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# Changes in bone mineralization pattern: a response to local stimulus in maxilla and mandible of dogs

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**Summary.** A pilot study was carried out in order to verify the pattern of changes in mineralization of bone in the maxillas and mandibles of dogs which had a tooth extraction or luxation. Bone mineral content was determined using computerized microdensitometry. Significant changes in patterns of mineralization were found for alveolar bone, cortical bone and trabecular bone at the sites adjacent to the area of operation. These findings suggest that the three envelopes of jaw bones of the dogs are influenced by Regional activation phenomenon (RAP). These results have important implications for the design of clinical studies of periodontium. A more detailed study should elucidate the cellular mechanisms by which these changes occur.

**Key words:** Bone, Mineralization, Luxation, Extraction, Microdensitometry

## Introduction

Alveolar bone has been shown to undergo cyclic activation-resorption-formation (ARF) activity (Van et al., 1982). This sequence of cellular events was first described by Frost in 1964 as three phases; an activation phase (A), a phase of osteoclastic resorption (R) and a phase of osteoblastic formation (F) (Frost, 1964). The ARF cycle of alveolar bone was found to be very short (6 days) as compared to other skeletal sites in the rat (Vignery and Baron, 1980). This normally high rate of turnover of alveolar bone would make this site an ideal location for the study of bone dynamics.

Alveolar bone is unique in that its primary function is the support of teeth. However, in rodents, alveolar bone has been shown to give a site dependant response to systemic stimulus and participate in mineral homeostasis (Lui and Baylink, 1984). One would expect that these unique features would predispose this bone to subtle differences in its pattern of response to local and systemic stimulation not seen in other bones. Indeed investigations of the response to mineral deprivation (Lui and Baylink, 1984), periodontal disease (Baron and Saffar, 1978) and calcitonin treatment (Baron and Saffar, 1977) have shown a differential response of alveolar bone. More information is necessary, especially for animal models which have a similar bone remodeling pattern to man in order to understand and accurately predict the outcome of many dental manipulations and treatment regimens.

Dogs have been used by numerous investigators in the investigations of pathogenesis of periodontal disease and to evaluate the effects of various surgical treatment modalities (Lindhe et al., 1975; Crigger et al., 1978; Williams et al., 1982; Jeffcoat et al., 1985; Watson et al., 1986).

Surprisingly, the existance of Regional activation phenomenon (RAP) has not been investigated in dog bones (maxilla and mandible) which are subjected to a wide variety of experimental procedures in periodontal research. Mineralization is an important part of bone metabolism. The accurate measurement of bone mineral content has been of interest in the assessment of skeletal changes that may be associated with normal skeletal development (Mazzess and Cameron, 1971) demineralization in aging (Riggs et al., 1981) altered bone metabolism in disease (Revak, 1980) and effectiveness of treatment (Anderson et al., 1984). Non-invasive techniques (photon absoptiometry, gamma ray computed tomography) have been extensively used for the determination of bone mineral content (Exner and Prader, 1979; Jorch and Anderson, 1982; Williams et al., 1982; Hausmann et al., 1983).

This project was undertaken to ascertain if changes in the pattern of bone mineralization at the sites adjacent to tooth extraction/luxation, in the mandible and maxilla of dogs, were demonstrable. Having obtained that information

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it would be possible to evaluate the mechanism by which the response occurred through further studies on alveolar bone remodelling.

These would help to understand changes in alveolar bone metabolism occurring in periodontal disease (Carranza et al., 1971), osteoporosis (Otero et al., 1967) endosseus implants (Powell et al., 1973; Watson et al., 1986) and orthodontic tooth movement (Roberts et al., 1981).

## Materials and methods

Two adult male mongrel dogs were utilized for this study. The dogs were subjected to tooth extractions under nembutal anaesthesia (0.44 ml/kg). The maxillary first premolar (tooth 15) and the mandibular second premolar (tooth 46) were luxated in the right quadrants of one dog. The maxillary right first premolar (tooth 15) was completely extracted from the other dog while the mandibular right first premolar (tooth 45) was luxated. Eleven weeks following the tooth manipulations the animals were sacrificed. Mandibles and maxillas were stored in 70% ethanol. They were bisected and x-rays were taken (30 sec. exposure at 30 kVp from a medial view) in order to guide the cutting of blocks of tissue.

The selection of areas for study was based upon the assumption that the right and left sides of the mandible and maxilla are autonomous in the sense that they receive a separate vascular and nervous supply with very little overlap. No extractions were performed on the left side, therefore, blocks of tissue cut from this side (contralateral sites) acted as controls. Blocks of tissue cut distal to the site of extraction on the same side comprised the test group (adjacent group).

The cut blocks were dehydrated by passing through a series of 95% and 100% ethyl alcohol. The blocks were infiltrated and embedded in LR White resin. Sections of approximately 180 ± 10.0 μm thickness were cut with the Isomet $^{R}$ rotary saw and lapped down to 100 μm ± 5 μm with the Marutto lapping machine.

### *Microradiography*

Microradiographs were prepared on a high resolution Kodak film (S30343). A step wedge was placed along side the specimens. The film was exposed for 105 minutes at 20 kVp in the Faxitron x-ray machine at a distance of 20 cm from the x-ray source. The film was developed with Kodak D-19 developer for 5 minutes and fixed with Kodak rapid fix.

### *Densitometry*

Microradiographs were viewed with a videocamera under a Zeiss photomicroscope III at 40 x magification. This magnification was selected because it isolated the largest field containing one type of bone only (Fig. 1 shows the areas of alveolar, cortical and trabecular bone on which analysis were made). The image picked up by the camera was analyzed with the IBM videocounting and microdensitometry program (Pugliese and Anderson, 1986). The videocounting program divided the image into 40832 pixels (rectangular picture elements) and read the brightness of each pixel on a scale of 0 to 255 (256 levels of brightness were distinguishable). The upper (255) and lower (0) limits of the grey scale were set for the brightest and darkest areas respectively on a standard area of the microradiograph of a control step wedge. Upper threshold values were chosen to be 255, 250, 230, 210, 180, and 150. At each threshold value the program counted the number of pixels which had a brightness below the threshold value. A constant lower threshold was set at 30 to eliminate the possibility of background due to bone dust or other debris (Pugliese and Anderson, 1986).

## Results

Fig. 2a and 2b show the typical observations, at the highest mineralization level ($ML_1$), of alveolar cortical and trabecular bone sites for controls and adjacent sites. The amount of total bone was not different at any of the sites. However, a significantly higher amount of bone was at highest mineralization level ($ML_1$) in the test groups as compared to the controls. The differences were most pronounced for cortical bone in mandible as well as in maxilla. The alveolar bone of adjacent sites in maxilla, however, was not significantly different from the controls. At adjacent sites, the major amount of bone was found to be at the highest mineralization level ($ML_1$) while in the controls all three envelopes of bone were at a wide spectrum of mineralization levels ($ML_1$ - $ML_6$, not shown).

Mean mineralization values were calculated for each field of all bone sites of the mandibles and maxillas. The following formula was used:

$$\frac{\text{E (mean min. level at MLn} \times \text{pixel count at MLn}}{\text{E (pixel count at MLn)}}$$

$$n = 1 - 6$$

Mean mineralization values are shown in Table 1. At each site the mandibular bone envelopes were found to be less mineralized than maxillary envelopes in the controls. (Alveolar Mand. 177.8 ± 5.7 vs. maxilla 190.8 ± 10.5; Cortical; Mand. 214.6 ± 4.7 vs. max. 223.3 ± 7.6; Trabecular; mand., 179.8 ± 5.0 vs. max. 208.3 ± 7.4). Furthermore the mean mineralization level of alveolar and trabecular envelopes were not significantly different in the controls at the mandiblar and maxillary sites. Each bone envelope (Alv., Cort. and Trab.) of adjacent site in mandible were found to be at significantly higher mineralization level as compared to the controls ($P < 0.001$). In maxilla, however, only cortical and alveolar sites showed significantly higher mean mineralization levels as compared to the controls.

**Table 1.** Mean mineralization levels of mandibular as well as maxillary alveolar (A), cortical (C) and trabecular (T) bone envelopes.

P VALUES

| MANDIBULAR | | | | | | |
|---|---|---|---|---|---|---|
| dog #115 | | extracted | | | adjacent | |
| | A | C | T | A | C | T |
| TMB | NS | NS | < 0.05 | NS | < 0.05 | NS |
| ML1 | < 0.005 | < 0.005 | < 0.05 | < 0.05 | < 0.005 | < 0.005 |
| ML2 | NS | < 0.005 | < 0.01 | < 0.005 | < 0.01 | NS |
| ML3 | < 0.05 | < 0.005 | < 0.005 | NS | < 0.005 | < 0.005 |
| ML4 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.01 | < 0.005 |
| ML5 | < 0.005 | < 0.005 | < 0.05 | < 0.005 | NS | < 0.05 |
| ML6 | < 0.05 | < 0.05 | NS | < 0.05 | NS | NS |
| dog #122 | | extracted | | | adjacent | |
| | A | C | T | A | C | T |
| TMB | < 0.01 | NS | NS | NS | NS | NS |
| ML1 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 |
| ML2 | < 0.05 | < 0.05 | < 0.05 | < 0.005 | < 0.05 | < 0.005 |
| ML3 | < 0.05 | < 0.005 | < 0.005 | < 0.05 | < 0.005 | < 0.01 |
| ML4 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 | < 0.005 |
| ML5 | < 0.005 | < 0.05 | < 0.005 | < 0.005 | < 0.05 | < 0.05 |
| ML6 | < 0.01 | < 0.005 | < 0.005 | < 0.05 | < 0.05 | NS |
| MAXILLARY | | | | | | |
| dog #115 | | extracted | | | adjacent | |
| | A | C | T | A | C | T |
| TMB | < 0.05 | < 0.05 | < 0.005 | < 0.05 | NS | < 0.05 |
| ML1 | < 0.005 | NS | < 0.005 | < 0.005 | < 0.01 | < 0.05 |
| ML2 | < 0.01 | NS | < 0.005 | NS | < 0.005 | NS |
| ML3 | NS | NS | NS | NS | < 0.005 | NS |
| ML4 | < 0.005 | NS | NS | < 0.005 | NS | NS |
| ML5 | < 0.005 | NS | NS | < 0.005 | NS | NS |
| ML6 | < 0.005 | NS | NS | < 0.05 | NS | < 0.05 |
| dog #122 | | extracted | | | adjacent | |
| | A | C | T | A | C | T |
| TMB | NS | < 0.05 | NS | NS | NS | NS |
| ML1 | < 0.005 | NS | < 0.005 | < 0.05 | < 0.005 | NS |
| ML2 | < 0.005 | < 0.01 | < 0.005 | NS | < 0.05 | < 0.01 |
| ML3 | < 0.05 | < 0.005 | NS | NS | < 0.005 | NS |
| ML4 | < 0.005 | NS | < 0.005 | NS | < 0.005 | < 0.005 |
| ML5 | < 0.005 | < 0.05 | NS | NS | < 0.05 | < 0.05 |
| ML6 | < 0.05 | < 0.05 | NS | NS | < 0.05 | NS |

A = alveolar C = cortical T = trabecular

**Table 2.** Mean Mineralization Levels.

| MANDIBULAR | | | |
|---|---|---|---|
| | alveolar | cortical | trabecular |
| control | 177.9 | 214.6 | 180.8 |
| dog #115 (X) | 250.7 | 246.5 | 214.1 |
| dog #115 (A) | 229.6 | 242.3 | 224.5 |
| dog #122 (X) | 250.5 | 249.9 | 234.9 |
| dog #122 (A) | 250.1 | 251.1 | 208.4 |
| MAXILLARY | | | |
| | alveolar | cortical | trabecular |
| control | 191.4 | 222.3 | 205.7 |
| dog #115 (X) | 247.2 | 241.8 | 236.6 |
| dog #115 (A) | 248.0 | 248.7 | 221.2 |
| dog #122 (X) | 193.0 | 180.6 | 188.5 |
| dog #122 (A) | 214.0 | 246.3 | 204.9 |

X = extracted/luxated group
A = adjacent group

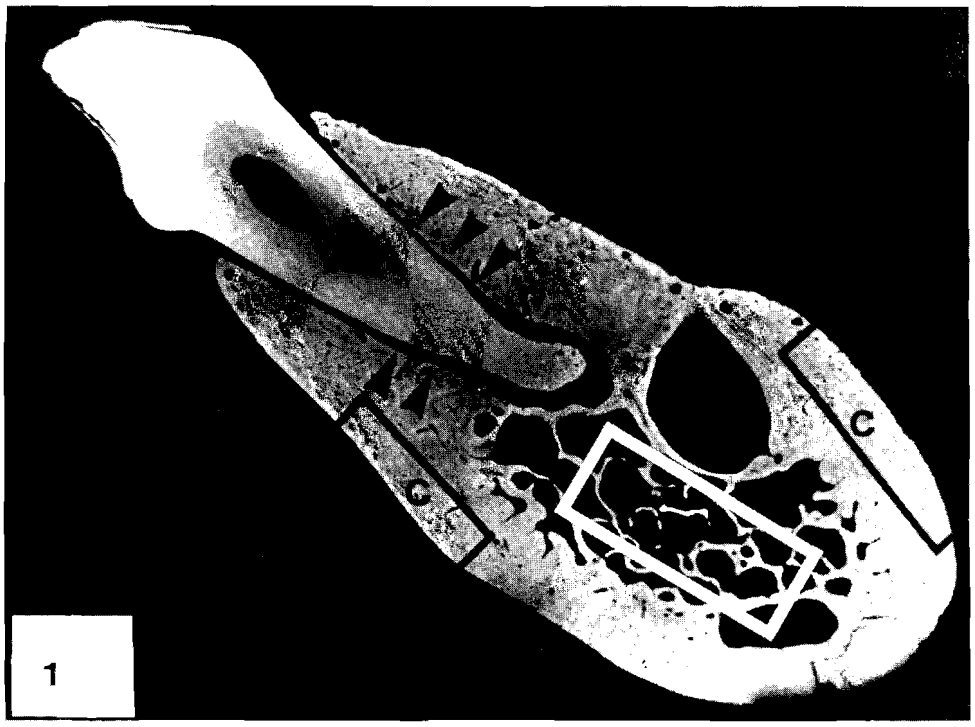


**Fig. 1.** Microradiograph of buccolingual section of a single rooted tooth showing alveolar bone (▲); cortical bone (C) and trabecular bone (Rectangle).

**Fig. 2a.** Percent volume of bone. A comparison of alveolar, trabecular and cortical bone envelopes of mandible between control and adjacent sites at the highest mineralization level (ML 1).

**Fig. 2b.** Percent volume of bone. A comparison of alveolar, trabecular and cortical bone envelopes of maxilla between control and adjacent sites at the highest mineralization levesl (ML 1).

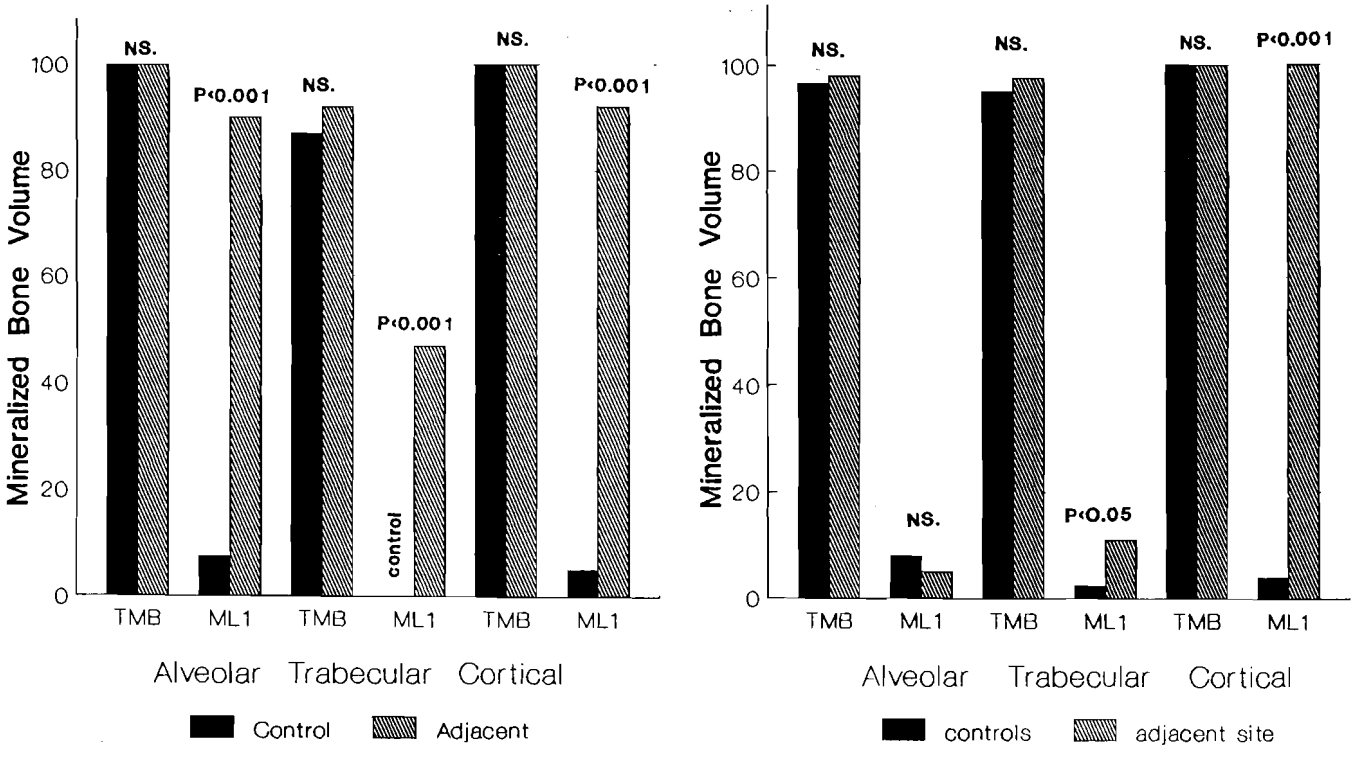


**Fig. 2a, b.** Bar graphs showing the total mineralized bone (TMB) as well as bone at the highest mineralization level ($Ml_1$) of the controls and adjacent sites.

## Discussion

The term 'Regional activation phenomenon (RAP)' was introduced by Frost (Frost, 1979). Since then many investigators have shown the existance of RAP in humans as well as animals (Andersson and Nilsson, 1979; Wendeberg, 1981; Obrant and Nilsson, 1984; ). It is believed that perfusion of area in response to the trauma to the adjacent structures is responsible for the activation of BMUs (Frost, 1979). In histomorphometric studies a wide range of results have been attributed to RAP. It is agreed upon, that the observed status of bone remodelling is dependent on the time of biopsy during sigma period (Andersson and Nilson, 1979).

In dog, the bone remodeling cycle (sigma) is approximately two months. In the present investigation the observations were made on the bones obtained at autopsy, eleven weeks following the traumatic stimulus to the adjoining areas. Our investigation showed that eleven weeks following trauma, significant changes in adjacent areas could be observed. The finding that a greater area of bone was found to be at higher mineralization levels in the test group as compared to the controls are highly significant. Finsen and Haave (1987) have shown similar changes after remodelling of fracture. However, previous studies have shown a picture of rapid bone remodelling as well as the presence of poorly mineralized bone in adjoining areas of the fracture (Obrant and Nilsson, 1984). The differences could be due to either: the time of biopsy; species differences; or due to the site of biopsy. A site dependent difference of response, to various surgical and chemical manipulations, have been reported in rodents (Baron and Saffer, 1977; Lui and Baylink, 1984). In the present study, the controls were found to have a wide range of mineral levels in all three bone envelopes. It could be explained on the basis that BMU's are asynchronized and are working at different levels of activity (Frost, 1979). The findings that major area of test sites were at one mineralization level ($ML_1$) can be attributed to activation and synchronization of BMU's at those sites. It remains a mystery, however, what number of sigma cycles it may take for those BMU's to revert back to the control situations.

The present investigation showed that bone, in maxilla of dogs, is denser than that of mandibles. Furthermore, the cortical bone is more highly mineralized than the alveolar and trabecular bone. This may be directly related to the inherent rate of bone turnover at these sites. Similar suggestions have been made by earlier investigators (Powell et al., 1973). Our study also showed that at the control maxillary and mandibular sites the alveolar and trabecular bones were similar in mineral density. These findings are important from the point of view of the radiological interpretation of the lamina dura in dental x-rays. The lamina dura has been described as a special entity in the arrangement of periodontium (Orban, 1957). However, it seems that it is the arrangement of bone rather than the contents which gives the lamina dura its characteristic radiological appearance. In conclusion, our investigation has shown that maxilla and mandible of dog exhibit regional activation phenomenon. The significance and the implications of these results lie in the fact that one has to be extremely cautious in interpreting the results of the studies of surgical periodontal and orthodontic treatments.

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