Alkaline phosphatase activity and tetracycline incorporation during initial orthodontic tooth movement in rats

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Lilja, E., Lindskog, S. & Hammarström, L. Alkaline phosphatase activity and tetracycline incorporation during initial orthodontic tooth movement in rats. *Acta Odontol. Scand.* 1984, 42, 1–11. Oslo. ISSN 0001–6357.

The activity of alkaline phosphatase and the incorporation of tetracycline as signs of bone formation were studied after orthodontic tooth movement for 10 h to 6 days in rats. Defined low or high forces were used. A moderate activity of non-specific alkaline phosphatase was found in the periodontal membrane (PDM) in untreated rats and in rats treated with low forces. In addition, all bone surfaces were outlined with a narrow band of intense non-specific alkaline phosphatase activity that was vanadate- and levamisole-resistant. Likewise, tetracycline was incorporated on all bone surfaces. The bone formation rate was low and uniform within the alveolus, indicating that no intra-alveolar drift of the molar occurred in the untreated rats. Orthodontic forces gradually inhibited vanadate- and levamisole-resistant alkaline phosphatases and tetracycline incorporation on the bone surfaces in the pressure zones of the PDM, depending on the magnitude of the force. It was suggested that the disappearance of these isoenzymes, in a limited area, as seen in the pressure zones, was associated with inhibited bone formation and subsequent initiation of bone resorption. On the tension side a slight reduction and redistribution of vanadate- and levamisole-resistant alkaline phosphatase activity could be noted irrespective of the magnitude of the applied force. □ *Bone formation; enzyme histochemistry; enzyme inhibitors*

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Orthodontic tooth movement induces bone apposition and bone resorption of the alveolar bone. Valuable information concerning the location and rate of bone formation has been obtained by in vivo labeling with bone tracers (10, 15, 16, 18, 24, 33). Such studies have shown that during the first days of orthodontic tooth movement bone formation is inhibited in the compressed areas of the periodontal membrane (PDM), whereas formation of new bone is stimulated in areas of tension (15, 39). However, the mechanisms by which mechanical forces influence the activity of bone formation cells are incompletely known.

Enzymes that are able to hydrolyze phosphate groups at an alkaline pH have been associated with the formation of mineralized tissues. The original idea of their action was that they hydrolyze phosphate groups at the site of mineralization (29). Several other mechanisms have been suggested. Recently, it was proposed that they act by removing inorganic pyrophosphate, which is an inhibitor of mineralization (8, 9). Alkaline phosphatases are not restricted to bone but are widely distributed throughout the body (1, 3) and have been associated with transport functions in, for example, the small intestine (14, 30).

The commonly used histochemical methods for demonstrating alkaline phosphatase activity are the azo-coupling method (27) and the method based on the precipitation of the liberated phosphate group (11). A wide range of enzymes, such as ATPases (EC 3.6.1.3), AMPase (EC 3.1.3.5), alkaline orthophosphoric monoesterase phosphohydrolase (EC 3.1.3.1), pyrophosphatase (no EC number assigned), and ADPase (EC 3.6.1.6), are capable of hydrolysing the substrates used in these methods. In this article this type of enzyme activity will be referred to as non-specific alkaline phosphatase activity. By means of various inhibitors several tissue-specific isoenzymes of alkaline phosphatase have been distinguished. A vanadate-resistant isoenzyme has been demonstrated in developing teeth and on bone surfaces (13). Levamisole is another potent inhibitor of alkaline phosphatase activity (1). Recent studies have shown that levamisole and related substances inhibit alkaline phosphatase activity on bone surfaces, whereas ATPases are levamisole-resistant (1). This is of interest, since Ca^{2+} ATPase has been shown to participate in calcium transport during enamel and dentin mineralization (5, 12).

Changes in activity of non-specific alkaline phosphatase in the PDM during experimental tooth movement have been studied in rats by inserting rubber dams between the maxillary first and second molars (35). Enzyme activity increased in areas of tension and decreased in areas of pressure in the PDM. There seem to be no studies of orthodontically induced changes of various isoenzymes of alkaline phosphatase in the alveolar bone.

The purpose of the present investigation was to study the activity and distribution of non-specific alkaline phosphatase and the effect of histochemical incubation with vanadate and levamisole after initial orthodontic tooth movement with low and high forces in rats and to study changes in bone formation in the alveolar bone as seen by incorporation by tetracycline.

Materials and methods

General procedure. The maxillary right first molars in 40 Sprague-Dawley rats were moved buccally by means of a fixed appliance with either low or high orthodontic forces for periods of from 10 h to 6 days. The orthodontically treated rats were divided into two groups. The first group was used for enzyme histochemical evaluation of non-specific alkaline phosphatase-, vanadate-, and levamisole-resistant alkaline phosphatase. The second group was labeled with tetracycline and the areas corresponding to those used for enzyme histochemical evaluation were step-serial sectioned and photographed in incident ultraviolet light to visualize tetracycline deposits. In addition, four untreated rats were given two injections of tetracycline to label bone sequentially and to measure the rate of bone formation in various locations of the alveolar bone.

Orthodontic procedure. Forty young adult female Sprague–Dawley rats weighing 280 ± 40 g each were treated orthodontically with low or high forces. The maxillary right first molar in each rat was moved in a buccal direction by means of a fixed appliance with predetermined forces as described previously (22). The magnitudes of the orthodontic forces were selected on the basis of previous studies, in which forces above 200 mN produced bleeding (22) and non-vital areas (23) in the pressure zones. They were designated high forces in this study. Forces ranging between 50 and 100 mN caused neither bleeding nor loss of vitality in the pressure zones and were designated low forces. Twenty rats were treated with high forces and 20 rats with low forces for 10 h or 1, 3, 4, or 6 days (Table 1). At the end of the experimental periods the clinical appearance of the gingiva of the upper jaws was examined through a dissecting microscope. The rats were killed by ether inhalation, and the orthodontic forces were measured as described previously (22). The upper jaw from each rat was dissected free and embedded in a 4% aqueous solution of carboxymethyl cellulose and frozen in hexane cooled with solid CO_2. To obtain sections or photographs in the same plane from all specimens, the jaws were reproducibly fixed during the embedding (22). The number of orthodontically treated rats was divided equally between evaluation with enzyme histochemistry and tetracycline labeling.

Histochemical procedure. Twenty orthodontically treated and two untreated rats were used for histochemical demonstration of non-specific alkaline phosphatase activity and of vanadate- and levamisole-resistant alkaline phosphatase activity. The frozen blocks were freeze-sectioned, and sections (10 μm) from the areas that were photographed in incident ultraviolet light were taken up on adhesive tape (No. 190, Min-

Table 1. Non-specific alkaline phosphatase activity (NAPase) in the connective tissue in the pressure zones of the periodontal membrane after orthodontic treatment with low (50–100 mN) or high (200–400 mN) forces. Vanadate-resistant alkaline phosphatase activity (VAPase) and levamisole-resistant alkaline phosphatase activity (LAPase) outlining the bone surfaces in the pressure zones and intensity of tetracycline fluorescence (TF) on the bone surfaces in the pressure zones of orthodontically treated rats

No. of rats	Treatment time	Force	NAPase	VAPase	LAPase	TF
6	0		++	+++	+++	+++
4	10 h	Low	++	+++	+++	++
4	10 h	High	++	++	++	+
4	1 day	Low	++	++	++	+
4	1 day	High	0	0	0	0
4	3 days	Low	++	0	0	0
4	3 days	High	0	0	0	0
4	4 days	Low	++	0	0	0
4	4 days	High	0	0	0	0
4	6 days	Low	++	0	0	0
4	6 days	High	0	0	0	0

+++ = Intense; ++ = moderate; + = weak; and 0 = none.

nesota Mining & Manufacturing Co., USA) (37, 38). Sections were incubated for non-specific alkaline phosphatase (EC 3.1.3.1) by the simultaneous azo-coupling method (2) with 0.1 mM naphthol-AS-MX-phosphate (Sigma N-5000) as substrate and 0.2 mM Fast Blue RR (Sigma F-0500) as coupling agent. Incubation was carried out at pH 8.2 for 30 min. Neighboring sections were preincubated with 5 mM sodium meta-vanadate (Kebo 1.3490, Sweden) in distilled water for 5 min, or 2 mM levamisole (Sigma L-9756) was added to their incubation medium. After incubation the sections were rinsed in distilled water *in vacuo* and mounted in glycerol. All concentrations indicate final concentrations in the incubation media. Sections incubated in an incubation medium without substrate showed no staining. The Sigma chemicals were purchased from Sigma Chemical Co., St. Louis, Mo., USA.

Tetracycline labeling. Twenty orthodontically treated rats received an intraperitoneal injection of tetracycline HCl (Sigma T-3383) dissolved in distilled water, using doses of 25 mg/kg body weight/24 h, before they were killed. The rats treated orthodontically for 10 h were injected with tetracycline immediately after the orthodontic appliances were cemented. To evaluate changes in tetracycline incorporation during the treatment periods the growth pattern of the alveolar bone was studied in untreated rats by sequential labeling with tetracycline. Four untreated rats received two tetracycline injections with a 20-day interval and were killed 24 h after the last injection. After being embedded, the upper jaws were step-serial sectioned in a frontal aspect, and macrophotographs were taken of the frozen surface in the area that was evaluated with enzyme histochemistry. The camera used was an Olympus OM2 with an Auto Bellow and a Zuiko Macro Objective 20 mm F 3.5 (Olympus Optical Co., Tokyo, Japan), as described previously (22). The photographs were taken on Ektachrome 400 film. To visualize the tetracycline deposits on the bone surfaces, incident ultraviolet light was used. The UV-light source (Penray, 22 3C-3S, Ultra-Violet Products, Inc., San Gabriel, USA) was attached to the objective on the camera. An excitation filter (KP 390, Ernst Leitz, Wetzlar, FRG) and a suppression filter (K430 or BG38) were used to improve contrast in the photographs. The first level to be photographed showed the entire mesial root of the first molar from the apex to the enamel–cementum border (22). Photographs were taken at 10 levels 50 μm apart in a distal direction (total distance, 500 μm). At the beginning and the end of each film a microscale with 0.1 mm division was photographed; this scale was later used as reference when bone formation rate was determined. The rate of bone formation was measured at the alveolar crests, on the periostal alveolar bone, on the intra-alveolar bone facing the root, and in the palatal median bone suture. Bone formation rate in the palatal median bone suture was measured at the level of the middle portion of the mesial root of the maxillary first molar. In addition, cementum formation in the apical part of the root was estimated in the same manner. In each location the perpendicular maximal distance between the two fluorescent lines was measured in each photograph and a mean value per day was calculated.

Results

Clinical inspection of the gingiva around the experimental teeth revealed that a mild inflammation had developed under the orthodontic spring and the arch wire in half of the rats that had been treated orthodontically for 6 days. The orthodontic force had produced a tipping movement of the maxillary right first molar and thus two pressure zones—a buccal cervical and a lingual apical—and two tension zones—a lingual cervical and a buccal apical—had developed in the PDM.

A moderate non-specific alkaline phosphatase activity was demonstrable in the connective tissue of the PDM. The bone surfaces were outlined with a narrow band of intense non-specific alkaline phosphatase activity that was vanadate- and levamisole-resistant. Incorporation of tetracycline was demonstrated on all bone surfaces. Bone formation rate was low and uniform in the alveolus. Non-specific alkaline phosphatase of the connective tissue in the PDM did not change after the application of low forces. High forces, however, inhibited enzyme activity of the connective tissue in the pressure zones of the PDM. Vanadate- and levamisole-resistant alkaline phosphatase activity on the bone surfaces and tetracycline incorporation into the bone were inhibited in the pressure zones of the PDM regardless of the magnitude of the force. The activity and distribution of alkaline phosphatases and tetracycline incorporation will be described in detail below.

Alkaline phosphatase

The connective tissue in the intra-alveolar part of the PDM and the connective tissue above the alveolar crests close to the tooth exhibited a moderate and uniform non-specific alkaline phosphatase activity (Fig. 1 and Table 1). A moderate enzyme activity was found in the palatal median bone suture and in the sutures of the skull. All bone surfaces were outlined by a continuous band of intense non-specific alkaline phosphatase

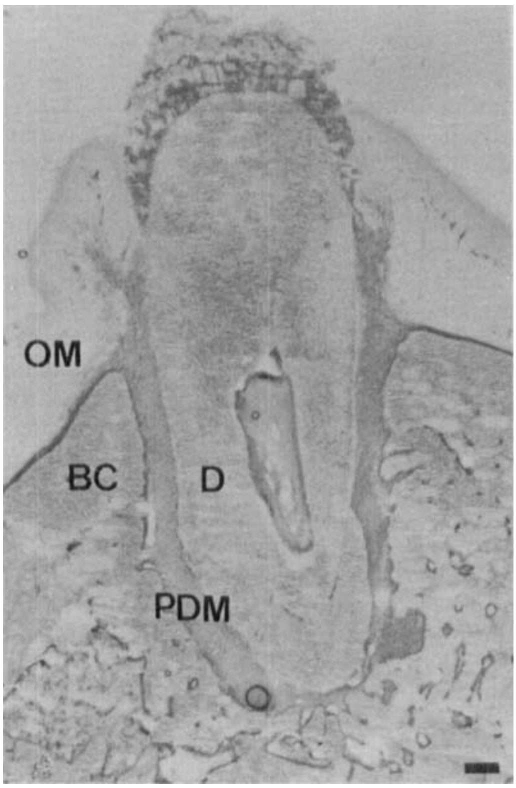

Fig. 1. Non-specific alkaline phosphatase activity in a frontal frozen section of an untreated tooth and surrounding alveolar bone. The enzyme activity was moderate and uniform in the periodontal membrane (PDM) below the crests and in the gingival connective tissue close to the tooth. Buccal alveolar crest (BC), dentin (D), oral mucosa (OM). Bar = 100 μm.

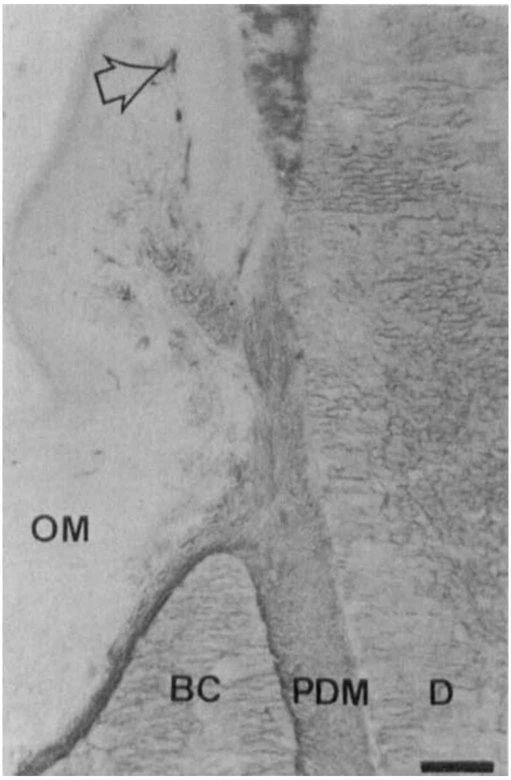

Fig. 2. Buccal alveolar crest (BC), buccal part of the tooth, and buccal oral mucosa (OM). A narrow band of connective tissue that covered the alveolar crests showed an intense non-specific alkaline phosphatase activity. In addition, some blood vessels (arrow) close to the oral epithelium showed an intense enzyme activity. Dentin (D), periodontal membrane (PDM). Bar = 100 μm.

activity (Fig. 2). The enzyme activity on the bone surfaces remained intense when levamisole was added to the incubation medium or preincubation with vanadate was performed (Fig. 3 and Table 1). Vanadate-resistant alkaline phosphatase activity could not be demonstrated in the connective tissue of the PDM and sutures, whereas a very weak activity of levamisole-resistant alkaline phosphatase was present.

Low orthodontic forces did not affect non-specific alkaline phosphatase activity in the connective tissue of the PDM. However, the intense activities of vanadate- and levamisole-resistant alkaline phosphatase on the bone surfaces in the pressure zones were gradually inhibited (Table 1). After 1 day of orthodontic treatment with low forces, moderate activities of vanadate- and levamisole-resistant alkaline phosphatase were demonstrated on the bone surfaces in the pressure zones (Fig. 4 and Table 1), and after 3, 4, and 6 days these enzyme activities were no longer demonstrable (Fig. 5 and Table 1).

After high orthodontic forces had been applied for 1 day, non-specific alkaline phosphatase activity was not demonstrable in the connective tissue of the pressure zones (Fig. 6 and Table 1). Changes in activity of vanadate- and levamisole-resistant alkaline phosphatase on the bone surfaces facing the pressure zones in rats treated with high forces were similar to those seen with low forces; however, they occurred more rapidly (Table 1).

The originally intense activities of vanadate- and levamisole-resistant alkaline phosphatase in the tension zones of the PDM in all the orthodontically treated rats were

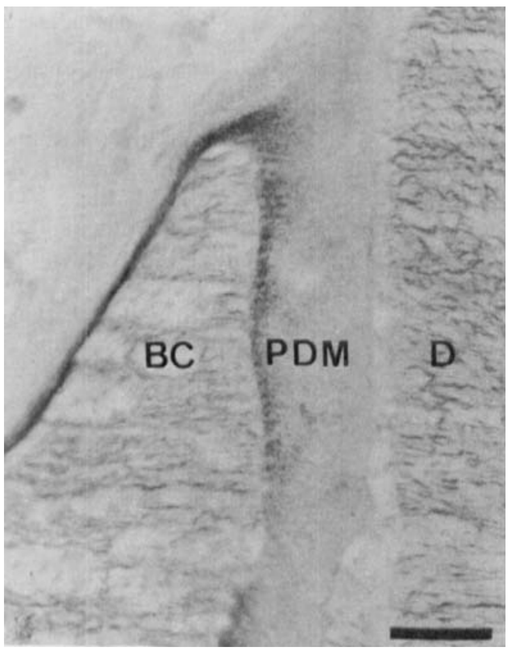

Fig. 3. Vanadate-resistant alkaline phosphatase activity in a frontal frozen section from an untreated rat. The buccal periostal bone surface and the buccal intra-alveolar bone were outlined by a narrow band of intense enzyme activity. Buccal alveolar bone (BC), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

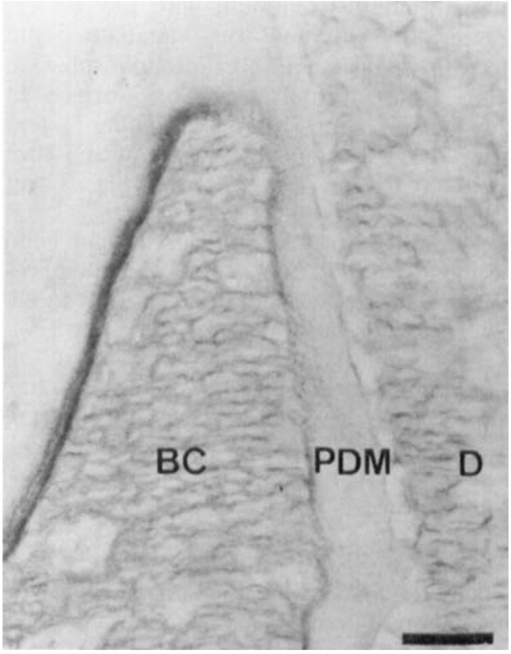

Fig. 4. Vanadate-resistant alkaline phosphatase activity in the buccal pressure zone after 1 day of orthodontic treatment with a low force. Enzyme activity along the alveolar bone surface was slightly reduced in the buccal pressure zone compared within the controls. Buccal alveolar crest (BC), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

reduced to moderate activities after 3, 4, and 6 days of orthodontic treatment (Fig. 7). At the palatal alveolar crests of these rats vanadate- and levamisole-resistant alkaline phosphatase activity could be seen extending along the stretched periodontal fibers into the PDM (Fig. 7). In the untreated rats these activities were confined to a narrow line at the bone surface. The palatal bone surface of the rats, where a mild inflammation was found under the orthodontic appliances, lacked demonstrable vanadate- and levamisole-resistant alkaline phosphatase activity (Fig. 8).

Tetracycline

The fluorescent lines were distinct and narrow in the analyzed area of the alveolar bone, indicating that the sections were more or less perpendicular to the surface of bone formation. The distance between the two fluorescent lines in the four rats given two doses of tetracycline could, thus, be used to calculate the average daily bone formation with a reasonable degree of accuracy and precision. The average rates of bone formation in various locations of the alveolar bone varied between 1 μm and 6 μm per day and are summarized in Fig. 9. Bone formation rate was low and uniform on the buccal and lingual bone surfaces of the alveolus. The palatal median bone suture showed a bone formation rate of 3 μm/day. The maximal deviation from the mean values was 0.6 μm/day.

When a single dose of tetracycline was administered, a narrow band of intense to moderate fluorescence appeared on all bone surfaces (Fig. 10). After the application of low orthodontic forces for 10 h the originally intense fluorescence on the bone surfaces facing the pressure zones and on the buccal periostal bone surfaces was reduced to a moderate activity (Fig. 11 and Table 1). Only

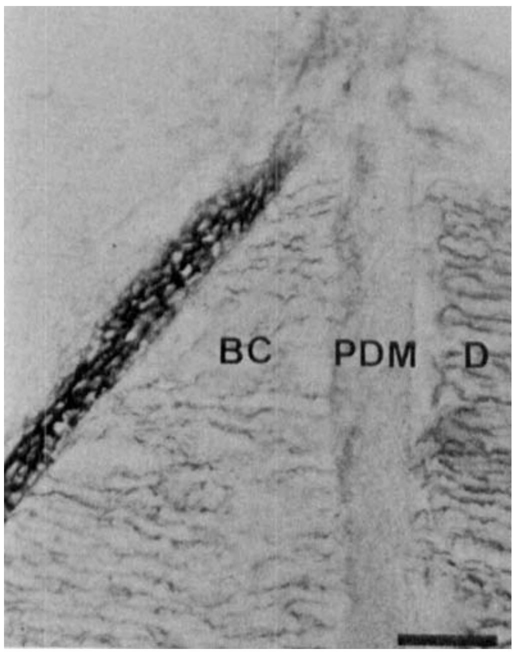

Fig. 5. Buccal pressure zone in a rat treated with a low orthodontic force for 3 days. The bone surface facing the buccal pressure zone lacked levamisole-resistant alkaline phosphatase activity, whereas the buccal periostal bone surface was covered with a cell layer, three to four cells thick, which exhibited an intense enzyme activity. Buccal alveolar crest (BC), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

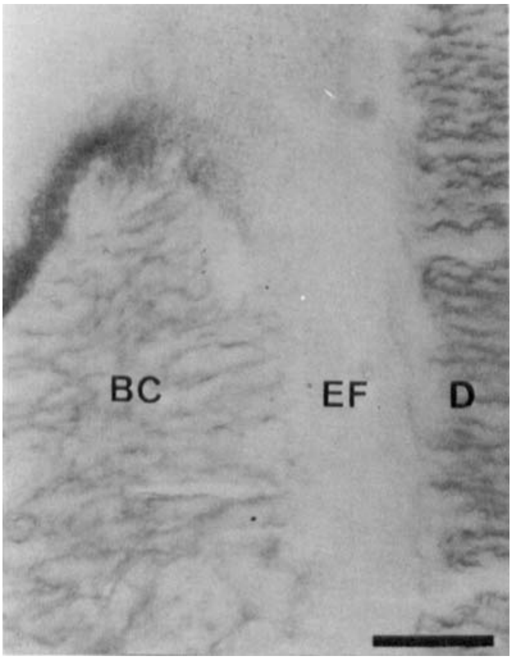

Fig. 6. Non-specific alkaline phosphatase activity in the buccal pressure zone after 1 day of orthodontic treatment with a high force. The buccal pressure zone lacked enzyme activity in the periodontal membrane and on the intra-alveolar bone surface. Buccal alveolar crest (BC), enzyme-free zone (EF), dentin (D). Bar = 100 µm.

a weak fluorescence in a non-continuous line could be detected in the pressure zones after the application of low forces for 1 day, and after 3, 4, and 6 days no tetracycline uptake could be demonstrated there (Fig. 12 and Table 1). Weak deposits of fluorescence were detected on the buccal periostal surface after 3, 4, and 6 days. In the rats treated with high forces the changes in intensity of fluorescence on the pressure sides and on the buccal periostal bone surfaces followed the same pattern as seen with low forces. However, the changes occurred more rapidly (Table 1). Incorporation of tetracycline on the bone surfaces in the tension zones neither increased nor decreased over the 6-day experimental period.

Discussion

Tetracycline forms a stable complex on the surface of growing apatite crystals at the front of mineralization (10, 18, 25). A few investigators have used tetracycline in combination with ground sections to demonstrate formation of bone in the tension zones and marrow spaces during orthodontic tooth movement (15, 21, 39). However, no correlation with the hyaline zone of the PDM has been made. The present method enables localization of areas of maximal compression and hyalinization in the PDM and a subsequent mapping of the tetracycline distribution in these areas. Macrophotographs of the frozen surfaces gave no information concerning cell morphology, and the resolution was not as high as in microphotographs of ground sections. A buccal drift of the rat molar within its alveolus has been discussed (34). In the present study the bone formation rate in the alveoli seemed to be uniform and did not exceed the resolution of the double

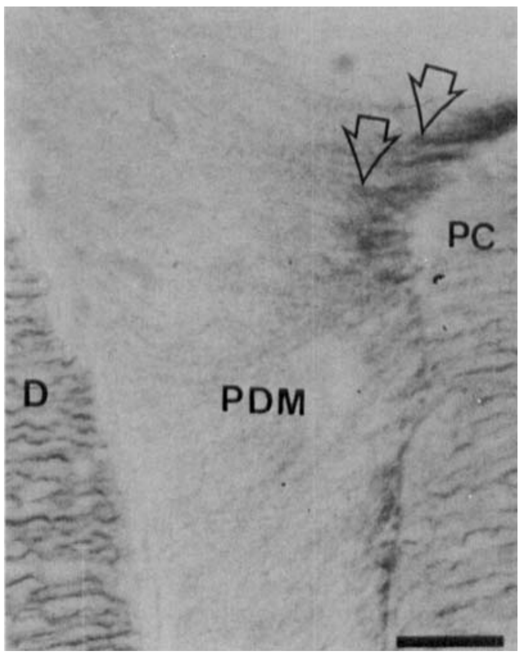

Fig. 7. Vanadate-resistant alkaline phosphatase activity in the lingual tension zone of a rat treated with a low force for 3 days. The originally intense enzyme activity on the lingual bone surface was reduced to a moderate activity. At the palatal alveolar crest (PC) the enzyme activity (arrows) could be seen extending along the stretched periodontal fibers into the periodontal membrane (PDM). Dentin (D). Bar = 100 µm.

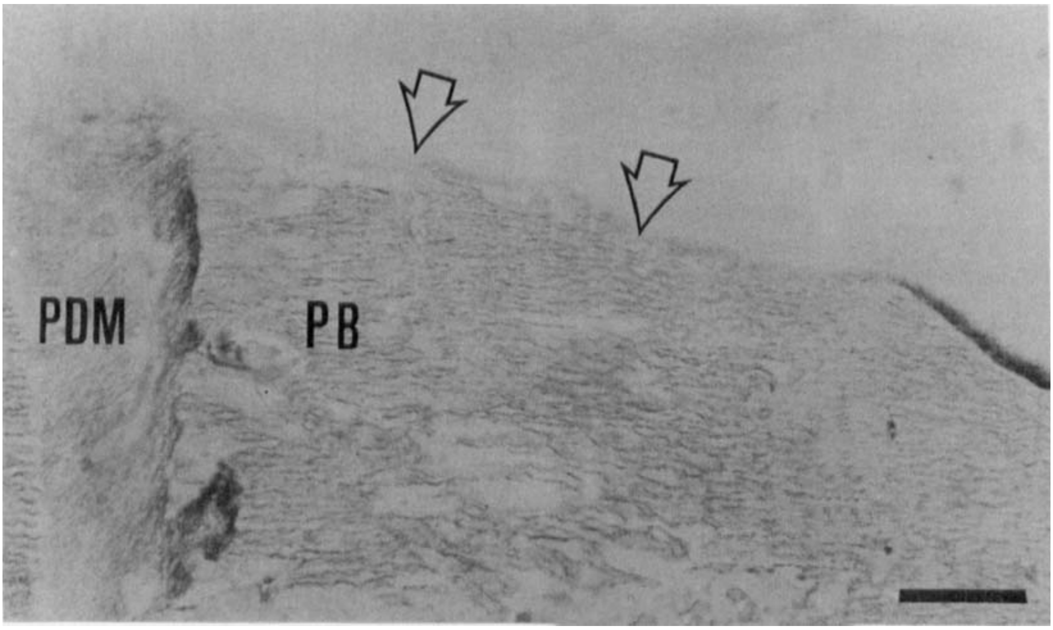

Fig. 8. Frontal frozen section of the palatal bone (PB) surface of a rat after 6 days of orthodontic treatment with a low force. Vanadate-resistant alkaline phosphatase had disappeared (arrows) on the bone surface below the location of the orthodontic appliance. Periodontal membrane (PDM). Bar = 100 μm.

labeling with tetracycline. The palatal median bone suture, however, showed a comparatively high bone formation rate. The low bone formation rate in the alveoli in the untreated rats and the lack of a buccal drift within the alveoli in combination with the sutural bone apposition probably resulted in a buccal movement of the entire alveolar processes including the molars.

In the present investigation tetracycline incorporation on the bone surfaces facing the pressure zones was gradually inhibited after both high and low orthodontic forces. A direct relationship between blood supply and incorporation of tetracycline into bone has been suggested (25). High orthodontic forces produce bleeding (22) and necrosis (23) in the most compressed areas in the PDM, and disturbances of the blood circulation may have prevented tetracycline from being distributed to these areas. An inhibited fluorescence also accompanied low forces, although neither bleeding (22) nor loss of vitality (23) has been found incident to these forces. In a previous study in the cat (26) it was shown that the blood circulation was not affected if forces were used that compressed the PDM less than one third of its width. Such low forces were used in the present study. It is thus possible that bone formation, as seen by tetracycline incorporation, was not only dependent on the local blood circulation but was influenced directly by mechanical forces. The fact that no changes in tetracycline incorporation could be demonstrated on the bone surfaces facing the tension zones was in accordance with a previous study in cats (15). Only a minor increase in tetracycline incorporation was demonstrated on the bone surfaces in the tension zones of the PDM during the first week of orthodontic treatment.

The activity of non-specific alkaline phosphatase in the connective tissue of the PDM did not change during orthodontic treatment except in areas of maximal compression, where local necrosis and hyalinization have previously been shown (20, 23, 31, 32). However, vanadate- and levamisole-resistant alkaline phosphatase activity on the bone surfaces of the PDM was influenced after both low and high orthodontic forces. The

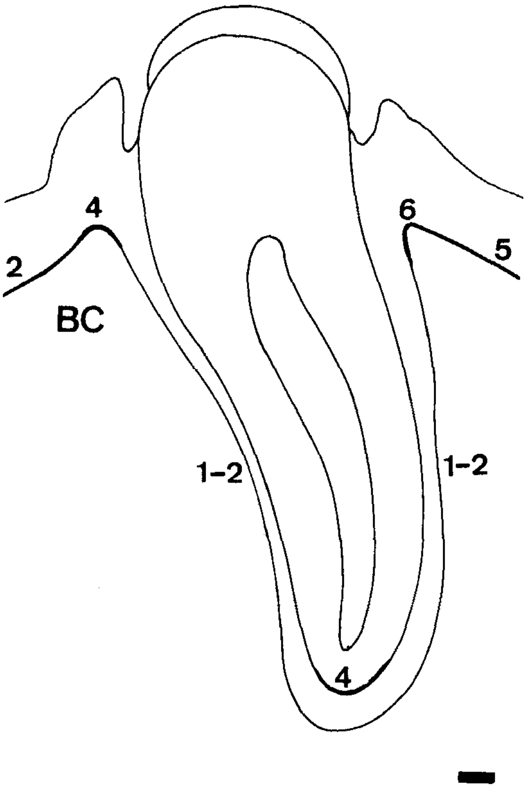

Fig. 9. Rate of bone and cementum formation at the alveolar crests, at the surfaces of the buccal and palatal bone, and at the apex of the root, expressed in micrometers per day. Measurements of bone formation of the buccal and palatal bone were made 400 μm from the crests. Bone formation rate in the central part of the alveoli varied between 1 and 2 μm/day, which was calculated from the total width of two tetracycline lines obtained by two injections 20 days apart. Buccal alveolar crest (BC). Bar = 100 μm.

activity of these tissue-specific isoenzymes on the bone surfaces and the incorporation of tetracycline into the bone were gradually inhibited in the pressure zones. The physiological significance of the intense alkaline phosphatase activity in the cell layers covering the bone is not fully understood. Resting and active bone surfaces have been suggested to be covered by a continuous membrane or 'bone membrane', which regulates the exchange of ions and nutrients between the interior of the bone and the extracellular fluid outside the bone (6, 7, 17, 19). The vanadate-resistant alkaline phosphatase activity in the present study seemed to form a continuous membrane on bone surfaces where bone formation took place, as indicated by tetracycline incorporation. Vanadate-resistant alkaline phosphatase may be identical to Ca^{2+}-activated ATPase (4, 28). It is possible that activity of Ca^{2+}-ATPase in the 'bone membrane' participates in bone mineralization and acts as a calcium pump to maintain the concentration gradient of calcium over the 'bone membrane' (36).

The presence of both levamisole- and vanadate-resistant alkaline phosphatase in the 'bone membrane' indicates that more than one isoenzyme of alkaline phosphatase

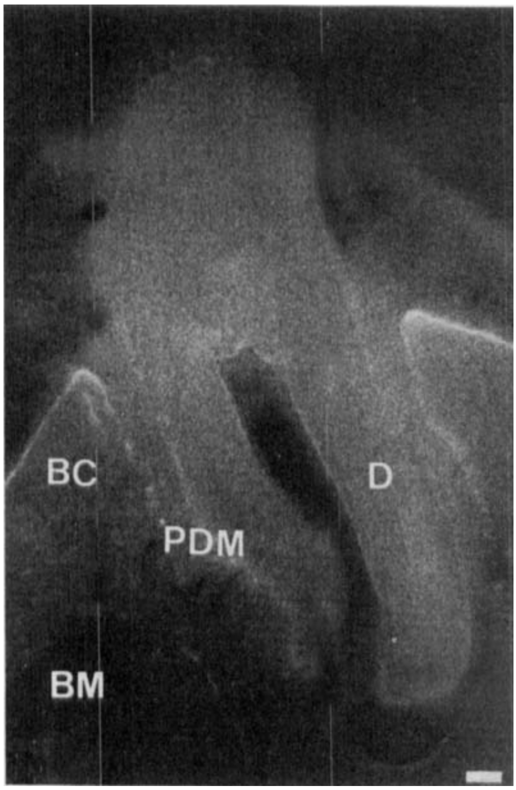

Fig. 10. Frontal macrophotograph of the right first molar and alveolar bone of an untreated rat that had been injected with tetracycline 24 h before it was killed. Tetracycline fluorescence was intense on the surfaces of the buccal and palatal bone, whereas the intra-alveolar bone surface sometimes exhibited a less intense fluorescence. Buccal alveolar crest (BC), bone marrow (BM), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

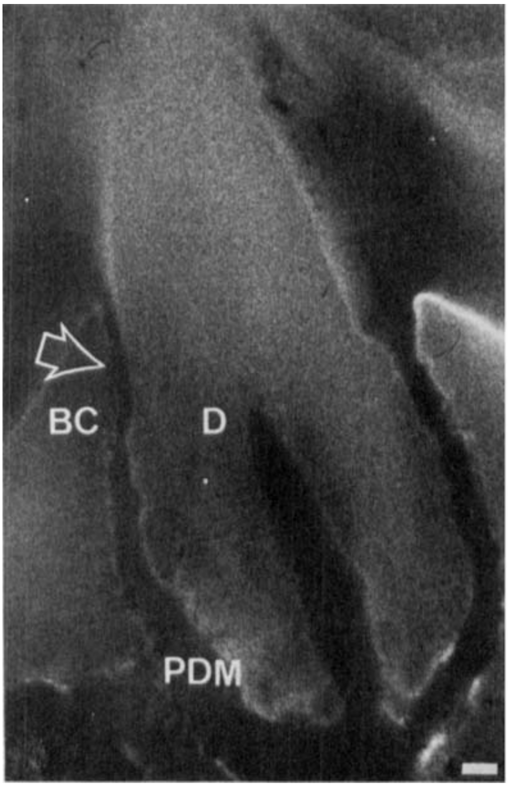

Fig. 11. Tetracycline fluorescence in a frontal macrophotograph after 10 h of a low orthodontic force. The fluorescence of the bone facing the buccal pressure zone (arrow) was reduced compared with the untreated rat. Note that the tetracycline fluorescence on the buccal periostal surface was lower than in the untreated rat. Buccal alveolar crest (BC), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

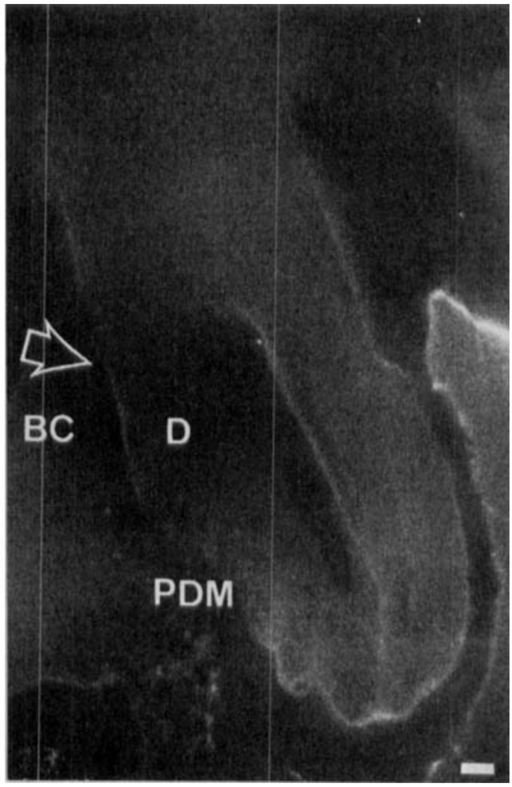

Fig. 12. Tetracycline fluorescence in a frontal macrophotograph after 3 days of a low orthodontic force. No fluorescence could be demonstrated on the bone surface facing the buccal pressure zone (arrow), and only weak traces of fluorescence remained on the buccal periostal surface. Buccal alveolar crest (BC), periodontal membrane (PDM), dentin (D). Bar = 100 μm.

may participate in bone mineralization. Activity of those isoenzymes could not be demonstrated in the pressure zone, which was an area where bone formation was inhibited. This area has previously been shown to undergo bone resorption (20, 23, 31, 32). The disappearance of the 'bone membrane' may be associated with an inhibited bone formation and the initiation of bone resorption.

Acknowledgements.—The authors gratefully acknowledge the secretarial help provided by Ms Christina Karlsson and Ms Eija Heinmetz. This investigation was supported by grants from the Swedish Medical Research Council (grant X06001-03A), from the School of Dentistry, Karolinska Institutet, and from Svenska Tandläkare-Sällskapet.

References

1. Borgers, M. The cytochemical application of new potent inhibitors of alkaline phosphatases. J. Histochem. Cytochem. 1973, 21, 812–824
2. Burstone, M. S. Histochemical comparison of naphtol AS-phosphates for the demonstration of phosphatases. J. Nat. Cancer Int. 1958, 20, 601–616
3. Burstone, M. S. Histochemical observations on enzymatic processes in bones and teeth. N.Y. Acad. Sci. 1969, 85, 431–444
4. Cantley, L. C., Jr., Josephson, L., Warner, R., Yanagisawa, M., Lechene, C. & Guidotti, G. Vanadate is a potent (Na, K)-ATPase inhibitor found in ATP derived from muscle. J. Biol. Chem. 1977, 252, 7421–7423
5. Chrenshaw, M. A. & Takano, Y. Mechanisms by which the enamel organ controls calcium entry into developing enamel. J. Dent. Res. (in press)

6. Davis, W. L., Mattew, J. L., Martin, J. H., Kennedy, J. W. & Talmage, R. V. The endosteum as a functional membrane. In: R. V. Talmage, M. Owen, & J. Parson, eds. Calcium Regulating Hormones. American Elsevier Publishing Co. Inc., New York 1975, pp. 274–283
7. Doty, S. B. Morphological evidence of gap junctions between bone cells. Calcif. Tiss. Int. 1981, 33, 509–512
8. Fleisch, H. & Neuman, W. F. Mechanisms of calcification: role of collagen, polyphosphates and phosphatase. Am. J. Physiol. 1961, 200, 1296–1300
9. Fleisch, H., Russel, R. G. G. & Strausman, F. Effect of pyrophosphate on hydroxyapatite and its implication in calcium homeostasis. Nature 1966, 212, 901–903
10. Frost, H. M., Villanueva, A. R., Roth, H. & Stanisavljevig, S. Tetracycline bone labeling. J. New Drugs 1961, 1, 206–216
11. Gomori, G. The study of enzymes in tissue sections. Am. J. Clin. Pathol. 1946, 16, 347–352
12. Granström, G. & Linde, A. ATP dependent uptake of Ca^{2+} by a microsomal fraction from rat incisor odontoblasts. Calcif. Tiss. Int. 1981, 33, 125–128
13. Hasselgren, G., Fransen, A. & Hammarström, L. E. Histochemical characterization of alkaline phosphatase in developing rat teeth and bone. Scand J. Dent. Res. 1978, 86, 325–336
14. Haussler, M. R., Nagode, L. A. & Rasmussen, H. Induction of intestinal bruch border alkaline phosphatase by vitamin D identity with Ca-ATPase. Nature 1970, 228, 1199–1201
15. Hermanson, P. Alveolar bone remodeling incident to tooth movement. Angle Orthod. 1972, 42, 107–115
16. Hoffman, M. M. & Schour, J. Quantitative studies in the development of the rat molar. Am. J. Orthod. & Oral Surg. 1940, 26, 854–874
17. Howard, J. E. Present knowledge of parathyroid function, with especial emphasis upon its limitations. In: G. E. Wolstenholme & C. M. O'Connors, eds. Ciba foundation of bone structure and metabolism. 1956, pp. 206–221
18. Johnson, R. H. The tetracyclines: A review of the literature—1948 through 1963. J. Oral Therap. Pharmacol. 1964, 1, 190–217
19. Jones, S. J. & Boyde, A. Experimental studies on changes in osteoblastic shape induced by calcitonin and parathyroid extract in an organ culture system. Cell. Tiss. Res. 1976, 169, 449–465
20. Kvam, E. Tissue changes incident to movement of rat molars. Thesis. Universitetsforlaget, Oslo 1967
21. Lemonie, C. & Petrovic, A. Le marquage a la tetracycline dans les experiences de transplantations dentaires et d'orthodontie ches le rat blanc. Rev. Franc. Odonto-Stomat. 1969, 16, 1191–1198
22. Lilja, E., Lindskog, S. & Hammarström, L. Orthodontic forces and periodontal compression – a new method and its application. Acta Odontol. Scand. 1981, 39, 367–378
23. Lilja, E., Lindskog, S. & Hammarström, L. Histochemistry of enzymes associated with tissue degradation incident to orthodontic tooth movement. Am. J. Orthod. (in press)
24. Meyer, J., Schneider, B. J. & Arup, K. Experimental studies on the interrelations of condylar growth and alveolar bone formation. Effect of growth hormone. Angle Orthod. 1967, 37, 309–319
25. Milch, R. A., Rall, D. P. & Tobie, J. E. Bone localization of the tetracyclines. J. Nat. Cancer Inst. 1957, 19, 87–91
26. Miura, F. Effect of orthodontic force on blood circulation in periodontal membrane. In: Cook, J. T., ed. Transactions of the third international congress. Granada Publishing Limited, London 1973, pp. 35–41
27. Pearse, A. G. E. Histochemistry. Theoretical and Applied. Churchill, London 1953, pp. 402–411
28. Quist, E. E. & Hokin, L. E. The presence of two $(Na^{+}+K^{+})$-ATPase inhibitors in equine muscle ATP: vanadate and a dithioerythritol – dependent inhibitor. Biochim. Biophys. Acta 1978, 511, 202–212
29. Robinson, R. The possible significance of hexosephosphoric esters in ossification. Biochem. J. 1923, 17, 286–293
30. Russel, R. G. G., Monod, A., Bonjour, J. P. & Fleisch, H. Relation between alkaline phosphatase and Ca^{2+} ATPase in calcium transport. Nature New Biology 1972, 240, 126–127
31. Rygh, P. Ultrastructural cellular reactions in pressure zones of rat molar periodontium incident to orthodontic tooth movement. Acta Odontol. Scand. 1972, 30, 575–593
32. Rygh, P. Ultrastructural vascular changes in pressure zones of rat molar periodontium incident to orthodontic movement. Scand. J. Dent. Res. 1972, 80, 307–321
33. Schneider, B. J. & Meyer, J. Experimental studies on the interrelations of growth and alveolar bone formation. Angle Orthod. 1965, 35, 187–199
34. Sicher, H. & Weinmann, J. Bone growth and physiologic tooth movement. Am. J. Orthod. Oral Surg. 1944, 30, 109–132
35. Takimoto, K., Deguchi, T. & Mori, M. Histochemical detection of acid and alkaline phosphatase in periodontal tissue following experimental tooth movement. J. Dent. Res. 1968, 47, 340
36. Trumbore, D. C., Heideger, W. J. & Beach, K. W. Electrical potential difference across bone membrane. Calcif. Tiss. Int. 1980, 32, 159–168
37. Ullberg, S. Studies on the distribution and fate of S^{35} labelled bencylpenicillin in the body. Acta Radiol. Suppl. 118, 1954
38. Ullberg, S. Autoradiographic studies on the distribution of labelled drugs in the body. Second U.N. Int. Conf. Peaseful uses of Atomic Energy 1958, 24, 248–254
39. Utley, R. K. The activity of alveolar bone incident to orthodontic tooth movement as studied by oxytetracycline induced fluorescence. Am. J. Orthod. 1968, 54, 167–207

Received for publication 8 September 1982