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Effect of Phenytoin on Periodontal Tissues Exposed to Orthodontic Force—An Experimental Study in Rats

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Abstract. *The influence of the anticonvulsive drug phenytoin on the periodontal tissues during orthodontic tooth movement in the rat was studied. The experimental and the control group each consisted of 10 Sprague–Dawley rats. The test group was injected daily with phenytoin during the experimental period of 6 weeks. A fixed appliance for expansion was applied on the first molars in both groups after 2 weeks (day 15). At the end of the experiment (day 42), radiographic measurements revealed less tooth movement in the phenytoin-treated rats. Compared to the control group, significant histologic changes in the periodontal tissues such as increased density of fibroblasts, decreased number of osteoclasts in contact with alveolar bone wall of the pressure side and deeper layer of non-mineralized osteoid on the tension side were observed in the phenytoin group.*

Index words: Diphenylhydantoin, Fibroblasts, Osteoclasts, Osteoid, Rats.

Introduction

Phenytoin (PHT,5·5-diphenylhydantion) has been used for the treatment of grand mal and psychomotor epilepsy since its first clinical trials on humans in 1938 (Merritt and Putnam, 1938). This anticonvulsant has been a first hand choice until lately and is still common in the treatment of adult epileptic patients. The first side-effect, gingival hyperplasia, was observed in 1939 and since then, side-effects affecting skeletal, hepatic, immunologic and endocrine tissues have been reported (Kimball, 1939; Hallell, 1981). Melsen *et al.* (1976) studied the effect of phenytoin on bone biopsies from the iliac crest in adult epileptic patients. They found an increase in parameters expressing new bone formation such as an increase in osteoid bone relative to trabecular bone and an increase in bone formation surfaces. They suggested that such changes within the alveolar bone, possibly in addition to a hindrance caused by hyperplastic gingivae could prolong orthodontic tooth movement. In a more recent work by Lau *et al.* (1994), phenytoin was shown to stimulate proliferation, differentiation and mature osteoblastic activity in normal human bone cells and to increase *in vivo* serum osteocalcin levels in epileptic patients. In agreement with this, *in vitro* tests performed by Nakade *et al.* in 1995, revealed increased proliferation, alkaline-phosphatase activity, collagen synthesis and osteocalcin secretion in human mandible-derived bone cells. Dahllöf *et al.* (1993) compared the periodontal condition in adult epileptic patients on long-term therapy with phenytoin to patients receiving other anticonvulsants and observed that patients medicating phenytoin did not exhibit increased marginal bone loss, despite an increase in gingival pocket depth, compared to patients medicating other anticonvulsants. Whether phenytoin elicitates changes in alveolar bone metabolism and/or cellular reactions within the periodontal ligament during orthodontic tooth movement has not been studied.

The aim of this histologic and radiographic study, was to investigate the effect of phenytoin on the alveolar bone and periodontal ligament (PDL) under influence of an orthodontic force applied on rat molars.

Materials and Methods

General Procedure

Animals. The sample consisted of 20 adult female Sprague–Dawley rats aged 3–5 months and averaging 250 g in weight. The rats were divided into two groups (Table 1). Group I, consisting of 10 animals was supplied with phenytoin during the whole experimental period of 6 weeks. Group II, also consisting of 10 animals, received no medication. Group II was used as a control to group I. A fixed orthodontic appliance for expansion of the upper first molars was applied on the rats of groups I and II. The rats were given a powdered diet (Evos R 3, Ewos AB, Södertälje, Sweden) and water *ad libitum.* In order to avoid cuttings from the litter interfering with the orthodontic appliance by way of impaction, the animals were kept in cages on net floors. The weight of the animals was verified before and after the experimental period.

Supply of phenytoin. The 10 animals in group I were injected daily subcutaneously with 30 mg/100 g bodyweight of phenytoin (5·5-diphenylhydantoin, Sigma Chemical Co, St Louis, U.S.A.) during the whole experimental period of 6 weeks. The phenytoin was dissolved in propylene glycol, ethyl alcohol and water as vehicle. The 10 animals in group II were not injected with phenytoin.

Orthodontic appliance. A fixed lingual appliance was constructed for a buccal movement of the upper first molars (Fig. 1). It consisted of an 0·011-inch Australian light wire (Wilcock) which was formed to fit between the molars and the curvature of the palate. Wire mesh was

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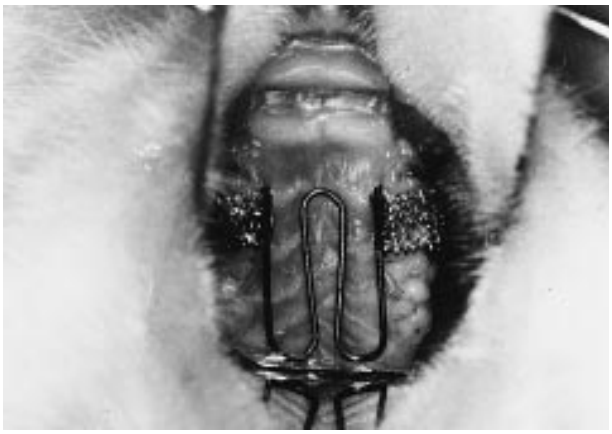

FIG. 1 The fixed appliance for expansion fitted on the upper first molars.

welded on the wire to cover the first molars. At the start of the experiment the appliance was expanded 1 mm more than the distance between the palatal surfaces of the first molars. This activation resulted in an initial force of 150 mN (15 g) delivered to the upper first molars. The force was checked using a constructed force measuring gauge as described by Hellsing and Hammarström (1991) which indicated that the spring with arm lengths of 6 mm delivered a bilateral force of 150 mN at 1 mm expansion.

Experimental protocol. After the first 2 weeks (day 15), the animals in the experimental group and control group, group I and II respectively, were anaesthesized with a subcutaneous injection of 0·3 ml Hypnorm vet (Kabi Pharmacia, Malmö, Sweden) per 1000 g body weight. The distances between the upper first molars were measured and the orthodontic appliance was adjusted transversely to 1 mm more than this distance. The individual orthodontic appliances were attached by acid-etch bonding (Transbond, Unitek/3 M, U.S.A.) to the palatal surfaces of the first molars.

Radiographs were taken on the day of application of the orthodontic force and at the end of the experiment after which the appliances were removed, the animals killed, and the maxillae prepared for histological examination (Table 1).

Radiography. The radiographic procedure followed the method used by Hellsing and Hammarström (1991): occlusal intra-oral film was used (Kodak Ultra-Speed, Eastman Kodak Company, U.S.A.). The radiographs were individually formed and adjusted to the size of the area between the maxillary incisors and molars. The animal was anaesthesized each time a radiograph was taken and stabilized lying on its face with the radiograph placed horizontally behind the incisors. The film-focus distance was constant and the enlargement was less than 2 per cent. When the maxillary fixed appliance had been attached, an intra-oral radiograph was taken (day 15) and another at the end of the experiment (day 42). The achieved expansion from day 15 to day 42 was calculated on the radiographs by measuring the transverse distance between the ends of the outer arms of the appliance using a digitizer with a resolution of ±0·1 mm. Each radiograph was measured three times and a mean value was calculated. The difference between the radiographs from day 15 and 42 showed the expansion during the experiment.

Histological examination. After decapitation on day 42, the heads were fixed in Histofix (Histolab., Göteborg, Sweden) for 5 days. The maxillae were removed and decalcified in 20 per cent formic acid for 3 weeks. Thereafter, the maxillae were dehydrated, embedded in paraffin and sectioned in a buccolingual direction as parallel as possible to the long axis of the upper first molars. The sectional thickness was 4 μm. The sections were stained with haematoxylin and eosin. The histological examination was performed with a light microscope (Aristoplan, Ernst Leitz Wetslar GMBH, Wetzlar, Germany) equipped with computerized image analysis facilities (Argus 10, Hamamatsu, Joko-cho, Japan). Ten central sections within a distance of 200 μm through the mesial roots on the right or left side were selected from each rat. The length of the cervical half of the alveolar bone on the pressure side of the PDL was measured (see Fig. 2). The number of multinucleated osteoclasts per micrometre in contact with the alveolar bone along this selected pressure zone was counted. Fibroblasts in homogenous regions of the pressure zone in the central part of the cervical half of the periodontal ligament were counted and calculated as cells per μm^2 (Fig. 2). The measurements were limited to the cervical half in order to ensure the area chosen represented a pressure zone. Areas closely related to root surface resorption and areas close to cementum, bone or blood vessels were avoided. Sections showing hyaline zones were discounted. The width of non-mineralized osteoid on the tension side was estimated (Fig. 2). The measurement was performed within a level of a tenth of the root length below the cervical margin. The width was measured in micrometres at the thickest part near the top of the alveolar septum.

TABLE 1 *Description of the experimental procedure from the start (day 1) to the end of the experiment (day 42). Group I consists of the experimental animals and group II the controls. Group I was treated daily with phenytoin. A fixed appliance for expansion was applied on the upper first molars og groups I and II on day 15. Radiographs for measurement of the expansion were taken on day 15 and day 42*

	n	Phenytoin injections	Expansion appliance	Radiographs
Group I	10	day 1–42	day 15–42	day 15 day 42
Group II	10	–	day 15–42	day 15 day 42

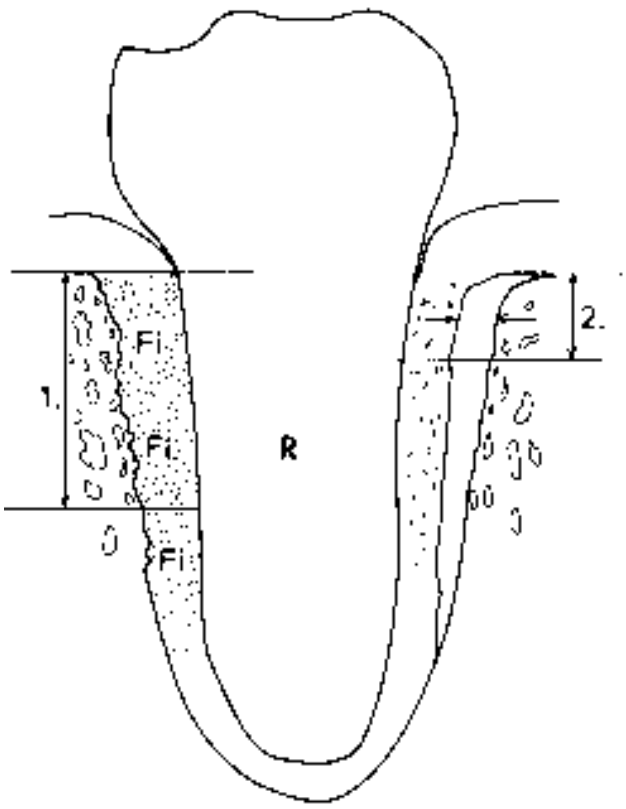

FIG. 2 Illustration of the effect of an orthodontic force on the rat molar. Pressure side on the left: The number of multinucleated osteoclasts in contact with the alveolar wall was measured along the distance from the top of the septum to a point opposite the root half way to the apex (1). Fibroblasts in homogenous regions of the pressure zone in the central part of the cervical half of the PDL were counted and calculated as cells per μm^2 (Fi). Tension side on the right: the highest value for the width of the non-mineralized osteoid (indicated by the horizontal arrows) was measured between the dark reversal line and the surface of the alveolar bone wall. Measurements were performed at the top of the septum opposite the cervical 10 per cent of the root length (2). R = mesial root of the first molar.

Method Error and Statistical Approach

Arithmetic means and 95 per cent confidence intervals (CI) for the number of osteoclasts and fibroblasts on the pressure side, and width of the non-mineralized osteoid on the tension side in the histological sections were calculated. The differences between group I and II were tested for significance using the Mann–Whitney *U*-test for the radiographic values, the number of osteoclasts, density of fibroblasts and width of the layer of non-mineralized osteoid. Differences were considered significant at $P<0{\cdot}01$. For estimation of the method error, 15 randomly selected sections from group I and group II were re-examined 2 months later in a blind test. The intra-observer error was calculated as a quotient of the means and determined as 0·96 for the osteoclast number, 0·94 for the fibroblast density, and 0·98 for the width of osteoid. The error of measurements for the radiographic values was calculated from duplicate measurements on 15 radiographs. The error variance was calculated using the formula

$$\delta^2 = \Sigma d^2/2n$$

where d is the difference between the first and second measurement, and n the number of double registrations. The intra-observer method error for the radiographic recordings was 0·06 mm.

Results

The achieved *expansion* consisted mainly of a tipping movement in the transversal plane. The mean value for the phenytoin group (group I) was 0·3 mm and for the control group (group II) 0·5 mm (Table 2). The difference in tooth movement between the phenytoin treated rats and the controls was almost significant.

The number of *osteoclasts* counted along the surface of the alveolar bone in the pressure zone of the PDL was less in the phenytoin group than in the controls (Table 2). The difference in mean values was significant at the level of $P<0{\cdot}001$.

A change in morphology of the surface of the alveolar bone on the pressure side was observed. The histologic sections of the pressure side exhibited a scalloped bone surface with active osteoclasts in the control rats (group II) after 1 month of orthodontic treatment while the administration of phenytoin in the rats of group I during the 6 weeks had resulted in a smooth bone surface (Fig. 3 A and B).

The difference in density of *fibroblasts* in the (PDL) on the pressure side was higher in the phenytoin group (group I) than in the control group (group II) (Table 2). The mean values were calculated as cells $\times 10^{-3}$ per μm^2 and the difference between the groups was significant at the level of $P<0{\cdot}01$.

TABLE 2 *Expansion: the achieved expansion was calculated on radiographs in mm. Osteoclasts: the number of multinucleated osteoclasts in contact with the alveolar wall on the pressure side was counted as cells *10^{-3} per μm. Fibroblasts: the density of fibroblasts in the central region of the periodontal ligament of the pressure side was counted as cells *10^{-3} per μm^2. Osteoid: the width of the non-mineralized bone was measured in mm.* n = *number of animals,* $\bar{x}$ = *arithmetic mean and 95 per cent confidence interval (CI) for the variables registered in groups. The Mann–Whitney* U*-test was used for testing differences for the radiographic values, differences in number of osteoclasts, and density of fibroblasts*

Observation period: 1 month	Group I (n = 10)		Group II (n = 10)		Sign
	$\bar{x}$	CI	$\bar{x}$	CI	
Expansion (in mm)	0·3	0·23–0·37	0·5	0·43–0·57	
Osteoclasts *10^{-3}/μm	3·4	2·33–4·47	9·0	6·79–11·21	***
Fibroblasts *10^{23}/μm^2	6·8	5·70–7·90	4·6	3·89–5·31	**
Width of osteoid (in μm)	176	150–202	119	83–155	**

Significance level: ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

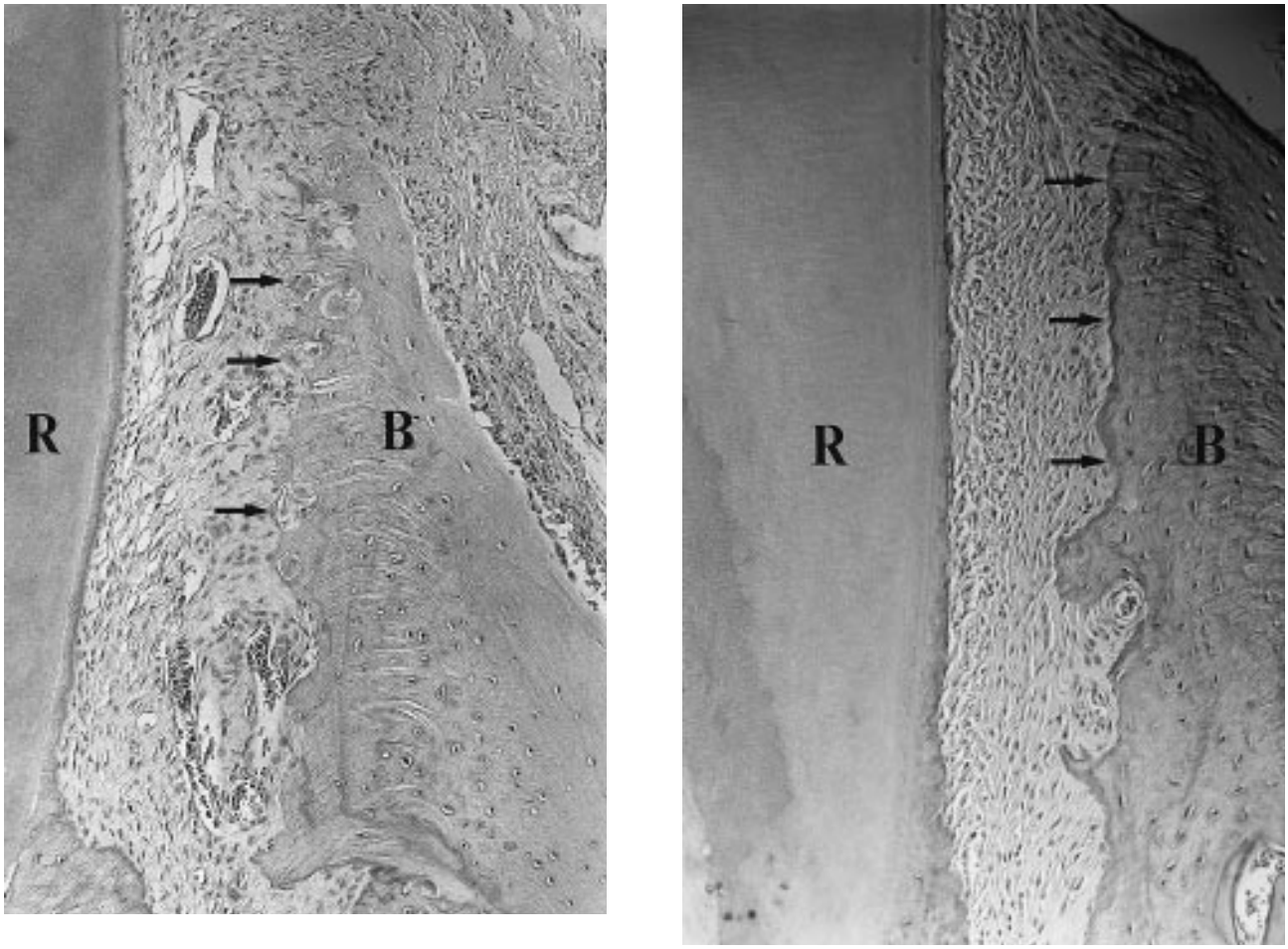

FIG. 3 (A) This section shows the appearance of the alveolar bone wall on the pressure side after application of an expansive force over a period of 4 weeks. Note the scalloped bone surface with seemingly active osteoclasts present indicated by the arrows. Bar = 100 μm. (B) The effect of PHT treatment can be seen in this section of a rat treated with the drug from day 1 to day 42. The alveolar bone wall has a smooth appearance with very few identifiable osteoclasts as shown by the arrows. Bar = 100 μm. B = alveolar bone; R = mesial root of first molar.

After 1 month of expansion, the width of non-mineralized osteoid along the alveolar bone wall of the tension side was larger in the rats treated with phenytoin (group I) compared to the control rats of group II. Figure 4 exemplifies this difference in widths between a control rat (A) and a rat treated with phenytoin (B). The Mann–Whitney *U*-test showed that the thickness of the newly formed bone layer on the alveolar bone was significantly greater ($P<0{\cdot}01$) in the phenytoin treated animals compared to the controls (Table 2). The mean value for group I was 176 μm (Ci 150–202) and for group II 119 μm (CI 83–155). The variation between the counted sections was, however, large.

Discussion

The phenytoin administrated in the experimental group was dissolved in propylene glycol, ethyl alcohol, and water. It was assumed that the vehicle could not have a significant influence on the results. However, high doses and chronic alcohol treatment in rats has been shown to result in disturbed vitamin D metabolism and inhibited bone matrix synthesis and mineralization (Turner, 1987, 1988). These effects were observed after treatment with a diet consisting of ethanol (15 g/l) constituting a third of the total caloric intake. In a long-term study of carcinogeneticity of increasing concentrations of alcohol, Holmberg *et al.* (1986) found no toxic reaction due to alcohol when administered in concentrations of 1–3 w/w% (max. 400 mg/250 g body weight). In the present study, the concentration of alcohol in the vehicle was 1/200th of the above-mentiond maximum concentration (1.84 mg/250 g body weight/day).

In the present study, the anticonvulsant drug phenytoin was found to modulate the effect of an orthodontic force on the periodontal tissues in the rat. This was seen on the pressure side as a decrease in the number of osteoclasts along the alveolar bone wall and as an increase in the density of the fibroblasts in the periodontal ligament. The alveolar bone of the tension side displayed a difference in morphology between the groups described as increased width of non-mineralized osteoid in the phenytoin group compared to the control group.

Since the distance moved in each rat was less than the 1 mm the appliance had been expanded when activated at the start of the experiment, a persisting active orthodontic

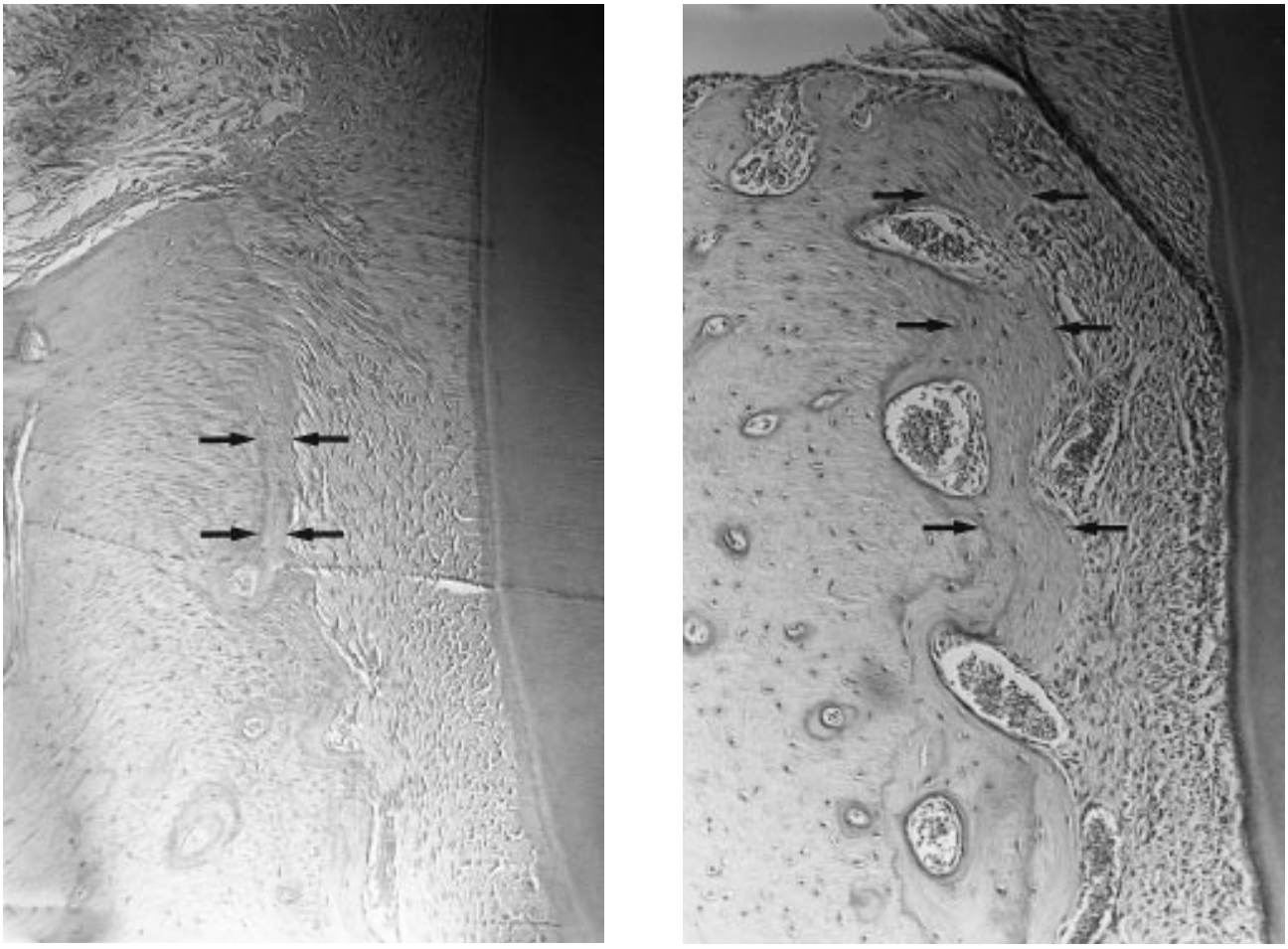

FIG. 4 (A) The section shows the width of the layer of non-mineralized bone tissue, indicated by the arrows, on the tension side in a control rat after one month of expansion. Bar = 100 μm. (B) The section shows the increased width of the non-mineralized osteod layer, indicated by the arrows, formed in a rat treated with phenytoin during one month of expansion. Bar = 100 μm. B = alveolar bone; R = mesial root of first molar.

force was present at the end of the period. The size of the force used for this tipping movement was 150 mN (15 g) at the start of the experiment. Remnants or signs of hyaline zones could still be seen in some sections after the experimental period of 1 month. The extent of the hyaline zone formed by this buccal tipping of the upper first molars in rats has been described in a scanning electron microscope study by Hellsing and Hammarström (1996). The hyaline zone was seen as an extended organic tissue covering the mesio-buccal side of the mesial root of the first molar. Brudvik and Rygh (1993) devised a method whereby molars in Wistar rats were moved mesially during 5 days under an initial force of 50 g in order to study root resorption. Using the same method in a subsequent study where the experimental period was extended to 10 days, they regularly observed a hyalinized zone on the pressure side (Brudvik and Rygh, 1994). The extent and amount of hyalinized tissue was significantly reduced between days 7 and 10 by which time the force had been reduced to 27 g.

The alveolar bone wall on the pressure side in the phenytoin treated rats was characterized by a smooth surface compared to the scalloped bone surface seen in the non-medication group exposed to the same orthodontic force. These findings on the pressure side in the phenytoin treated rats could be interpreted as signs of decreased osteoclast activity. The radiographic measurements exhibited a tendency of decrease in tooth movement which was in line with the histologic observations.

Midgett *et al.* (1981) demonstrated, by altering the bone metabolism systemically in beagle dogs, that orthodontic tooth movement is not only dependent on the applied force, but also on the state of calcium metabolism in the alveolar bone. Whether phenytoin is capable of influencing bone metabolism during orthodontic tooth movement directly or indirectly is unclear. It has been postulated that phenytoin accelerates the breakdown of vitamin D by liver enzyme induction (Dent *et al.*, 1970; Richens and Rowe, 1970; Hahn *et al.*, 1972). This could, indirectly, lead to an inhibition of the mechanically stimulated osteoclast activation. According to Livingston (1973), however, this theory is dubious. It may only be speculated at this stage on whether there exists a connection between a drug-induced stimulatory effect on PDL fibroblasts and the inhibitory effect seen on osteoclasts of the alveolar bone. A possible direct way phenytoin might influence osteoclast activity is by activation of subclones of PDL fibroblasts capable of inhibiting osteoclasts as suggested by Giniger *et al.* (1991). Another is the creation of a localized folate deficiency in

the PDL fibroblasts which Brown *et al.* (1991) suggested would result in a decrease in active collagenase (Vogel, 1977; Ariel *et al.*, 1982).

On the tension side, a significantly increased width of a non-mineralized osteoid, was observed in the phenytoin treated rats. This new layer of pre-bone coated the dark reversal line in the adjacent alveolar bone and appeared more homogeneous than in the controls. This agrees with Melsen *et al.* (1976) who concluded an increased covering of osteoid in bone biopsies of epileptic adult patients treated with phenytoin and Lau *et al.* (1994) who found phenytoin to be osteogenic in humans and, furthermore, Ohta *et al.* (1995) demonstrated in a more recent report osteogenic action by phenytoin in the rat *in vivo*. Indications of osteogenic effects on human bone metabolism after anticonvulsant therapy have been reported earlier by Wright (1965), Vas and Parsonage (1967), Kattan (1970) and Lefebvre *et al.* (1972).

The reaction on phenytoin medication observed in the periodontal ligament (PDL) could be a direct effect. Roberts and Jee (1974) reported that orthodontic force stimulates proliferation in rat periodontal ligament fibroblasts. One of the effects of phenytoin found in the present study was an accentuation of this reaction. Phenytoin is known to induce growth in gingival fibroblasts in conjunction to an inflammatory component, but whether a similar reaction could be expected in the periodontal ligament has been unclear (Hassell *et al.*, 1976, 1978; Modéer and Dahllöf, 1987). Saito *et al.* (1990, 1991) reported that human PDL fibroblasts respond *in vitro* to the application of mechanical stress by elevating the synthesis of prostaglandin and Davidovitch *et al.* (1988) observed that the periodontal ligament of the rat responded to mechanical force by increased production of the cytokine IL-1. Also, Dinarello *et al.* (1983) reported that one of the particular properties of the cytokine IL-1 is the induction of PGE_2 synthesis in fibroblasts. The presence of these proinflammatory mediators in the periodontal ligament during orthodontic treatment could provide the inflammatory component needed for phenytoin to induce the accentuation of fibroblast growth.

Approximately half of patients medicating phenytoin develop gingival hyperplasia implying increased pocket depth (Angelopoulos and Goaz, 1972). It has, accordingly, been suggested that phenytoin may contribute to periodontal disease (Lundström *et al.*, 1982). If this was true, then in combination with other factors that need to be considered in adult orthodontics such as marginal bone loss, reduced mineralization, and increased fenestratin in the lamina dura with age, it would seem reasonable to fear that the adult epileptic patient about to undergo orthodontic treatment, is going to risk increased breakdown of periodontal tissues (Williams *et al.*, 1982). Seymour *et al.* (1985), however, published contradicting results of less marginal bone loss in adult epileptic patients treated with phenytoin for 4 years compared to other anticonvulsants and Dahllöf *et al.* (1993) reached the same conclusion in a long-term study. The present study does not support the view that application of an orthodontic force increases the risk for periodontal destruction when applied in conjunction with anticonvulsive therapy in the rat. What the orthodontist might encounter when treating a patient is a prolonged treatment time.

Conclusions

The anticonvulsive drug phenytoin would appear to inhibit osteoclast activity on the alveolar bone surface and increase the density of fibroblastin the periodontal ligament on the pressure side during orthodontic tooth movement in the rat. On the tension side, phenytoin increases the apposition of non-mineralized bone. Phenytoin may decrease the speed of orthodontic tooth movement in the rat.

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