.*, 1982; Madri *et al.*, 1983). There has been much speculation concerning the mechanisms by which information residing in the matrix is transduced to the cell, and evidence supports the existence of cell-surface matrix binding proteins in many cell types (Lesot *et al.*,

1983; Malinoff *et al.*, 1983; Terranova *et al.*, 1983, 1984; Brown *et al.*, 1983). Furthermore, investigators have speculated on the existence of a cell surface matrix receptor integral membrane protein cortical protein cytoskeletal nuclear transduction network capable of signal transduction to cytoplasmic compartments and nucleus (Bissell *et al.*, 1982; Hay, 1981). The existence of such receptors and a transducing network responsive to changes in matrix in endothelial cells, is generally supported (Pratt *et al.*, 1984; Madri *et al.*, 1987). Greater knowledge of the organization of extracellular matrices and of how microvascular endothelial cells and pericytes perceive and interact with the extracellular matrix will provide a better appreciation of angiogenesis and insight as to the optimal manipulation of the process so as to allow the most beneficial and optimal repair.

Neovascular inhibition and capillary regression also are important subjects for study. By learning more about these processes, strategies may be devised to permit reduction of scarring following some injuries.

During and subsequent to orthodontic tooth movement there is injury to and inflammation within the periodontal ligament. As a result of this injury and inflammation it is suggested that the angiogenic response occurs during and continues to some extent following orthodontic tooth movement, within the ligament. The effect that the angiogenic response may have during and subsequent to tooth movement has never been studied, however it may play some part in tooth movement and hence should be considered.

CHAPTER II

STATEMENT OF THE PROBLEM

CHAPTER II**STATEMENT OF THE PROBLEM**

Blood vessels have been implicated in having an effect on tooth position due to changes in hydraulic/interstitial pressure. Thus changes in blood vessels number, size, and proportional area may change interstitial pressure assuming the periodontal ligament volume remains constant assuming this is a closed system.

As a first step, this study will determine baseline data for changes in periodontal ligament blood vessel number, size, and proportional area with regard to level (apical, middle, cervical) of periodontal ligament, zone (bone to tooth), and presence or absence of orthodontic force at different times points after force change.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODSAnimals:

Twenty eight male Sprague Dawley rats, 190 ± 15 grams, were sedated with diethyl ether then anaesthetized by intramuscular injection of a 4:1 solution of ketamine/xylazine (0.23mg/100grams body weight). Four animals were assigned to each of the experimental groups: 0, 3, 5, 7, 10, 14, and 21 days. In each experimental animal the molar on the left half of the maxilla was not treated, or used as an internal control. A comparable group of age and weight matched animals were untreated and served as non-sham external controls. Hence in the entire project a total of 56 animals was used, this includes both experimental and control animals. The contralateral maxillary molar was not used as an internal control since it has been suggested that manipulation of a maxillary molar on one side causes changes in the other side (Row and Johnson, 1990).

The animals were fed standard rat chow and tap water ad libitum. The experimental and control animals were kept in cages with alternating periods of artificial light, e.g. LD 12:12 with light from 0600 to 1800 hours.

Appliance:

The appliance consisted of a 0.022" stainless steel coil, 0.007" stainless steel ligature wire, and stainless steel fine mesh material. The coil was soldered to the mesh pad posteriorly, while anteriorly it was attached through one of its loops to the steel ligature wire which was then twisted to secure it to the coil. (See Fig. 1 and 2).

Fig. 1 and Fig. 2

THE ORTHODONTIC APPLIANCE

Fig. 1

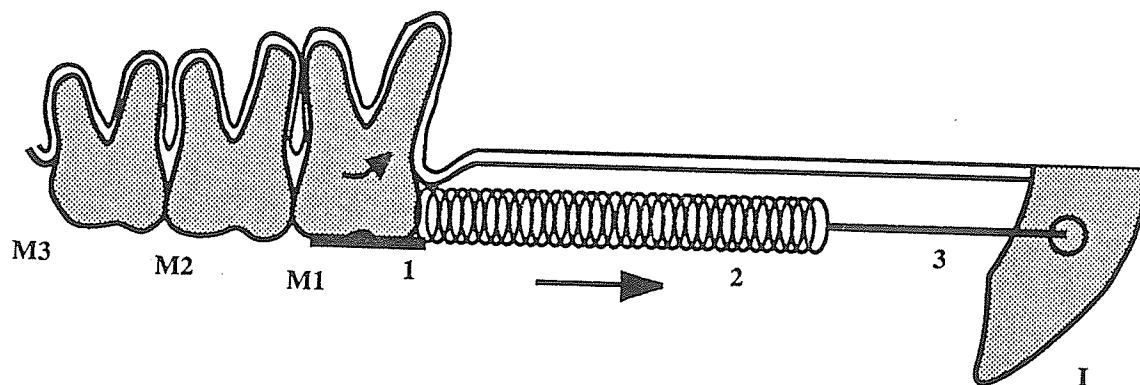
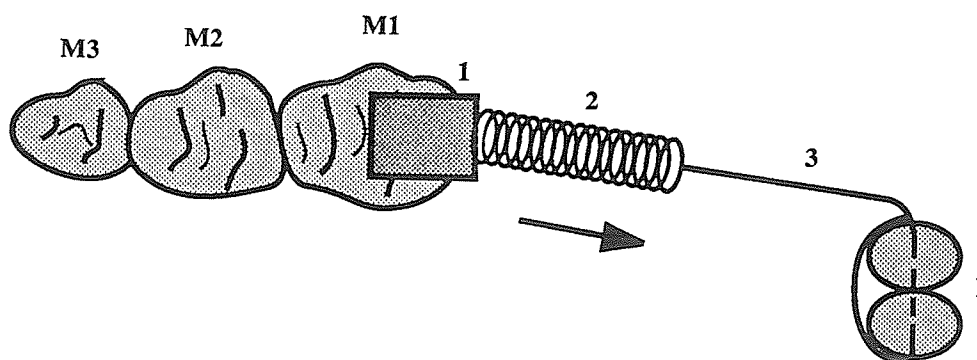


Fig. 2



-
- M1 = First molar
M2 = Second molar
M3 = Third molar
- I = Central incisor
- 1 = Occlusal mesh pad
2 = 0.022 closed coil (stainless steel)
3 = 0.007 stainless steel ligature wire

→ Force in a mesial direction

↗ Tipping of the molar in a mesial direction

Appliance Placement and Force Measuring Device Activation:

Following anaesthesia of the experimental animals, the animal is laid on its back on the retraction bed (a unit of the force measuring device). This allows for the elevation of the mandible and placement of the maxilla in a maximum opening position to allow placement of and subsequent activation of the spring. At the same time the cheeks and tongue of the animal can be retracted by 0.03" stainless steel wire looped at each end. In total there are four retractors on this "bed" one each for the mandible and maxilla, and two lateral ones for the cheeks. Once access to the molar was achieved the fine mesh pad was attached to the occlusal surface of the first molar, by cyanoacrylate cement. The molar and central incisors were etched for 90 seconds with a 35% phosphoric acid gel, washed and dried prior to bonding the fine mesh pad. The mesh pad was left for 30 minutes to allow sufficient bond to the tooth, although the polymerization reaction time is virtually instantaneous. The coil was then activated by pulling on the steel ligature anteriorly to achieve a 30 gram force. Once activated, the ligature was passed through a hole in the central incisors, which had been drilled with a 1/4" retention burr. The ligature was then wrapped around the incisor teeth and overlaid with 3M System One resin bonding material. Following appliance activation, the occlusal surface of the agonist molar and incisal edge of the lower incisor was reduced, using a 330 friction grip burr held in a high speed dental drill; to prevent any masticatory interference with the appliance. In addition the toe nails on all four feet were trimmed to prevent any attempt to dislodge the appliance. The net result of placing

the spring appliance on the maxillary first molar, is mesial tipping (Row and Johnson, 1990) with a force of 30 ± 1 g (experimental side).

Attaining a $30 \pm$ g force is accomplished by means of a force measuring device. This device is based on the calibration of an internal spring following Hooke's Law regarding load versus deflection. The spring is enclosed in a plexiglass cylinder which is attached at one end to a lever arm and the other end to a screw for tension adjustment. A scale on the wall of the cylinder denotes the amount of deflection produced by the spring. The ratio between the lever arm length and the pivot point indicates the ratio of force between the internal spring to force felt by the lever arm, that is the force delivered to the animal.

The pivot point of the lever and base are wired to a contact detector. A noise is produced by an electronic connection which is stopped when the desired force of 30 grams is produced. The appliance spring is connected to the lever arm of the device and the platform is moved posteriorly to activate the spring anteriorly.

Tissue Collection:

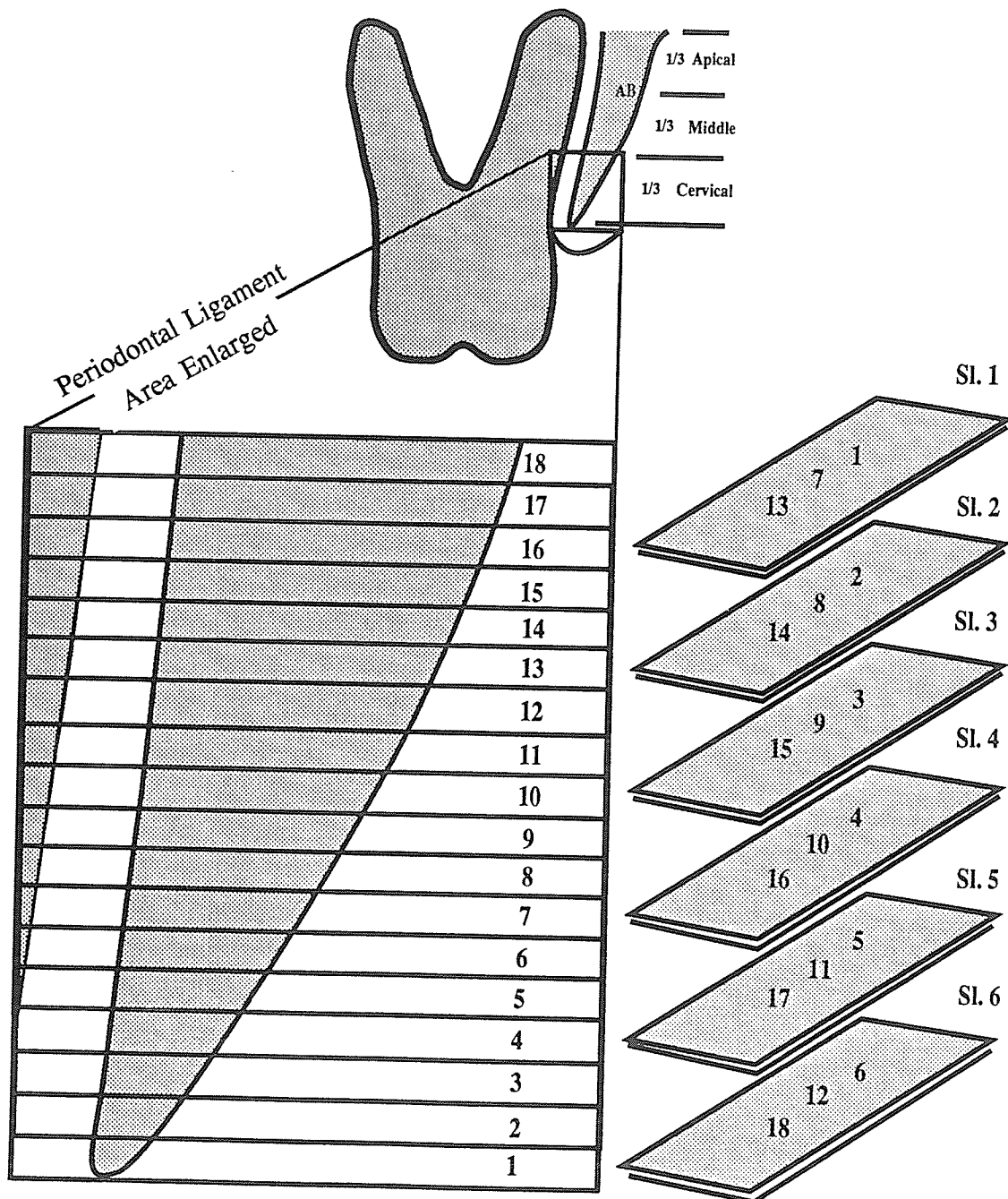
After two weeks, the springs were deactivated in the experimental quadrants, by cutting the wire between the central incisor and first molar tooth. Four animals were killed at the time of deactivation and 3, 5, 7, 10, 14, and 21 days after appliance deactivation. An equivalent number of external control animals were killed at corresponding time periods.

Prior to sacrifice all animals were anaesthetized as per appliance insertion and perfused via the inferior vena cava with Krebs-Ringer's bicarbonate buffer (pH 7.4)

(Sigma) supplemented with glucose (Sigma) (5 mm) and bovine albumin (Cohn, fraction V) (Sigma) (4g/100ml). The perfusion was for 30 minutes at room temperature under a constant flow of 0.6 ml/minute and a constant pressure of 45 cm H₂O. Each animal was then perfused with Karnovsky's fixative (Karnovsky, 1965) through the left ventricle. The right maxilla was removed, immersed in Karnovsky's fixative for 12 hours, and demineralized in 4.13% EDTA for approximately three weeks, pH 7.0 (Warshawsky *et al.*, 1967). Specimens were dehydrated in ethanol and embedded in paraffin wax.

Serial sections (6 μ m thick) in a horizontal plane from the base of the gingival sulcus to the apex of the tooth were made. The sections were assigned to three regions: representing, cervical, middle and apical thirds of the root. The first 18 sections from the cervical third, the middle 18 sections from the middle third and the last 18 sections from the apical third were used for further analysis. These sections were affixed to glass slides with a gelatin chrome alum composition. The first section from the cervical third was affixed to slide number one, section two onto slide two etc. to slide number six, then the procedure was repeated, assuring that each slide had three sections representing fields 36 μ m apart. This procedure was repeated for the middle third, and apical thirds of the tooth (Fig. 3).

Fig. 3
REPRESENTATION SHOWING HOW EACH HISTOLOGICAL SECTION
WAS MOUNTED FROM EACH LEVEL (CERVICAL MIDDLE APICAL)



Numbers = Histological section
 SL = Slide
 AB = Alveolar bone

Staining Procedures:

The second slide from each third of the tooth was stained with hemotoxylin and eosin (Luna, 1968). This stain readily allows differentiation of blood vessels and surrounding periodontal membrane.

The first slide from each third was used to study the permeability of the blood vessels using antibodies to bovine albumin and the protein A-Gold/Silver enhancement technique (Roth *et al.*, 1978; Bendayan, 1980; Bendayan *et al.*, 1980; Springall *et al.*, 1984; Ghitescu *et al.*, 1990), to localize the presence of extravascular bovine albumin. In following the staining techniques advocated by Bendayan (1980), it became evident that the amount of background staining present in the sections was too great. I then attempted to further dilute the concentrations of both the anti-bovine serum albumin (BSA) and the Protein A Gold, further dilutions were also insufficient as was omission of silver enhancement since visual was impossible. Thus vascular permeability was not included as part of the study.

Osteoclast like cells and their precursors were identified and counted from the slides stained for tartrate resistant acid phosphatase (TRAP) positive cells. The technique used to identify TRAP positive cells was according to that advocated by Cole *et al.*, 1987. This staining technique is specific for TRAP, a cytochemical marker for osteoclast cells and their immediate precursors. In this study this technique was used to identify osteoclasts which are the cells involved in bone resorption, a necessary step for orthodontic and physiologic tooth movement to occur.

The third slide from each third of the periodontal membrane was used in order to identify any TRAP positive cells within the periodontal membrane. TRAP positive cells were counted and assigned to a sector within the periodontal ligament (sectors will be explained in the next section) next to the bone or next to the tooth.

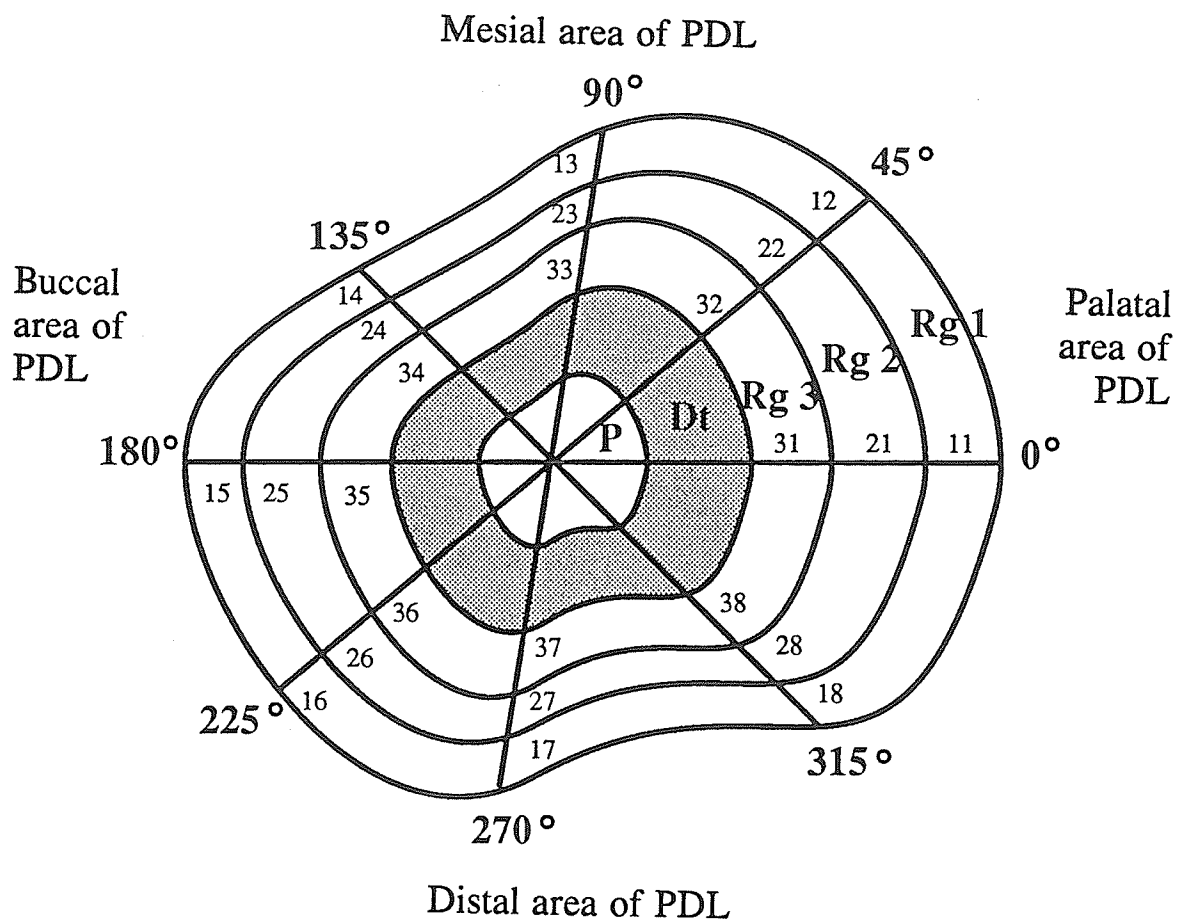
Histomorphometric Analysis of the Blood Vessels Within the Periodontal Ligament:

The slides stained with H & E were analyzed using the Kontron IBAS AT image analysis system (Kontron Eching, Munich, W. Germany). This system consisted of a Macroscope 5x-150x (M400-Leitz-W. Germany), digital T.V. Camera (Videk by Kodak). The image is held in high speed image memory by the system. The user/operator then defines his/her region of interest, in this case the inner and outer borders of the periodontal ligament of the mesio-buccal root of the maxillary right first molar, which is traced using a cursor. The system then automatically identifies the centre of gravity of the image and sends out radii from the centre of gravity at 4 degree intervals, starting at the mid-palatal region of the root. Isolated segments of radii that intersected the periodontal region are then identified as image coordinates for each end of the radii segments (inner and outer aspects of the periodontal ligament). From the end-points calculated the coordinates of two internal equidistant boundaries (bands) for each radius are created. Hence the periodontal ligament at each radius is divided into three equal distances thus creating three concentric zones within the periodontal ligament at each radius. To prevent errors, a minimum radius length of 42 μm was set. If the radius was shorter, it was extended to this minimal distance automatically. Once the three concentric circles within the periodontal ligament were established, the user then selected

the grey band that identified blood vessels (0=black, 255=white). The bands that are derived by the system are used as a mask through which the blood vessels are measured. Blood vessels on the boundaries of each circle are counted in the outer band. The entire periodontal ligament with its concentric circles is further subdivided into sectors by the radii which extend at approximately 0, 45, 90, 135, 180, 225, 270, and 315 degrees; thus each circle is divided. This effectively divides each periodontal ligament specimen into 24 sectors which are coded, as per (Fig. 4).

Fig. 4

DIAGRAM OF SECTORS CREATED BY THE IMAGING COMPUTER FOR
BLOOD VESSEL ANALYSIS (H & E STAINED SPECIMEN)



-
- P** = Pulp chamber of the tooth
Dt = Dentin and cementum of the tooth
Rg1 = Area of the periodontal ligament closest to the alveolar bone
Rg2 = Middle area of the periodontal ligament
Rg3 = Area of the periodontal ligament closest to the tooth
PDL = Periodontal ligament

Histomorphometrically, the IBAS counts the total number of blood vessels within the periodontal ligament and assigns them to one of the 24 sectors defined. The total area of each circle and each of the 24 sectors is also calculated, this allows for the proportional area of blood vessels within each sector to be calculated by the machine. It further calculates the following histomorphometric parameters of each vessel: area, maximum and minimum diameter, circumference, and shape. This procedure was carried out for each specimen at each level of the periodontal ligament from the cervical to the apical zone.

All of the analyzed specimens were oriented for analysis by the machine by the same individual, as a result it was felt that any error in orientation would be uniform for all specimens. Each specimen was oriented so that 90 degrees and 270 degrees were straight up and down.

Histomorphometry of the TRAP Cells:

Trap cells were counted (using a light microscope) in each specimen at each level and assigned to one of the 24 sectors previously described for the blood vessel histomorphometric section. This was done so as to allow some type of blood vessel to osteoclast like cell correlation.

STATISTICAL ANALYSIS

The raw data available for analysis are the characteristics of 27117 blood vessels found in specimens of tissue. The parameters recorded for each vessel found include dimension (length of major and minor axis) and location of vessel relative to a predefined origin. Each vessel was labelled as coming from an experimental or control site

(EXPCNTL) and which time Period from which it was examined 1-7 (PERIOD in time ie: 0, 3, 5, 7, 10, 14, or 21 days). Four animals (ANIMAL) were sacrificed at each of the fourteen Experimental/Control by PERIOD combinations. Nine histological sections were examined from each animal with a label identifying which replication (REPL) of the three taken from each of the three regions (REGION; that is cervical, middle or apical aspect of the periodontal ligament). Thus, a total of 504 histological specimens were examined.

Vessels found within each specimen were further identified as having been located in the inner (Ring 3), middle (Ring 2), or outer (Ring 1); with each ring divided into eight sectors (Sector) of 45 degrees each (Fig. 4).

Each vessel found was uniquely classified into one category by each of these factors. In summary:

Grouping factors (between animals):

EXPCNTL 2 levels

PERIOD 7 times (0, 3, 5, 7, 10, 14, 21 days)

Animals identified within each grouping factor combination:

ANIMAL 4 (within each of the 14)

Sub-units within each animal:

REGION 3 regions

RING 3 rings

Sector 8 forty-five degree sectors defining location

Sampling within sub-units:

REPL 3 replications of each REGION by RING by SECTOR
combination

The vessels found were summarized within the 12096 ($2 \times 7 \times 4 \times 3 \times 3 \times 8 \times 3$) categories of these factors. This summary of the vessels was made in terms of the following characteristics:

Number of vessels found per unit area.

Average area of vessels found per unit area.

Average area of vessels found.

Proportion of total area covered by a vessel.

Average ratio of major to minor axis length.

Proportion of the total number of vessels found that were at least 11 μm in minimum diameter.

To analyze whether the classification factors had any effect on determination of these vessel characteristics a split-unit repeated measures analysis of variance model was used. During exploratory univariate analysis large variation in patterns were observed in all variables across ring and region. Because of this, the dimension of the analysis was reduced by stratification on both ring and region. That is, separate models would be examined and interpreted for each of the ring-region combinations. This would reduce the 12096 combinations by a factor of 9 to 1344. Further examination suggested examination of four 90 degree sectors rather than eight 45 degree sectors, thus reducing each model to $(1344/2)$ 672 observations.

The level of significance for testing effects in the ANOVA (analysis of variance) models was set at 5% ($p < 0.05$). The level of statistical significance for comparison of mean values within level of significant main effects or interactions was adjusted using the Bonferroni correction. That is, the level of significance is divided by the number of comparisons being made. For example, when comparing mean differences between the four regions (mesial, palatal, distal and buccal) a total of six comparisons are made. Consequently, the level of significance of 0.05 (5%) will be adjusted to 0.05 divided by

6 to equal 0.008. From this point on it shall be understood that all statistical tests cited are statistically significant at $p < 0.05$ with the appropriate Bonferonni correction.

The outline of the ANOVA summary table for the models examined, with appropriate degrees of freedom, for a balanced (no missing observations) design is as follows:

Source of Variability	degrees of freedom
Between Animal (56-1)	55
EXPCNTL	1
PERIOD	6
EXPCNTL*PERIOD	6
ANIMAL (EXPCNTL*PERIOD)	42
Sub-units within each animal (56*(12-1))	616
Sector	3
SECTOR*EXPCNTL	3
SECTOR*PERIOD	18
SECTOR*EXPCNTL*PERIOD	18
Residual variability from replications	
Residual	574
TOTAL variability (672-1)	671

CHAPTER IV

RESULTS

CHAPTER IV

RESULTSTARTRATE RESISTANT ACID PHOSPHATASE DATA

Although TRAP positive cells were evident in the histological specimens, there were no statistically significant findings with regard to main effects or interactions. TRAP positive cells are an indication of osteoclasts or osteoclasts-like cells (Cole and Walters, 1987). The raw data for the TRAP positive cells may be found in Appendix A.

BLOOD VESSEL DATA

In the raw data a total of 27,115 blood vessels were found. The blood vessels were then separated into the following groups: control and experimental data, number of blood vessels found in each Sector, proportional area of blood vessels in each Sector, proportion of blood vessels 11 μm or greater, and ratio of maximum to minimum diameter.

Due to a resolution limitation 1011 vessels found in 127 quadrants were deleted. The limitation was created because in situations where the inner and outer boundaries of the periodontal ligament touched, the image analysis computer was unable to artificially extend this 'touching' area to the artificial minimal width, if a radius did not cross at this point. If the boundary was not extended, instead of dividing the periodontal ligament into three rings the computer would form some other number of rings or semi-circles greater than three. Forming more than three rings meant that each of the pieces formed would be less in area than the area expected to represent a ring. Therefore a situation

was created whereby it then became possible that the area of blood vessel occupation within each ring/piece would be greater than the area ring/piece itself, a non-sensible situation. This limitation explains why some blood vessels were deleted from statistical testing.

A summary of all the vessels found (raw data) in the study minus the 1011 which were deleted is as follows and may be found in Appendix B:

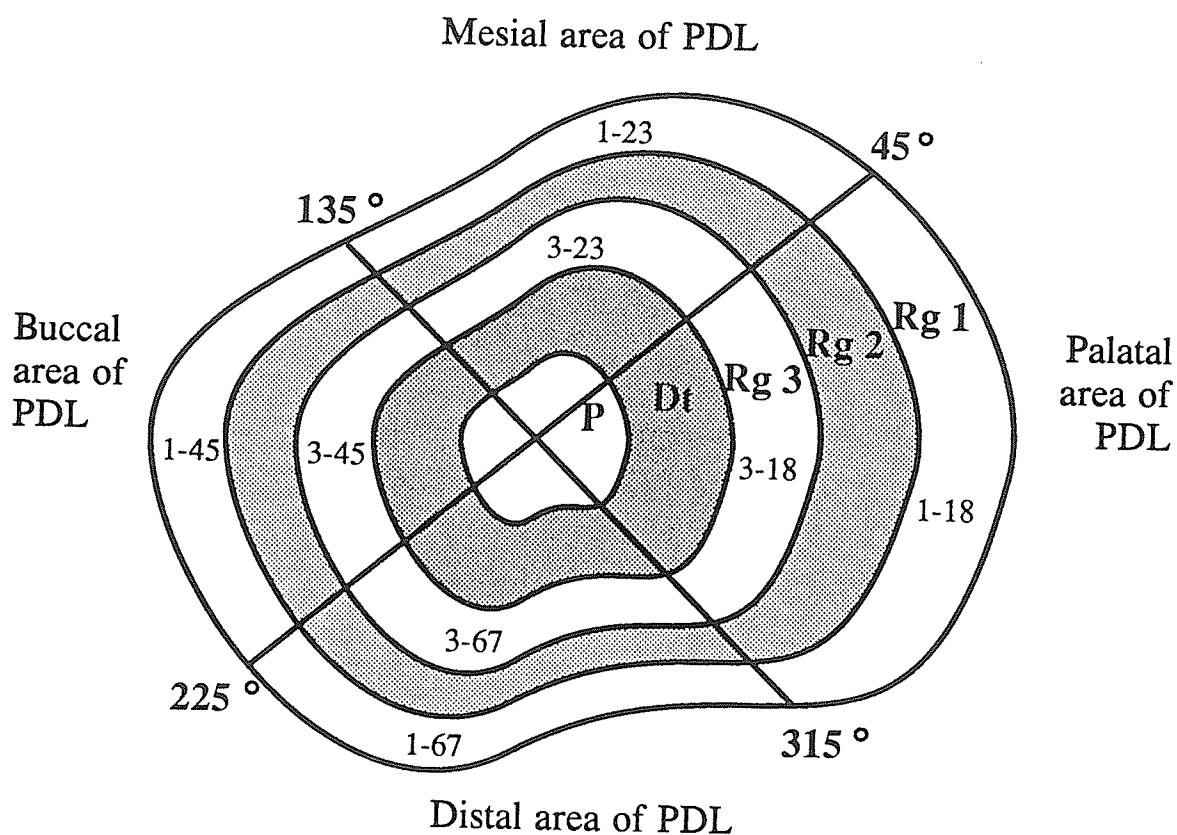
- (a) overall, it was found that of the remaining 26106 vessels which were analyzed, 44.2 % (12570) were found in the control group, while 47.7 % (13536) were found in the experimental group;
- (b) summarizing the 26106 vessels with regard to Period it was shown that at Period one 9.4 % (2463), Period two 17.6 % (4606), Period three 12.2 % (3183), Period four 12.2 % (3171), Period five 17.6 % (4584), Period six 17.2 % (4476), and Period seven 13.9 % (3623) vessels were found respectively;
- (c) with regard to blood vessels found at each region, in the apical region 31 % (8091); middle 43.91 % (11464); and cervical 25 % (6551) vessels were found respectively;
- (d) regarding which ring each vessel was found in, in ring one 60.1 % (15695); ring two 18.5 % (4825); and ring three 21.4 % (5586) vessels were found respectively;
- (e) with regard to vessel size it was found that approximately 50 % of all the vessels were less than 11 μm in minimum diameter.

Summarizing this data before applying any statistical tests indicated that there were more blood vessels in the Experimental group, at Periods two, five and six, in the middle and apical regions in Rings one and three, of all the vessels 50 % were approximately 11 μm or less. Raw data regarding the number of blood vessels found, proportional area covered by blood vessels, and the proportion of vessels 11 μm (or greater) may be found in Appendices C and D respectively.

After collecting the data in 8 sectors per ring it was decided to combine Sectors within each ring as follows: 1 and 8 to form sector 18; 2 and 3 to form sector 23; 4 and 5 to form sector 45; and 6 and 7 to form sector 67. This decision to combine Sectors was based on the fact that similarity of blood vessel characteristics was evident between the region that were combined. Also, histologically very few vessels were seen in the cervical region and middle rings in both the experimental and control animals. Hence in analyzing the data for statistical significance, efforts were concentrated on rings 1 and 3, and the apical and middle regions (Fig. 5).

Fig. 5

DIAGRAM OF COMBINED SECTORS FOR THE STATISTICAL
ANALYSIS OF THE BLOOD VESSELS



Combined mesial sectors (45°-135°)
 Combined palatal sectors (45°-315°)
 Combined buccal sectors (135°-225°)
 Combined distal sectors (225°-315°)

P = Pulp chamber of the tooth
 Dt = Dentin and cementum of the tooth
 PDL = Periodontal ligament

Shaded Rg2 = was not analysed
 Rg1 and Rg3 = were analysed

In order that the analyzed data may be more clearly understood, the various computer terms used in the analysis have been changed for the remainder of this thesis as follows:

- (a) sectors will be regions of the periodontal ligament, palatal for 18, mesial for 23, buccal for 45 and distal for 67;
- (b) rings within the periodontal ligament will be alveolar ring of the periodontal ligament for ring 1 and tooth ring of the periodontal ligament for ring 3;
- (c) periods will be referred to as time in days 0, 3, 5, 7, 10, 14, and 21; and
- (d) regions will be levels of the periodontal ligament, middle and apical.

Any unit of measure could have been chosen in analyzing the number of blood vessels found per $10,000 \mu\text{m}^2$, however the tooth ring is very small and upon dividing it into four regions the areas involved become smaller. As a result $10,000 \mu\text{m}^2$ appeared to be a good unit of area to use in analyzing the data.

From the raw data, statistical tests were run to answer the following questions:

1. Does the number of blood vessels found per $10,000 \mu\text{m}^2$ of periodontal ligament differ between different regions within each ring?
2. Does the proportion of the area covered by blood vessels differ between different regions within each ring?

The proportional area is equal to the total blood vessel area (of all diameters) divided by the total area of that region.

3. Does the proportion of the total number of blood vessels above $11 \mu\text{m}$ differ between the different regions within each ring?

The proportion of blood vessels $11 \mu\text{m}$ (or greater) is equal to the number of blood vessels $11 \mu\text{m}$ (or greater) divided by the total number of blood vessels in that region.

STATISTICAL FINDINGS FOLLOWING RAW DATA ANALYSIS

- 1) One of the questions asked was: Does the number of blood vessels found per $10,000 \mu\text{m}^2$ of periodontal ligament differ between different regions of each ring?

Alveolar Ring Apical Level: There were no significant differences between Experimental/Control by Time by Region, nor did any two factor differences exist between pairs of these three factors. That is, there were no differences between the Time by Region, Experimental/Control by Time, or Experimental/Control by Region. However, there were significant differences in the Regions. This implies that the Regions behave in the following way (Fig. 6- table-1):

The number of blood vessels found per $10,000 \mu\text{m}^2$ in the palatal region of the alveolar ring (1.923 ± 0.093) was significantly less than that found in the mesial and distal regions of the alveolar ring at the apical level.

The number of blood vessels found per $10,000 \mu\text{m}^2$ in the mesial region of the alveolar ring (2.59 ± 0.09) was significantly greater than that found in the buccal region of the alveolar ring at the apical level.

Alveolar Ring Middle Level: There was a significant two factor difference between Time by Region. This implies that there was a difference in the number of blood vessels found per $10,000 \mu\text{m}^2$ in each Region depending on the Time looked at as follows (Fig. 7- table 2):

- At 0 days: There was no significant difference.
- At 3 days: The mesial region of the alveolar ring (3.422 ± 0.03) was significantly greater than that found in the palatal region of the alveolar ring.
- At 5 days: There was no significant difference.
- At 7 days: There was no significant difference.
- At 10 days: The mesial region of the alveolar ring (2.5 ± 0.05) was significantly less than that found in the buccal and distal regions of the alveolar ring.
- At 14 days: There was no significant difference.
- At 21 days: There was no significant difference.

Tooth Ring Apical Level: There were no significant main effects, nor significant interactions.

Tooth Ring Middle Level: There were no significant main effects, nor significant interactions.

2) **Another Question: Does the proportion of the area covered in μm^2 by blood vessels differ between different regions in each ring?**

Alveolar Ring Apical Level: There was a significant interaction between the Time by Region. This implies that differences between Regions depends on which Time is being looked at, as follows (Fig. 8- table 3).

At 0 days: There was no significant difference.

At 3 days: The area covered in the mesial region of the alveolar ring (0.355 ± 0.036) was significantly greater than all other regions.

At 5 days: There was no significant difference.

At 7 days: The area covered in the palatal region of the alveolar ring (0.196 ± 0.035) was significantly less than the mesial and distal regions of the alveolar ring.

At 10 days: There was no significant difference.

At 14 days: The area covered by the mesial region of the alveolar ring (0.366 ± 0.035) was significantly greater than the distal region of the alveolar ring.

At 21 days: The area covered by the palatal region of the alveolar ring (0.150 ± 0.035) was significantly less than all other regions.

There was a significant difference between the Experimental/Control by Region. This implies that differences between the Regions depends on whether Experimental or Control group is looked at, as follows (Fig. 9- table 4):

Between Experimental/Control Groups: The area covered by the palatal region of the alveolar ring (0.265 ± 0.019) and distal region of the alveolar ring (0.319 ± 0.019) in the Experimental group was significantly greater than that in the Control group.

There was also a significant difference between Experimental/Control by Time. This implies that differences at different Times depend on whether Experimental or Control group is looked at, as follows (Fig. 10- table 5):

Between Experimental/Control: The area covered at 0 days (0.356 ± 0.05) and 7 days (0.403 ± 0.05) was significantly greater than in the Control group at the same Times.

Alveolar Ring Middle Level: There were no significant difference between Experimental/Control by Time by Region. However, the proportional area covered by blood vessels varied between Regions. This difference was unaffected by Time or Experimental/Control groups (ie: the Regions stayed in the same relative proportions across these factors) as follows (Fig. 11- table 6):

The area covered by the mesial region of the alveolar ring (0.228 ± 0.013) and distal region of the alveolar ring (0.253 ± 0.013) was

significantly greater than the area covered by the buccal and palatal regions of the alveolar ring.

There was an interaction between the Experimental/Control group by Time. This implies differences will be found across Time depending on whether Experimental or Control group is looked at, as follows (Fig. 12- table 7):

Between Experimental/Control: The area covered at 0 days (0.322 ± 0.039), 7 days (0.376 ± 0.0376) and 10 days (0.369 ± 0.04) for the Experimental group was significantly greater than the Control group.

Tooth Ring Apical Level: There were no significant interactions between Region and either Experimental/Control or Time. However, the proportional area of each Region was statistically significant. This implies that the proportional area covered by blood vessels between Regions was unaffected by either Experimental/Control or Time as follows: (Fig. 13- table 8).

The area covered by the mesial region of the tooth ring at the apical level was significantly greater (0.051 ± 0.007) than the buccal region of the tooth ring at the apical level.

Tooth Ring Middle Level: There were no significant differences.

3) Another Question Asked: Does the proportion of the total number of blood vessels above $11 \mu\text{m}$ differ between regions in each ring?

Alveolar Ring Apical Level: There were no significant interactions or main effects.

Alveolar Ring Middle Level: There was a significant difference between Experimental/Control by Region. This implies that the proportion of blood vessels $11\ \mu\text{m}$ or greater differs between the Regions depending on whether the Experimental or Control group was looked at, as follows (Fig. 14- table 9):

Between Experimental/Control: The proportion $11\ \mu\text{m}$ (or greater) in the Experimental group mesial region of the alveolar ring (0.692 ± 0.021) was significantly greater than the Control group mesial region of the alveolar ring.

Tooth Ring Apical Level: There were no significant differences.

Tooth Ring Middle Level: There was a significant difference between Region by Time. This implies that differences between regions depends on the Time looked at, as follows (Fig. 15- table 10):

At 0 days: The proportion $11\ \mu\text{m}$ (or greater) in the distal region of the tooth ring (0.889 ± 0.093) was significantly greater than all other regions.

At 3 days: There was no significant difference.

At 5 days: The proportion $11\ \mu\text{m}$ (or greater) in the mesial region of the tooth ring (0.622 ± 0.07) was significantly greater than the distal region of the tooth ring.

- At 7 days: There was no significant difference.
- At 10 days: There was no significant difference.
- At 14 days: There was no significant difference.
- At 21 days: The proportion $11 \mu\text{m}$ (or greater) in the mesial region of the tooth ring (0.548 ± 0.072) was significantly greater than all other regions.

Summary of Analysis of Variance

Tables A through L

	Dependent Variable	Ring	Level	Effect of Interaction Summarized
Table A	# Found	Alveolar	Apical	Region
Table B	# Found	Alveolar	Middle	Region x Time
Table C	Tp.	Alveolar	Apical	Region x Time
Table C	Tp.	Alveolar	Apical	Region x Group
Table C	Tp.	Alveolar	Apical	Time x Group
Table D	Tp.	Alveolar	Middle	Region
Table D	Tp.	Alveolar	Middle	Time x Group
Table E	Tp.	Tooth	Apical	Region
Table F	D11	Alveolar	Middle	Region x Group
Table G	D11	Tooth	Middle	Region x Time
Table H	D11	Alveolar	Apical	No Effect No Interaction
Table I	D11	Tooth	Apical	No Effect No Interaction
Table J	# Found	Tooth	Apical	No Effect No Interaction
Table K	# Found	Tooth	Middle	No Effect No Interaction
Table L	Tp.	Tooth	Middle	No Effect No Interaction

Found = Number of blood vessels found per 10,000 μm^2
 Tp. = Proportional Area covered by blood vessels
 D11 = Proportion of blood vessels 11 μm or greater

Table A

Analysis of Variance for the Alveolar Ring at the Apical Level

(Number of Blood Vessels Found per 10,000 μm^2)
(see Table 1)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	24.340	24.340	2.750	0.105
Time	6	29.236	4.873	0.550	0.767
Expcntl x Time	6	82.389	13.731	1.550	0.186
Anima (Expcntl x Time)	42	372.105	8.860		
Region	3	42.496	14.165	10.250	0.000
Expcntl x Region	3	0.923	0.308	0.220	0.881
Time x Region	18	32.849	1.825	1.320	0.168
Expcntl x Time x Region	18	19.658	1.092	0.790	0.713
Residual (Error)	553	764.242	1.382		
TOTAL	650	1371.843			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	24.266	24.266	2.730	0.106
Time	6	29.008	4.835	0.540	0.771
Expcntl x Time	6	83.618	13.936	1.570	0.180
Anima (Expcntl x Time)	42	372.927	8.879		
Region	3	41.178	13.726	9.930	0.000
Residual (Error)	592	818.260	1.382		
TOTAL	650	1371.843			

Table 1

Number of Blood Vessels Found Per 10,000 μm^2 in each Region of the Alveolar Ring at the Apical Level		
Region	#	S.E.
Palatal	1.926*	0.092
Mesial	2.600*/ \diamond	0.092
Buccal	2.087 \diamond	0.092
Distal	2.313*	0.092

= Number of blood vessels found per 10,000 μm^2

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

S.E. = Standard error

There was no significant difference between the regions, when comparing the control to experimental group so the data has been pooled for the entire ring for both groups.

The mesial and distal regions were significantly greater than the palatal region at $p < 0.05$ (*).

The mesial region was significantly greater than the buccal region at $p < 0.05$ (\diamond).

Fig. 6

Number of Blood Vessels Found per 10,000 μm^2 in each Region of the Alveolar Ring at the Apical Level

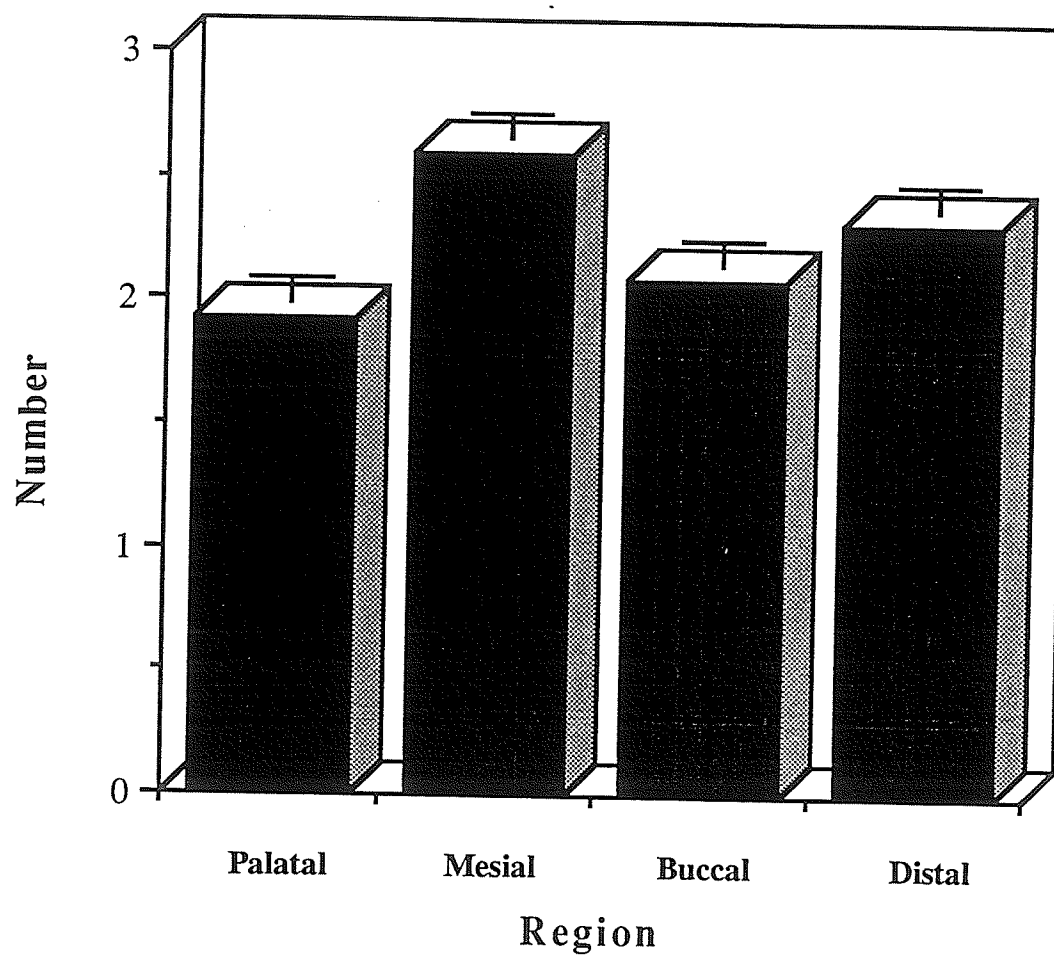


Table B

Analysis of Variance for the Alveolar Ring at the Middle Level

(Number of Blood Vessels Found per 10,000 μm^2)
(see Table 2)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	25.813	25.813	2.650	0.111
Time	6	145.396	24.233	2.490	0.038
Expctl x Time	6	88.948	14.825	1.520	0.195
Anima (Expctl x Time)	42	409.360	9.747		
Region	3	30.543	10.181	5.320	0.001
Expctl x Region	3	4.936	1.645	0.860	0.462
Time x Region	18	66.443	3.691	1.930	0.012
Expctl x Time x Region	18	31.864	1.770	0.930	0.548
Residual (Error)	510	975.942	1.914		
TOTAL	607	1794.892			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	27.194	27.194	2.790	0.103
Time	6	148.314	24.719	2.530	0.035
Expctl x Time	6	89.858	14.976	1.530	0.191
Anima (Expctl x Time)	42	409.999	9.76		
Region	3	31.999	10.666	5.590	0.001
Time x Region	18	64.181	3.566	1.870	0.016
Residual (Error)	531	1012.660	1.907		
TOTAL	607	1794.892			

Table 2

Number of Blood Vessels Found per 10,000 μm^2 in each Region of the Alveolar Ring at the Middle Level								
Time	Palatal #	S.E.	Mesial #	S.E.	Buccal #	S.E.	Distal #	S.E.
0	1.946	0.300	2.342	0.300	1.466	0.300	1.735	0.300
3	1.979*	0.300	3.421*	0.300	2.877	0.300	2.588	0.300
5	1.367	0.300	1.619	0.300	2.205	0.300	2.077	0.300
7	1.586	0.300	2.304	0.300	1.744	0.300	1.726	0.300
10	2.807	0.300	2.500♦	0.300	3.847♦	0.300	3.784♦	0.300
14	1.508	0.300	1.804	0.300	1.951	0.300	2.191	0.300
21	1.563	0.300	2.502	0.300	2.430	0.300	2.355	0.300

= Number of blood vessels found per 10,000 μm^2

Time = Number of days of spring deactivation

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

S.E. = Standard error

There was no significant difference between the regions across time, when comparing the control to experimental group. The data for the control and experimental group has been pooled due to the lack of difference.

The mesial region at 3 days was significantly greater than the palatal region, at $p < 0.05$ (*).

The buccal and distal regions at 10 days were significantly greater than the mesial region, at $p < 0.05$ (♦).

Fig. 7

Number of Blood Vessels Found per 10,000 μm^2 in each Region of the Alveolar Ring at the Middle Level

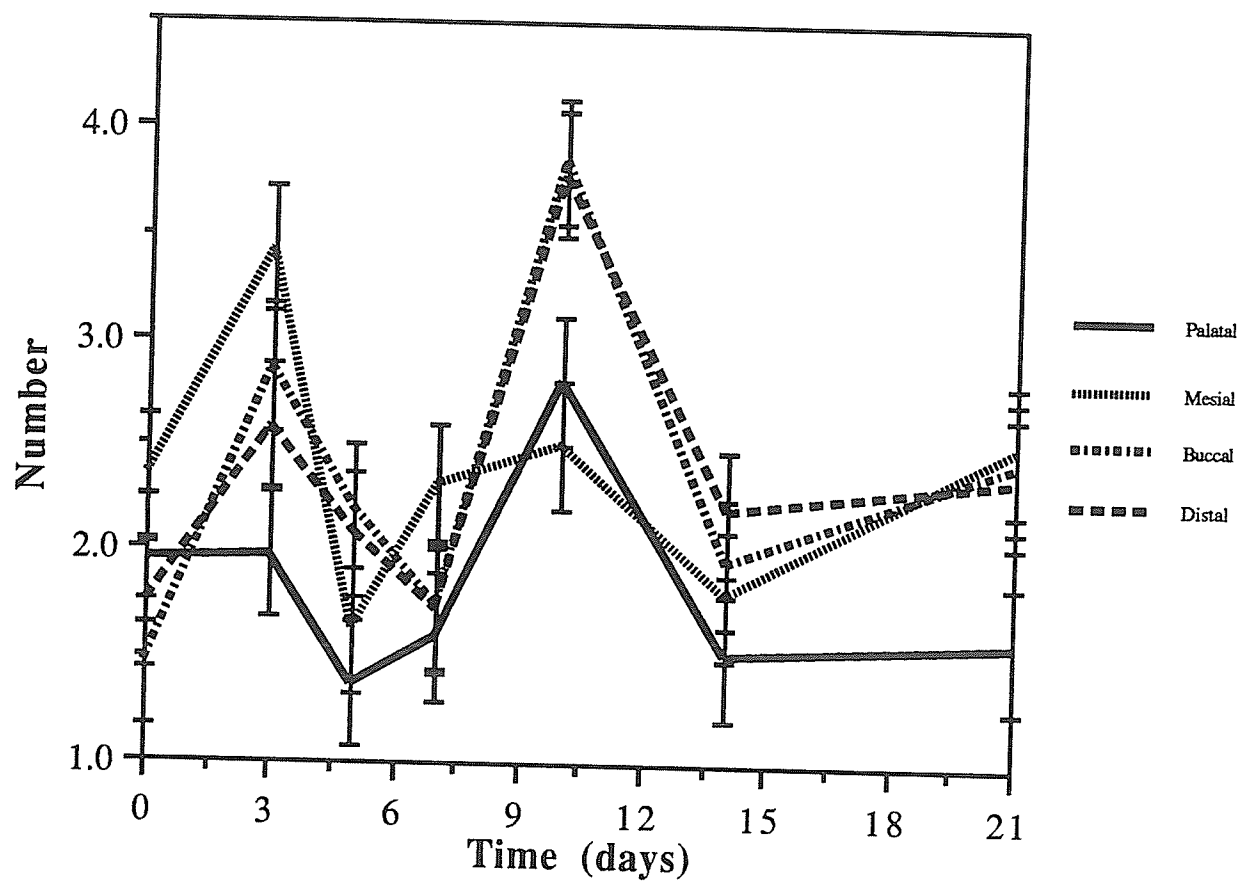


Table C

Analysis of Variance for the Alveolar Ring at the Apical Level

(Proportional Area Covered by Blood Vessels)
(see Tables 3, 4, and 5)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	1.148	1.148	11.290	0.002
Time	6	1.290	0.215	2.110	0.072
Expctl x Time	6	1.621	0.270	2.660	0.028
Anima (Expctl x Time)	42	4.271	0.102		
Region	3	0.766	0.255	8.740	0.000
Expctl x Region	3	0.689	0.230	7.870	0.000
Time x Region	18	1.309	0.073	2.490	0.001
Expctl x Time x Region	18	0.598	0.033	1.140	0.311
Residual (Error)	561	16.376	0.029		
TOTAL	658	28.249			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	1.168	1.168	11.520	0.002
Time	6	1.280	0.213	2.110	0.073
Expctl x Time	6	1.617	0.269	2.660	0.028
Anima (Expctl x Time)	42	4.257	0.101		
Region	3	0.779	0.260	8.860	0.000
Expctl x Region	3	0.689	0.230	7.840	0.000
Time x Region	18	1.301	0.072	2.470	0.001
Residual (Error)	579	16.974	0.029		
TOTAL	658	28.249			

Table 3

Proportional Area Covered by Blood Vessels in each Region of the Alveolar Ring at the Apical Level								
Time	Palatal (Tp.)	S.E.	Mesial (Tp.)	S.E.	Buccal (Tp.)	S.E.	Distal (Tp.)	S.E.
0	0.204	0.036	0.274	0.036	0.289	0.036	0.245	0.036
3	0.223*	0.036	0.355*	0.036	0.161*	0.036	0.202*	0.036
5	0.134	0.036	0.199	0.036	0.184	0.036	0.226	0.036
7	0.196♦	0.036	0.393♦	0.036	0.276	0.036	0.360♦	0.036
10	0.214	0.036	0.166	0.036	0.206	0.036	0.130	0.036
14	0.239	0.036	0.366⊕	0.036	0.319	0.036	0.217⊕	0.036
21	0.150★	0.036	0.288★	0.036	0.292★	0.036	0.306★	0.036

Tp. = Proportional area covered by blood vessels
 Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal
 Time = Number of day of spring deactivation
 S.E. = Standard error

There was no significant difference between the regions across time, when comparing the control to experimental group. Therefore, data for control and experimental groups has been pooled due to the lack of difference.

The mesial region at 3 days was significantly greater than all other regions, at $p < 0.05$ (*).

The mesial and distal regions at 7 days were significantly greater than the palatal region, at $p < 0.05$ (♦).

The mesial region at 14 days was significantly greater than the distal region, at $p < 0.05$ (⊕).

The mesial, buccal and distal regions at 21 days were significantly greater than the palatal region, at $p < 0.05$ (★).

Fig. 8

Proportional Area Covered by Blood Vessels in each
Region of the Tooth Ring at the Apical Level

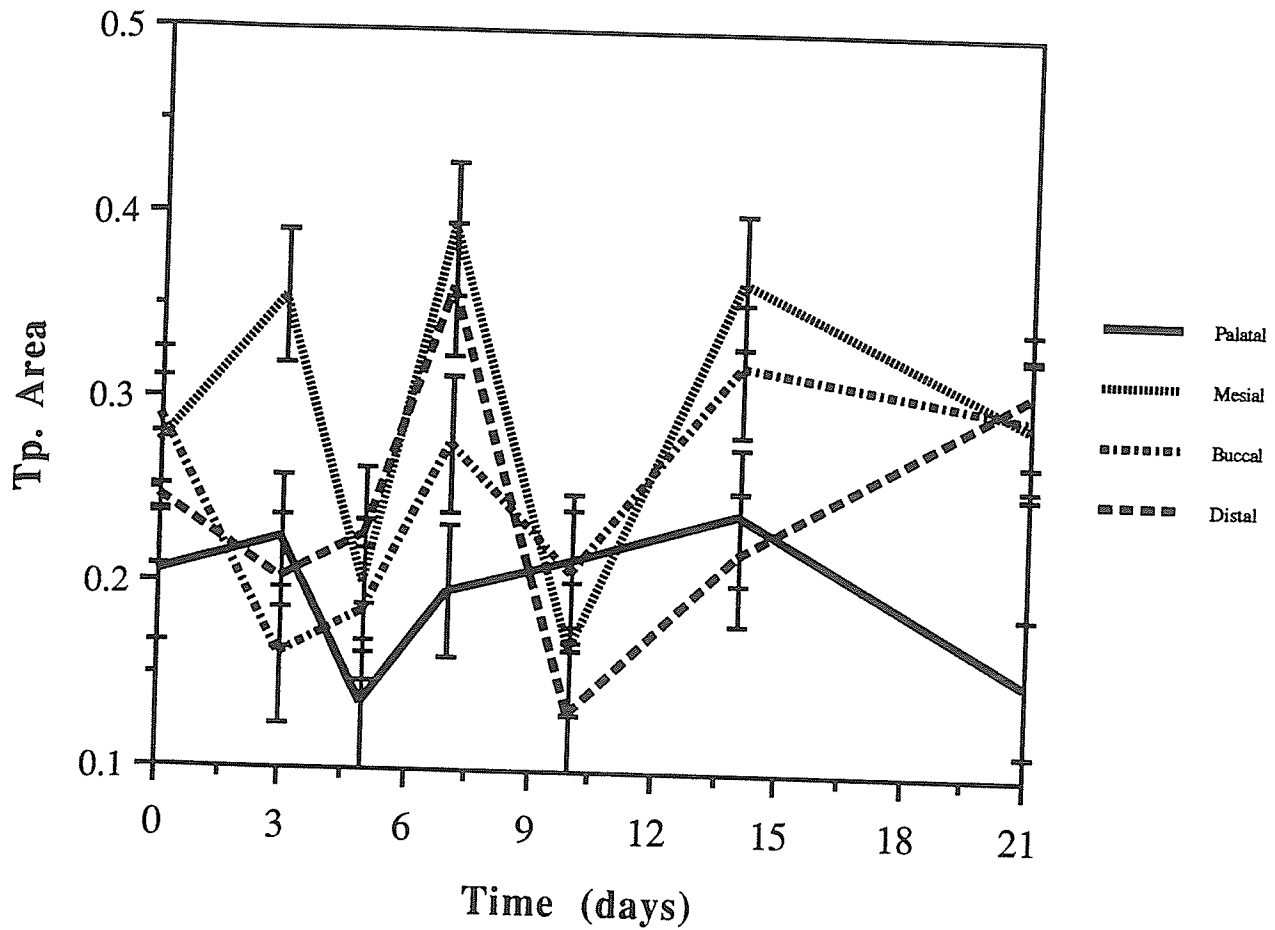


Table 4

Proportional Area Covered by Blood Vessels in each Region of the Alveolar Ring at the Apical Level (Control vs. Experimental)			
Group	Region	Tp.	S.E.
Cont.	Palatal	0.124*	0.190
Cont.	Mesial	0.286	0.190
Cont.	Buccal	0.233	0.190
Cont.	Distal	0.163♦	0.190
Expt.	Palatal	0.265*	0.190
Expt.	Mesial	0.297	0.190
Expt.	Buccal	0.261	0.190
Expt.	Distal	0.319♦	0.190

Tp. = Proportional area covered by blood vessels

Group = Experimental (Expt.) or control (Cont.)

S.E. = Standard error

Regional of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

The time (days) had no effect on either the control or experimental group when looking at the behaviour in each region, therefore the data has been pooled for all times.

The palatal region in the experimental group was significantly greater than the palatal region in the control group, at $p < 0.05$ (*).

The distal region in the experimental group was significantly greater than the distal region in the control group, at $p < 0.05$ (♦).

Fig. 9

Proportional Area Covered by Blood Vessels in each
Regional of the Alveolar Ring at the Apical Level
(Control vs. Experimental)

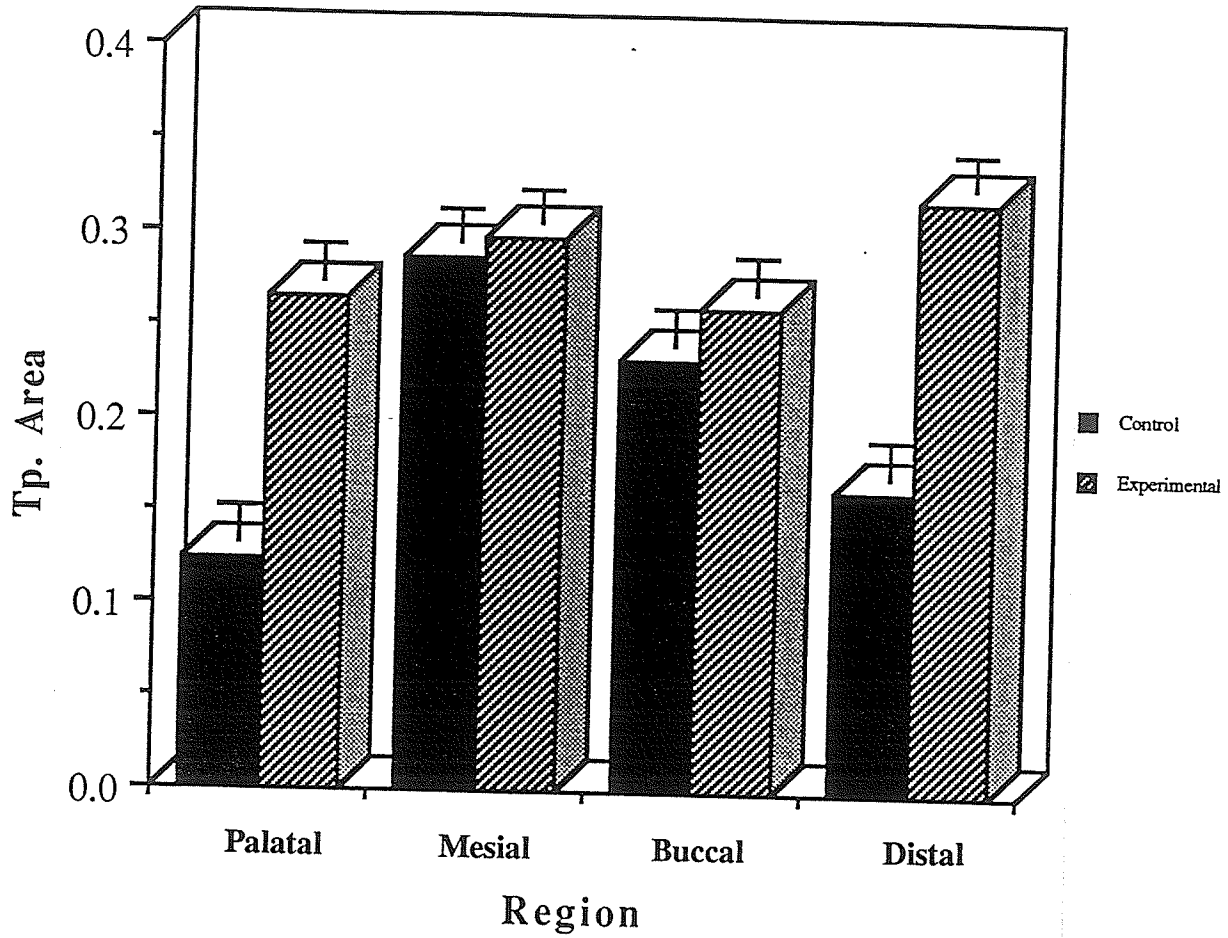


Table 5

Proportional Area Covered by Blood Vessels in the Alveolar Ring at the Apical Level (Control vs. Experimental)				
Time	Tp.(Cont.)	S.E.	Tp.(Expt.)	S.E.
0	0.150*	0.046	0.356*	0.046
3	0.205	0.046	0.266	0.046
5	0.160	0.046	0.211	0.046
7	0.209♦	0.046	0.403♦	0.046
10	0.106	0.046	0.253	0.046
14	0.336	0.046	0.235	0.046
21	0.243	0.046	0.276	0.046

Time = Number of days of spring deactivation

Tp. = Proportional area covered by blood vessels

Cont. = Control group

Expt. = Experimental group

S.E. = Standard error

The data for each region within the ring has been pooled for both the control and experimental group across time.

The experimental group at 0 days was significantly greater than the control group, at $p < 0.05$ (*).

The experimental group at 7 days was significantly greater than the control group, at $p < 0.05$ (♦).

Fig. 10

Proportional Area Covered by Blood Vessels in the
Alveolar Ring at the Apical Level
(Control vs. Experimental)

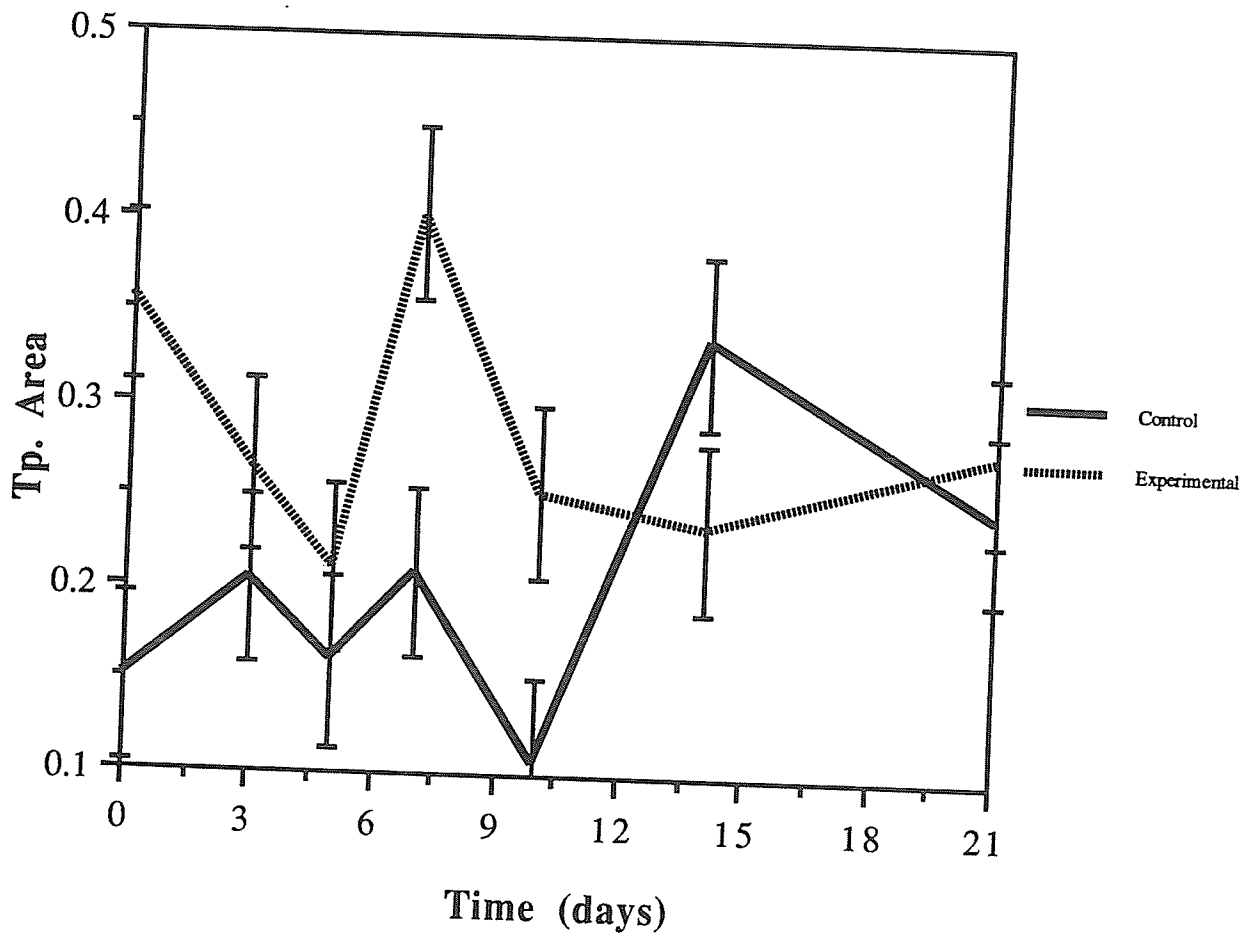


Table D

Analysis of Variance for the Alveolar Ring at the Middle Level

(Proportional Area Covered by Blood Vessels)
(see Tables 6 and 7)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	1.225	1.225	18.160	0.000
Time	6	2.083	0.347	5.150	0.001
Expcntl x Time	6	1.465	0.244	3.620	0.001
Anima (Expcntl x Time)	42	2.833	0.067		
Region	3	0.472	0.157	6.170	0.000
Expcntl x Region	3	0.113	0.038	1.480	0.218
Time x Region	18	0.330	0.018	0.720	0.793
Expcntl x Time x Region	18	0.694	0.039	1.510	0.079
Residual (Error)	546	13.907	0.026		
TOTAL	643	23.064			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	1.261	1.261	18.800	0.000
Time	6	2.081	0.347	5.170	0.001
Expcntl x Time	6	1.481	0.247	3.680	0.001
Anima (Expcntl x Time)	42	2.818	0.067		
Region	3	0.492	0.164	6.380	0.000
Residual (Error)	585	15.041	0.026		
TOTAL	643	23.064			

Table 6

Proportional Area Covered by Blood Vessels in each Region of the Alveolar Ring at the Middle Level		
Region	Tp.	S.E.
Palatal	0.180*	0.012
Mesial	0.228*	0.012
Buccal	0.202♦	0.012
Distal	0.253*/♦	0.012

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

Tp. = Proportional area covered by blood vessels

S.E. = Standard error

There was no significant difference when comparing the control to experimental group so the data for both groups has been pooled.

The mesial and distal regions were significantly greater than the palatal region, at $p < 0.05$ (*).

The distal region was significantly greater than the buccal region, at $p < 0.05$ (♦).

Fig. 11

Proportional Area Covered by Blood Vessels in each
Region of the Alveolar Ring at the Middle Level

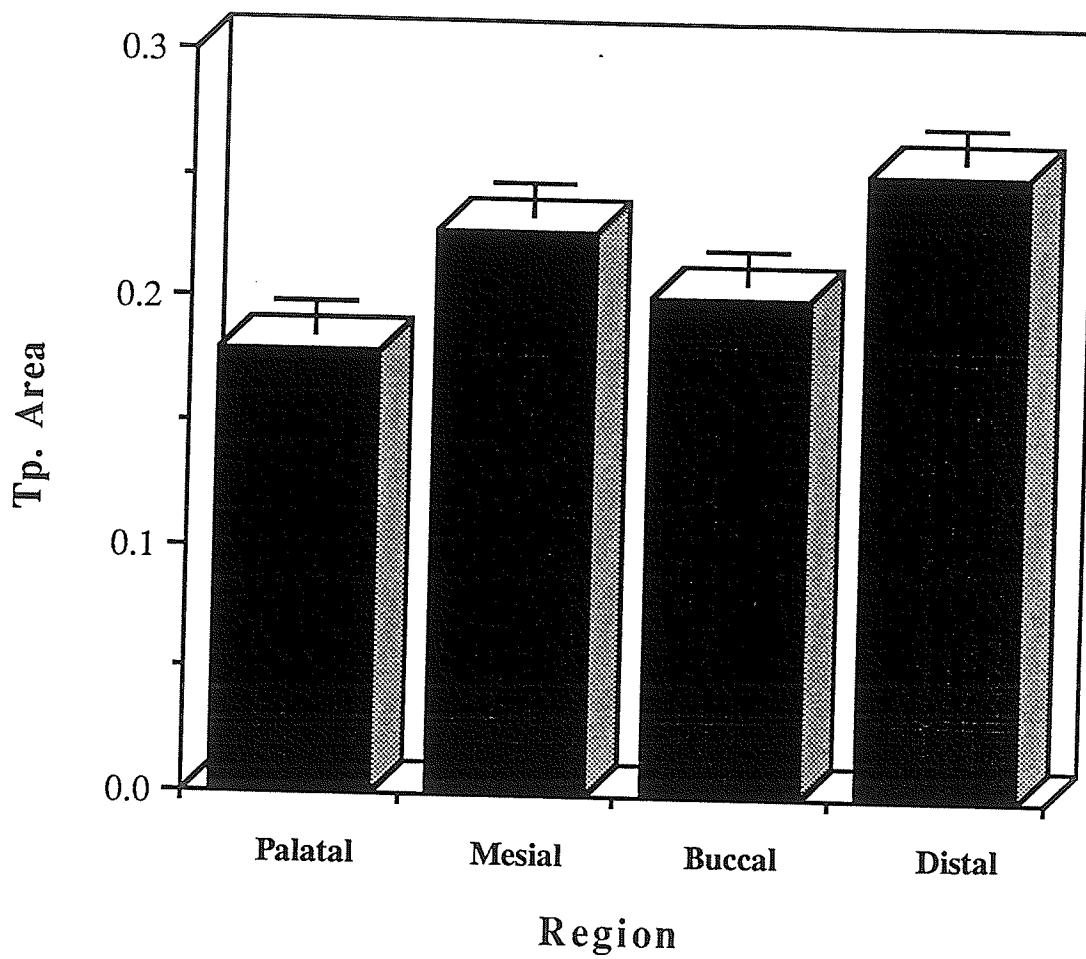


Table 7

Proportional Area Covered by Blood Vessels in the Alveolar Ring at the Middle Level Across Time (Control vs. Experimental)				
Time	Tp.(Cont.)	S.E.	Tp.(Expt.)	S.E.
0	0.126*	0.037	0.322*	0.037
3	0.177	0.037	0.237	0.037
5	0.059	0.037	0.133	0.037
7	0.168(◆)	0.037	0.376◆	0.037
10	0.210⊕	0.037	0.369⊕	0.037
14	0.245	0.037	0.196	0.037
21	0.213	0.037	0.187	0.037

Time = Number of days of spring deactivation

Tp. = Proportional area of blood vessels

Cont. = Control group

Expt. = Experimental group

S.E. = Standard error

The data for each region within the ring has been pooled for both the control and experimental group across time.

The experimental group at 0, 7 and 10 days was significantly greater than the control group, at $p < 0.05$ for 0 days (*).

7 days (◆) and 10 days (⊕).

Fig. 12

Proportional Area Covered by Blood Vessels in the
Alveolar Ring at each Level Across Time
(Control vs. Experimental)

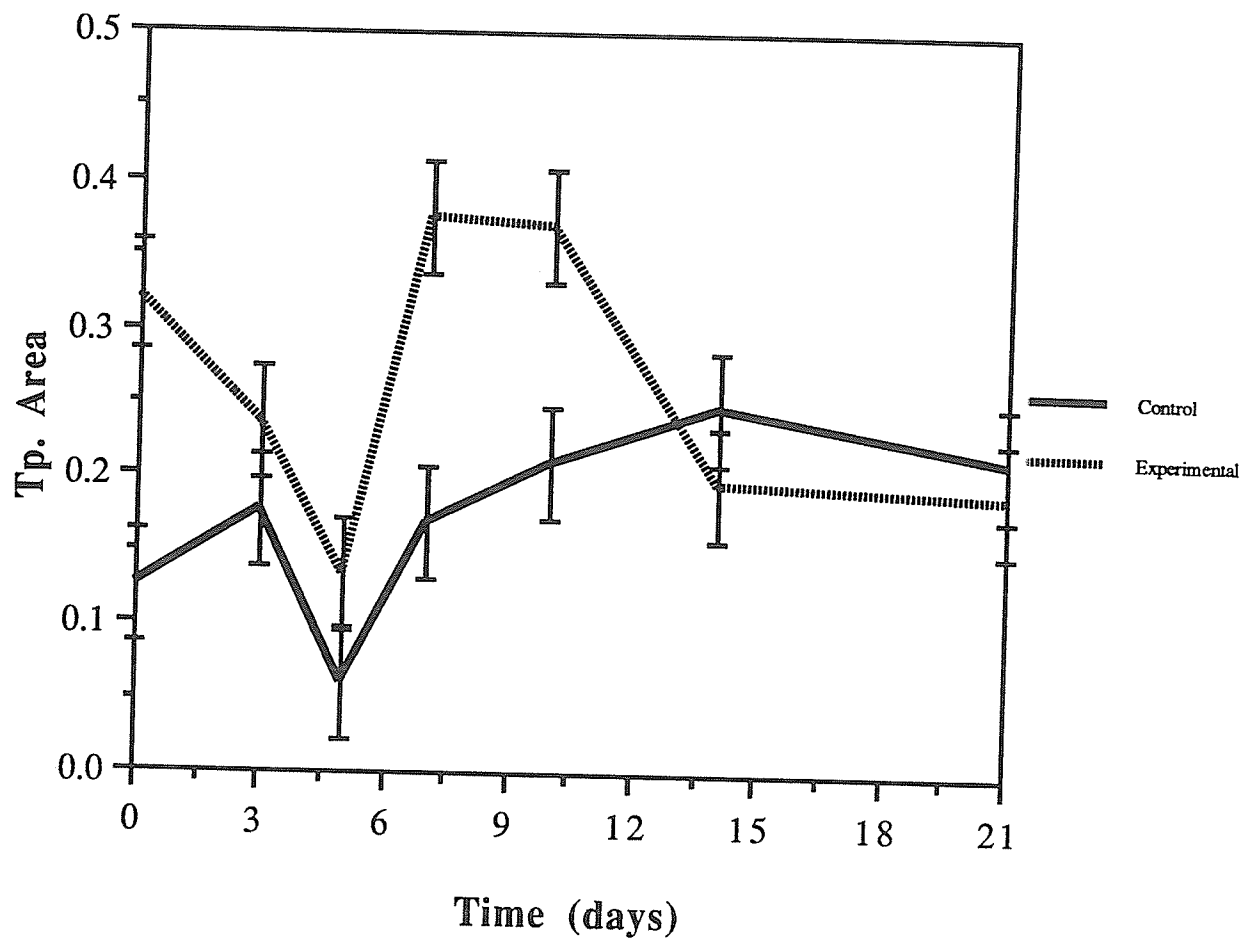


Table E

Analysis of Variance for the Tooth Ring at the Apical Level

(Proportional Area Covered by Blood Vessels)
(see Table 8)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.059	0.059	2.240	0.142
Time	6	0.042	0.007	0.270	0.950
Expcntl x Time	6	0.131	0.022	0.830	0.550
Anima (Expcntl x Time)	42	1.098	0.026		
Region	3	0.090	0.030	0.320	0.020
Expcntl x Region	3	0.021	0.007	0.790	0.501
Time x Region	18	0.134	0.007	0.830	0.669
Expcntl x Time x Region	18	0.154	0.009	0.950	0.521
Residual (Error)	561	5.057	0.009		
TOTAL	658	6.781			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.060	0.060	2.290	0.138
Time	6	0.042	0.007	0.270	0.949
Expcntl x Time	6	0.129	0.022	0.820	0.561
Anima (Expcntl x Time)	42	1.105	0.026		
Region	3	0.086	0.029	3.220	0.022
Residual (Error)	600	5.367	0.009		
TOTAL	658	6.781			

Table 8

Proportional Area Covered by Blood Vessels in each Region of the Tooth Ring at the Apical Level		
Region	Tp.	S.E.
Palatal	0.025	0.007
Mesial	0.051*	0.007
Buccal	0.021*	0.007
Distal	0.035	0.007

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

Tp. = Proportional area covered by blood vessels

S.E. = Standard error

There was no significant difference when comparing the control to experimental group so the data for both groups has been pooled.

The mesial region was significantly greater than the buccal region, at $p < 0.05$ (*).

Fig. 13

Proportional Area Covered by Blood Vessels in each
Region of the Tooth Ring at the Apical Level

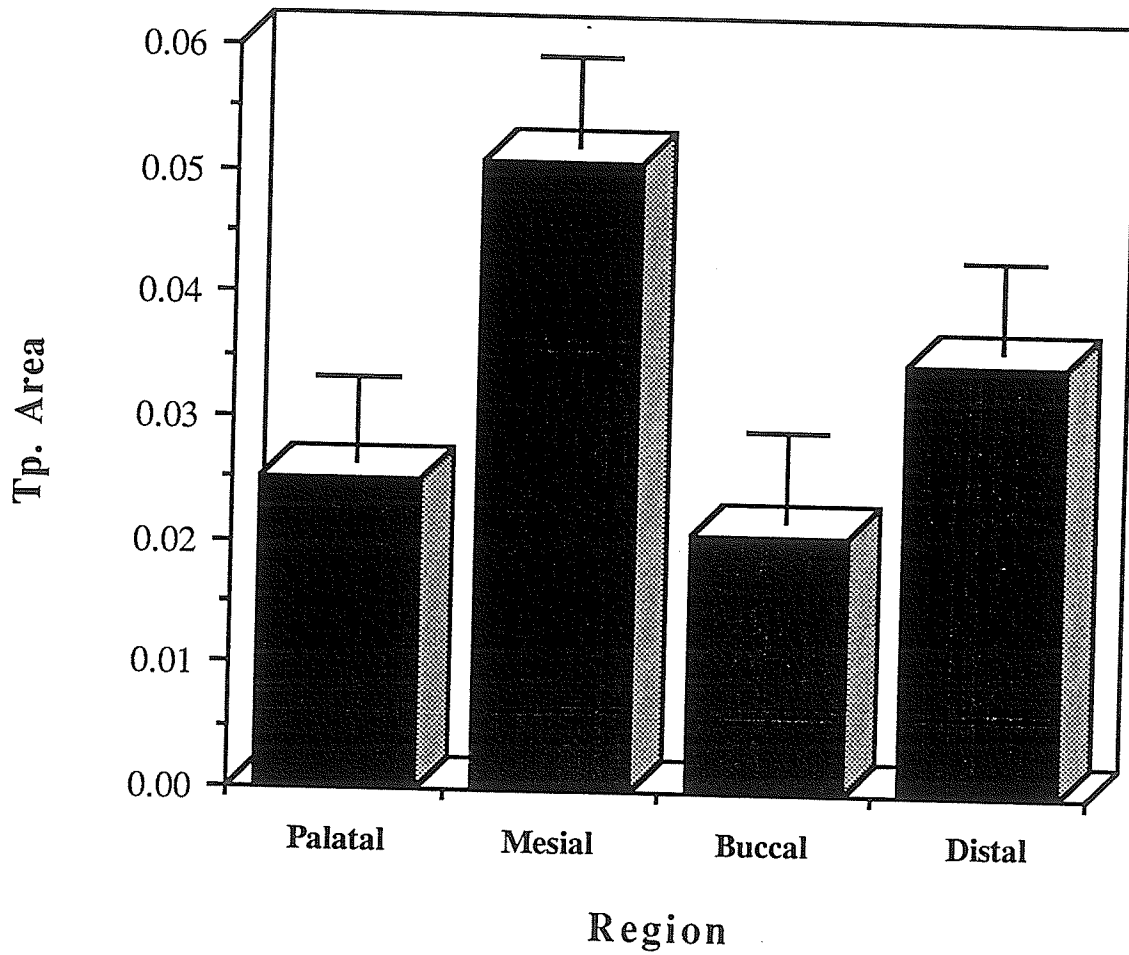


Table F

Analysis of Variance for the Alveolar Ring at the Middle Level

(Proportion of Blood Vessels 11 μm or Greater)
(see Table 9)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	0.214	0.214	1.690	0.201
Time	6	1.173	0.195	1.550	0.187
Expctl x Time	6	0.954	0.159	1.260	0.298
Anima (Expctl x Time)	42	5.311	0.126		
Region	3	0.533	0.178	5.350	0.001
Expctl x Region	3	0.241	0.080	2.420	0.065
Time x Region	18	0.503	0.028	0.840	0.651
Expctl x Time x Region	18	0.191	0.011	0.320	0.997
Residual (Error)	457	15.179	0.033		
TOTAL	554	24.585			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expctl	1	0.193	0.193	1.520	0.225
Time	6	1.223	0.204	1.600	0.171
Expctl x Time	6	0.989	0.165	1.290	0.282
Anima (Expctl x Time)	42	5.356	0.128		
Region	3	0.627	0.209	6.500	0.000
Expctl x Region	3	0.262	0.087	2.710	0.044
Residual (Error)	493	15.848	0.032		
TOTAL	554	24.585			

Table 9

Proportion of Blood Vessels 11 μm or Greater in each Region of the Alveolar Ring at the Middle Level (Control vs. Experimental)				
Region	D '11'+ (Cont.)	S.E.	D '11'+ (Expt.)	S.E.
Palatal	0.670	0.024	0.684	0.021
Mesial	0.579*	0.024	0.692*	0.021
Buccal	0.625	0.024	0.660	0.021
Distal	0.721	0.022	0.720	0.021

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal

D'11'+ = Proportion of blood vessels 11 μm or greater

Cont. = Control group

Expt. = Experimental group

S.E. = Standard error

The regions did not differ in their proportions with time between the control and experimental group.

The mesial region in the experimental group was significantly greater than the mesial region in the control group, at $p < 0.05$ (*).

Fig. 14

Proportional of Blood Vessels $11\ \mu\text{m}$ or Greater in each
Region of the Alveolar Ring at the Middle Level
(Control vs. Experimental)

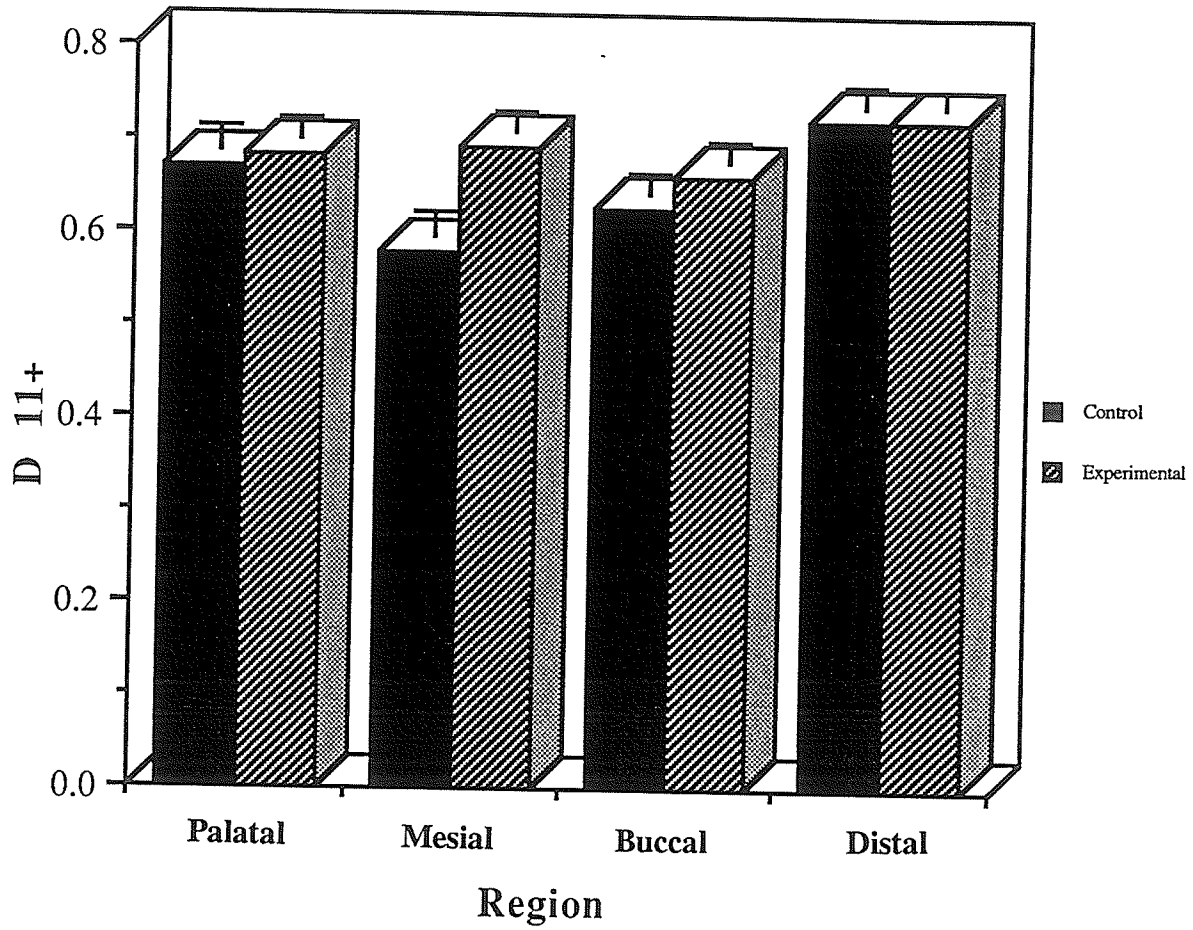


Table G

Analysis of Variance for the Tooth Ring at the Middle Level

(Proportion of Blood Vessels 11 μm or Greater)
(see Table 10)

**Full Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.064	0.064	0.670	0.420
Time	6	1.388	0.231	2.400	0.047
Expcntl x Time	6	1.625	0.271	2.810	0.024
Anima (Expcntl x Time)	36	3.468	0.096		
Region	3	0.202	0.067	1.350	0.259
Expcntl x Region	3	0.048	0.016	0.320	0.812
Time x Region	18	1.820	0.101	2.030	0.010
Expcntl x Time x Region	18	1.269	0.071	1.420	0.127
Residual (Error)	193	9.603	0.050		
TOTAL	284	20.054			

**Reduced Model
(Analysis of Variance)**

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.040	0.040	0.780	0.379
Time	6	1.381	0.230	4.480	0.000
Expcntl x Time	6	1.774	0.296	2.820	0.023
Anima (Expcntl x Time)	36	3.769	0.105		
Region	3	0.280	0.093	1.820	0.145
Time x Region	18	2.133	0.118	2.310	0.003
Residual (Error)	214	10.998	0.051		
TOTAL	284	20.054			

Fig. 15

Proportional of Blood Vessels $11\ \mu\text{m}$ or Greater in each Region of the Tooth Ring at the Middle Level

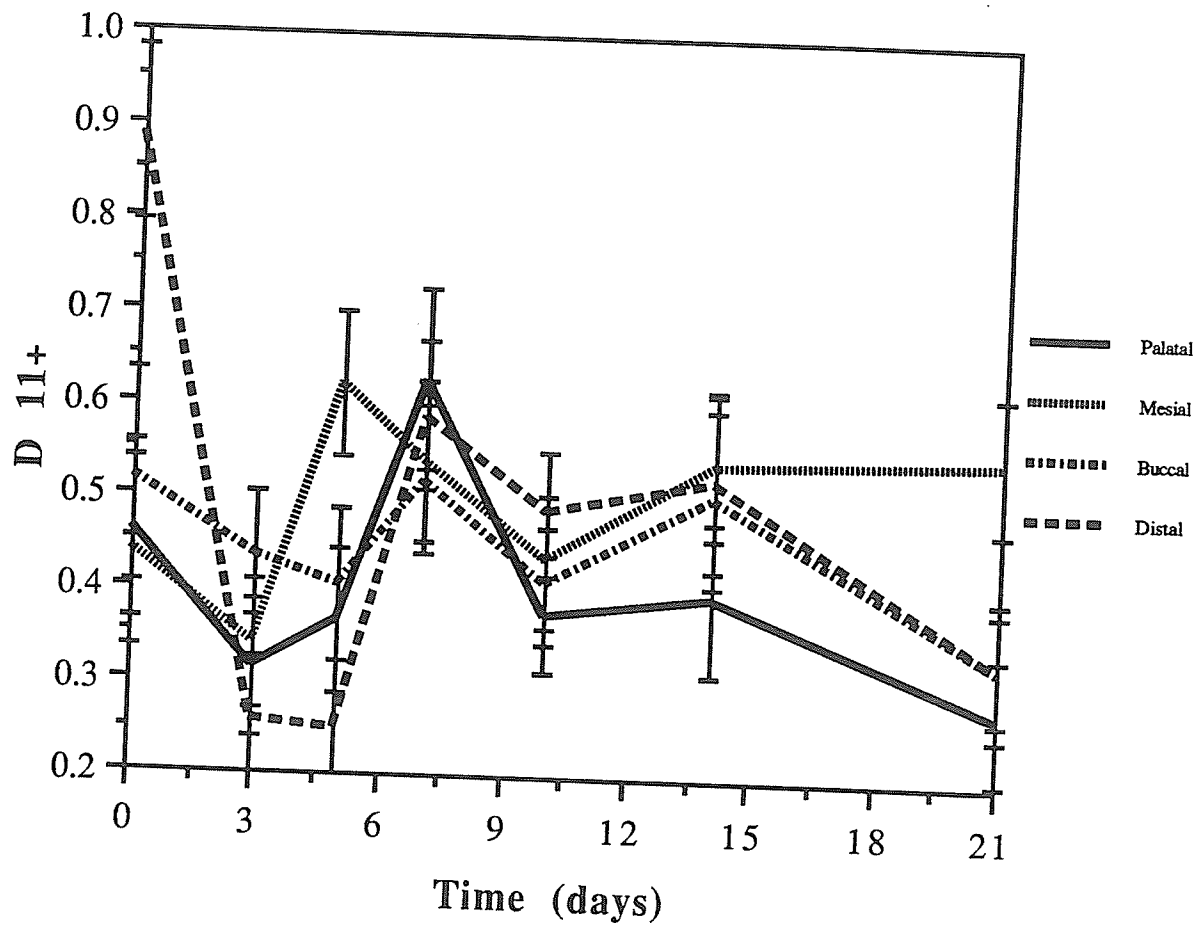


Table 10

Proportion of Blood Vessels 11 μm or Greater in each Region of the Tooth Ring at the Middle Level								
Time	Palatal D'11'+	S.E.	Mesial D'11'+	S.E.	Buccal D'11'+	S.E.	Distal D'11'+	S.E.
0	0.460*	0.100	0.438*	0.100	0.519*	0.110	0.890*	0.090
3	0.313	0.070	0.338	0.070	0.436	0.070	0.258	0.070
5	0.365	0.070	0.622 \diamond	0.080	0.405	0.080	0.246 \diamond	0.080
7	0.627	0.090	0.538	0.080	0.519	0.080	0.589	0.080
10	0.375	0.060	0.433	0.070	0.407	0.060	0.486	0.070
14	0.395	0.080	0.538	0.080	0.505	0.100	0.522	0.100
21	0.272 \otimes	0.070	0.548 \otimes	0.070	0.326	0.070	0.329	0.060

Region of the Periodontal Ligament = Palatal, Mesial, Buccal, Distal
 D11 = Proportion of blood vessels 11 μm or greater
 Time = Number of days of spring deactivation
 S.E. = Standard error

There was no significant difference between the regions across time, when comparing the control to experimental group. The data for the control and experimental group has been pooled due to the lack of difference.

The distal region at 0 days was significantly greater than all other regions, at $p < 0.05$ (*).

The mesial region at 5 days was significantly greater than the distal region, at $p < 0.05$ (\diamond).

The mesial region at 21 days was significantly greater than the palatal region, at $p < 0.05$ (\otimes).

Table H

Analysis of Variance for the Alveolar Ring at the Apical Level

(Proportion of Blood Vessels 11 μm or Greater)

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.078	0.078	0.510	0.478
Time	6	0.207	0.034	0.230	0.966
Expcntl x Time	6	0.658	0.110	0.720	0.637
Anima (Expcntl x Time)	42	6.409	0.153		
Region	3	0.085	0.028	0.640	0.592
Expcntl x Region	3	0.145	0.048	1.090	0.351
Time x Region	18	1.045	0.058	1.310	0.175
Expcntl x Time x Region	18	0.675	0.038	0.850	0.645
Residual (Error)	518	22.950	0.044		
TOTAL	615	32.265			

Table I
 Analysis of Variance for the Tooth Ring at the Apical Level
 (Proportion of Blood Vessels 11 μm or Greater)

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.496	0.496	4.030	0.053
Time	6	0.396	0.066	0.540	0.777
Expcntl x Time	6	0.864	0.144	1.170	0.347
Anima (Expcntl x Time)	31	3.813	0.123		
Region	3	0.253	0.084	1.280	0.283
Expcntl x Region	3	0.335	0.112	1.700	0.170
Time x Region	18	1.013	0.056	0.860	0.632
Expcntl x Time x Region	18	1.318	0.073	1.110	0.345
Residual (Error)	139	9.134	0.066		
TOTAL	225	18.190			

Table J

Analysis of Variance for the Tooth Ring at the Apical Level

(Number of Blood Vessels Found per 10,000 μm^2)

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	8.488	8.488	0.800	0.377
Time	6	24.385	4.064	0.380	0.887
Expcntl x Time	6	96.384	16.064	1.510	0.199
Anima (Expcntl x Time)	42	447.167	10.647		
Region	3	9.314	3.105	1.860	0.135
Expcntl x Region	3	2.651	0.884	0.530	0.662
Time x Region	18	15.108	0.839	0.500	0.957
Expcntl x Time x Region	18	11.699	0.650	0.390	0.990
Residual (Error)	561	935.535	1.668		
TOTAL	658	1549.054			

Table K

Analysis of Variance for the Tooth Ring at the Middle Level

(Number of Blood Vessels Found per 10,000 μm^2)

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	1.467	1.467	0.090	0.763
Time	6	171.006	28.501	1.800	0.123
Expcntl x Time	6	129.980	21.663	1.370	0.250
Anima (Expcntl x Time)	42	665.564	15.847		
Region	3	4.084	1.361	0.440	0.724
Expcntl x Region	3	7.894	2.631	0.850	0.466
Time x Region	18	21.632	1.202	0.390	0.990
Expcntl x Time x Region	18	62.254	3.459	1.120	0.328
Residual (Error)	543	1675.941	3.086		
TOTAL	640	2720.805			

Table L

Analysis of Variance for the Tooth Ring at the Middle Level

(Proportional Area Covered by Blood Vessels)

Source	DF	Sum of Squares	Mean Square	F Value	P Value
Expcntl	1	0.031	0.031	0.810	0.372
Time	6	0.108	0.018	0.480	0.822
Expcntl x Time	6	0.106	0.018	0.470	0.826
Anima (Expcntl x Time)	42	1.580	0.038		
Region	3	0.045	0.015	1.250	0.290
Expcntl x Region	3	0.073	0.024	2.040	0.107
Time x Region	18	0.314	0.017	1.470	0.096
Expcntl x Time x Region	18	0.214	0.012	1.000	0.457
Residual (Error)	543	6.465	0.012		
TOTAL	640	8.957			

CHAPTER V

DISCUSSION - CONCLUSION

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DISCUSSION - CONCLUSION

Physiologic drift of the dentition is in the mesial direction in humans but distal in the rat; with respect to the molars. In this study the first maxillary molar of the rat was moved for two weeks in a mesial direction. Following mesial movement for two weeks the spring was deactivated so as to allow the normal distal physiologic drift to resume. Since physiologic drift is distal with respect to rat molars, the mesial periodontal ligament space of the maxillary first molar is normally under tension while the distal periodontal ligament space is normally under compression. In the normal physiologic situation the periodontal ligament space is usually uniform around the roots of the teeth, in the study the mesio-buccal root of the maxillary right first molar (Fig. 16) was studied. However, in the study in response to the mesial force of the spring the mesial periodontal ligament space became the region of compression while the distal ligament space became the region of tension. In response to the mesial force the uniform/ even distribution of the ligament space around the mesio-buccal root of the maxillary right first molar, was lost. Instead the mesial space appeared very compressed while the distal region which was under tension appeared to be very much widened (Fig. 17).

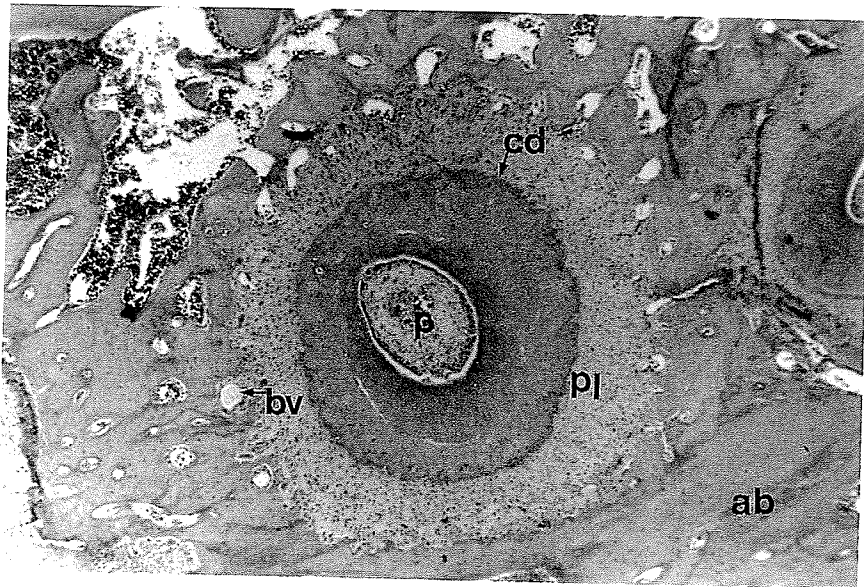


Fig. 16 Cross section of a control root specimen from the middle level of the periodontal ligament (pl): period (0 days) of spring deactivation. The (pl) area is evenly distributed around the root between the alveolar bone (ab), and cementum and dentin (cd); pulp of root (p). The number of observable blood vessels (bv), within the (pl) is limited.

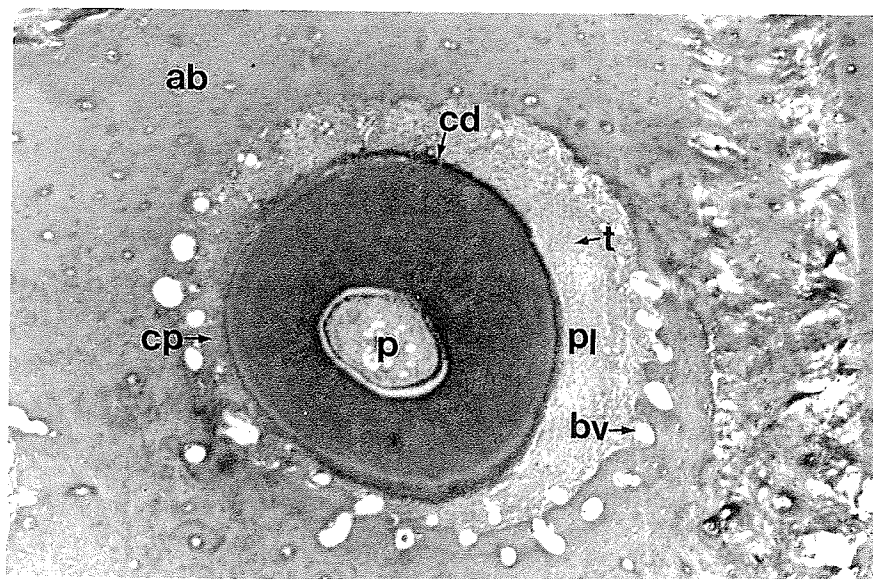


Fig. 17 Cross section of an experimental specimen from the middle level of the periodontal ligament (pl); period (0 days) of spring deactivation. The (pl) area is no longer evenly distributed around the root. There is an area of tension (t) and an area of compression (cp). The mesial area of the ligament has been compressed due to influence of the spring, while the distal area of the ligament has been increased due to tension. The number of observable blood vessels (bv) has also increased as compared to the control specimen. Most of the (bv) appear to be on the mesial and distal surfaces or areas of compression and tension. Cementum and dentin is identified as (cd).

In humans an array of causes have been postulated to account for undesirable tooth movements (relapse) seen following orthodontic therapy. These include: a slowness or absence of gingival and periodontal fibre remodelling; unstable tooth positions (Little *et al.*, 1981; Profitt, 1986); apical base discrepancies (Koole, 1990); third molars (Kaplan, 1988); incisor shape (Peck *et al.*, 1972); and musculature of the tongue and cheeks (Reitan, 1969). The role of each of these factors on adverse tooth movements is at best poorly understood. Furthermore, the mechanism of control at a cellular level is also not well understood (Wagemans *et al.*, 1988; Southard *et al.*, 1988).

It has been hypothesized that teeth may be moved as in the eruption process and maintained by means of the vasculature within the periodontal ligament (Bryer, 1957; Korber, 1970; Berkovitz, 1971; Ng *et al.*, 1981). Furthermore Kuflinec (1968) and Lew (1987) have suggested that the periodontal ligament vascularity could act as a shock absorber, capable of maintaining teeth in response to loading. In spite of this, past studies have failed to test vascular changes within the ligament as a potential agent capable of causing adverse tooth movement (relapse). Furthermore in the literature, histological data concerning the periodontal ligament with regard to the proportional area covered by blood vessels, the number of blood vessels per unit area, and the size of vessels, is sparse.

This study was histomorphometric in nature. The model was designed to ascertain baseline data on the blood vessels (Fig. 18) within the ligament, subsequent to relapse. Following the summarization of the baseline histomorphometric data on the blood

vessels, the aim was to speculate on how relapse may occur due to changes in blood vessel characteristics.

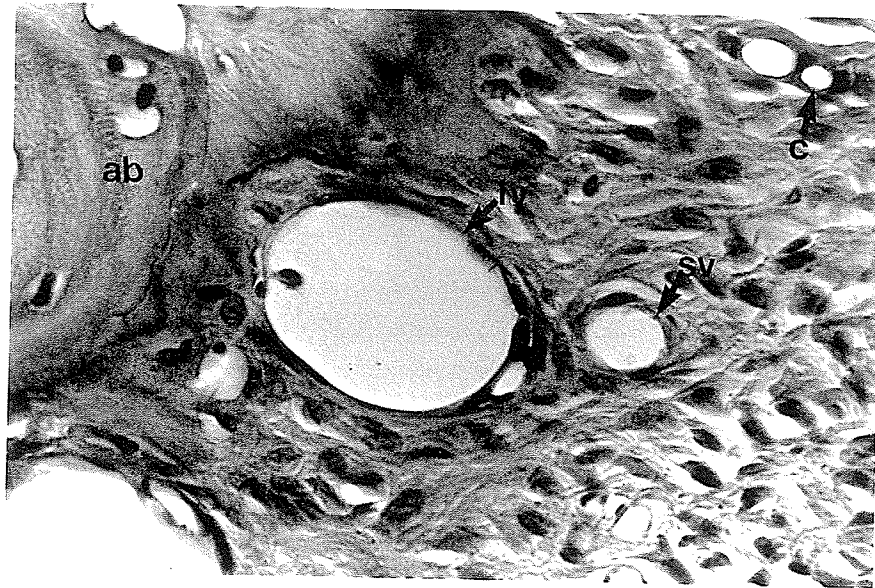


Fig. 18 Periodontal Ligament

Showing a large venule (lv), smaller venule (sv), capillary (c) and alveolar bone (ab).

It was assumed that mesial movement (tipping) of the maxillary first molar in the study did in fact occur in response to the mesial force placed on the molar by the spring, as per Row and Johnson, 1990. As a result of the tipping, following spring deactivation the molar would have been freed to resume its normal distal more upright position as well as normal distal physiologic drift. The resumption of the more upright position of the maxillary first molar in the study may be regarded as relapse as outlined in the relapse section.

One may speculate that the biological activity or energy required for relapse to occur in addition to the resumption of normal distal physiologic drift in the rat molar would be greater than for distal physiologic drift alone. This increase in biological activity within the ligament or increase in energy demand by the ligament would place a greater demand on the microvasculature (metarterioles, capillaries, post-capillary venules and venules) (Guyton, 1991), so as to provide more nutrient to and remove excreta from within the ligament (Guyton, 1991).

The tooth movement studied was of a normal distal physiologic nature, once the initial period of relapse was completed following spring deactivation. The force used to move the molar was uniform for the experimental animals and was maintained for the same amount of time in each experimental animal. From 0 days of spring deactivation to 3, 5, 7, 10, 14, and 21 days of spring deactivation data was collected and analyzed regarding the vascular changes within the periodontal ligament.

Tooth movement causes wounding of the periodontal ligament, and subsequent inflammation (Rygh, 1974, 1976). With inflammation and wounding to the periodontal

ligament new vessels are required to meet the nutritional requirements of the injured tissue, replace the injured/destroyed vessels and remove excreta (Rygh, 1974; Follin *et al.*, 1986). The creation of new vessels in response to injury is called wound healing angiogenesis and occurs within days (Folkman, 1985).

In the study several significant findings were made along and across the periodontal ligament, some of which were unknown prior to this study. In spite of statistical significance under each of the parameters studied at different levels and regions, a few conclusions of this histomorphometric data may be drawn with confidence as follows:

- (a) the mesial and distal regions when looked at irrespective of time appear to have more blood vessels per unit area (Fig. 6) and also a greater proportional area covered by blood vessels as compared to the palatal and buccal regions (Fig. 11 and 13);
- (b) the experimental group appears to show greater proportional coverage (initially) at 0, 7, and 10 days when compared to the control group. However at 14 and 21 days there are no differences (Fig. 10 and 12);
- (c) in regions of tension there appears to a greater proportion of blood vessel coverage initially (Fig. 8) and also an increase in the proportion of blood vessels $11 \mu\text{m}$ (or greater) (Fig. 15);
- (d) a cyclic pattern appears to be evident during the 21 day study (Fig. 7, 8, 10, 12, and 15). Each of these conclusions warrant further discussion as to why the separate vascular features may have behaved as they did.

In all of the statistically significant data across time irrespective of the parameter being viewed, that is number of blood vessels per unit area, proportional area covered by blood vessels or proportion of blood vessels $11 \mu\text{m}$ (or greater) (Fig. 7, 8, 10, 12, and 15), a cyclic pattern seemed to be evident. This cyclic pattern with regard to blood vessels has previously never been discussed in the literature. The 'peaks' and the 'valleys' of the cycles are not uniform within the 21 day study period, however there are two and sometimes three cycles evident. The mesial and distal regions appear to show the greater values for the given parameters. Throughout the study the only movements have been basically along the mesial and distal (mesio-distal) axis. Hence the cyclic pattern may be in some way related to this movement. During distal physiologic drifting, tooth movement probably consisted of a series of small tipping movements. It should be kept in mind that the initial distal tipping following spring deactivation (0 days) was probably larger than any subsequent tipping since the force tipping the tooth mesially was likely greater than the physiologic distal force. The series of tipping motions during the physiologic movement likely create alternating regions of tension and compression in the mesial and distal ligament space, thereby subjecting blood vessels in these regions to compression and tension. The tension/compression likely restricts blood flow (compression area) and enhances blood flow (tension area), alternately resulting in changes in nutritional requirements, thus creating the cycles.

The experimental group seemed to show greater proportional areas of coverage by blood vessels when compared to the control group across time (Fig. 10 and 12). This was a significant finding at 0, 7 and 10 days following spring deactivation, however at

14 and 21 days there was no significant difference. The greater values for the experimental group early in time as compared to the control group may be a reflection of a heightened ligament nutritional demand. The biological activity in the experimental group may be greater than that in the control group initially, due to the fact that the experimental group had undergone active tooth movement by the spring. Since wounding likely occurred in response to the mesial movement by the spring then it would be likely that the experimental group would initially have an increase in nutrient demand and need to remove excreta. During times of increased demand such as for oxygen the intermittent periods of blood flow (vasomotion) (Guyton, 1991) occur more readily and for longer periods of time so as to satisfy the needs of the ligament until repair is completed. At the same time instead of vascular shunting from the metarterioles to venules, during this time of greater demand in the experimental group blood is channelled to the capillaries, post-capillary venules and then venules. Under normal conditions when tissue nutritional demands are low, post-capillary venules have ellipsoid lumens which may be very small due to vascular shunting (Guyton, 1991). As more blood flows through the post-capillary venules they increase in diameter thereby occupying more area, hence when analyzed by the image analysis computer (IBAS) a greater proportional area will be measured as compared to the control group where shunting of blood is likely occurring to a greater degree. However, as the experimental group approaches 14 and 21 days the differences between control and experimental groups cease to exist, likely because by this time the tissues in the experimental group have been repaired. Also by 14 and 21 days the relapse component of the distal

movement of the experimental teeth may be largely completed and hence the teeth are probably drifting physiologically much the same as the control teeth, hence the tissue demands are probably comparable.

In areas of tension there appears to be not only an increase in the proportion of blood vessels $11\ \mu\text{m}$ (or greater) (Fig. 15) but also an increase in the proportional area covered by blood vessels (Fig. 8). Oxytalan fibres within the periodontal ligament among other things attach blood vessels within the ligament to the cementum of the tooth (Sims, 1975, 1979). The exact purpose of this is not clear, however it may be related to vascular supply for the tooth. As the rat molar undergoes distal physiologic drift a region of tension within the ligament is created within the mesial periodontal ligament space. With distal drift the lumens of post-capillary venules within the ligament in the tension region become more opened which allows for a greater amount of blood vessel coverage within the ligament and hence an increase in the proportional area in the tension region. Also initially (3 days) the compressive force which at one time was in the mesial region is now released. Hence any blood vessels which were constricted are now able to become patent again, thereby further increasing the proportional area covered by blood vessels in the tension region. The proportion of vessels $11\ \mu\text{m}$ (or greater) in the distal region of the tooth appears to be increased at 0 days of spring deactivation (Fig. 15). The IBAS system measured vessels which were patent, therefore some vessels with collapsed lumina would be undetected. In areas of tension such as in the distal region at 0 days (Fig. 15), the lumens of vessels which were once collapsed would become more patent, therefore they would be more readily detectable by the IBAS system. Therefore

it is likely than the increase in the proportion of vessels $11 \mu\text{m}$ (or greater) is a result of patency and hence detectability.

The number of blood vessels per $10,000 \mu\text{m}^2$ (Fig. 1) and the proportional area covered by blood vessels (Fig. 11 and 13) appears to be greater in the mesial and distal regions as compared to the buccal and palatal regions as reported by Birn (1966) and Ten Cate *et al.* (1980). In areas where this was not statistically significant the trend was still evident. Physiologic drift as well as active tooth movement in the study (by the spring) was in a mesio-distal direction. In order for tooth movement to occur there must be apposition and resorption of bone in the mesial and distal regions of the periodontal ligament depending on which way tooth movement is occurring (Kuitert *et al.*, 1988). Since the mesial and distal surfaces were the regions that experienced the greatest amounts of biological activity it would seem reasonable that they had more blood vessels and a greater proportional area than the buccal and palatal region, again in response to the demands of the ligament for nutrition and waste removal.

Each animal in the study was perfused with albumin and also fixed with fixative. Perfusion of each animal both experimental and control was for 30 minutes under a constant flow of 0.6 ml/minute and a constant pressure of $45 \text{ cm H}_2\text{O}$. It is possible that the pressure involved could have caused blood vessel dilation and hence created some of the findings with regard to blood vessel characteristics within the study. However, both the control and experimental groups were subjected to the same pressure parameters therefore, the relative results between control and experimental animals should not have been affected.

TRAP positive cells were counted and analyzed, however no significant main effect or interaction between the various parameters (Time, Experimental/Control, or Region) were found. This was not a surprising finding since osteoclasts may require a pathologic situation in order to attract them to a given area (area of compression or tension) (Chambers, 1991). It was speculated that osteoclast/osteoclast-like cells may migrate freely in the ligament during physiologic conditions, with little specificity to a given area. In the study tooth movement was physiologic in a distal direction once the spring had been deactivated. It is likely that the distal physiologic drift following active tooth movement in the experimental group was insufficient to stimulate the osteoclasts. Similarly the distal physiologic drift in the control animals was also unlikely to stimulate the osteoclasts to any particular area. It has been stated that a residual effect may have been in progress with regard to the experimental animals relative to the controls. This residual process (if it exists) with regard to TRAP positive cells may also have been insufficient to stimulate the osteoclasts to any given area at any time. The histologic sections of TRAP positive cells were nevertheless very interesting, there appeared to be an association between these cells and nearby blood vessels (Fig. 19). This association may have some underlying biologic significance, such as a hormonal activation of the osteoclasts via the blood vessels or a mechanical activation of the osteoclasts by means of blood vessel stretching.

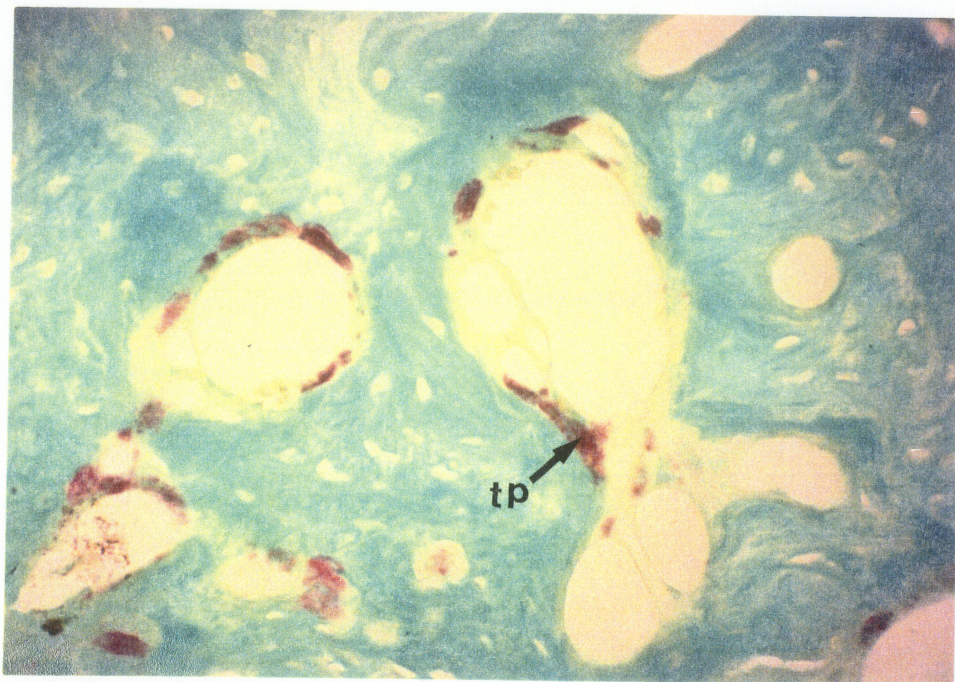


Fig. 19

Tartrate resistant acid phosphatase stain (TRAP) with red TRAP positive cells (tp). Note the association of TRAP positive cells to the blood vessels.

CLINICAL RELEVANCE

The data collected in the study was histomorphometric in nature and will significantly add to the literature presently available regarding the periodontal ligament. Due to the morphometric nature of the data the opportunity to prove cause and effect or correlation was not possible, however this does not mitigate the potential to speculate on the clinical applications of the data.

It has been assumed that the periodontal ligament is a closed system of fixed volume. With this assumption any increase in fluid or tissue in the ligament would result in an increase in interstitial/hydraulic pressure. If the volume is fixed and the interstitial/hydraulic pressure increases due to an increase in vascularity or an increase in edema then this may act as the impetus to cause teeth to move adversely or relapse post-orthodontically. The increase in vascularity may be due to an increase in number of blood vessels, increase in patency of some vessels such as post-capillary venules (Guyton, 1991) or an increase in vascular distension (arterioles) (Nakamura *et al.*, 1986; Rygh *et al.*, 1989). Also it has been reported that new vessels (capillaries) as well as venules are more 'leaky' (Madri *et al.*, 1988). Increased permeability of vessels hence may contribute to the interstitial pressure and hence relapse. It was shown in this study that in certain regions there were changes in the number of blood vessels found per unit area, changes in size and changes in the proportional area covered by blood vessels. If the number of blood vessels per unit area and proportional area of blood vessel coverage increases this may facilitate an increase in blood flow within the ligament thereby affecting a change in pressure thereby resulting in relapse.

If the periodontal ligament continues to be injured then the demands of the ligament will be slow to subside; as a result little shunting of blood from metarterioles to venules will occur. As a result the blood flow within the ligament will be increased thereby possibly causing relapse. Furthermore, if something after treatment continues to cause wounding or continued inflammation the angiogenic process may not subside and vascular regression may be slowed.

With the removal of fixed orthodontic appliances the teeth are free from any constraints. Generally at this point the teeth are more mobile than under normal non-treated circumstances. The mobility is due to a widened periodontal ligament space, as well as a lack of total calcification of the alveolar bone. It has been suggested that osteoid bone takes approximately 3 months to become calcified (Crumley, 1964; Lew, 1987). In the case where no retainer is used to maintain the teeth, then the normal occlusal load may result in a state of inflammation great enough to sustain the angiogenic process. This sustained process then works within the 'fixed volume closed system' periodontal ligament possibly causing an increase in vessel patency and hence relapse.

If however, a retainer is used, then the mobility of the teeth to normal occlusal load is reduced. This reduction in turn allows for downregulation of the inflammatory process and subsequent vascular regression. The number of vessels within the ligament returns to a normal number for the given volume and the interstitial pressure effect is removed. By this time osteoid bone has calcified and the width of the ligament space has reduced to normal, the result being that the vascular contribution to relapse is removed. In addition to this the reduced mobility may act to reduce the amount of injury. Reduced

injury then results in a reduce nutritional demand by the ligament, vascular shunting between the arterial and venous system occurs and the potential for relapse may be reduced. Hence the message from this hypothesis is: a month or two prior to removing the braces, there should be no wire activation, therefore the amount of wounding and inflammation is reduced and the regression process can begin. Also vascular shunting can start to occur. Furthermore, following the removal of the braces a retainer should be worn initially so as to decrease mobility.

Another clinical application of the findings deals with anchorage segments is as follows. Orthodontically anchorage (the ability to maintain teeth in a desired position while using them to pull or push) is a major concern. Because of this concern it has been advocated by some that segmental approaches be employed to correct some cases. Many times in a segmental approach the posterior segments are prepared (straightened) separately from the anterior segment, then the posterior segment is used as an anchor to pull the anterior segment posteriorly. However, if teeth were moved in the posterior segment then wounding and inflammation may occur which may turn on the angiogenic process creating an increase in vascularity within the ligament as previously explained. Also tooth movement results in osteoid formation on bone deposition surfaces. This being the case in using the posterior teeth as an anchor the segment may be too susceptible to movement anteriorly which would be undesirable. If on the other hand, the posterior teeth were allowed to rest for some period of time, then the mobility would be reduced, as well the regression process would have a chance to occur and as a result the posterior teeth would then be able to act as a more sound anchorage segment.

Another situation may be that movement of the posterior teeth may create a heightened vascular demand by the ligament, thereby acting as previously explained. Hence, if the posterior segment were prepared before the anterior segment, then as the posterior segment 'rested' allowing recalcification, vascular regression and shunting then one could work on aligning the anterior teeth so that by the end of the rest period the anterior teeth would be ready to be moved but they would not be afforded a rest period hence the anterior teeth would be easier to move than the posterior anchor segment, thus making the posterior teeth a better anchor segment.

SUGGESTED FUTURE STUDIES

In this study there are several changes that would be undertaken so as to improve on what was done. One change would be to measure the distance the teeth moved in response to the spring. The distance was not measured since the parameters used to create tooth movement in the study had been successful for other authors (Rygh *et al.*, 1986; Row and Johnson, 1990). Had the distance been measured initially and then at the time of sacrifice then one would be able to say with certainty whether or not the molar moved and by how much. This measure could be accomplished by methods such as, by using a quick set putty material while the animal is anaesthetized and being fitted with the spring. The material could be placed into a small custom tray and placed over both the incisors and first molar. The same procedure could be carried out at the time the animal is sacrificed. Another method might be to measure the distance between the disto-buccal root of the maxillary first molar and the mesio-buccal root of the maxillary second molar tooth in the experimental animals and compare this to the control animals

at Period 1 so as to determine on the average how far the molar had been moved before being left to resume physiologic drift.

Another limitation to the study is that no animals were sacrificed while active tooth orthodontic movement was occurring. However it was felt that the data obtained at 0 days would be comparable to data had animals been killed during tooth movement. Also time was a factor in the study therefore all parts of the study were not feasible from a practical perspective. However data during active tooth movement would have been useful in showing whether or not there are differences between physiologic and orthodontic tooth movements on vascular changes.

Another limitation was in the Protein A-gold staining procedure. Further work might have been attempted in an effort to resolve the problem.

A sham control also might have been done so as to make sure that the spring placement itself does not introduce any adverse changes to the vascular elements in the ligament. Although not evidenced spring placement alone could be causing inflammation.

Future studies which could be undertaken in relation to this study are numerous and would include the following:

- (a) Instead of using Protein A-gold then one might choose to use microspheres of different diameters. This way any diffusion into the tissues could be detected but also the size of the endothelial gap could be detected.
- (b) The incisor teeth of rabbits might be used and spread apart to attempt to demonstrate the data obtained here. That is the incisors would be spread

apart for some period of time and then allowed to drift back together. At selective times they could be sacrificed, much the same as the rats were in this study. This could also be done with rats incisors, however the rat is somewhat smaller which makes the work a lot more tedious due to the reduced access relative to larger animal.

- (c) A greater force might be used in the rat model so as to move the teeth and intentionally tear the tissue between the first and second molar. This tearing would again require wound healing angiogenesis. At various time periods following tooth movement and tissue damage then animals could be sacrificed and studied over a longer period of time to investigate the changes in vascularity but also to see how long the regression period takes. The difference here is that the level of injury would be heightened and definitively require a more active reparative process to occur.
- (d) The use of the IBAS system used in study allows for many potential uses. During the embedding process prior to histologic sectioning a vertical reference marker might be placed along side the specimen to be cut. This could be a piece of rubber poured perpendicular to the axis that sectioning of the specimen is going to occur. Each histologic section could be saved even if they were not all used as in the authors study. By having all of the sections then the IBAS machine could then be used to recreate a three-dimensional image of the vascularity of the areas of study.

- (e) One could test for proteoglycan/glycosaminoglycans in the soft tissues of the periodontal ligament. Proteoglycans/glycosaminoglycans have been shown to be hydrophilic, therefore if the vascular permeability can be shown to occur in the ligament then perhaps there is leakage of the 'glycans' into the ligament space, capable of attracting and binding water. This in turn would result in increases in hydraulic pressure within the ligament and possibly affect tooth position.
- (f) My hypothesis on tooth movement deals with among other things the angiogenic/inflammatory response. It would be an interesting study to find an animal model with anterior teeth similar to man, that readily undergoes a heightened, sustained chronic inflammatory process. This being the case one might expect some degree of tooth movement which would help to support the hypothesis.

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APPENDICES

LEGEND FOR RAW DATA APPENDICES

- TRAP 1 Rat = Tartrate Resistant Acid Phosphatase positive cells
- RSector = Region (Palatal, buccal, mesial, distal)
- Period = Time (0, 3, 5, 7, 10, 14, 21 days)
- NFound = Number of blood vessels found (minus 1011 - see text)
- ExpCntl = Experimental (e), control (c)
- Region }
 a = apical } levels of the periodontal ligament
 c = cervical }
 m = middle }
- DRatio 1 = Maximum diameter of the blood vessel divided by the minimum diameter
- Tp Area 1 = Proportional area covered by blood vessels
- DEleven 1 = Proportion of blood vessels 11 μ m (or greater)
- NFound (0) = Regions where no blood vessels were found
- NFound (1) = Regions where blood vessels were found
- Section }
 1 = Alveolar ring
 2 = Middle ring
 3 = Tooth ring }
- Dint = Blood vessel size in microns

APPENDIX A

Raw Data for Tartrate Resistant Acid Phosphatase

RSECTOR	PERIOD	EXPCNTL							
		c				e			
		REGION				REGION			
		a		m		a		m	
		TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT
		N	MEAN	N	MEAN	N	MEAN	N	MEAN
1_18	1	12	0.000	11	0.091	11	4.591	12	0.486
	2	12	0.121	11	0.426	12	0.109	12	0.201
	3	12	0.068	8	0.047	12	0.514	12	0.489
	4	11	0.381	11	0.565	12	0.124	12	0.108
	5	12	0.232	12	0.195	12	0.219	12	0.704
	6	12	0.622	12	0.912	12	0.436	12	0.524
	7	12	0.358	12	0.752	12	0.593	12	0.301
1_23	1	12	0.000	11	0.000	12	0.000	12	0.273
	2	12	0.000	10	0.192	12	0.348	12	0.271
	3	12	0.000	8	0.000	12	0.568	12	0.614
	4	11	0.000	12	0.884	12	0.159	12	0.391
	5	12	0.000	12	0.075	12	0.137	12	0.554
	6	12	0.063	12	0.336	12	0.016	12	0.551
	7	12	0.060	12	0.531	12	0.467	12	0.072
1_45	1	12	0.000	11	0.000	11	0.000	12	0.853
	2	12	0.000	11	0.264	12	0.250	12	0.472
	3	12	0.000	8	0.000	12	0.496	12	0.572
	4	11	0.363	12	0.534	12	0.000	12	0.136
	5	12	0.078	12	0.093	12	0.027	12	0.030
	6	12	0.349	12	0.280	12	0.000	12	0.180

Raw Data for Tartrate Resistant Acid Phosphatase (Continued)

RSECTOR	PERIOD	EXPCNTL							
		c				e			
		REGION				REGION			
		a		m		a		m	
		TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT	TRAPIRAT
N	MEAN	N	MEAN	N	MEAN	N	MEAN		
1_45	7	12	0.000	12	0.242	12	0.623	12	0.016
1_67	1	12	0.000	11	0.048	11	0.000	12	0.438
	2	13	0.278	11	0.684	12	0.441	12	0.868
	3	12	0.000	8	5.520	12	1.069	12	1.006
	4	12	2.007	12	1.509	12	0.282	12	0.203
	5	12	0.368	12	0.257	12	0.634	12	1.521
	6	12	2.547	12	1.570	12	0.000	12	0.486
	7	12	0.252	12	0.670	12	1.094	12	0.297
3_18	1	12	0.000	12	0.000	12	0.125	12	0.000
	2	11	0.000	12	0.000	12	0.000	12	0.000
	3	12	0.000	12	0.000	12	0.018	12	0.000
	4	12	0.000	11	0.000	11	0.000	11	0.019
	5	12	0.000	12	0.000	12	0.000	12	0.000
	6	12	0.000	12	0.000	10	0.000	8	0.211
	7	12	0.000	12	0.000	12	0.000	12	0.033
3_23	1	12	0.000	12	0.000	12	0.846	12	0.000
	2	11	0.000	12	0.000	12	0.000	12	0.000
	3	12	0.000	12	0.000	12	0.124	12	2.263
	4	12	0.000	11	0.000	11	0.000	11	0.000
	5	12	0.000	12	0.000	12	0.000	12	0.000

Raw Data for Tartrate Resistant Acid Phosphatase (Continued)

RSECTOR	PERIOD	EXPCNTL							
		c				e			
		REGION		REGION		REGION		REGION	
		a	m	a	m	a	m	a	m
TRAPIRAT		TRAPIRAT		TRAPIRAT		TRAPIRAT			
N	MEAN	N	MEAN	N	MEAN	N	MEAN		
3_23	6	12	0.000	12	0.000	10	0.000	8	0.068
	7	12	0.000	12	0.111	12	0.000	12	0.000
3_45	1	12	0.000	12	0.000	12	0.000	12	0.000
	2	11	0.000	12	0.000	12	0.033	12	0.036
	3	12	0.000	12	0.000	12	0.462	12	0.055
	4	12	0.040	11	0.000	11	0.000	11	0.237
	5	12	0.000	12	0.020	12	0.000	12	0.056
	6	12	0.000	12	0.000	10	0.000	8	0.000
	7	12	0.044	12	0.319	12	0.000	12	0.000
3_67	1	12	0.000	12	0.000	12	0.000	12	0.013
	2	11	0.000	12	0.000	12	0.215	12	0.125
	3	12	0.080	12	0.000	12	0.831	12	0.077
	4	12	0.224	11	0.305	11	0.000	11	0.052
	5	12	0.030	12	0.000	12	0.000	12	0.046
	6	12	0.000	12	0.198	10	0.000	9	0.000
	7	12	0.065	12	0.000	12	0.000	12	0.000

APPENDIX B

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study

TABLE OF NFOUND BY EXPCNTL

NFOUND	EXPCNTL		Total
	c	e	
0	1194	1120	2314
	4.20	3.94	8.14
	51.60	48.40	
	8.67	7.64	
1	12570	13536	26106
	44.23	47.63	91.86
	48.15	51.85	
	91.33	92.36	
Total	13764	14656	28420
	48.43	51.57	100.00

TABLE OF NFOUND BY PERIOD

NFOUND	PERIOD							Total
	1	2	3	4	5	6	7	
0	348	320	366	316	310	326	328	2314
	1.22	1.13	1.29	1.11	1.09	1.15	1.15	8.14
	15.04	13.83	15.82	13.66	13.40	14.09	14.17	
	12.38	6.50	10.31	9.06	6.33	6.79	8.30	
1	2463	4606	3183	3171	4584	4476	3623	26106
	8.67	16.21	11.20	11.16	16.13	15.75	12.75	91.86
	9.43	17.64	12.19	12.15	17.56	17.15	13.88	
	87.62	93.50	89.69	90.94	93.67	93.21	91.70	
Total	2811	4926	3549	3487	4894	4802	3951	28420
	9.89	17.33	12.49	12.27	17.22	16.90	13.90	100.00

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

1011 VESSELS FOUND IN 127 QUADRANTS(WITHIN RINGS) ARE DELETED

TABLE OF NFOUND BY REGION

NFOUND	REGION			Total
	a	c	m	
0	832 2.93 35.96 9.32	722 2.54 31.20 9.93	760 2.67 32.84 6.22	2314 8.14
1	8091 28.47 30.99 90.68	6551 23.05 25.09 90.07	11464 40.34 43.91 93.78	26106 91.86
Total	8923 31.40	7273 25.59	12224 43.01	28420 100.00

TABLE OF NFOUND BY SECTION

NFOUND	SECTION			Total
	1	2	3	
0	797 2.80 34.44 8.72	780 2.74 33.71 8.63	737 2.59 31.85 7.19	2314 8.14
1	8340 29.35 31.95 91.28	8258 29.06 31.63 91.37	9508 33.46 36.42 92.81	26106 91.86
Total	9137 32.15	9038 31.80	10245 36.05	28420 100.00

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

TABLE OF NFOUND BY RSECTOR

NFOUND	RSECTOR												Total	
	1_18	1_23	1_45	1_67	2_18	2_23	2_45	2_67	3_18	3_23	3_45	3_67		
0	58	34	40	42	312	273	291	279	263	221	256	245	2314	
	0.20	0.12	0.14	0.15	1.10	0.96	1.02	0.98	0.93	0.78	0.90	0.86		8.14
	2.51	1.47	1.73	1.82	13.48	11.80	12.58	12.06	11.37	9.55	11.06	10.59		
1.77	0.77	1.05	0.97	23.73	17.12	19.73	17.49	17.36	13.08	16.85	13.26			
1	3228	4410	3773	4284	1003	1322	1184	1316	1252	1468	1263	1603	26106	
	11.36	15.52	13.28	15.07	3.53	4.65	4.17	4.63	4.41	5.17	4.44	5.64		91.86
	12.36	16.89	14.45	16.41	3.84	5.06	4.54	5.04	4.80	5.62	4.84	6.14		
98.23	99.23	98.95	99.03	76.27	82.88	80.27	82.51	82.64	86.92	83.15	86.74			
Total	3286	4444	3813	4326	1315	1595	1475	1595	1515	1689	1519	1848	28420	
	11.56	15.64	13.42	15.22	4.63	5.61	5.19	5.61	5.33	5.94	5.34	6.50	100.00	

TABLE OF NFOUND BY SECTOR

NFOUND	SECTOR				Total	
	18	23	45	67		
0	633	528	587	566	2314	
	2.23	1.86	2.07	1.99		8.14
	27.36	22.82	25.37	24.46		
10.35	6.83	8.62	7.29			
1	5483	7200	6220	7203	26106	
	19.29	25.33	21.89	25.34		91.86
	21.00	27.58	23.83	27.59		
89.65	93.17	91.38	92.71			
Total	6116	7728	6807	7769	28420	
	21.52	27.19	23.95	27.34	100.00	

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

1011 VESSELS FOUND IN 127 QUADRANTS(WITHIN RINGS) ARE DELETED

TABLE OF NFOUND BY RING

NFOUND	RING			Total
	1	2	3	
Frequency				
Percent				
Row Pct				
Col Pct				
0	174 0.61 7.52 1.10	1155 4.06 49.91 19.31	985 3.47 42.57 14.99	2314 8.14
1	15695 55.23 60.12 98.90	4825 16.98 18.48 80.69	5586 19.66 21.40 85.01	26106 91.86
Total	15869 55.84	5980 21.04	6571 23.12	28420 100.00

TABLE OF REGION BY SECTION

REGION	SECTION			Total
	1	2	3	
Frequency				
Percent				
Row Pct				
Col Pct				
a	2839 9.99 31.82 31.07	2977 10.48 33.36 32.94	3107 10.93 34.82 30.33	8923 31.40
c	2707 9.52 37.22 29.63	2276 8.01 31.29 25.18	2290 8.06 31.49 22.35	7273 25.59
m	3591 12.64 29.38 39.30	3785 13.32 30.96 41.88	4848 17.06 39.66 47.32	12224 43.01
Total	9137 32.15	9038 31.80	10245 36.05	28420 100.00

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study (Continued)

1011 VESSELS FOUND IN 127 QUADRANTS (WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
2	126 0.48 54.31 1.00	106 0.41 45.69 0.78	232 0.89
3	182 0.70 51.12 1.45	174 0.67 48.88 1.29	356 1.36
4	1767 6.77 50.18 14.06	1754 6.72 49.82 12.96	3521 13.49
5	680 2.60 51.36 5.41	644 2.47 48.64 4.76	1324 5.07
6	648 2.48 48.76 5.16	681 2.61 51.24 5.03	1329 5.09
7	991 3.80 47.81 7.88	1082 4.14 52.19 7.99	2073 7.94
8	469 1.80 50.43 3.73	461 1.77 49.57 3.41	930 3.56
9	773 2.96 49.30 6.15	795 3.05 50.70 5.87	1568 6.01
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
10	442 1.69 52.25 3.52	404 1.55 47.75 2.98	846 3.24
11	499 1.91 47.25 3.97	557 2.13 52.75 4.11	1056 4.05
12	245 0.94 48.90 1.95	256 0.98 51.10 1.89	501 1.92
13	370 1.42 49.20 2.94	382 1.46 50.80 2.82	752 2.88
14	325 1.24 44.77 2.59	401 1.54 55.23 2.96	726 2.78
15	232 0.89 51.67 1.85	217 0.83 48.33 1.60	449 1.72
16	329 1.26 49.55 2.62	335 1.28 50.45 2.47	664 2.54
17	167 0.64 47.31 1.33	186 0.71 52.69 1.37	353 1.35
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
18	349 1.34 49.29 2.78	359 1.38 50.71 2.65	708 2.71
19	119 0.46 47.41 0.95	132 0.51 52.59 0.98	251 0.96
20	197 0.75 50.90 1.57	190 0.73 49.10 1.40	387 1.48
21	215 0.82 49.43 1.71	220 0.84 50.57 1.63	435 1.67
22	161 0.62 45.87 1.28	190 0.73 54.13 1.40	351 1.34
23	204 0.78 45.64 1.62	243 0.93 54.36 1.80	447 1.71
24	96 0.37 43.64 0.76	124 0.47 56.36 0.92	220 0.84
25	193 0.74 45.73 1.54	229 0.88 54.27 1.69	422 1.62
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study (Continued)

1011 VESSELS FOUND IN 127 QUADRANTS (WITHIN RINGS) ARE DELETED.

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
26	103 0.39 47.91 0.82	112 0.43 52.09 0.83	215 0.82
27	92 0.35 42.79 0.73	123 0.47 57.21 0.91	215 0.82
28	174 0.67 48.33 1.38	186 0.71 51.67 1.37	360 1.38
29	74 0.28 38.54 0.59	118 0.45 61.46 0.87	192 0.74
30	162 0.62 44.51 1.29	202 0.77 55.49 1.49	364 1.39
31	69 0.26 41.07 0.55	99 0.38 58.93 0.73	168 0.64
32	142 0.54 46.41 1.13	164 0.63 53.59 1.21	306 1.17
33	86 0.33 47.78 0.68	94 0.36 52.22 0.69	180 0.69
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
34	84 0.32 45.90 0.67	99 0.38 54.10 0.73	183 0.70
35	98 0.38 44.14 0.78	124 0.47 55.86 0.92	222 0.85
36	62 0.24 41.61 0.49	87 0.33 58.39 0.64	149 0.57
37	114 0.44 49.78 0.91	115 0.44 50.22 0.85	229 0.88
38	86 0.33 52.44 0.68	78 0.30 47.56 0.58	164 0.63
39	55 0.21 48.25 0.44	59 0.23 51.75 0.44	114 0.44
40	85 0.33 46.70 0.68	97 0.37 53.30 0.72	182 0.70
41	57 0.22 49.57 0.45	58 0.22 50.43 0.43	115 0.44
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
42	73 0.28 41.01 0.58	105 0.40 58.99 0.78	178 0.68
43	43 0.16 41.75 0.34	60 0.23 58.25 0.44	103 0.39
44	56 0.21 46.28 0.45	65 0.25 53.72 0.48	121 0.46
45	51 0.20 47.66 0.41	56 0.21 52.34 0.41	107 0.41
46	37 0.14 50.68 0.29	36 0.14 49.32 0.27	73 0.28
47	52 0.20 40.63 0.41	76 0.29 59.38 0.56	128 0.49
48	39 0.15 39.39 0.31	60 0.23 60.61 0.44	99 0.38
49	40 0.15 39.22 0.32	62 0.24 60.78 0.46	102 0.39
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
50	46 0.18 45.10 0.37	56 0.21 54.90 0.41	102 0.39
51	37 0.14 36.63 0.29	64 0.25 63.37 0.47	101 0.39
52	34 0.13 59.65 0.27	23 0.09 40.35 0.17	57 0.22
53	40 0.15 56.34 0.32	31 0.12 43.66 0.23	71 0.27
54	38 0.15 43.68 0.30	49 0.19 56.32 0.36	87 0.33
55	33 0.13 58.93 0.26	23 0.09 41.07 0.17	56 0.21
56	35 0.13 50.00 0.28	35 0.13 50.00 0.26	70 0.27
57	33 0.13 50.00 0.26	33 0.13 50.00 0.24	66 0.25
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
58	32 0.12 41.03 0.25	46 0.18 58.97 0.34	78 0.30
59	23 0.09 42.59 0.18	31 0.12 57.41 0.23	54 0.21
60	10 0.04 28.57 0.08	25 0.10 71.43 0.18	35 0.13
61	31 0.12 47.69 0.25	34 0.13 52.31 0.25	65 0.25
62	24 0.09 58.54 0.19	17 0.07 41.46 0.13	41 0.16
63	22 0.08 39.29 0.18	34 0.13 60.71 0.25	56 0.21
64	10 0.04 33.33 0.08	20 0.08 66.67 0.15	30 0.11
65	18 0.07 41.86 0.14	25 0.10 58.14 0.18	43 0.16
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
66	11 0.04 36.67 0.09	19 0.07 63.33 0.14	30 0.11
67	16 0.06 47.06 0.13	18 0.07 52.94 0.13	34 0.13
68	17 0.07 51.52 0.14	16 0.06 48.48 0.12	33 0.13
69	9 0.03 36.00 0.07	16 0.06 64.00 0.12	25 0.10
70	17 0.07 45.95 0.14	20 0.08 54.05 0.15	37 0.14
71	9 0.03 39.13 0.07	14 0.05 60.87 0.10	23 0.09
72	19 0.07 46.34 0.15	22 0.08 53.66 0.16	41 0.16
73	10 0.04 47.62 0.08	11 0.04 52.38 0.08	21 0.08
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study (Continued)

1011 VESSELS FOUND IN 127 QUADRANTS (WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
74	10 0.04 55.56 0.08	8 0.03 44.44 0.06	18 0.07
75	15 0.06 36.59 0.12	26 0.10 63.41 0.19	41 0.16
76	7 0.03 23.33 0.06	23 0.09 76.67 0.17	30 0.11
77	11 0.04 40.74 0.09	16 0.06 59.26 0.12	27 0.10
78	9 0.03 52.94 0.07	8 0.03 47.06 0.06	17 0.07
79	6 0.02 40.00 0.05	9 0.03 60.00 0.07	15 0.06
80	7 0.03 43.75 0.06	9 0.03 56.25 0.07	16 0.06
81	5 0.02 31.25 0.04	11 0.04 68.75 0.08	16 0.06
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
82	11 0.04 61.11 0.09	7 0.03 38.89 0.05	18 0.07
83	11 0.04 61.11 0.09	7 0.03 38.89 0.05	18 0.07
84	5 0.02 31.25 0.04	11 0.04 68.75 0.08	16 0.06
85	8 0.03 42.11 0.06	11 0.04 57.89 0.08	19 0.07
86	1 0.00 14.29 0.01	6 0.02 85.71 0.04	7 0.03
87	9 0.03 47.37 0.07	10 0.04 52.63 0.07	19 0.07
88	4 0.02 28.57 0.03	10 0.04 71.43 0.07	14 0.05
89	6 0.02 40.00 0.05	9 0.03 60.00 0.07	15 0.06
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
90	5 0.02 41.67 0.04	7 0.03 58.33 0.05	12 0.05
91	7 0.03 58.33 0.06	5 0.02 41.67 0.04	12 0.05
92	5 0.02 31.25 0.04	11 0.04 68.75 0.08	16 0.06
93	2 0.01 22.22 0.02	7 0.03 77.78 0.05	9 0.03
94	5 0.02 45.45 0.04	6 0.02 54.55 0.04	11 0.04
95	4 0.02 66.67 0.03	2 0.01 33.33 0.01	6 0.02
96	3 0.01 42.86 0.02	4 0.02 57.14 0.03	7 0.03
97	4 0.02 50.00 0.03	4 0.02 50.00 0.03	8 0.03
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study (Continued)

1011 VESSELS FOUND IN 127 QUADRANTS (WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
98	6 0.02 42.86 0.05	8 0.03 57.14 0.06	14 0.05
99	0 0.00 0.00 0.00	4 0.02 100.00 0.03	4 0.02
100	4 0.02 80.00 0.03	1 0.00 20.00 0.01	5 0.02
101	2 0.01 25.00 0.02	6 0.02 75.00 0.04	8 0.03
102	5 0.02 71.43 0.04	2 0.01 28.57 0.01	7 0.03
103	6 0.02 40.00 0.05	9 0.03 60.00 0.07	15 0.06
104	3 0.01 75.00 0.02	1 0.00 25.00 0.01	4 0.02
105	5 0.02 35.71 0.04	9 0.03 64.29 0.07	14 0.05
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
106	4 0.02 80.00 0.03	1 0.00 20.00 0.01	5 0.02
107	6 0.02 50.00 0.05	6 0.02 50.00 0.04	12 0.05
108	1 0.00 14.29 0.01	6 0.02 85.71 0.04	7 0.03
109	1 0.00 16.67 0.01	5 0.02 83.33 0.04	6 0.02
110	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
111	2 0.01 40.00 0.02	3 0.01 60.00 0.02	5 0.02
112	1 0.00 25.00 0.01	3 0.01 75.00 0.02	4 0.02
113	4 0.02 57.14 0.03	3 0.01 42.86 0.02	7 0.03
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
114	4 0.02 57.14 0.03	3 0.01 42.86 0.02	7 0.03
115	2 0.01 50.00 0.02	2 0.01 50.00 0.01	4 0.02
116	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
117	2 0.01 50.00 0.02	2 0.01 50.00 0.01	4 0.02
118	7 0.03 58.33 0.06	5 0.02 41.67 0.04	12 0.05
119	4 0.02 57.14 0.03	3 0.01 42.86 0.02	7 0.03
120	1 0.00 20.00 0.01	4 0.02 80.00 0.03	5 0.02
121	1 0.00 25.00 0.01	3 0.01 75.00 0.02	4 0.02
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

1011 VESSELS FOUND IN 127 QUADRANTS (WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
122	1 0.00 33.33 0.01	2 0.01 66.67 0.01	3 0.01
123	1 0.00 25.00 0.01	3 0.01 75.00 0.02	4 0.02
124	3 0.01 75.00 0.02	1 0.00 25.00 0.01	4 0.02
126	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
127	2 0.01 40.00 0.02	3 0.01 60.00 0.02	5 0.02
128	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
129	0 0.00 0.00 0.00	4 0.02 100.00 0.03	4 0.02
130	0 0.00 0.00 0.00	3 0.01 100.00 0.02	3 0.01
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
131	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
132	2 0.01 50.00 0.02	2 0.01 50.00 0.01	4 0.02
133	2 0.01 66.67 0.02	1 0.00 33.33 0.01	3 0.01
134	0 0.00 0.00 0.00	2 0.01 100.00 0.01	2 0.01
135	2 0.01 50.00 0.02	2 0.01 50.00 0.01	4 0.02
136	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
137	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
138	0 0.00 0.00 0.00	3 0.01 100.00 0.02	3 0.01
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
139	0 0.00 0.00 0.00	3 0.01 100.00 0.02	3 0.01
140	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
141	1 0.00 25.00 0.01	3 0.01 75.00 0.02	4 0.02
142	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
143	2 0.01 33.33 0.02	4 0.02 66.67 0.03	6 0.02
145	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
146	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
147	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

Raw Data Summary of All Blood Vessels (Period, RSector, Region) Found in the Study (Continued)

1011 VESSELS FOUND IN 127 QUADRANTS(WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
148	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
149	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
150	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
151	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
152	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
154	0 0.00 0.00 0.00	2 0.01 100.00 0.01	2 0.01
155	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
157	2 0.01 100.00 0.02	0 0.00 0.00 0.00	2 0.01
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
158	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
159	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
161	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
163	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
164	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
165	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
166	1 0.00 50.00 0.01	1 0.00 50.00 0.01	2 0.01
168	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
169	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
170	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
171	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
172	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
176	2 0.01 66.67 0.02	1 0.00 33.33 0.01	3 0.01
177	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
180	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
183	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

1011 VESSELS FOUND IN 127 QUADRANTS(WITHIN RINGS) ARE DELETED

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
184	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
185	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
186	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
187	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
188	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
189	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
190	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
191	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
192	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
193	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
195	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
196	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
197	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
209	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
212	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
216	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
217	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
218	2 0.01 100.00 0.02	0 0.00 0.00 0.00	2 0.01
221	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
230	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
245	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
246	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
258	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
262	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

(Continued)

**Raw Data Summary of All Blood Vessels
(Period, RSector, Region) Found in the Study (Continued)**

TABLE OF DINT BY EXPCNTL

DINT	EXPCNTL		Total
	c	e	
266	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
268	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
298	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
299	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
306	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
310	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
347	0 0.00 0.00 0.00	1 0.00 100.00 0.01	1 0.00
407	1 0.00 100.00 0.01	0 0.00 0.00 0.00	1 0.00
Total	12570 48.15	13536 51.85	26106 100.00

APPENDIX C

Raw Data of Number of Blood Vessels Found in the Study (Period x Sector)

		EXPCNTL												
		c						e						
		REGION						REGION						
		a		c		m		a		c		m		
NFOUND		NFOUND		NFOUND		NFOUND		NFOUND		NFOUND				
PERIOD	RSECTOR	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	
1	1_18	34	1.0	42	0.9	30	0.9	33	1.0	64	1.0	77	1.0	
	1_23	102	1.0	88	1.0	52	1.0	90	1.0	89	1.0	94	1.0	
	1_45	100	1.0	77	1.0	24	0.9	58	1.0	46	1.0	80	1.0	
	1_67	76	1.0	66	1.0	28	0.9	66	1.0	67	1.0	110	1.0	
	2_18	7	0.4	15	0.7	1	0.1	17	0.8	17	0.7	14	0.7	
	2_23	18	0.6	36	0.9	4	0.3	36	0.9	18	0.8	12	0.7	
	2_45	7	0.4	41	0.9	2	0.2	11	0.6	5	0.4	21	0.8	
	2_67	36	0.8	43	1.0	5	0.3	19	0.8	16	0.7	24	0.8	
	3_18	7	0.4	16	0.7	7	0.4	4	0.3	17	0.8	17	0.8	
	3_23	23	0.8	59	0.9	11	0.6	17	0.7	13	0.8	28	0.8	
	3_45	31	0.8	29	0.8	7	0.5	7	0.5	11	0.6	18	0.8	
	3_67	25	0.8	14	0.7	17	0.7	9	0.6	20	0.8	38	0.9	
	2	1_18	93	1.0	47	1.0	83	1.0	129	1.0	63	1.0	152	1.0
		1_23	118	1.0	81	1.0	189	1.0	130	1.0	122	1.0	210	1.0
1_45		92	1.0	67	1.0	138	1.0	176	1.0	82	1.0	149	1.0	
1_67		111	1.0	53	1.0	175	1.0	204	1.0	54	1.0	111	1.0	
2_18		24	0.8	7	0.5	45	0.9	4	0.3	38	0.9	63	0.9	
2_23		23	0.8	20	0.8	62	0.9	9	0.5	47	0.9	81	0.9	
2_45		25	0.8	34	0.9	54	0.9	6	0.4	30	0.8	69	0.9	
2_67		24	0.8	27	0.8	68	0.9	3	0.2	20	0.7	74	0.9	

**Raw Data of Number of Blood Vessels
Found in the Study (Period x Sector)(Continued)**

PERIOD	RSECTOR	EXPCNTL											
		c						e					
		REGION						REGION					
		a		c		m		a		c		m	
		NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND
		SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN
2	3_18	29	0.8	17	0.8	50	0.9	30	0.8	34	0.9	30	0.8
	3_23	41	0.9	26	0.9	82	1.0	41	0.8	19	0.8	52	0.9
	3_45	20	0.7	24	0.8	66	1.0	49	0.9	35	0.9	40	0.9
	3_67	30	0.8	18	0.7	59	1.0	74	0.9	26	0.8	28	0.8
3	1_18	85	1.0	21	0.8	47	0.9	106	1.0	6	0.5	101	1.0
	1_23	77	1.0	53	0.9	59	0.9	118	1.0	48	0.9	101	1.0
	1_45	67	1.0	58	0.9	70	0.9	86	1.0	57	1.0	116	1.0
	1_67	90	1.0	43	1.0	48	0.9	130	1.0	41	1.0	171	1.0
	2_18	42	0.9	6	0.4	26	0.8	27	0.7	11	0.6	16	0.6
	2_23	21	0.8	29	0.8	34	0.9	22	0.7	21	0.8	17	0.6
	2_45	6	0.4	23	0.8	42	0.9	12	0.5	14	0.7	41	0.8
	2_67	39	0.9	19	0.8	22	0.8	31	0.8	10	0.6	36	0.8
	3_18	18	0.7	18	0.9	42	0.9	48	0.9	8	0.5	62	0.9
	3_23	6	0.4	24	0.8	27	0.9	79	1.0	8	0.5	44	0.9
	3_45	4	0.3	15	0.7	26	0.8	41	0.9	22	0.8	89	1.0
	3_67	9	0.5	16	0.7	16	0.8	53	0.9	17	0.8	125	1.0
4	1_18	77	1.0	30	1.0	65	1.0	72	1.0	52	1.0	112	1.0
	1_23	109	1.0	62	1.0	66	1.0	107	1.0	97	1.0	179	1.0
	1_45	75	1.0	53	1.0	71	1.0	71	1.0	65	1.0	112	1.0
	1_67	96	1.0	39	1.0	52	1.0	93	1.0	92	1.0	157	1.0

**Raw Data of Number of Blood Vessels
Found in the Study (Period x Sector)(Continued)**

PERIOD	RSECTOR	EXPCNTL												
		c						e						
		REGION			REGION			REGION			REGION			
		a	c	m	a	c	m	a	c	m	a	c	m	
		NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	
SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN			
4	2_18	55	0.9	11	0.6	7	0.4	11	0.6	20	0.8	13	0.7	
	2_23	27	0.8	23	0.8	11	0.6	15	0.7	23	0.8	46	0.9	
	2_45	44	0.9	19	0.9	17	0.7	7	0.4	29	0.9	19	0.8	
	2_67	31	0.8	29	0.9	17	0.7	19	0.7	36	0.9	40	0.9	
	3_18	39	0.8	8	0.5	10	0.6	15	0.8	29	0.9	40	0.9	
	3_23	29	0.8	16	0.8	6	0.5	20	0.8	27	1.0	49	0.9	
	3_45	37	0.8	18	0.8	24	0.8	12	0.7	16	0.9	29	0.8	
	3_67	34	0.9	11	0.6	27	0.8	39	0.9	37	1.0	26	0.8	
	5	1_18	53	1.0	70	1.0	119	1.0	90	1.0	50	1.0	180	1.0
		1_23	59	1.0	143	1.0	150	1.0	84	1.0	94	1.0	105	1.0
1_45		73	1.0	85	1.0	193	1.0	71	1.0	68	1.0	219	1.0	
1_67		70	1.0	104	1.0	170	1.0	72	1.0	45	0.9	250	1.0	
2_18		15	0.7	27	0.8	58	0.9	9	0.5	19	0.7	82	1.0	
2_23		12	0.6	63	1.0	53	0.9	8	0.5	51	0.9	83	1.0	
2_45		15	0.7	31	0.9	53	0.9	18	0.7	30	0.9	72	0.9	
2_67		13	0.7	71	1.0	61	0.9	15	0.7	33	0.8	89	1.0	
3_18		13	0.6	21	0.8	33	0.8	20	0.8	19	0.8	128	1.0	
3_23		13	0.6	56	0.9	30	0.8	23	0.8	33	0.9	96	1.0	
3_45		12	0.7	9	0.5	60	0.9	31	0.9	17	0.7	82	1.0	
3_67		22	0.8	26	0.8	68	0.9	29	0.8	17	0.7	128	1.0	

**Raw Data of Number of Blood Vessels
Found in the Study (Period x Sector)(Continued)**

PERIOD	RSECTOR	EXPCNTL												
		c						e						
		REGION			REGION			REGION			REGION			
		a	c	m	a	c	m	a	c	m	a	c	m	
		NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	NFOUND	
SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN			
6	1_18	109	1.0	98	1.0	140	1.0	85	1.0	59	1.0	109	1.0	
	1_23	142	1.0	122	1.0	154	1.0	119	1.0	87	1.0	89	1.0	
	1_45	114	1.0	66	1.0	104	1.0	94	1.0	78	1.0	98	1.0	
	1_67	113	1.0	97	1.0	180	1.0	119	1.0	83	1.0	212	1.0	
	2_18	30	0.8	73	0.9	24	0.7	27	0.8	20	0.8	8	0.5	
	2_23	40	0.8	64	0.9	32	0.8	36	0.9	11	0.7	31	0.8	
	2_45	36	0.8	45	0.9	41	0.8	21	0.7	30	0.9	23	0.7	
	2_67	27	0.8	71	1.0	29	0.8	46	0.9	7	0.4	46	0.8	
	3_18	106	0.9	28	0.8	59	0.9	13	0.7	7	0.4	30	0.8	
	3_23	60	0.9	69	0.9	84	1.0	17	0.8	10	0.5	35	0.8	
	3_45	71	0.9	35	0.9	48	0.9	7	0.5	6	0.4	14	0.6	
	3_67	112	1.0	57	1.0	73	0.9	17	0.7	7	0.4	22	0.7	
	7	1_18	68	1.0	76	1.0	93	1.0	66	1.0	32	0.9	110	1.0
		1_23	80	1.0	101	1.0	122	1.0	100	1.0	73	0.9	145	1.0
		1_45	81	1.0	82	1.0	129	1.0	72	1.0	45	0.9	111	1.0
1_67		112	1.0	28	0.9	128	1.0	111	1.0	37	1.0	180	1.0	
2_18		12	0.6	10	0.6	10	0.5	2	0.2	13	0.6	67	1.0	
2_23		11	0.6	35	0.9	14	0.6	9	0.5	53	0.9	61	0.9	
2_45		18	0.7	33	0.9	30	0.8	5	0.4	32	0.9	73	0.9	
2_67		23	0.8	9	0.6	16	0.6	4	0.3	15	0.8	53	0.9	

**Raw Data of Number of Blood Vessels
Found in the Study (Period x Sector)(Continued)**

PERIOD	RSECTOR	EXPCNTL											
		c						e					
		REGION						REGION					
		a		c		m		a		c		m	
		NFOUND	MEAN	NFOUND	MEAN	NFOUND	MEAN	NFOUND	MEAN	NFOUND	MEAN	NFOUND	MEAN
		SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN	SUM	MEAN
7	3_18	33	0.9	13	0.6	53	0.9	6	0.4	14	0.7	61	0.9
	3_23	35	0.9	28	0.9	30	0.9	16	0.7	16	0.7	70	0.9
	3_45	46	0.9	29	0.8	56	0.9	5	0.3	17	0.8	48	0.9
	3_67	97	0.9	4	0.3	53	1.0	9	0.5	14	0.7	80	0.9

APPENDIX D

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)**

EXPCNTL	PERIOD	RSECTOR	REGION																	
			a						c						m					
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1	DELEVEN1		
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN		
c	1	1_18	11	2.016	12	0.039	12	0.644	9	2.221	12	0.028	12	0.356	9	2.876	12	0.135	12	0.577
		1_23	12	2.099	12	0.238	12	0.565	12	2.041	12	0.156	12	0.489	10	2.209	12	0.153	12	0.516
		1_45	12	2.303	12	0.222	12	0.671	10	2.016	12	0.102	12	0.439	10	2.961	12	0.144	12	0.597
		1_67	12	1.742	12	0.100	12	0.615	10	2.130	12	0.069	12	0.651	10	2.121	12	0.072	12	0.586
		2_18	3	1.545	12	0.001	12	0.117	6	1.752	12	0.023	12	0.326	1	3.778	12	0.006	12	0.083
		2_23	2	1.683	12	0.008	12	0.052	9	2.190	12	0.029	12	0.334	2	2.529	11	0.014	11	0.091
		2_45	3	2.766	12	0.009	12	0.111	8	1.673	12	0.052	12	0.467	2	1.730	12	0.003	12	0.083
		2_67	4	1.599	12	0.013	12	0.066	10	1.886	12	0.081	12	0.340	2	2.382	12	0.007	12	0.104
		3_18	2	1.624	12	0.001	12	0.000	5	1.938	12	0.039	12	0.173	3	2.438	12	0.007	12	0.125
	3_23	7	2.396	12	0.011	12	0.163	7	1.972	12	0.040	12	0.208	3	1.725	12	0.011	12	0.042	
	3_45	4	2.329	12	0.035	12	0.183	5	2.127	12	0.013	12	0.143	4	2.299	12	0.018	12	0.222	
	3_67	6	2.045	12	0.007	12	0.107	5	2.093	12	0.012	12	0.183	3	2.357	12	0.016	12	0.173	
	2	1_18	11	2.050	12	0.191	12	0.652	10	1.861	12	0.038	12	0.530	9	1.892	11	0.136	11	0.574
		1_23	12	1.937	12	0.388	12	0.821	11	2.367	12	0.206	12	0.596	10	1.878	12	0.186	12	0.389
		1_45	12	1.631	12	0.116	12	0.770	12	1.894	12	0.101	12	0.595	9	2.010	11	0.140	11	0.540
		1_67	12	1.742	12	0.124	12	0.699	10	1.919	12	0.079	12	0.709	10	1.798	12	0.246	12	0.574
		2_18	5	2.023	12	0.032	12	0.109	3	1.947	11	0.002	11	0.121	6	1.785	12	0.004	12	0.066
		2_23	5	1.997	12	0.049	12	0.251	6	1.819	11	0.015	11	0.252	6	1.637	12	0.025	12	0.242
2_45		4	1.697	12	0.017	12	0.196	6	1.916	11	0.016	11	0.283	6	1.827	12	0.027	12	0.233	
2_67		5	1.594	12	0.007	12	0.213	6	1.675	11	0.009	11	0.107	6	1.721	12	0.015	12	0.180	
3_18		3	1.821	11	0.008	11	0.076	7	2.411	12	0.051	12	0.246	7	1.984	10	0.008	10	0.271	
3_23	7	1.682	11	0.030	11	0.427	8	2.109	11	0.110	11	0.358	6	1.810	10	0.043	10	0.116		

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																	
			a						c						in					
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1	
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN
c	2	3_45	3	1.858	11	0.008	11	0.117	7	1.756	12	0.023	12	0.174	7	1.690	10	0.055	10	0.356
		3_67	4	1.826	11	0.011	11	0.201	5	1.674	12	0.008	12	0.175	8	1.660	11	0.095	11	0.264
	3	1_18	10	2.004	12	0.104	12	0.366	7	2.206	12	0.039	12	0.174	7	1.984	11	0.049	11	0.452
1_23		12	2.185	12	0.181	12	0.723	9	2.345	12	0.035	12	0.352	6	1.753	11	0.048	11	0.336	
1_45		12	2.131	12	0.192	12	0.755	7	1.902	12	0.051	12	0.392	7	1.869	11	0.055	11	0.322	
1_67		12	2.037	12	0.165	12	0.637	10	1.812	12	0.049	12	0.556	5	1.601	11	0.066	11	0.273	
2_18		5	1.872	12	0.018	12	0.164	3	2.092	12	0.002	12	0.125	6	1.888	12	0.005	12	0.199	
2_23		7	1.767	12	0.011	12	0.108	4	2.041	12	0.012	12	0.233	7	1.937	11	0.026	11	0.354	
2_45		3	2.102	12	0.010	12	0.083	6	1.705	12	0.014	12	0.222	7	1.636	11	0.013	11	0.196	
2_67		7	1.697	12	0.011	12	0.121	6	1.820	12	0.012	12	0.297	4	1.629	11	0.007	11	0.246	
3_18		4	2.082	12	0.006	12	0.109	9	3.377	12	0.142	12	0.556	6	1.709	11	0.105	11	0.147	
3_23		1	1.854	12	0.003	12	0.042	6	2.194	12	0.038	12	0.405	7	1.993	11	0.004	11	0.315	
3_45		3	1.809	11	0.002	11	0.136	6	2.125	12	0.019	12	0.146	4	1.854	9	0.016	9	0.143	
3_67		2	3.386	12	0.004	12	0.104	5	1.539	12	0.008	12	0.196	5	1.820	9	0.004	9	0.074	
4		1_18	8	1.919	12	0.101	12	0.475	10	2.061	11	0.079	11	0.709	10	1.782	12	0.137	12	0.668
		1_23	11	2.235	12	0.367	12	0.653	10	2.202	12	0.077	12	0.583	9	2.005	12	0.213	12	0.520
		1_45	11	2.377	12	0.167	12	0.707	10	2.498	12	0.122	12	0.536	11	2.069	12	0.171	12	0.727
	1_67	10	1.875	12	0.204	12	0.643	10	2.356	11	0.147	11	0.647	10	1.888	12	0.150	12	0.724	
	2_18	5	1.957	12	0.012	12	0.073	5	2.062	12	0.035	12	0.208	1	1.886	12	0.003	12	0.060	
	2_23	5	1.812	12	0.025	12	0.182	6	1.962	12	0.016	12	0.311	3	1.935	12	0.015	12	0.097	
	2_45	6	2.580	12	0.054	12	0.242	8	2.254	11	0.016	11	0.312	3	2.006	12	0.022	12	0.091	
	2_67	4	1.942	12	0.008	12	0.130	8	2.050	12	0.080	12	0.179	6	1.731	12	0.013	12	0.381	

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																		
			a						c						m						
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1	DELEVEN1			
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN			
c	4	3_18	4	1.995	11	0.010	11	0.071	5	2.014	12	0.005	12	0.167	5	2.216	11	0.042	11	0.318	
		3_23	5	1.906	11	0.060	11	0.155	7	2.753	12	0.089	12	0.181	3	2.177	9	0.005	9	0.167	
		3_45	4	1.826	11	0.018	11	0.171	6	2.425	12	0.024	12	0.160	5	2.008	11	0.047	11	0.186	
		3_67	5	2.087	11	0.072	11	0.244	6	2.232	12	0.018	12	0.236	4	1.694	11	0.009	11	0.176	
		5	1_18	11	2.230	12	0.086	12	0.746	11	2.055	11	0.185	11	0.760	9	1.853	10	0.177	10	0.555
	1_23		12	2.057	12	0.086	12	0.784	12	1.961	12	0.183	12	0.510	10	2.090	12	0.220	12	0.482	
	1_45		12	2.319	12	0.184	12	0.691	12	2.200	12	0.088	12	0.619	11	1.831	12	0.153	12	0.549	
	1_67		12	1.843	12	0.067	12	0.651	9	1.849	12	0.161	12	0.574	11	1.830	12	0.267	12	0.620	
	2_18		4	1.940	12	0.018	12	0.219	7	1.822	12	0.005	12	0.243	5	1.813	12	0.064	12	0.157	
	2_23		3	1.702	12	0.015	12	0.067	9	1.778	12	0.060	12	0.330	3	1.672	12	0.043	12	0.094	
	2_45		5	2.273	12	0.029	12	0.200	7	1.897	12	0.023	12	0.267	3	1.905	12	0.035	12	0.087	
	2_67		5	1.760	12	0.007	12	0.264	9	1.843	12	0.042	12	0.394	5	1.884	12	0.031	12	0.224	
	3_18		4	1.859	12	0.004	12	0.069	5	1.940	12	0.004	12	0.135	5	2.173	12	0.064	12	0.176	
	3_23		4	1.770	12	0.011	12	0.075	8	2.022	12	0.088	12	0.354	4	1.832	11	0.013	11	0.096	
	3_45		6	3.051	12	0.037	12	0.408	3	1.764	12	0.003	12	0.028	5	2.458	12	0.034	12	0.199	
	3_67		6	2.099	12	0.012	12	0.163	5	2.092	12	0.007	12	0.105	5	1.768	12	0.052	12	0.260	
	6		1_18	12	1.987	12	0.276	12	0.668	9	1.845	12	0.077	12	0.299	11	1.872	11	0.285	11	0.731
			1_23	12	1.978	12	0.409	12	0.743	11	2.831	12	0.117	12	0.536	11	2.371	11	0.149	11	0.673
			1_45	12	2.065	12	0.401	12	0.759	8	3.242	11	0.090	11	0.370	10	1.822	10	0.247	10	0.641
		1_67	12	1.795	12	0.258	12	0.737	10	2.039	11	0.162	11	0.623	12	1.712	12	0.300	12	0.757	
		2_18	4	1.592	12	0.023	12	0.082	8	1.784	12	0.030	12	0.229	2	1.883	12	0.008	12	0.042	
		2_23	3	1.810	12	0.047	12	0.092	7	1.849	12	0.072	12	0.192	5	2.004	12	0.012	12	0.137	

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																	
			a						c						m					
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1	DELEVEN1		
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN		
c	6	2_45	3	1.744	12	0.021	12	0.118	5	1.783	12	0.035	12	0.135	4	1.699	12	0.039	12	0.131
		2_67	4	1.675	12	0.033	12	0.117	9	1.814	12	0.058	12	0.264	5	1.680	12	0.020	12	0.196
		3_18	6	1.773	12	0.020	12	0.116	6	1.728	11	0.028	11	0.081	5	1.776	12	0.014	12	0.104
		3_23	6	1.767	12	0.014	12	0.120	8	1.901	12	0.053	12	0.189	9	2.067	12	0.043	12	0.405
		3_45	6	1.801	12	0.028	12	0.178	6	2.050	11	0.052	11	0.200	7	1.940	12	0.017	12	0.133
		3_67	7	2.018	12	0.032	12	0.206	9	1.875	12	0.078	12	0.292	8	1.681	12	0.022	12	0.373
		7	1_18	10	1.981	12	0.069	12	0.420	12	1.964	12	0.119	12	0.638	12	2.131	12	0.147	12
	1_23	12	1.992	12	0.332	12	0.675	12	2.220	12	0.134	12	0.604	11	2.148	11	0.300	11	0.632	
	1_45	12	1.700	12	0.347	12	0.758	12	2.445	12	0.184	12	0.425	12	1.793	12	0.150	12	0.649	
	1_67	12	1.700	12	0.223	12	0.802	8	2.210	12	0.014	12	0.210	12	1.796	12	0.257	12	0.816	
	2_18	2	1.929	11	0.075	11	0.030	4	1.689	12	0.001	12	0.056	3	2.138	12	0.016	12	0.125	
	2_23	6	1.657	12	0.022	12	0.389	7	1.768	12	0.017	12	0.266	3	1.841	12	0.052	12	0.122	
	2_45	3	1.780	12	0.008	12	0.051	7	1.872	12	0.013	12	0.192	3	1.735	12	0.012	12	0.069	
	2_67	6	1.745	12	0.006	12	0.145	5	1.992	12	0.001	12	0.100	3	1.663	12	0.010	12	0.104	
3_18	6	1.867	11	0.028	11	0.134	3	1.756	12	0.002	12	0.023	9	2.076	12	0.023	12	0.245		
3_23	8	1.753	11	0.123	11	0.426	8	1.936	12	0.010	12	0.163	8	1.938	11	0.045	11	0.409		
3_45	6	1.914	12	0.018	12	0.159	6	2.143	12	0.018	12	0.253	9	1.867	12	0.069	12	0.282		
3_67	6	2.386	12	0.034	12	0.071	4	4.040	12	0.022	12	0.250	11	1.773	12	0.019	12	0.422		
e	1	1_18	9	3.599	10	0.380	10	0.835	10	3.022	12	0.121	12	0.587	11	2.350	11	0.160	11	0.672
		1_23	10	2.105	11	0.328	11	0.676	12	2.243	12	0.310	12	0.702	10	1.917	10	0.343	10	0.759
		1_45	10	1.827	11	0.376	11	0.731	11	2.013	12	0.159	12	0.749	10	2.054	11	0.293	11	0.673
		1_67	10	2.301	11	0.412	11	0.618	12	2.141	12	0.358	12	0.857	11	1.860	12	0.473	12	0.754

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																	
			a						c						m					
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1	
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN
e	1	2_18	7	1.835	12	0.014	12	0.142	5	2.399	12	0.008	12	0.153	5	1.843	12	0.071	12	0.244
		2_23	10	1.595	12	0.038	12	0.502	7	2.347	12	0.014	12	0.250	6	2.037	12	0.005	12	0.274
		2_45	6	1.648	12	0.027	12	0.319	3	1.578	12	0.020	12	0.139	6	1.734	12	0.007	12	0.144
		2_67	7	1.644	12	0.016	12	0.133	6	2.339	12	0.021	12	0.346	6	2.151	12	0.025	12	0.289
		3_18	3	1.971	12	0.065	12	0.083	6	1.777	11	0.009	11	0.073	8	1.930	12	0.009	12	0.278
		3_23	6	1.839	12	0.117	12	0.297	7	1.628	11	0.018	11	0.341	7	1.819	12	0.015	12	0.214
		3_45	3	1.502	11	0.002	11	0.045	5	2.031	12	0.010	12	0.236	6	1.769	12	0.005	12	0.243
		3_67	5	1.496	12	0.046	12	0.208	6	1.901	12	0.037	12	0.297	8	1.909	12	0.198	12	0.487
		2	1_18	12	1.882	12	0.255	12	0.707	12	2.136	12	0.082	12	0.598	12	1.801	12	0.187	12
	1_23		11	1.888	11	0.309	11	0.735	11	2.090	12	0.134	12	0.483	12	1.900	12	0.250	12	0.720
	1_45		12	1.876	12	0.206	12	0.607	10	2.137	12	0.077	12	0.464	12	1.864	12	0.252	12	0.728
	1_67		12	1.819	12	0.280	12	0.674	12	2.051	12	0.110	12	0.605	10	1.929	10	0.244	10	0.800
	2_18		1	1.794	12	0.006	12	0.042	7	1.713	12	0.020	12	0.240	4	1.706	11	0.033	11	0.167
	2_23		1	1.819	12	0.005	12	0.037	9	1.952	12	0.012	12	0.291	6	1.851	12	0.074	12	0.155
	2_45		1	2.420	12	0.011	12	0.069	6	1.863	12	0.022	12	0.175	5	2.068	12	0.023	12	0.223
2_67	1		1.942	12	0.003	12	0.028	5	1.651	12	0.025	12	0.151	6	1.878	12	0.030	12	0.234	
3_18	4		1.934	12	0.036	12	0.110	7	2.667	12	0.010	12	0.205	5	1.898	12	0.005	12	0.138	
3_23	4		1.713	12	0.032	12	0.177	7	1.908	12	0.047	12	0.174	6	1.927	12	0.071	12	0.234	
3_45	4		1.923	12	0.023	12	0.178	8	2.388	12	0.066	12	0.365	6	2.123	12	0.026	12	0.242	
3_67	5		1.973	12	0.096	12	0.186	6	2.224	11	0.031	11	0.168	5	1.832	12	0.009	12	0.144	
3	1_18		11	1.854	11	0.164	11	0.773	5	2.335	12	0.002	12	0.125	11	1.783	12	0.085	12	0.632
	1_23		12	1.807	12	0.218	12	0.701	9	2.394	12	0.042	12	0.424	10	2.205	11	0.106	11	0.594

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																		
			a						c						m						
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	
c	3	1_45	11	1.905	11	0.168	11	0.684	11	2.287	12	0.096	12	0.605	11	1.750	12	0.148	12	0.612	
		1_67	12	1.884	12	0.286	12	0.776	11	2.316	12	0.060	12	0.571	11	1.869	12	0.187	12	0.697	
		2_18	2	1.804	12	0.029	12	0.132	4	1.780	12	0.005	12	0.217	2	1.780	12	0.002	12	0.056	
		2_23	2	1.741	11	0.023	11	0.104	7	2.256	12	0.016	12	0.405	2	1.757	12	0.007	12	0.121	
		2_45	2	1.632	12	0.010	12	0.117	6	1.874	12	0.004	12	0.236	2	1.781	12	0.022	12	0.053	
		2_67	2	2.000	12	0.010	12	0.033	5	2.227	12	0.007	12	0.222	2	1.800	12	0.017	12	0.056	
		3_18	8	1.911	12	0.031	12	0.184	3	1.941	12	0.010	12	0.100	6	1.918	11	0.084	11	0.341	
		3_23	9	1.803	12	0.050	12	0.324	4	1.882	12	0.013	12	0.146	7	1.886	10	0.117	10	0.516	
		3_45	8	1.746	12	0.026	12	0.219	8	1.879	12	0.061	12	0.153	7	1.863	11	0.053	11	0.280	
		3_67	8	1.771	12	0.033	12	0.234	7	2.594	12	0.068	12	0.200	8	1.844	11	0.046	11	0.189	
		4	1_18	12	2.790	12	0.291	12	0.690	11	2.712	11	0.268	11	0.680	12	2.542	12	0.337	12	0.765
			1_23	11	2.094	11	0.410	11	0.756	11	2.310	11	0.418	11	0.716	12	2.010	12	0.377	12	0.741
			1_45	12	2.259	12	0.386	12	0.746	9	2.211	11	0.178	11	0.625	12	2.117	12	0.352	12	0.720
			1_67	12	2.149	12	0.516	12	0.825	11	1.965	11	0.217	11	0.752	12	1.940	12	0.439	12	0.783
			2_18	3	1.787	12	0.015	12	0.153	6	2.547	11	0.076	11	0.231	6	2.758	12	0.009	12	0.271
	2_23		3	1.948	11	0.018	11	0.201	5	1.681	11	0.036	11	0.265	6	1.916	12	0.020	12	0.159	
	2_45		2	1.625	12	0.006	12	0.017	6	1.516	11	0.020	11	0.265	6	1.885	12	0.016	12	0.360	
	2_67		4	1.762	12	0.013	12	0.143	6	2.091	11	0.036	11	0.225	6	1.790	12	0.042	12	0.333	
	3_18		7	2.206	12	0.017	12	0.382	8	2.078	11	0.071	11	0.418	5	1.866	12	0.059	12	0.261	
	3_23		7	1.963	12	0.112	12	0.358	9	2.095	10	0.030	10	0.373	6	2.075	12	0.063	12	0.244	
	3_45		6	2.510	12	0.018	12	0.167	9	1.588	11	0.030	11	0.419	5	2.053	12	0.037	12	0.282	
	3_67		8	2.010	12	0.031	12	0.372	10	1.786	11	0.032	11	0.473	5	1.731	12	0.020	12	0.338	

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCHTL	PERIOD	RSECTOR	REGION																			
			a						c						m							
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1	DELEVEN1				
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN				
e	5	1_18	12	2.189	12	0.343	12	0.756	10	2.332	12	0.050	12	0.457	11	1.865	11	0.345	11	0.536		
		1_23	12	1.877	12	0.246	12	0.637	10	2.068	12	0.097	12	0.466	9	1.929	9	0.347	9	0.646		
		1_45	12	1.839	12	0.229	12	0.677	8	2.120	10	0.080	10	0.530	11	2.008	11	0.406	11	0.530		
		1_67	10	2.209	10	0.192	10	0.686	8	1.955	12	0.069	12	0.365	11	1.976	11	0.329	11	0.569		
		2_18	3	1.938	12	0.021	12	0.181	5	1.905	12	0.084	12	0.150	8	1.852	12	0.052	12	0.305		
		2_23	3	1.981	12	0.005	12	0.097	6	1.901	11	0.028	11	0.350	8	1.807	12	0.060	12	0.239		
		2_45	4	1.907	12	0.023	12	0.171	9	1.716	12	0.014	12	0.464	7	1.680	12	0.026	12	0.220		
		2_67	4	1.922	12	0.019	12	0.135	5	1.873	12	0.008	12	0.158	8	1.795	12	0.031	12	0.262		
		3_18	6	1.791	11	0.059	11	0.255	6	1.938	12	0.012	12	0.303	10	2.091	12	0.053	12	0.282		
		3_23	5	1.724	12	0.112	12	0.167	8	2.988	12	0.141	12	0.441	10	1.883	12	0.064	12	0.297		
		3_45	7	1.863	12	0.052	12	0.230	5	2.202	12	0.014	12	0.203	10	1.924	12	0.027	12	0.244		
		3_67	5	1.905	12	0.037	12	0.227	5	2.132	12	0.014	12	0.222	10	1.819	12	0.150	12	0.295		
		6	6	1_18	11	2.449	11	0.201	11	0.696	12	2.880	12	0.074	12	0.787	11	1.964	12	0.140	12	0.660
				1_23	12	2.325	12	0.324	12	0.777	12	2.674	12	0.098	12	0.763	10	2.349	10	0.208	10	0.762
				1_45	12	1.917	12	0.237	12	0.770	12	2.616	12	0.064	12	0.558	12	3.329	12	0.158	12	0.695
1_67	11			1.848	11	0.175	11	0.811	10	2.087	12	0.028	12	0.403	12	2.192	12	0.273	12	0.781		
2_18	5			2.633	12	0.009	12	0.263	8	2.048	12	0.025	12	0.646	3	3.021	12	0.010	12	0.160		
2_23	6			1.909	12	0.068	12	0.381	7	2.257	12	0.018	12	0.181	4	1.865	12	0.024	12	0.186		
2_45	4			1.660	12	0.005	12	0.161	9	2.404	12	0.017	12	0.300	4	1.775	12	0.030	12	0.190		
2_67	6			1.539	12	0.022	12	0.334	3	2.188	12	0.006	12	0.042	3	1.713	12	0.012	12	0.082		
3_18	6			2.002	12	0.015	12	0.250	3	2.294	12	0.005	12	0.111	5	1.952	12	0.057	12	0.136		
3_23	8			1.820	12	0.008	12	0.238	3	2.578	12	0.006	12	0.119	4	1.866	11	0.035	11	0.200		

**Raw Data for Proportional Area Covered by Blood Vessels
and Proportion of Blood Vessels 11 μ m (or Greater)(Continued)**

EXPCNTL	PERIOD	RSECTOR	REGION																	
			a						c						m					
			DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1		DRATIO1		TPAREA1		DELEVEN1	
			N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN
e	6	3_45	5	1.609	12	0.008	12	0.222	3	1.747	12	0.002	12	0.181	3	2.177	11	0.055	11	0.209
		3_67	6	2.334	12	0.072	12	0.300	3	2.519	12	0.005	12	0.200	4	1.965	12	0.083	12	0.083
	7	1_18	12	2.427	12	0.232	12	0.704	6	2.366	11	0.039	11	0.289	10	2.096	12	0.151	12	0.498
		1_23	12	2.166	12	0.244	12	0.723	8	1.955	12	0.074	12	0.400	12	2.171	12	0.240	12	0.646
		1_45	11	2.050	12	0.239	12	0.700	8	2.181	11	0.046	11	0.371	10	2.433	12	0.153	12	0.478
		1_67	12	2.255	12	0.388	12	0.691	10	1.872	11	0.116	11	0.661	11	1.962	12	0.204	12	0.526
		2_18	2	2.503	11	0.007	11	0.182	4	1.821	12	0.004	12	0.153	9	2.311	12	0.077	12	0.470
		2_23	2	1.744	11	0.011	11	0.095	7	2.030	12	0.023	12	0.202	7	2.192	11	0.059	11	0.333
		2_45	3	2.126	11	0.004	11	0.182	7	1.826	12	0.008	12	0.193	8	2.050	12	0.113	12	0.263
		2_67	3	1.836	11	0.004	11	0.091	7	1.956	12	0.030	12	0.375	5	1.592	10	0.016	10	0.214
		3_18	3	2.279	12	0.031	12	0.111	4	1.836	11	0.003	11	0.027	6	2.650	12	0.021	12	0.155
		3_23	5	1.761	12	0.025	12	0.306	5	2.238	11	0.012	11	0.250	6	1.840	11	0.079	11	0.277
		3_45	2	1.502	12	0.003	12	0.042	6	2.553	11	0.029	11	0.409	5	2.253	12	0.029	12	0.142
		3_67	4	2.183	12	0.008	12	0.153	5	2.223	11	0.025	11	0.212	6	2.203	12	0.063	12	0.150