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Compensatory bone formation in young and old rats during tooth movement

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SUMMARY The aim of this study was to investigate compensatory lingual alveolar bone formation during tooth movement in young and old rats, using the vital bone marker tetracycline. Wistar male rats were separated into the following groups: 13-week-old rats without appliances (13C: control, $n = 5$), 60-week-old rats without appliances (60C: control, $n = 5$), 13-week-old rats with appliances (13E: experimental, $n = 10$), and 60-week-old rats with appliances (60E: experimental, $n = 10$). The upper first molars of the 13E and 60E groups were moved lingually using fixed appliances. On the third day of tooth movement, tetracycline (TC) was intra-peritoneally injected in all animals including the controls. On the 21st day of tooth movement, the animals were killed and unfixed, and undecalcified, 5-μm frozen frontal sections of the rat first molar areas in both control and experimental groups were examined under light and fluorescent microscopes.

In the 13C group without tooth movement, tetracycline labelling lines were obvious in the alveolar crest, apical areas, and interradicular septum, indicating vertical alveolar bone growth. However, in the 60C control group, tetracycline labelling was almost undetectable throughout the alveolar bone.

Although the lingual alveolar crest was resorbed from the periodontal side after lingual tooth movement, the sharp, bright labelling lines were still present from the crest to the lingual periosteal alveolar bone in the 13E group. In the 60E group the lines appeared in the lingual periosteal alveolar bone containing the crest, indicating considerable new bone formation. The results indicate that compensatory bone formation occurs in the alveolar crest area and, consequently, alveolar bone height is maintained, even in aged rats.

Introduction

Many investigations on the histology of tooth movement have been reported (King *et al*., 1991; Brudvik and Rygh, 1995; Nakamura *et al*., 1996; Bridges *et al*., 1998; Verna *et al*., 1999; Melsen, 1999) and have contributed to the clinical development of orthodontic treatment for young growing patients. The frequency of orthodontic treatment for adult patients, including the elderly, has increased with interest in the concept of 'quality of life' (Nuttal *et al*., 2001), and 'a positive self image' (Szymanski, 2000). The effect of age on tooth movement is very important, because bone does not grow during adulthood.

Bridges *et al*. (1998) investigated the effect of age on tooth movement in rats. They found that the amount and rate of tooth movement was greater in younger rats, and that differences between young and aged rats were reflected as a result of the reduction in tissue mineral density. Jäger and Radlanski (1991) observed the remodelling process of aged rats during tooth movement, and concluded that the processes of periodontal tissues were qualitatively similar in aged and younger animals. Harris and Baker (1990) clinically examined the loss of crestal bone height in adolescent and adult patients, and suggested that treatment does not place adults at greater risk.

Periodontal reaction during tooth movement in adult patients appears similar to that seen in young growing patients. However, whether alveolar bone is modelled at the crestal area when bone is exposed to pressure and resorbed from the periodontal side during lingual or labial tooth movement remains uncertain, because the responsiveness of bone to some stimuli seems to decrease with age (Frost, 2000) and active bone-forming capacity also decreases with age (Jäger, 1996).

Clear evidence is required to solve this issue. The aims of this study were to investigate the alveolar bone, especially the crestal area, to determine the reactions that occur during tooth movement, using the vital bone marker tetracycline (TC) (Urist and Ibsen, 1963; Suga, 1973; Kawasaki and Fearnhead, 1975).

Materials and methods

Male Wistar rats, 13 (young) and 60 (old) weeks of age, were used in this study. The rats were housed in stainless steel cages in an air-conditioned environment and lighting according to the guidelines of the Tsurumi University for Animal Research. The animals had free access to a

standard diet and water. The rats were divided into four groups as follows:

1. Young without appliance (13C: control, $n = 5$).
2. Old without appliance (60C: control, $n = 5$).
3. Young with appliance (13E: experimental, $n = 10$).
4. Old with appliance (60E: experimental, $n = 10$).

Fixed appliances (Noda *et al*., 2000; Yoshii *et al*., 2000) were bonded to the upper incisors of both experimental groups with light curing resin. An initial force of 10 *g* was used to move the maxillary molars in a lingual direction (Figure 1). On the third day of tooth movement, TC (1.5 mg/100 g b/w) was injected intra-peritoneally into all rats, including the controls.

On the 21st day of tooth movement (18 days after TC injection), the animals were killed under ether anaesthesia, the upper jaws were excised, cut in half along the sagittal plane and immersed rapidly in liquid nitrogen. The enamel above the gingival sulcus was trimmed away from the frozen tissues by carefully grinding the enamel parallel to the occlusal plane with a dental diamond disk, then the specimens were further trimmed into smaller blocks. The frozen blocks were carefully positioned in cooled metal containers with the enamel surface, parallel to the occlusal plane, lying on the bottom and embedded with pre-cooled OCT compound (Miles Inc., Elkhart, Indiana, USA). The containers were returned to liquid nitrogen until the OCT compound was completely frozen. The frozen blocks were trimmed to $6 \times 6 \times 8$ mm, and mounted on stubs pre-cooled to –20°C in a cryostat (Raitz, Wetzlar, Germany) with the enamel surface lying on the top.

The blocks were then cut vertically to the enamel surface and several sections were examined for minute adjustment of the specimen stage of the cryostat during the sectioning. Serial frontal, unfixed, and undecalcified 5 μm sections were collected individually using adhesive tape, and freeze-dried for 1 hour in the cryostat (Nakamura *et al*., 1994). All sections were fixed with 10 per cent buffered formalin for 30 seconds, washed with distilled water, stained with 0.5 per cent toluidine blue (pH 4.5) for 1 minute at room temperature and mounted under a coverslip with glycerine. Sections were examined under light and fluorescent microscopy (Olympus Co., Ltd, Tokyo, Japan AX80T) using ultraviolet illumination.

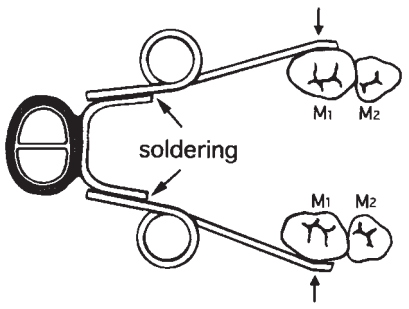

Figure 1 The orthodontic appliance used in this study. Initial force was 10 *g*, moving both maxillary first molars in lingual direction (arrow).

Results

Histology and TC labelling of the periodontal tissues were simultaneously visualized in individual sections that revealed good structural relationships among the root, periodontal ligament (PDL), and alveolar bone.

Control groups

Thirteen-week-old rats (13C rats). The toluidine blue-stained frontal section showed the entire dento-alveolar region of the maxillary first molar (Figure 2a). The section was cut at the disto-buccal and disto-lingual roots almost perpendicular to the occlusal plane. The PDL was interposed between the entire surface of roots and the alveolar bone. The periodontal bone surface was slightly undulated, but almost parallel to the root surface even in the interradicular septum. The surface of the periosteal bone was much flatter than that on the periodontal side.

In the alveolar crest area, a layer of metachromatically stained osteoid was identified on the calcified bone surface from the top of the crest to the alveolar bone on the lingual side, and many osteoblasts were present (Figure 2b). These are characteristic features of normal PDL.

Fluorescent light microscopy revealed bone labelling in the section. The lines demarcated the new bone produced over the course of 18 days following TC injection (Figure 2c). Sharp, bright TC labelling lines were evident in both periosteal sides of the alveolar bone. The lines on the periodontal side differed from those on the periosteal side, in that the configuration was complex. The lines reached from the alveolar crests to the height of cervical third of the roots on both sides, then faded out. However, they reappeared in the apical areas. A line was also obvious in the interradicular septum. The thickness of the bone produced over the 18 days varied in a site-specific manner, being very thick in both alveolar crests, at the top of the septum and in the apical areas. The lines on the periosteal side were smooth along the calcified bone surface and gave the impression that a large volume of new bone was formed in the areas where the lines were clear and thick (Figure 2d).

Sixty-week-old rats (60C rats). The toluidine blue-stained section showed that the findings were similar in the 60C and 13C rats except for a layer of metachromatically stained osteoid, which was evident on the periosteal surface in the 13C rats. There was no sign of

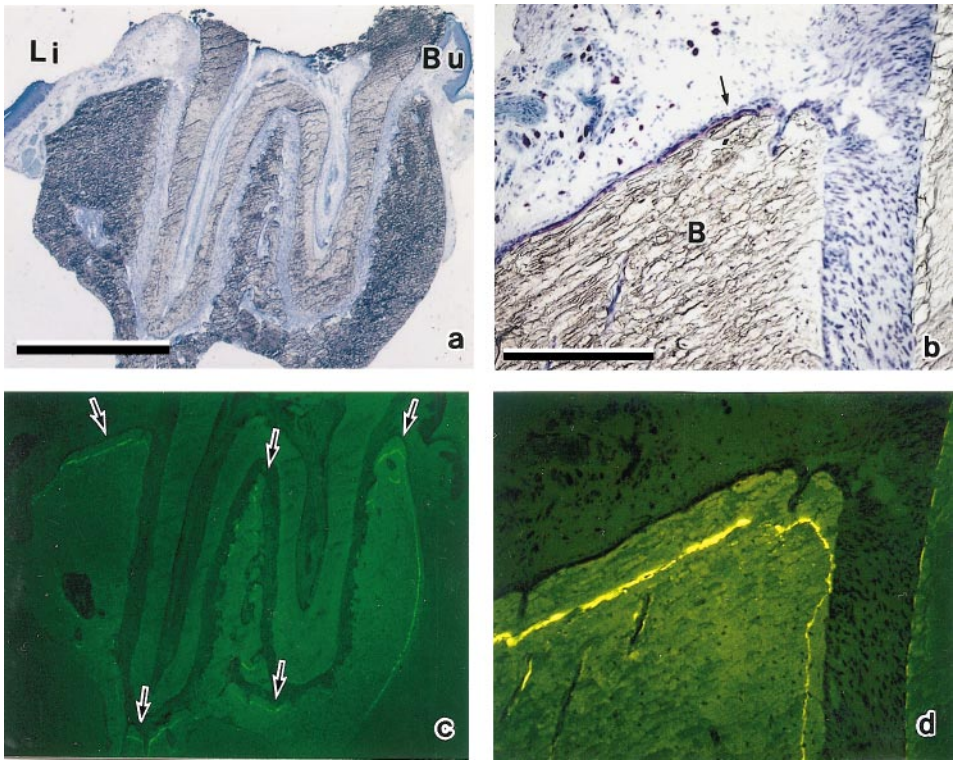

Figure 2 Periodontal tissues of the upper first molar in 13-week-old rats. (a) Overall view, toluidine blue stain; Li, lingual side; Bu, buccal side: B, bone. Bar = 1 mm, ×20. (b) Lingual alveolar crest area. Metachromatically stained osteoid (arrow) is present along the calcified bone on the periosteal side. Bar = 100 µm, ×100. (c) Tetracycline labelling lines (arrow) are obvious in alveolar bone of the upper first molar, especially in alveolar crest, apical areas and on top of interradicular septum. Magnification ×20. (d) Bright, sharp labelling line clearly demarcates new from old lingual alveolar bone. Magnification ×100.

periodontal disease such as round cell infiltration. These features seemed normal in 60C rats (Figure 3a,b). However, TC uptake in the 60C and 13C rats differed considerably. Sharp, bright TC labelling lines were not detected in the 60C (Figure 2c) and only a trace of the labelling was seen in the alveolar crest area (Figure 3d).

Experimental groups

The present study examined changes in the alveolar crest areas on the pressure side, namely, whether or not alveolar bone is modelled in the crestal area during tooth movement. Therefore, most of the findings described are focused on the changes in these regions.

Thirteen-week-old rats (13E rats). When the first molars were moved for 21 days, typical histological changes appeared in the PDL. Figure 4a shows some characteristic aspects of the PDL during tooth movement. The periodontal space was narrower on the lingual and wider on the buccal sides in the upper third of the PDL, indicating a lingual tipping movement. The periodontal bone surface became notched because of bone remodelling, while the periosteal bone surface remained smooth. Degenerating tissues had appeared in the lingual PDL and osteoclasts were seen on the bone surface adjacent to them (Figure 4b). In contrast, a thick layer of metachromatically stained osteoid was present on the calcified bone surface, which was covered by many osteoblasts on the periosteal side.

The TC labelling lines differed from those in the control. Although bright, sharp TC labelling lines were still present in the periosteal side of the alveolar bone, they were absent on the periodontal side, even in the tension zone. Only traces of labelling were detected in the interradicular septum (Figure 4c). In the lingual alveolar crest area a bright, sharp TC labelling line demarcated new from old bone (Figure 4d). The distance from the labelling line to the bone surface seemed longer than that in the 13C rats.

Sixty-week-old rats (60E rats). The toluidine blue-stained section showed that the findings after 21 days of tooth movement were similar in the 60E and 13E rats

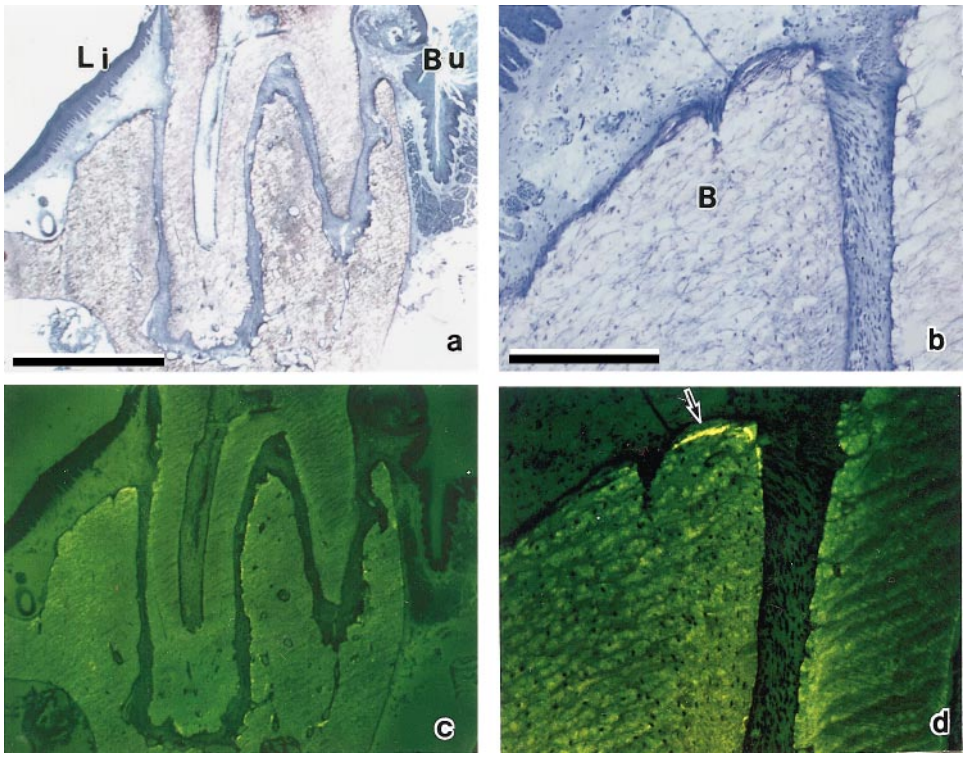

Figure 3 Periodontal tissue of the upper first molar in 60-week-old rats. (a) Overall view, toluidine blue stain; Li, lingual side; Bu, buccal side; B, bone. Bar = 1 mm, ×20. (b) Lingual alveolar crest area. Osteoid is absent along bone surface on the periosteal side. Bar = 100 μm, ×100. (c) Tetracycline labelling is scarcely detectable in alveolar bone. Magnification ×20. (d) Trace of the labelling (arrow) is seen in the alveolar crest area. Magnification ×100.

(Figure 5a). Degenerating tissues had appeared in the PDL and osteoclasts were seen on the bone surface near the tissues (Figure 5b). On the periosteal side, a layer of metachromatically stained osteoid was formed on the calcified bone. Many osteoblasts were present along the osteoid.

TC labelling in the bone on the periodontal side was similar to that in the 13E rats (Figure 5c). A sharp, bright line appeared in the lingual alveolar bone on the periosteal side (Figure 5d), which began at the top of the crest and ran in a lingual direction. A considerable amount of lingual alveolar bone had been formed over the 18 days of tooth movement.

Discussion

In histological examination of bone formation and bone growth, it is convenient to use vital bone markers such as TC. However, TC labelling is usually less sharp in ground sections of plastic-embedded specimens and it is difficult to obtain clear histological findings from ground sections under light microscopy (Jäger and Radlanski, 1991) because of the section thickness causing the results to be somewhat unreliable. With the method employed in this study it was possible to obtain 5 μm sections of the whole alveolar region of the upper first molar. Consequently, the labelling lines were very sharp and the periodontal tissues were histologically obvious in the same section. This increases the reliability of the study (Nakamura *et al.*, 2000).

The histological findings between the 13 and 60C rats were similar (Figures 2a and 3a). However, TC uptake differed significantly in the alveolar bone in the two groups. In the 13C rats, TC labelling lines were sharp and bright in the alveolar bone, especially in the alveolar crest areas on both sides and in the apical areas and interradicular septum. The distance from the lines to the bone surface in these three areas was almost identical. These results indicate that alveolar bone grows vertically in 13C rats. Hanada (1967) examined the growth of Wistar rats cephalometrically and noted that an increase in molar height was observed in vertical facial growth in 13-week-old rats. Bone apposition in the three areas plays a substantial role in the vertical facial growth, especially in the increase in molar height. Moreover, alveolar bone turnover is highly active in 13C rats.

In contrast, the labelling was almost undetectable in the alveolar bone of the 60C rats (Figure 3c). Only a trace of labelling was identified in the alveolar crest area (Figure 3d), suggesting that the labelling did not indicate

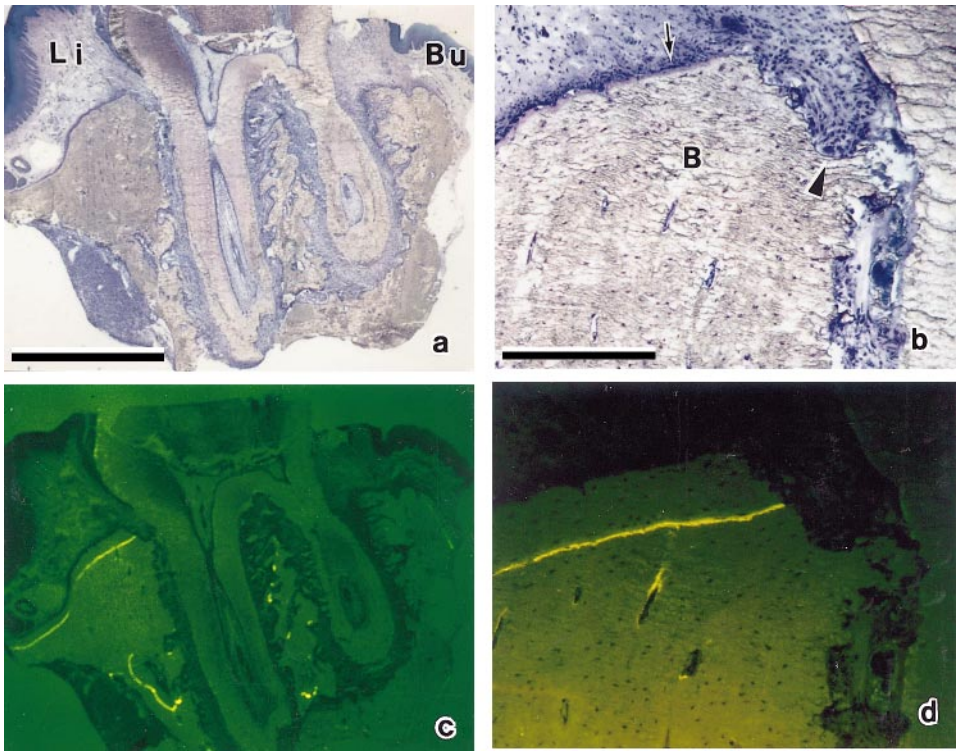

Figure 4 Periodontal tissues of the upper first molar after 21 days of tooth movement in 13-week-old rats. (a) Lingual and buccal periodontal spaces are reduced and widened, respectively, in the upper third of the periodontal ligament. Toluidine blue stain; Li, lingual side; Bu, buccal side; B, bone. Bar = 1 mm, ×20. (b) Lingual alveolar crest area. Degenerating tissues are present in the periodontal ligament and osteoclasts (arrowhead) are seen on the bone surface near degenerating tissues. Metachromatically stained osteoid (arrow) is evident on periosteal bone surface. Toluidine blue stain. Bar = 100 μm, ×100. (c) Tetracycline labelling lines are seen in alveolar bone of the upper first molar. Labelling pattern, except for that of periosteal bone, differs from that of control. Magnification ×20. (d) A bright, sharp labelling line clearly demarcates new from old bone. Magnification ×100.

alveolar bone growth, but only surface bone remodelling, i.e. alveolar bone growth was already complete in the 60C rats and turnover of the alveolar bone was not sufficiently high to detect with TC labelling (Jäger, 1996). Hence, 13C and 60C rats are good models of young and adult/elderly patients, respectively.

The effect of age on the reaction of periodontal tissues during tooth movement has been described (Weiss, 1972; Jäger and Radlanski, 1991; Bridges *et al*., 1998). However, the reaction of crestal alveolar bone to orthodontic stimuli has not been elucidated. Whether or not alveolar bone modelling occurs during tooth movement is a critical factor in adult orthodontics, because loss of crestal bone results in a reduction of tooth support in adults.

The histological findings after 21 days of the tooth movement in the 13E rats were similar to those reported by Nakamura *et al.* (1996) and Verna *et al.* (1999); that is a reduced periodontal space in the pressure zone and a broader space in the tension zone (Figures 4a and 5a). Degenerating tissues remained in the upper third of the lingual PDL and osteoclasts were present on the bone surface near the degenerating tissues in the pressure zone (Figures 4b and 5b). On the periosteal side, many osteoblasts were present along the layer of metachromatically stained osteoid on the calcified bone surface. In other words, the osteoblastic bone formation and osteoclastic bone resorption simultaneously continued on both sides of the lingual alveolar bone even on the 21st day of tooth movement. With regard to alveolar bone growth in the 13C rats (Figure 2d), the periosteal bone formation of lingual alveolar bone during tooth movement (Figure 4d) probably resulted from both alveolar bone growth and compensatory bone formation. Consequently, alveolar bone modelling at the crestal area was maintained during tooth movement in the young rats. However, it is difficult to distinguish between compensatory bone formation and bone growth.

Young rats around 13 weeks old are sound experimental models for studying orthodontic tooth movement. Many important conclusions have been derived concerning alveolar bone remodelling from such investigations (Utley, 1968; Reitan and Kvam, 1971; Tran Van *et al*., 1982). The results of the present study suggest that alveolar bone growth should be considered when bone

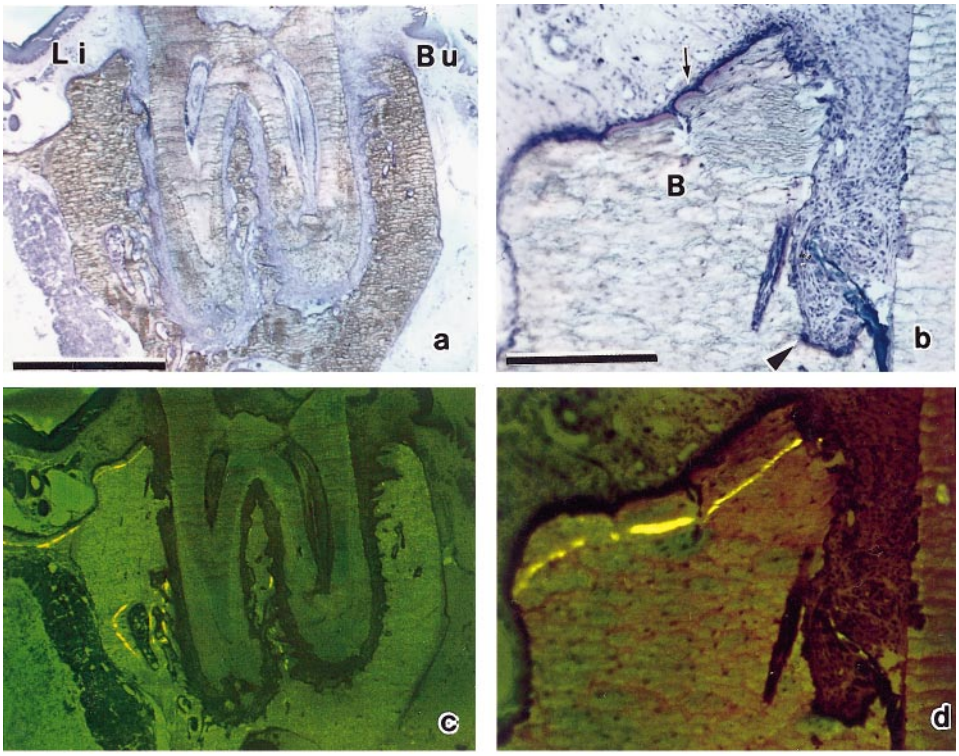

Figure 5 Periodontal tissue of the upper first molar after 21 days of tooth movement in 60-week-old rats. (a) Lingual and buccal periodontal space are reduced and widened, respectively, in the upper third of the periodontal ligament. Toluidine blue stain; Li, lingual side; Bu, buccal side; B, bone. Bar = 1 mm; ×20. (b) Lingual alveolar crest area. Degenerating tissues are present in the periodontal ligament, and metachromatically stained osteoid (arrow) is seen on the periosteal bone surface. Toluidine blue stain. Bar = 100 μm, ×100. (c) Tetracycline labelling lines are seen in lingual alveolar bone of the upper first molar. Magnification ×20. (d) A bright, sharp labelling line clearly demarcates new from old bone. Magnification ×100.

remodelling in the periodontal tissues is examined during tooth movement, since bone growth could be mistaken for bone formation.

The histological findings were similar in both experimental groups. In the 60E rats, the bone was resorbed on the periodontal side and newly formed with a new layer of osteoid on the periosteal side in the alveolar crest areas. The results indicate that tooth movement causes periosteal bone formation even in old rats (Figure 5b). In addition, a bright, sharp TC labelling line (Figure 5d) clearly demonstrated that a considerable amount of bone was formed during tooth movement and that this formation had commenced by the third day after the initiation of tooth movement. Kabasawa *et al*. (1996) reported that mechanically stressed alveolar bone revealed no evidence of a numerical difference in number, size, or activity of osteoclasts and osteoblasts between young and old rats. Jäger and Radlanski (1991) also noted that the remodelling processes of periodontal tissues in aged animals (500-day-old rats) was qualitatively similar to that in younger rats. The results in this study indicate that alveolar bone is fairly reactive to orthodontic stimuli, irrespective of age.

This periosteal bone formation represents genuine compensatory bone formation, and can result from the physical behaviour of loaded bone and/or bone adaptation to resist occlusal force (Epker and Frost, 1965). The mechanism of this bone formation is unclear. However, considering the degeneration of the PDL in alveolar crestal areas, this bone formation is probably due to the regional acceleratory phenomenon (RAP) in which injuries and other noxious stimuli usually increase all ongoing biological activities in the affected region of the body (Frost, 2000).

Periosteal reaction appears to be faster than that in alveolar bone on the periodontal side, as a bright sharp labelling line was not detected in the tension zones, such as lingual root apex and buccal alveolar crest areas. This indicates a difference in reactivity between the periosteum and PDL to orthodontic stimuli. Verna *et al*. (1999) also noted that TC labels were scarce in alveolar bone, whereas on the buccal cortex (direction of tooth movement) they were detectable by the fourth day of tooth movement. Reitan (1957) also described, in a histological observation of adult patients, that bone apposition had not started in the PDL after 4 days of tooth movement. Furthermore,

scarce labelling in the buccal alveolar bone of the tension zone may be due to inflammatory responses in the upper area of the buccal PDL, which delays the onset of bone formation (Lee and Nakamura, 1999). The appliance positioned at the cervical margin of the buccal side appeared to pose a risk of external injury to the buccal periodontium.

Conclusions

The results of the present investigation strongly indicate that compensatory bone formation occurs in the alveolar crestal area and, consequently, alveolar bone height is maintained, even in aged rats.

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