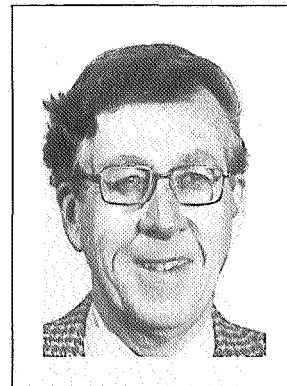


A model of the anisotropic distribution of microvascular volume in the periodontal ligament of the mouse mandibular molar

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Abstract: Histological sections of periodontal ligament from the mesial root of ten randomly selected mouse mandibular molars were digitised for luminal vascular volume. Total vascularity differed markedly between mice. Statistical analysis, using a logistic model, revealed significant quadrant and depth effects at the 1 percent level with regional vascular volumes varying from 3 to 35 percent. Maximum vascular volume occurred in the lingual quadrants and minimum volume characterised the buccal quadrants. Depth effects were maximal at the apical limit and minimal in the mid root regions, whereas the cervical ligament had a vascular volume midway between the other two regions. The overall average for the vascular volume was 10.9 per cent. This ligament model demonstrates marked volumetric gradients in distribution of the microvascular bed.

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MeSH words: Blood volume determination; periodontal ligament/blood supply; mouse; molar/anatomy and histology
Key words: Microvascular volume, periodontal ligament, mouse molar, statistical model

INTRODUCTION

The microvascular system is considered to be a vital component in tooth support (Bien, 1966; Wills, Picton and Davies, 1976) and orthodontic tooth movement (Reitan, 1951; Lindskog and Lilja, 1984; Rygh et al., 1986). Nevertheless, few attempts have been made to quantify the vascular volume within large samples of intact periodontal ligament (Götz, 1986, 1976, 1980) to provide comparison with the data obtained from small tissue quadrants employed in transmission electron microscopy (TEM) stereological investigations (Freezer and Sims, 1987).

Vascular volumes are reported to range from 0.8 to 3.88 percent in histological sections of human periodontal ligament, depending on the age and region examined (Götz, 1965, 1976, 1980). Light microscopic studies of restricted sites in mouse molar ligament indicate volume densities of 7.7 ± 0.6 percent (Gould, Melcher and Brunette, 1977) and 7.25 ± 0.75 percent (McCulloch and Melcher, 1983). These findings contrast with localised vascular volumes of 11 and 17 percent determined for human and mouse ligament, respectively (Sims, 1980).

Because of the diverse vascular volumes reported in the literature, this investigation was undertaken to provide animal, quadrant and depth data for the microvascular bed of the mouse molar using the mesial root ligament as a large sample model. Such knowledge will contribute to the integration of normal vascular morphology with functional hypotheses in different species (Wills et al., 1976; Sobin and Tremer, 1977; Rygh et al., 1986).

MATERIALS AND METHODS

Ten male mice, aged 30 days, were anaesthetised intraperitoneally with sodium pentobarbitone (Abbott) using 0.3mg/3.0 gm body weight. The mandibles were removed, hemisected, fixed in 10 percent buffered formalin, demineralised and processed with the celloidin-paraffin double embedding technique. One half mandible was randomly selected from each mouse and serially sectioned at 8 μ m through the first molar, parallel to the occlusal plane. Pollack's trichrome was used to delineate vessels. All animals were histologically free from periodontal disease.

Five equally spaced sections at 200 μ m intervals, spanning each mesial molar root, were analysed. Zero point was the epithelial attachment and the end point the mid apical ligament extremity. The investigation was based on quadrant ligament divisions (Fig. 1) to enable comparison of the mesial, distal, buccal and lingual regions and subdivided vertically into occlusal, middle, and apical thirds to compare

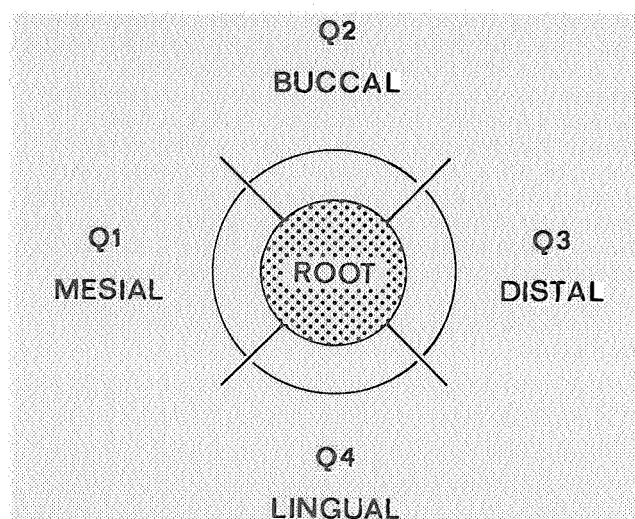


Figure 1. Quadrant divisions of the mesial root periodontal ligament used for the pilot study and principal statistical analyses.

Figures 2-4. Vascular volume at varying depths for all quadrants plotted from the model data in Tables 2-4 depth effects. Stained sections were screened with a neo-PROMAR projection microscope (Ernst Leitz), at a magnification of x300 on to the tablet of a semi-automatic MOP 1 digitiser (Carl Zeiss) for computation of the total ligament and luminal vascular areas in each quadrant. Where a vessel penetrated the alveolar wall, a line joining opposite margins of the foramen demarcated the computed vessel area. Individual vessel types were not categorised.

Exploratory statistical procedures of graphing and tabulating the data in various ways determined that a general logistic model would be suitable for a formal statistical analysis of vascular volume. This model showed that a sample of 5 equally spaced mesial root sections from the attachment to the apical limit would be representative of the vascular volumes in each ligament.

The principal analysis was conducted using a randomly selected half mandible from each animal. Ligament vascular volume was analysed using a logistic model and non-linear maximum likelihood curve fitting techniques. The model had parameters corresponding to possible animal, quadrant and depth effects. A full range of multiple regression and analysis of variance techniques were employed to determine this model (Draper and Smith, 1966). Calculations were performed using programs IV and

1R of the BMDP-81 Biomedical Statistical Software Package (The Department of Biostatistics, School of Health, U.C.L.A., Los Angeles).

RESULTS

Statistical analysis of the data demonstrated significant differences in ligament vascular volumes between animals. Quadrant effects were statistically significant at the 5 percent level. The mesial (Q1) and distal (Q3) quadrants were found to be statistically the same, with slightly larger vascular volumes than the buccal quadrant (Q2). Vascular volume of the lingual quadrant (Q4) was significantly higher than the other three quadrants at the 1 percent level. The vascular volume for each quadrant, over all mice and all depths, as calculated from the basic data (Table 1), had an overall average of 10.9 percent.

Table 1

	Q1	Q2	Q3	Q4
N=	9.9%	6.8%	8.9%	17.8%
	103	102	110	109

Basic data for the calculation of an overall average vascular volume. N is the number of observations.

The effect of depth was found to be quadratic in nature. That is, larger vascular volumes at the cervical and apical regions and lower vascular volumes opposite the mid third of the root. For all animals maximum vascular volume occurred apically, whereas the mid root region had minimum vascular volume and the ligament opposite the epithelial attachment had a vascular content midway between the other two regions. Using the fitted model parameters for animal, quadrant and depth, tables were constructed to show the general features of vascular change. Tables 2 and 4 illustrate the model for mice with above and below average vascularity, respectively. Table 3 shows the situation for the average animal. Data are displayed graphically in Figures 2, 3 and 4. The range of variation is indicated in the fitted models presented in the Tables and Figures.

Table 2.

Depth (μ m)	Q1	Q2	Q3	Q4
0	16.03	12.61	16.03	25.43
100	13.88	10.86	13.88	22.36
200	12.39	9.66	12.39	20.16
300	11.41	8.87	11.41	18.71
400	10.87	8.44	10.87	17.88
500	10.70	8.31	10.70	17.64
600	10.91	8.47	10.91	17.94
700	11.49	8.94	11.49	18.82
800	12.51	9.76	12.51	20.35
900	14.07	11.01	14.07	22.62
1000	16.29	12.82	16.29	25.79
1100	19.37	15.37	19.37	30.02
1200	23.55	18.89	23.55	35.49

Derived percentage vascular volumes for ligament quadrants (Q1-Q4) using the logistic model to illustrate an above average mouse.

Table 3.

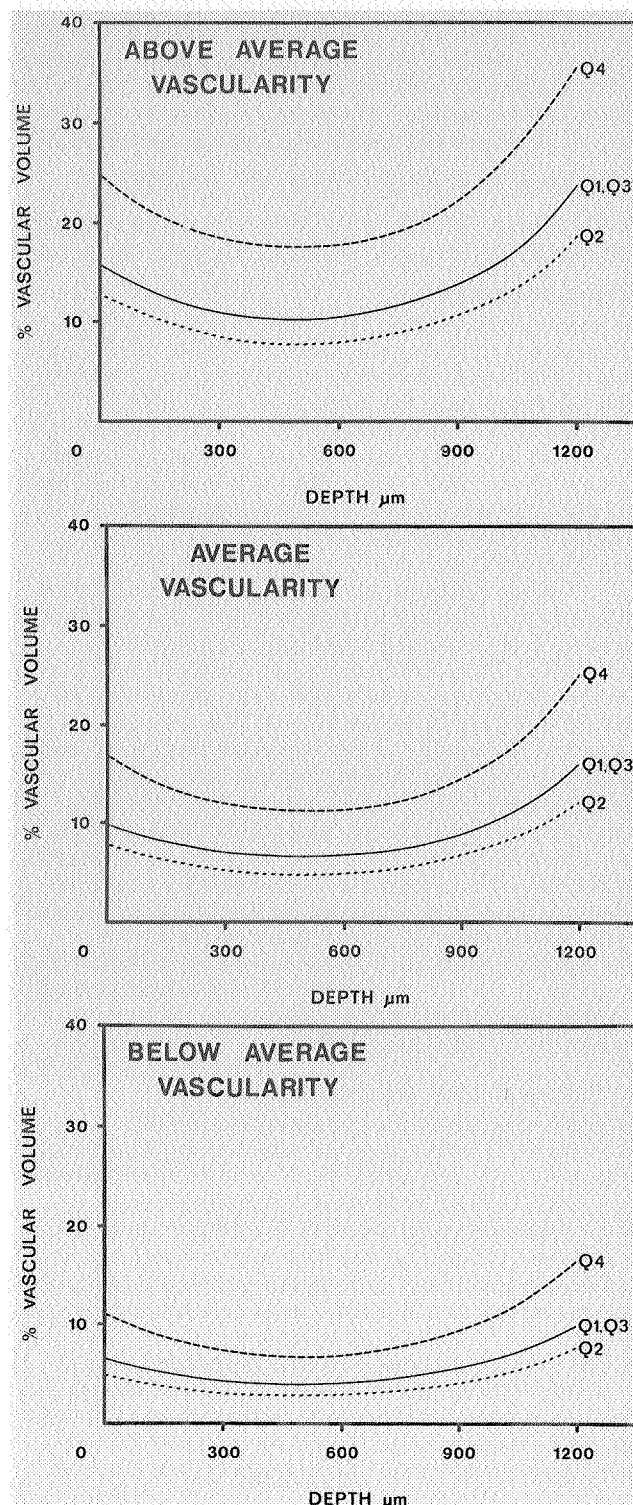
Depth (μ m)	Q1	Q2	Q3	Q4
0	10.38	8.05	10.38	17.14
100	8.91	6.88	8.91	14.87
200	7.90	6.09	7.90	13.28
300	7.25	5.58	7.25	12.25
400	6.89	5.29	6.89	11.67
500	6.78	5.21	6.78	11.49
600	6.91	5.31	6.91	11.71
700	7.30	5.62	7.30	12.33
800	7.98	6.15	7.98	13.42
900	9.03	6.98	9.03	15.06
1000	10.56	8.19	10.56	17.41
1100	12.72	9.92	12.72	20.65
1200	15.74	12.37	15.74	25.02

Model of the ligament vascular volume for an average mouse.

Table 4.

Depth (μ m)	Q1	Q2	Q3	Q4
0	6.56	5.04	6.56	11.15
100	5.60	4.29	5.60	9.58
200	4.95	3.78	4.95	8.50
300	4.53	3.46	4.53	7.81
400	4.29	3.28	4.29	7.42
500	4.22	3.23	4.22	7.30
600	4.31	3.29	4.31	7.44
700	4.56	3.48	4.56	7.86
800	5.00	3.82	5.00	8.59
900	5.68	4.35	5.68	9.71
1000	6.68	5.13	6.68	11.33
1100	8.12	6.26	8.12	13.63
1200	10.18	7.89	10.18	16.83

Ligament vascular volume for a below average mouse.



Figures 2-4. Vascular volume at varying depths for all quadrants plotted from the model data in Tables 2-4

DISCUSSION

Despite the fact that Johnson and Highison (1985) regard the mouse as an important research model, information on vascular volume and its distribution is deficient. Previous estimates of mouse molar vascularity provide only limited data because they are based on tissues from a single animal (Gould et al., 1977), or restricted to coronal samples from an isolated mesial site (McCulloch and Melcher, 1983). Nevertheless these localised assessments of plastic embedded ligament only accord with the present findings for the corresponding mid-buccal region of an above average mouse (Gould et al., 1977), or the mesial quadrant below the alveolar crest of an average mouse (McCulloch and Melcher, 1983). Moreover, between animal, quadrant and depth effects were not considered in previous morphometric studies of the mouse ligament, although vascular pattern changes are said to occur down the root length in the adult rat molar (Garfunkel and Sciaky, 1971).

Parfitt (1967) reports that ligament blood volume is 2 percent and Wills et al., (1976) calculate that 0.5 to 1.0 percent of Macaque monkey ligament is occupied by displaceable blood. By contrast, a study in our laboratory of the marmoset (*Callithrix jacchus*) mandibular second molar by Douvartzidis (1984) reveals a ligament vascular volume of 8.3 ± 0.4 percent. Additionally, there is an increasing volume gradient from the cervical to apical regions. Across the width of the marmoset periodontal ligament the middle third has the greatest vascular volume being 13.1 percent, whereas the alveolar third ligament vascularity is 8.9 percent. In man, the estimates of vascular volume vary from 0.5 to 3.5 percent (Götz 1965, 1976, 1980) with specific sites recorded as exceeding 20 percent (Sims, 1980). Species differences are further highlighted by the report of ligament mean vascular volumes varying from 10-20 percent opposite the mid root region of beagle premolars (Follin and Nilsson, 1986). The work of Götz (1965, 1976, 1980) implies that the ligament of man contains an inherently low vascular volume. Should this conclusion be confirmed, then the direct extrapolation of vascular volume data from animal models to explain vascular phenomena in man will prove to be arbitrary. These diverse findings clearly indicate the need for further studies to provide definitive vascular volume information for a wide range of heterogeneous dentitions.

A feature of the present mouse material is the wide variability in vascular volume relating to animal, quadrant and depth effects, thus indicating the importance of utilizing maximum sample sizes. Differences between the minimum and maximum vascular regions can vary by a factor of X5 in mouse ligament and X4 in man (Götz, 1976). In the mouse these vascular extremes coincide with the mid-root and apical regions, compared with the coronal and apical regions in man. Marked variation in regional vascular volume between animals has also been noted by Follin and Nilsson (1986) who, furthermore, define volumetric differences between the mesial and distal roots of dog premolars.

The maximum vascular volume between quadrants varies by X2 in both mouse and man. This difference is based on the comparison of all mouse quadrants, but has only been detected in the mesial and distal ligament of man (Götz, 1976). In man the mesial and distal vascular volumes are also said to coincide (Götz, 1976), whereas a progressive increase in vascular volume occurs in the buccal and lingual regions, respectively. The mouse ligament, however, proved to be least vascular in the buccal quadrant. As a further comparison, Götz (1976) reports a progressive increase in the vascular volume of man from the coronal to apical thirds, which differs from the present mouse data, but corresponds with marmoset findings (Douvartzidis, 1984).

Values for vascular volume in the present study are markedly higher than those determined from other mouse investigations (Gould et al., 1977; McCulloch and Melcher, 1983; Freezer, 1984). These reported discrepancies in ligament blood volume may be due to a variety of factors. Both histological and TEM sections may incorporate

anaesthetic vascular effects (Severs et al., 1981), processing changes (Kushida, 1962) and compression factors (Weibel, 1979). Recently, Steigman, Weinreb and Michaeli (1983) analysed histologically processed, rat incisor ligament and concluded that no statistically significant dimensional changes occurred when compared with glycol methacrylate embedded sections. Like previous investigations (Götz, 1965, 1976, 1980; Gould et al., 1977; McCulloch and Melcher, 1983) the present study assumed that ligament components were proportionally affected by preparation and area density measurements were not, therefore, compromised.

Site and sampling techniques are clearly important factors in assessing the ligament vascular volume. Furthermore, vascular volume differs between the mesial and distal roots of some teeth (Follin and Nilsson, 1986). However, finite studies (Gould et al., 1977; McCulloch and Melcher, 1983; Freezer and Sims, 1987) do not allow for the anisotropic distribution of the microvascular bed on different sides of the root, nor animal differences in larger samples. Although arterial, capillary and venous vessels were not differentiated in the present study, the vascular volume reflects the predominately venous pool and the subsidiary capillary network of the ligament microvascular bed (Sims, 1984; Freezer and Sims, 1987). Arterioles do not comprise a significant volumetric component in rat molar ligament (Weekes and Sims, 1986; Lew, 1986) and this morphology may be analogous in the mouse (Wong and Sims, 1987).

The structure - function interrelationship of the periodontal ligament and its microvascular bed in tooth support has been emphasised by many investigators (Bien, 1966; Parfitt, 1967; Melcher and Walker, 1976; Wills et al., 1976; Walker, 1980; Kardos and Simpson, 1980; Wills and Picton, 1981; Moxham and Berkovitz, 1982; Edwall, 1982; Picton, 1984). Studies suggest that differences in ligament interstitial pressure (Edwall, 1982) may reflect regional fluid compartmentalization associated with changes in ground substance electrolyte balance (Tyler and Burn-Murdoch, 1976) and biochemistry (Ferrier and Dillon, 1983) induced by tooth loading. But, as yet, the contribution of the permeability characteristics of the principal vascular components, capillaries and postcapillary-sized venules (Freezer and Sims, 1987), to interstitial fluid flow remains undetermined. A further factor to be accounted for is that mouse periodontal ligament is 7 to 10 times more vascular than other fibrous connective tissues (Freezer, 1984). It is pertinent to note that Sobin and Tremer (1977) believe that the three-dimensional architecture of microcirculations must first be defined in order to understand their operational mechanisms.

Since the mouse molar ligament exhibits volumetric and vascular gradients, it can be inferred that this distribution also reflects operational gradients of tooth support in a structurally anisotropic ligament. If other species, for example the marmoset, possess not only comparable vascular volumes to the mouse ligament, but are also shown to have a predominately venous microcirculation, then hypotheses of tooth support need to reconcile these structural attributes with physiological concepts. Should further studies determine fluid pools larger than previously accepted for human and animal periodontal ligament (Götz, 1980; Wills et al., 1976), existing theories of tooth support will require reassessment.

Operational concepts of periodontal vessels as functional units may be elucidated by comparing them with other animal and human microvascular beds where the micro-physiology of capillary permeability, and fluid exchange, is well documented (Casley-Smith, 1983). The functional characteristics of a microcirculation reflect the dynamic properties of its structural components (Intaglietta and De Plomb, 1973; Zweifach, 1977). Therefore, there is a need to establish ultrastructural parameters of quantitative morphology for the normal animal periodontal ligament so that the basic mechanisms underlying fluid exchange across

the microvascular wall to the interstitial tissues can be determined. With this background information, the effects of orthodontic treatment on microvascular bed integrity can then be assessed and extrapolated to man.

CONCLUSION

This investigation documents the anisotropic distribution of the mouse molar microvascular bed (Sims, 1987; Wong and Sims, 1987). The findings demonstrate the need for further ultrastructural studies, since TEM analyses based on restricted sites within the mesial portion of the molar ligament (Gould et al., 1977; McCulloch and Melcher, 1983; Freezer and Sims, 1987) do not provide data representative of the ligament as a whole. Furthermore, it must be pointed out that statistical studies of vascular ultrastructure based upon undefined regional sampling within molar periodontal ligament (Moxham, Shore and Berkovitz, 1985) are also subject to challenge (Lew, 1986; Clark, 1986).

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