Effect of experimental traumatic occlusion on periodontal and pulpal blood flow

Steinar Kvinnsland, Ansgar B. Kristiansen, Inger Kvinnsland and Karin J. Heyeraas

Department of Anatomy, Department of Physiology, and Department of Cariology and Endodontics, University of Bergen, Bergen, Norway

Kvinnsland S, Kristiansen AB, Kvinnsland I, Heyeraas KJ. Effect of experimental traumatic occlusion on periodontal and pulpal blood flow. Acta Odontol Scand 1992;50:211–219. Oslo. ISSN 0001-6357.

Fluorescent microspheres (FM) were used to visualize and semi-quantify flood flow in the periodontal ligament (PDL) and dental pulp during experimental traumatic occlusion of the maxillary and mandibular molar teeth in young rats. At different observation points FM were injected systemically, and the number of FM was counted in serial sections from the jaws in the PDL and pulp of the molar teeth in a fluorescent microscope. Blood flow was related to the number of FM in the tissues and in a reference blood sample. In the early stages an increase in blood flow in the PDL and dental pulp was found on the experimental side compared with the contralateral side. Furthermore, there was an increase in blood flow on both sides of the jaws compared with an unoperated control material. The study thus indicates that a local unilateral occlusal trauma initiates blood flow responses in the total molar dentition in rats. □ *Blood flow; fluorescent microspheres; occlusal trauma*

Steinar Kvinnsland, Department of Anatomy, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

Mechanical forces applied to intact teeth cause changes in blood flow in the pulp and periodontium (1, 2), increase the periodontal tissue pressure (3), and activate sensory receptors in the pulp and periodontium (1, 4). The periodontal ligament (PDL) and dental pulp are highly vascular tissues. Because direct venous blood sampling from these regions is not possible, various indicators, such as ^{86}Rb and ^{125}I, have been used to quantify blood flow in the teeth of experimental animals (5, 6). The hydrogen polarographic approach has been used in the dental pulp by Tønder & Aukland (7), Meyer & Path (8, 9), and Heyeraas Tønder (10). Folke & Stallard (11) used plastic microspheres to investigate the vascular pattern in periodontal microcirculation. Radioactively labeled microspheres have been used to quantify blood flow in the dental pulp and alveolar bone (5, 12), and lately fluorescent microspheres (FM) have been used to semi-quantify blood flow in the PDL and dental pulp (2). The results of measuring pulp blood flow per unit mass (in ml/min g tissue) with the various indicators vary widely (13), probably due to differences in the methods of indicator application. Few characteristics of the PDL apart from its morphology have been investigated, partly owing to the technical difficulties involved in studying the small tissues enclosed within rigid structures, and so far little information has accumulated on the blood flow of the PDL under both normal and experimental conditions (2, 6).

FM was first used as a new tool for quantifying and visualizing blood flow in ischemic myocardium in rats by Hale et al. in 1986 (14) and has also been used in visualizing and semi-quantifying blood flow in PDL and dental pulp during experimental mesial movement of the first maxillary molar in rats (2). In the present study FM were used to visualize and semi-quantify blood flow in the PDL and dental pulp during experimental traumatic occlusion of the maxillary and mandibular molars in rats.

Materials and methods

A total of 45 Møll–Wistar rats (140–150 g) 55 ± 6 days old were anesthetized with subcutaneous injection of fentanyl/fluanison midazolam (Hypnorm-Dormicum®), 0.3 ml/100 g body weight. The occlusal surface of the first maxillary right molar was raised 1 mm with the composite material P-30 after acid etch with Scotchbond etching gel for 30 sec, followed by water rinsing and air drying. The untreated contralateral side served as operated control. During the experimental period the animals received a standard pellet diet with tap water ad libitum. One, 2, 5, 10, 15, 20, and 30 days later the animals were re-anesthetized, the heart was surgically exposed, and 0.2 ml FM (Fluoresbrite Plain Microspheres, Polysciences Inc., Warrington, U.K.), suspended in 10% Ficoll-70 in 0.9% saline, was injected directly into the apex of the left ventricle in the course of 20 sec. The diameter of the microspheres was 9.5 μm ± 15%, which means the majority will be lodged in the precapillary arterioles in the rat. Ten rats served as unoperated parallel control animals and received FM as described for the experimental group. In addition, these animals had withdrawal of arterial reference blood sample from the femoral artery, carried out at a rate of 0.08 ml/min (Harward pump) starting 10 sec before microsphere injection and continued for 50 sec after completing the injection. The reference blood sample was diluted until hemolysis, filtered, and mounted on a microscopic slide, whereupon the number of microspheres was counted in a counting chamber in a fluorescent microscope.

The animals were killed with an overdose of anesthetics 1–2 min after the injection of FM. The jaws were excised, fixed in 10% buffered formalin, demineralized in 4 N formic acid and 0.05 sodium formate at 4°C for 10 days, and finally sectioned sagitally at 40 μm in a cryostat. The sections were mounted serially and examined unstained in a fluorescent microscope. The FM were clearly visible in the fluorescent microscope (Fig. 1), and the total numbers were counted in the PDL and dental pulp in all maxillary and mandibular molars on both the experimental side and the contralateral side. Owing to loss of composite material in the first molars in 10 animals in late stages we were left with 35 experimental animals (420 teeth), and, in addition, 10 unoperated controls (120 teeth).

To obtain a fairly accurate measure of the volume of the PDL and pulp space, the areas were measured on serial sections under a

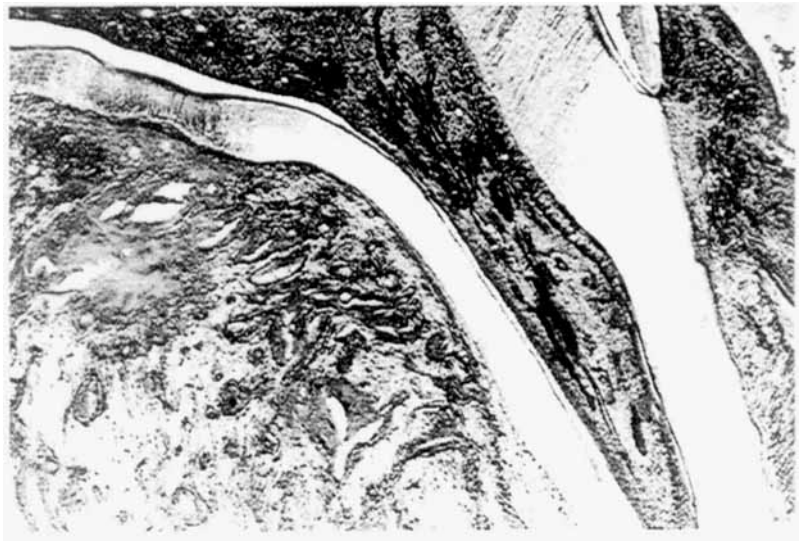

Fig. 1. Fluorescent microspheres in the periodontal ligament and dental pulp.

microscope and divided into several triangles (2, 15). Local blood flow was then calculated as the ratio between the number of FM counted in the dental tissues under investigation and the reference sample (per cm^3 and per ml, respectively) multiplied by the reference sampling rate (ml/min), giving the blood flow in ml/min × cm^3 tissue (2) according to the equation:

$$BF = \frac{FM_T \times F_R}{FM_R},$$

where BF is blood flow in ml/min × cm^3 tissue, FM_T is the number of fluorescent microspheres per cm^3 (g) of oral tissue, F_R is the reference blood sample withdrawal rate (0.08 ml/min), and FM_R is the number of FM in the reference blood sample (Fig. 2).

To ensure sufficient number of FM in the small areas under investigation, a relatively large quantity was used in suspension for each injection (4×10^6), giving 200–500 FM in each tissue sample. This, together with the pneumothorax created by opening of the thorax during microsphere injection, caused a drop in systemic blood pressure which must be taken into account when referring to the absolute blood flow values. It was assumed, however, that the drop in blood pressure was equal in the two sides of the jaws; thus the difference in blood flow found on the experimental side compared with the contralateral side will be relatively representative. The microspheres were injected directly into the left ventricle to avoid the systematic error involved in passing a catheter into the same site via the common carotid artery, which by this method will invariably cause different arterial blood pressure in the two sides of the jaws (2).

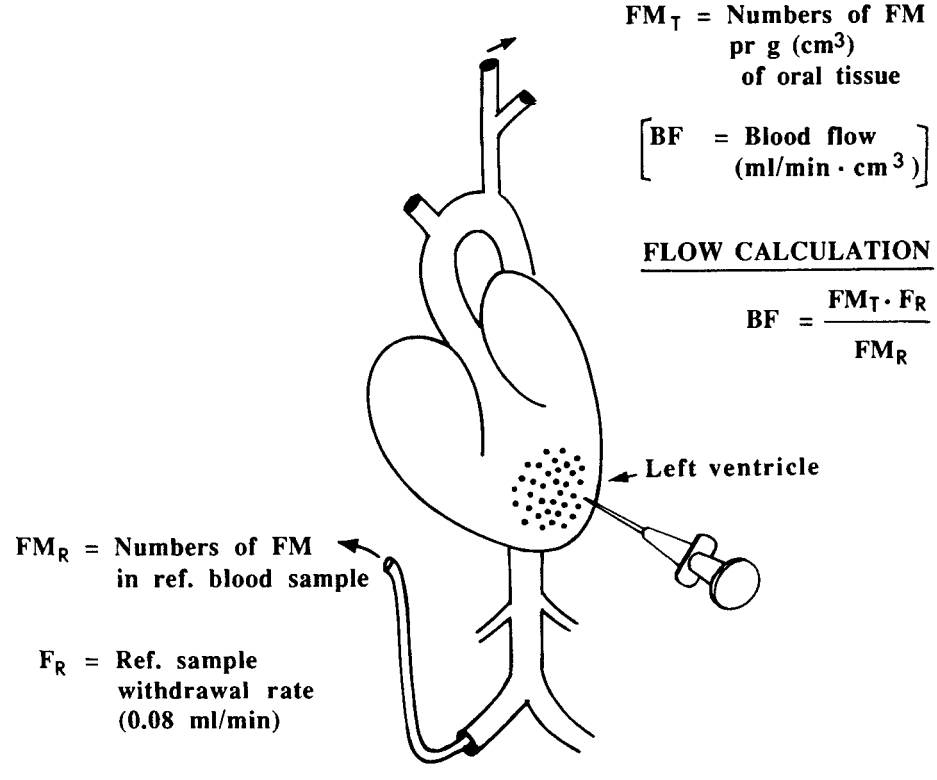

Fig. 2. Schematic illustration of injection of fluorescent microspheres (FM) into the left ventricle of the heart and withdrawal of reference blood sample from the femoral artery.

To evaluate blood flow, the number of FM was expressed as mean percentage increase or decrease of the experimental side compared with the contralateral mean values at the different stages. In addition, by expressing the mean blood flow values in ml/min × cm^3 from the unoperated control material as unity, blood flow from the experimental and contralateral sides was calculated as 'hypothetical' increase or decrease in values from this norm. This was undertaken to ascertain whether the contralateral side and the experimental side were affected by the experimental procedure. These methods will give a semi-quantitative measure of blood flow in the aforementioned regions under experimental conditions.

The results are presented in graphs. Observations from the contralateral and unoperated control blood flow values are presented as arithmetic mean ($\bar{x}$) ± 1 SD. Blood flow values from the experimental molars are presented as arithmetic mean values ± 1 SD. SD bars are plotted when there are non-overlapping intervals between the experimental and control/contralateral materials.

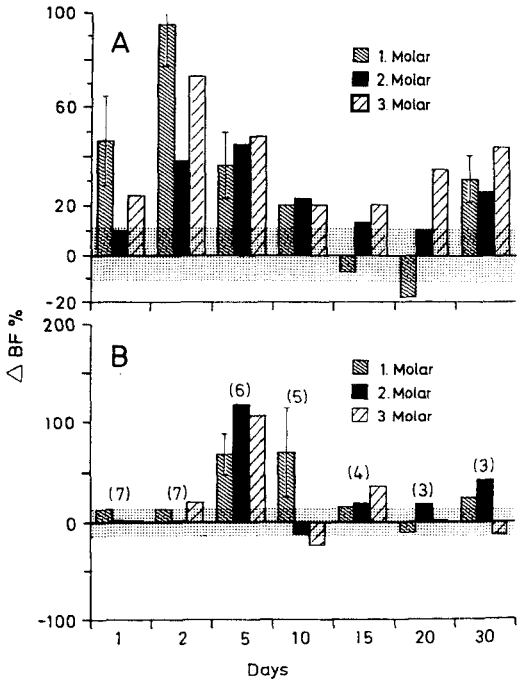

Fig. 3. Periodontal ligament (PDL). Maxillary molars (A) and mandibular molars (B). Mean percentage change in blood flow (BF) in the molar PDL on the experimental side compared with the contralateral side at different observation points. The contralateral BF values were defined as unity at zero (a hypothetical axis at 0 parallel to the abscissa). Shaded area represents ±1 SD of mean contralateral BF values. Bars indicate ±1 SD of mean experimental BF values. Note the increase in BF in the PDL of the experimental side first molars compared with the contralateral side molars at 2 days for the upper jaw (A) and at 5–10 days for the lower jaw (B). The number of animals in each group (n) is indicated in parentheses; n is identical in Figs. 3–5.

Results

Unoperated control animals

Mean blood flow measured in 120 molars from 10 rats was 1.7 ± 0.6 ml/min × cm^3 in the PDL and 0.4 ± 0.16 ml/min × cm^3 in the dental pulp. We found no difference between first, second, and third molars or between the two sides. This is in good agreement with previous findings (2, 10, 12).

Experimental animals

Periodontal ligament. At 24 h there was a mean percentage increase in blood flow in the PDL of the maxillary first molars on the experimental side compared with the contralateral side. The greatest increase was seen at 2 days; thereafter there was a fluctuating decrease during the rest of the experimental period. The same tendency, although less pronounced, was seen in the second- and third-molar PDL on the experimental side in the maxilla (Fig. 3A).

The tendency was the same in the mandible, but the greatest percentage increase in the blood flow of the first molar on the experimental side compared with the contralateral side appeared later, at 5–10 days, but thereafter the trend was the same as in the upper jaw (Fig. 3B).

Comparison between mean blood flow in the experimental PDL related to the mean values of the unoperated controls (1.7 ± 0.6 ml/min × cm^3), expressed as an increase or

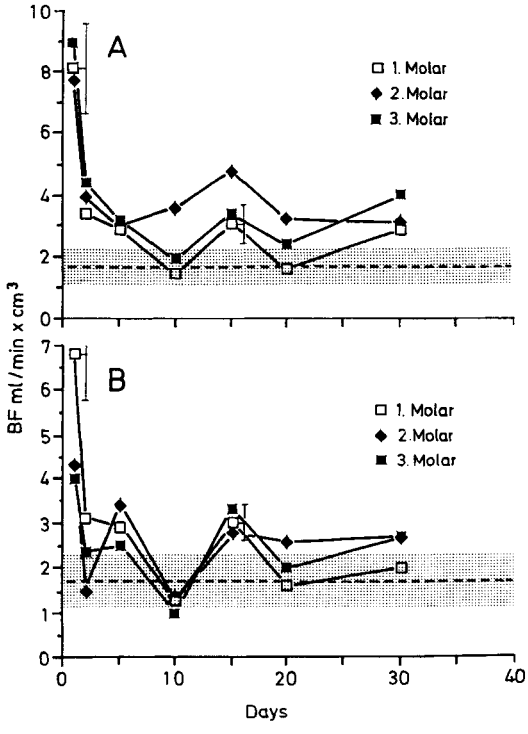

Fig. 4. Periodontal ligament (PDL). Maxillary molars (A) and mandibular molars (B). Mean blood flow (BF) in the molar PDL per ml/min × cm^3 tissue on the experimental side calculated as an increase or decrease relative to mean BF values from the unoperated control animals (1.7 ± SD, 0.6 ml/min × cm^3; stippled line and shaded area). Bars indicate ±1 SD of mean experimental values. Note the increase at 24 h both in the upper (A) and in the lower (B) jaws.

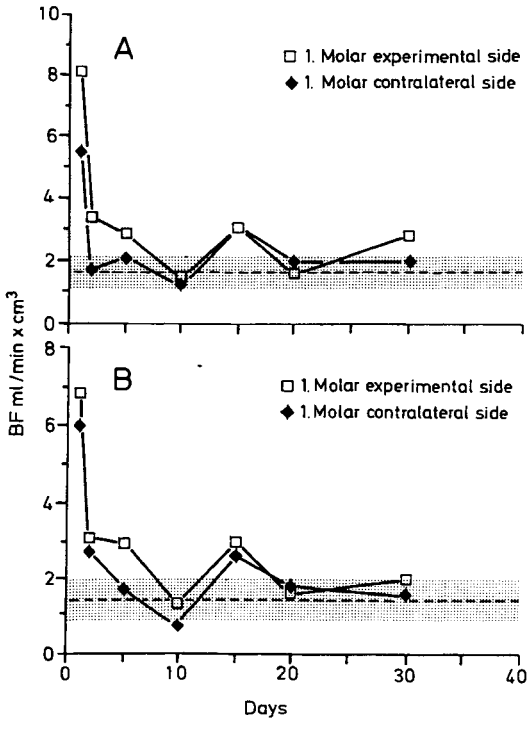

Fig. 5. Periodontal ligament (PDL). Maxillary first molar (A) and mandibular first molar (B). Mean blood flow (BF) of the first molar PDL (experimental and contralateral sides) relative to mean BF values from the unoperated control animals (1.7 ± SD, 0.6 ml/min × cm^3; stippled line and shaded area). Note the similar behavior of BF changes on the experimental and contralateral sides.

decrease in blood flow relative to this value, showed that the greatest increase was as early as 24 h in both the upper and lower jaws, thereafter decreasing, possibly showing a slight increase around 15 days (Figs. 4A and B).

When the mean blood flow values of the experimental and contralateral first molars were compared with the corresponding unoperated control mean values (1.7 ± 0.6 ml/min × cm^3), both showed an increase at 24 h (Figs. 5A and B).

Dental pulp. Blood flow in the first maxillary molar on the experimental side showed a mean percentage increase compared with the contralateral side at 2 days and thereafter a fluctuating decrease towards 30 days. The third molars seemed to show a rather similar tendency but, curiously, not the second molars (Fig. 6A).

In the mandible there was a small increase in the blood flow of the first molar at 24 h and at 5–10 days on the experimental side compared with the contralateral side (Fig. 6B).

When the blood flow in the pulps of the experimental side were compared with the mean values of the unoperated controls (0.4 ± 0.16 ml/min × cm^3), the blood flow was markedly increased at 24 h both in the upper and in the lower jaws (Figs. 7A and

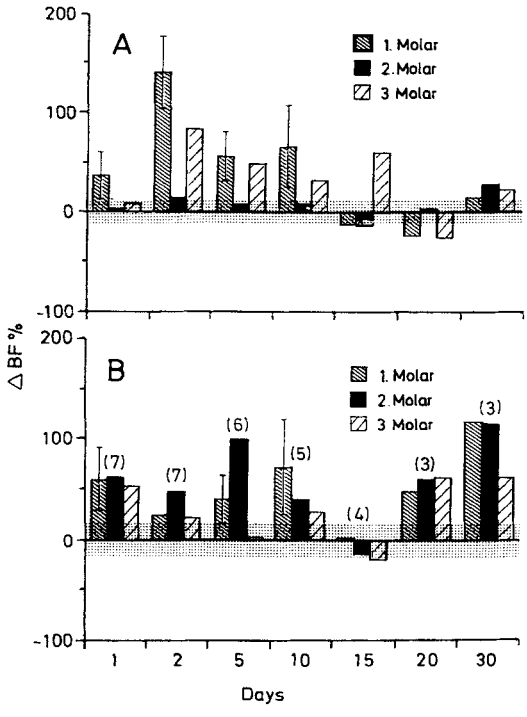

Fig. 6. Pulp. Maxillary molars (A) and mandibular molars (B). Mean percentage change in blood flow (BF) in the molar dental pulps on the experimental side compared with the contralateral side at different observation periods. The contralateral BF values are defined as unity at zero (a hypothetical line at 0 parallel to the abscissa). Shaded area represents ±1 SD of the mean contralateral BF values, and bars indicate ±1 SD of the mean experimental BF values. Note the increase in BF on the experimental side at 2 days for the upper first molar and at 24 h and at 5–10 days for the lower first molar. Number of animals in each group (*n*) is indicated in parentheses; *n* is identical in Figs. 6–8.

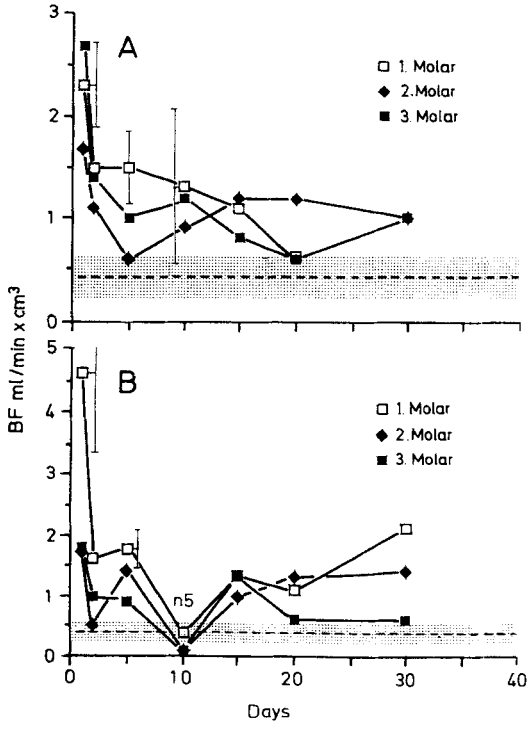

Fig. 7. Pulp. Maxillary molars (A) and mandibular molars (B). Blood flow (BF) in the dental pulp in ml/min × cm^3 tissue on the experimental side relative to mean BF values from the unoperated control animals (0.4 ± SD, 0.16 ml/min/cm^3; stippled line and shaded area). Bars indicate ±1 SD of mean experimental values. Note the increase already at 24 h in both the upper (A) and lower (B) jaws.

B). When the experimental and contralateral mean blood flow values of the maxillary and mandibular first molars were compared with the corresponding unoperated control mean values, both sides showed an increase at 24 h (Figs. 8A and B).

Discussion

It must be emphasized that owing to the great individual variations found in any biologic material of the present experimental model, stringent statistical significance may be hard to justify, and the present findings are therefore defined as general tendencies during the experimental period. Nevertheless, this study indicates that unilateral experimental occlusal trauma initiates blood flow responses in the total molar dentition in rats. Previous studies on the effect of occlusal trauma on blood circulation have been mostly morphologic. Transmission and scanning electron microscopic observations indicate that cellular conditions in both fibrous and vascular tissues have been markedly influenced by experimental compressive or extrusive forces. Lew et al. (16) found increase in vascular volume during extrusion, whereas Gaengler & Merte (17) found that

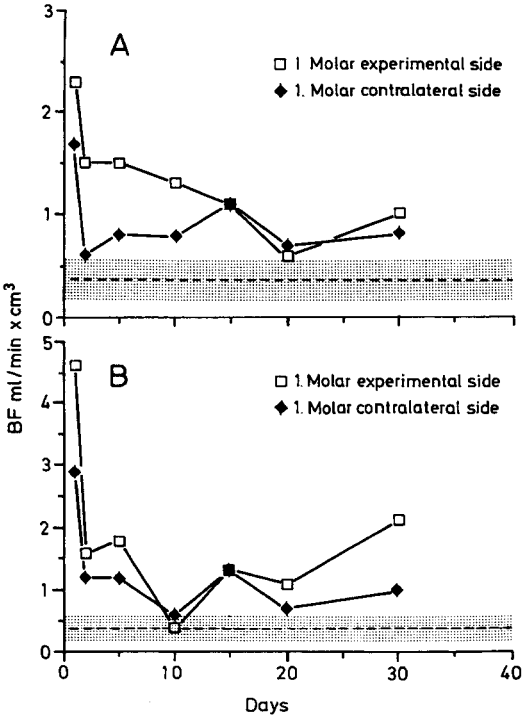

Fig. 8. Pulp maxillary first molar (A) and pulp mandibular first molar (B). Blood flow (BF) in the dental pulp (experimental and contralateral) per ml/min × cm^3 tissue relative to mean BF values for the unoperated controls. The mean BF values for the unoperated control animals are defined as unity; that is, BF per ml/min × cm^3 = 0.4 ± SD 0.16 (stippled line and shaded area). Note the similarity in behavior of the experimental and contralateral sides.

both compression and tension led to ischemic areas in venules, arterioles, and capillaries. Furthermore, after continuous force application, the periodontal vessels showed irreversible thrombosis. These studies were short-term observations, from 10 sec to 180 min, on anesthetized animals. Ng et al. (18), using intermittent intrusive force and recovery (2 sec and 8 min, respectively), found that loading created a 10% reduction of blood flow in the PDL, followed by hyperemia after removal of the load. By applying continuous traumatic intrusive force for 48 h, Palcanis (3) found a small increase in tissue fluid pressure in the PDL. Short-term experimental tooth movement in rats has revealed compression of vessels or, alternatively, dilated vessels packed with erythrocytes, endothelial leakage, stasis, and cellular degeneration (19–23). There is anatomic evidence that arteriovenous anastomosis (A-V shunts) exist in the pulp. If appreciable shunting occurs, such as non-entrapment of microspheres, it is likely that the absolute blood flow values will be underestimated more than overestimated, and this underestimation is most likely evenly distributed on both jaw sides (24). Larger spheres (15–18 μm) have been shown to cause overestimation of blood flow (25). The use of 9.5-μm size therefore seems justifiable in this study.

In the present investigation with unilateral experimental traumatic occlusion we found a percentage increase in blood flow at 2 and 5 days postoperatively in the PDL and pulp in the maxillary and mandibular molars, respectively, on the experimental side compared with the contralateral side. The difference in blood flow between the two sides gradually equalized during the experimental period, but the experimental side still tended to show a small overall increase compared with the contralateral side. Furthermore, it was demonstrated that unilateral occlusal trauma led to an increase in blood flow in the total molar dentition (experimental and contralateral sides) compared with an unoperated control material. Increase in pulpal blood flow in response to load application has also been reported by Olgart et al. (1), who described this as an 'unexpected finding'. An increase in blood flow is dependent on vasodilation, absence of gross vascular leakage, and absence of stasis. Vasodilation is probably initiated by stimulation of sensory nerves with release of neuropeptides like substance P (SP) and calcitonin gene-related peptide (CGRP) (26). In response to electric tooth stimulation, cavity preparation, or application of capsaicin or bradykinin in dental cavities, afferent nerves were demonstrated to contribute to pulpal blood flow increase (27). This neurogenic inflammation may initiate release of chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins, which will create reactionary processes in the tissues

(28–30). Increased vascular permeability and, eventually, the resultant stasis will occur if the inflammatory process continues and worsens. PDL and dental pulp are both low-compliance systems, and, although vascular permeability is an important factor in inflammation, little work has been done in oral tissues in this field to date (12). Results of the present study in pulp and PDL might seem to contradict some previous findings in that a tendency to an increase in blood flow was found on the experimental traumatic side, compared both with the contralateral side and with the unoperated controls. It is, however, quite conceivable that small, localized vasculature will show compression or stasis, as seen in transmission electron and scanning electron microscopy using semithin and thin sections after orthodontic movement of teeth (22). However, a generalized stasis of the vasculature in the tissues in the present investigation would have led to a decrease in blood flow and not an increase, as was actually found initially. This agrees well with our previous findings during mesial movement of the first maxillary molar in rats, in which an increase in blood flow was found after 5 days of orthodontic treatment (2). It is therefore probable that, although local areas of compression and stasis will be found in dental tissues during occlusal trauma, there will probably be a general vasodilation to permit the increased blood flow subsequent to the traumatic stimulus. The tendency for both the experimental and the contralateral teeth to show increased blood flow compared with the unoperated controls indicates that traumatic occlusion is not purely a local occurrence. In an identical experimental model it has recently been shown that morphologic changes and proliferation of CGRP and SP-immunoreactive nerves were found in the pulp and in the periodontal ligament and that traumatic occlusion induced morphologic nerve responses in the total molar dentition (31). These results support the present findings and may in part explain the blood flow increase. Concomitant nerve proliferation and blood flow increase has also been demonstrated in response to experimental orthodontic tooth movement in rats (2, 4).

Owing to the primary occlusal trauma the mandible will probably change to a new pattern of occlusive activity, which in itself can cause trauma to the teeth both on the 'non-experimental' side and on the affected side. This 'secondary' trauma may induce an increase in blood flow on the contralateral side. This is in accordance with previous studies, which have shown that when stimuli of different types are directed to experimental teeth with intact innervation, a prompt increase in blood flow is produced (1, 27).

From the results of this study it may be deduced that experimental traumatic occlusion in rats causes a relative increase in blood flow on the experimental side compared with the contralateral side. Furthermore, it causes blood flow increase in the total molar dentition, suggesting that traumatic occlusion is not purely a local phenomenon but probably a more general inflammatory reaction in pulp and periodontium, induced by a local stimulus.

Acknowledgements.—The authors express their gratitude to Ingrid Sandvik Gavlen for histologic tissue processing and to Liv Skarstein for help with the histograms. This study was supported by the Norwegian Research Council for Science and the Humanities (NAVF).

References

1. Olgart L, Gazelius B, Sundström S. Intradental nerve activity and jaw opening reflex in response to mechanical deformation of rat teeth. Acta Physiol Scand 1988;133:399–406.
2. Kvinnsland S, Heyeraas K, Øfjord ES. Effect of experimental tooth movement on periodontal and pulpal blood flow. Eur J Orthod 1989;11:200–5.
3. Palcanis KG. Effect of occlusal trauma on interstitial pressure in the periodontal ligament. J Dent Res 1973; Suppl 5.
4. Kvinnsland I, Kvinnsland S. Changes in CGRP-immunoreactive nerve fibres during experimental tooth movement in rats. Eur J Orthod 1990;12:320–9.
5. Path MG, Meyer MW. Quantification of pulpal blood flow in developing teeth of dogs. J Dent Res 1977;56:1245–54.
6. Edwall B. Experimental studies of blood flow regulation in oral tissues [thesis]. Stockholm: Karolinska Institutet, 1987.
7. Tønder K, Aukland K. Blood flow in the dental pulp in dogs measured by local H_2 gas desaturation techniques. Arch Oral Biol 1975;20:345–9.

8. Meyer MW, Path MG. Blood flow in the dental pulp of dogs determined by hydrogen polarography and radioactive microsphere methods. Arch Oral Biol 1979;24:601–5.
9. Meyer MW, Path MG. Heterogeneity of blood flow in the canine tooth in the dog. Arch Biol 1980;25:83–6.
10. Heyeraas Tønder K. Blood flow and vascular pressure in the dental pulp. Acta Odontol Scand 1980;38:1–10.
11. Folke LEA, Stallard RE. Periodontal microcirculation as revealed by plastic microspheres. J Periodont Res 1967;2:53–63.
12. Kim S. Regulation of pulpal blood flow. J Dent Res 1985;64:590–6.
13. Meyer MW. Methodologies for studying pulpal hemodynamics. J Endodont 1980;6:466–72.
14. Hale SL, Vivaldi MT, Kloner RA. Fluorescent microspheres: a new tool for visualization of ischemic myocardium in rats. Am J Physiol 1986;251:863–8.
15. Fosse G. The number of prisms on the inner and outer surface of the enamel mantle of human teeth. J Dent Res 1964;43:57–63.
16. Lew KK, Sims MR, Leppard PI. Tooth extrusion effects on microvessel volumes, endothelial areas, and fenestrae in molar apical periodontal ligament. Am J Orthod Dentofac Orthop 1989;96:221–31.
17. Gaengler P, Merte K. Effect of force application on periodontal blood circulation. J Periodont Res 1983;18:86–92.
18. Ng GC, Walker TW, Zingg W, Burke PS. Effect of tooth loading on the periodontal vasculature of the mandibular fourth premolar in dogs. Arch Oral Biol 1981;26:189–95.
19. Hirashita A. Electron-microscopic studies of the osteoblasts and osteocytes appeared in experimental tooth movement. Bull Tokyo Med Dent Univ 1973;40:75–102.
20. Nakumura Y, Hirashita A, Kuwabara Y. Scanning electron microscopy of the periodontium in physiological tooth movement in rats. J Jpn Orthod Soc 1980;39:194–207.
21. Okumura E. Light and electron microscopic of multinucleated giant cells related with resorption of hyalinized tissues. J Jpn Orthod Soc 1982;41:531–55.
22. Nakumura M, Nakukura TK, Yoshikawa M, Kiomura H. Vascular changes in pressure zones of rat molar periodontium following orthodontic tooth movement. J Jpn Orthod Soc 1986;45:126–34.
23. Göz G, Rakosi T, Rahn BA. Die Bedeutung der paradontalen Zirkulationsstörung für Umbau und mögliche paradontale Schädigung im Laufe einer Kieferorthopädischen Behandlung. Fortschr Kieferorthop 1987;48:34–40.
24. Takahashi K. Vascular architecture of dog pulp using corrosion resin cast examined under a scanning electron microscope. J Dent Res 1985;64:579–84.
25. Fung YC. Stochastic flow in capillary blood vessels. Microvasc Res 1973;5:34–48.
26. Brodin E, Gazelius B, Olgart L, Nilsson G. Tissue concentration and release of substance P- like immunoreactivity in the dental pulp. Acta Physiol Scand 1981;111:141–9.
27. Olgart L, Edwall L, Gazelius B. Involvement of afferent nerves in pulpal blood flow reactions in response to clinical and experimental procedured in the cat. Arch Oral Biol 1991;36:575–81.
28. Weiss RC, Tansy MF, Chaffe RB, Kendell FM. Functional control of intrapulpal vasculature. J Dent Res 1972;51:1350–8.
29. Wakisaka S, Nishikawa S, Ichikawa H, Matsuo S, Takano Y, Akai M. The distribution and origin of sustance P-like immunoreactivity in the rat molar pulp and periodontal tissues. Arch Oral Biol 1985;30:813–8.
30. Gazelius B, Edwall B, Olgart L, Lundberg JM, Høkfelt T, Fisher JA. Vasodilatory effects and coexistence of calcitonine gene related peptide (CGRP) and substance P in sensory nerves of rat dental pulp. Acta Physiol Scand 1987;108:181–6.
31. Kvinnsland I, Heyeraas KJ. Effect of traumatic occlusion on CGRP and SP immunoreactive nerve fibre morphology in rat molar pulp and periodontium. Histochemistry 1992. In press.

Received for publication 11 November 1991