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The Effect of Prostaglandin E_2 Applied to an Extraction Site, on Orthodontically Induced Tooth Movement in Cats

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The Effect of Prostaglandin E_2 Applied to an Extraction Site, on Orthodontically Induced Tooth Movement in Cats

by

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DEDICATION

To my parents, Roni and Jeanette Gitter, for offering their love and support while I pursued my orthodontic training so far away from home.

ACKNOWLEDGEMENTS

I would like to express my gratitude to those who contributed their help in the making of this thesis.

My sincere appreciation is extended to the following people:

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To all my teachers and faculty members for their guidance during my orthodontic education.

My wife, Atalie, for her patience and endurance thoughout the typing of this manuscript.

VITA

Reuven Gitter was born on August 20, 1954 in Tiberias, Israel, to Mr. and Mrs. Roni Gitter, being the second of nine children. He graduated from Katzenelson High School, Kfar Saba, in Israel, in 1972.

In October 1975, Dr. Gitter entered the Hebrew University School of Dentistry and obtained a degree of Doctor in Dental Medicine in July 1981, graduating with summa cum laude honors.

From 1981 until 1983 he had a successful private practice in Tel-Aviv, Israel.

He began Graduate studies in the department of Oral Biology and Postgraduate studies in the Orthodontics department at Loyola University School of Dentistry in Maywood, IL. in July, 1983 leading to a Master of Science degree in Oral Biology and a Postgraduate Certificate in Orthodontics. In May 1985, Dr. Gitter obtained a specialty certificate in Orthodontics.

Dr. Gitter published his D.M.D. thesis entitled "Tooth enamel dissolution from erosion or etching and subsequent caries development" in the Journal of Pedodontics 7(2) 100-8, 1983.

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CHAPTER I

INTRODUCTION

When a mechanical force is delivered to a tooth and thereby transmitted to the adjacent investing tissues the periodontal membrane (PDM) and the alveolar bone are gradually remodelled and ultimately movement of teeth to new positions occur.

The cellular events leading to the remodeling of the PDM and alveolar bone following orthodontic treatment have been studied mainly histologically (Oppenheim: 1944; Macapanpan: 1954; Reitan: 1947, 1951, 1960, 1964; Rygh: 1972, 1973, 1974). These studies have resulted in an extensive morphologic knowledge of the different tissue and cells which participate in the reorganization of the tooth supporting structures. However, the biological mechanisms which are responsible for transformation of a force to a specific tissue reaction are largely unknown.

A variety of mechanisms which regulate the activity of bone cells have been discussed. Some investigators have suggested that bone forming cells have membrane receptors for direct mechanical pressure

(Somjen: 1979; Harell:1977). Mechanical deformation of the cell membrane has been found to affect the intracellular cAMP level which in turn transmits the stimulus to the cell nucleus where an appropriate cell response is initiated. As a final step this may result in an increased bone formation by osteoblasts or bone lining cells (Davidovitch: 1975, 1976, 1978).

Others proposed that the bone possesses piezoelectric properties, i.e., the bone tissue generates electrical currents as a response to mechanical deformation (Basset: 1962, 1968, 1971). During the past ten years a great number of investigations have shown that a negative net charge stimulates bone formation while a positive net charge seems to stimulate bone resorption (Basset: 1962,1968,1971). Some studies have shown that these phenomena may also play a role during orthodontic tooth movement. (Basset: 1971; Gillooly: 1968; Zengo: 1973, 1974; Davidovitch: 1984)

Local humoral substances such as prostaglandins are produced at sites of injury (Elattar:1978). Prostaglandins stimulate both formation and activity of bone resorbing cells (Klein: 1970; Setayesh: 1981; Dietrich, et al.: 1975, Gomes, et al.:1976; Tasjipan: 1978). Davidovitch and Shanfeld (1980, 1984) reported

the involvement of prostaglandin E_2 in bone remodeling of orthodontically treated cats, showing the rise of PGE_2 levels in alveolar bone. Indirect evidence in animals and in humans have shown that prostaglandins are involved in orthodontically induced bone resorption (Yamasaki: 1980, 1982, 1984; Shumbley: 1981; Arendorf: 1981).

The purpose of the present study is:

(1) To develop a method for primarily bodily tooth movement in cats which allows the application of well defined orthodontic forces.

(2) To expore the effect of exogeneous PGE_2 on orthodontically induced "physiologic" tooth movement in cats. In contrary to inflamatory involved tooth movement.

CHAPTER II

REVIEW OF THE LITERATURE

Early Theories of Orthodontic Tooth Movement

Edward H. Angle claimed in 1907 that absorption of the alveolar process occurred in advance of the moving tooth and that deposition of bone followed behind it, but that the first and principle response to orthodontic force was the bending of alveolar bone by noting that the bony septum closely followed a moving tooth.

Breitner refuted Angle's theory when he stated in 1940 that, during tooth movement, the bone tissue which formed the septa between the teeth did not travel along with the moving teeth. Instead, he said, it was progressively reformed of entirely new bone.

Histologic investigation of the effect of orthodontic force on alveolar bone was initiated in 1904 by Carl Sandstedt. He experimented on the teeth of dogs and reported that, with the application of force, both weak and strong, there was deposition of bone on the tension side of the old socket wall. On the pressure side the alveolar bone was equally resorbed

by weak forces. Strong forces, however, resulted in "undermining resorption".

Since the experimental work by Stanstedt, there is general agreement that orthodontic tooth movement causes resorption of bone on the side of pressure and apposition of bone on the side of traction. This statement is based on experiments on animals by Gottlieb and Orban (1932), Schwartz (1931), Oppenheim (1911, 1942, 1944), Reitan (1947, 1951, 1960, 1964), Macapan-pan, Weinmann and Brodie (1954), and experiments on humans by Herzberg (1932), Reitan (1950, 1960 1964).

Magnitude of Orthodontic Force

The exact magnitude of force that is most ideal for physiologic tooth movement has been a subject of controversy for many years among the members of the orthodontic specialty.

In 1929 McKey was one of the earliest investigators to propose a specific force value for the orthodontic movement of teeth. He claimed that the initial pressure should be 2 ounces for each tooth.

In 1942 Oppenheim conducted histologic investigation on tissue changes due to orthodontic forces on macaca monkeys. He claimed that intermittent light

forces administered over long periods of time constituted the best orthodontic treatment.

Storey and Smith conducted a study in 1952 to determine the magnitude of forces to be applied to get the optimal rate of movement of a tooth without causing damage to the tissues. They found that the optimal range of pressure on the tooth-bone interface which produced the maximum rate of movement of the cuspid teeth was 150-200 grams. Increasing the force above this range results in a slower rate of tooth movement.

In 1962 Burstone described three phases of orthodontic tooth movement: (1) the initial phase, which represented the displacement of the tooth in the periodontal membrane space, (2) the lag phase, a period in which the tooth did not move or had a relatively low rate of displacement, (3) the post lag phase, in which the rate of movement gradually or suddenly increased.

In 1965 Brian Lee used the same experimental apparatus as the one used by Storey and Smith, to find out the relation between tooth movement rate and estimated pressure applied. His results supported Storey and Smith's finding that the optimal force levels ranged between 150-260 grams.

In 1973 Storey stated that within the optimal

range of force bone remodeling continues toward a more mature state even after treatment, so that the potential for relapse is high. In the low range of forces, the quality of new bone is better and the potential for relapse is less.

In Vivo Effect of Prostaglandin E on Bone

Evidence that local PG's stimulate bone resorption in Vivo has been reported by Goodson Et Al. (1974). They demonstrated that repeated injections of 50 mg of PGE_1 directly into rat alveolar bone produced marked changes in bone morphology with increased resorption, such as extensive loss of bone matrix, fibrous replacement, and increased vascularity.

In 1980, Yamasaki, Miura, and Suda, injected Prostaglandins E_1 or E_2 solutions in rat gingiva lying near the upper first molar. They found that the prostaglandins caused the appearance of osteoclasts and bone resorption. Yamasaki also examined the mechanism of alveolar bone resorption in experimental tooth movement in rats by inserting an elastic band interproximally between the first and second upper molars. Administration of indomethacin, a specific inhibitor of PGs synthetase, suppressed the appearance of osteoclasts

and alveolar bone resorption that was induced by experimental tooth movement.

In a more recent study by Yamasaki, Shibata, and Fukuhara (1982), effects of PGE_2 were shown on experimental tooth movement in two Macaca monkeys. PGE_2 was injected into the submucosa of the right canine. They reported that local administration of PGE_2 combined with orthodontic tooth movement induce more rapid bone remodeling and have additional effect on the rate of tooth movement.

In 1981 Arendorf injected 8 week old hamsters submucosally over the buccal surface of the alveolar plate adjacent to the roots of the mandibular molars. His microscopic observations were that there was resorption of the alveolar bone, cementum and dentin. He states that PGE_2 induced bone resorption.

In 1980 a study by Davidovitch and Shanfeld has shown an increase in PGE_2 level in compression sites of orthodontically induced tooth movement in cats. The increase was 50% and 90% at day one and fourteen respectively.

In 1981 Chumbley and Tuncay showed that indomethacin, a specific inhibitor of prostaglandin synthesis reduced the rate of orthodontic tooth movement in

cats. They also agreed with the importance of production of PGs during tooth movement.

The first clinical application of prostaglandin upon orthodontic tooth movement in humans was done in 1984 by Yamasaki et al.. They injected PGE_1 into the submucosa near the orthodontically treated teeth. This local administration of PGE_1 caused almost double the rate of tooth movement seen on the control side.

In Vitro Effect of Prostaglandin E_2 on Bone

Data from several experiments indicate that PGs stimulate bone resorption. Klein and Raisz (1970) have shown that PGs are potent stimulators of bone resorption in tissue culture.

Tashjian et al. (1972) have demonstrated that the potent bone resorption stimulating factor in the HSDM transplantable mouse fibrosarcoma is PGE_2.

Goldhaber et al. (1973) have shown that growing of whole human gingival fragments in serum-free media resulted in formation of a potent bone resorption stimulating factor. Indomethacin added to gingival fragment media inhibited bone resorption in dose response fashion. It has also been demonstrated by Goldhaber et al. (1973) that the addition of PGE_2 to

bone culture media caused significant decrease in the synthesis of collagen.

Harris and his co-workers in 1973 found that prostaglandin could be detected in all three of the commonest jaw cysts-periodontal cysts, dentigerous cysts and the odontogenic keratocysts, as well as in a ameloblastoma. All of these lesions may be associated with bone and root resorption.

In 1974 Raisz and Koolemans - Beynen found that prostaglandin E_2 inhibited incorporation of labeled proline into collagenase digestible protein, but not noncollagen protein, in calvaria of 21-day fetal rats cultured in a chemically defined medium.

In 1975 Dietrich, Goodson and Raisz demonstrated the ability of E, F, A, and B Prostaglandins to stimulate bone resorption in fetal rat bone culture. Prostaglandins of the E series were 10-to 100- fold more potent thatn F, A, or B prostaglandins.

In 1976 Gomes, Hausmann, Weinfeld and DeLuca found that gingival fragments from monkeys released prostaglandin-like material. The prostaglandins contributing to the bone resorptive activity have been found to be prostaglandins E_1 and E_2.

CHAPTER III

PILOT STUDY

The significance of the pilot study was to (1) determine effective grams of force to be applied to the premolar with the appliance, and (2) assist in proper design of the appliance and the method of the experiment. The appliance should stimulate mostly bodily movements with minimum tipping with frontal resorption rather than undermining resorption.

Methods and Materials of Pilot Study

Animals

Three young male domestic cats were used in the pilot study, similar to the cats that were used in the experiment itself.

The maxillary first premolars were extracted five days prior to the beginning of the experiment. All treatments were done under general anesthesia with an intramuscular injection of ketamine (Ketaset 30 mg/kg, Bristol, Syracuse, N.Y.).

The animals were divided into three groups as follows:

Cat I - 30 grams force was delivered to right maxillary premolar, and 60 grams to left maxillary premolar.

Cat II - 90 grams force was delivered to right maxillary premolar, and 30 grams to left maxillary premolar.

Cat III -60 grams force was delivered to right maxillary premolar, and 90 grams to left maxillary premolar.

Method of Tooth Movement

The appliance used and the method of tooth movement were the same as in the study itself (Figure 4). The orthodontic forces were delivered to the teeth by means of stretched elastics which were delivered at 30 grams, 60 grams and 90 grams of force respectively. The elastics were changed every fourth day. Tooth movement was measured with a Helios Caliper each time the elastics were changed.

Histological Study

On the twelfth day, all three cats were sacrificed and the premolars segments of bone were prepared for histological study as will be described in the methods portion for the experiment.

Histological examination to determine the influence of the different forces focused on the PDL surrounding the premolar, its cellularity, fibers arrangement, bolld vessels, hyalinization zones, and on the alveolar bone adjacent to the mesial surface of the second premolar. For the purpose of estimating the degree of bone resorption, the number of osteoclasts lacunae were counted.

Results

The average tooth movement at the twelfth day of the 30 gram force segments was 0.70 mm.. For the 60 gram force it was 0.55 mm., and for the 90 gram 0.42 mm..

The PDL of the premolar with the 30 gram force looked the most physiological, that is no necrosis, no inflammatory cells, blood vessels intact, and clear cellular detail (See Figure 1), while the PDL of the 60 gram force had the same inflammatory cells but no

necrosis (See Figure 2).

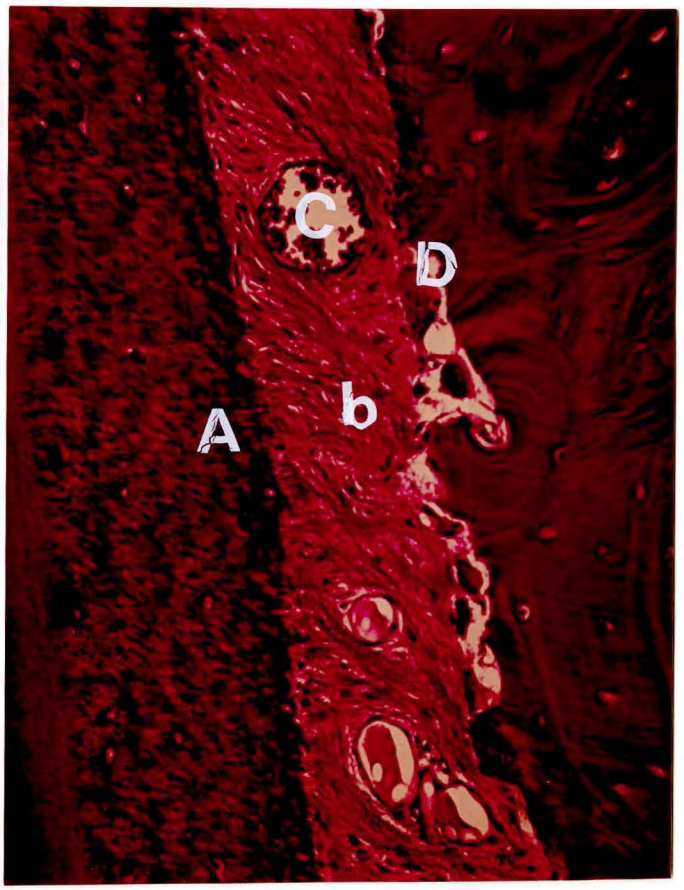
The PDL of the premolar with the 90 gram force was with necrosis, loss of cell detail, hemosiderin along the PDL, and crushing blood vessels (See Figure 3). The number of osteoclasts lacunae were for the 30, 60, and 90 grams of force 156, 122, and 82 respectively.

From the data of the pilot study it was clear to us that the 30 grams force was the most physiological and the most effective one.

FIGURE 1

Pilot Study

Pressure side of premolar moved with 30 gram force.



A. Root surface

B. Normal Compressed PDL

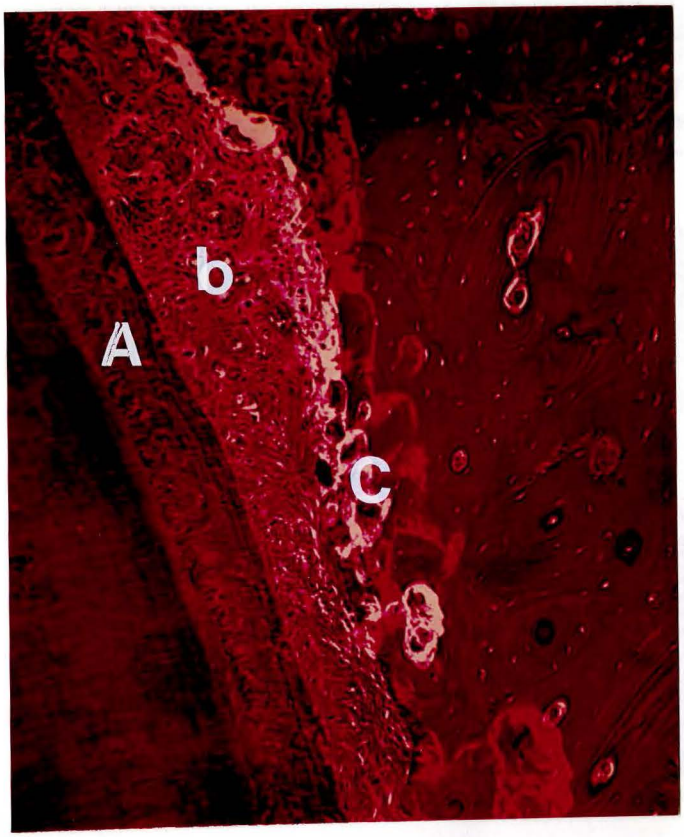
C. Blood vessels intact

D. Frontal bone resorption - many osteoclasts are present

FIGURE 2

Pilot Study

Pressure side of premolar moved with 60 gram force.



A. Root surface

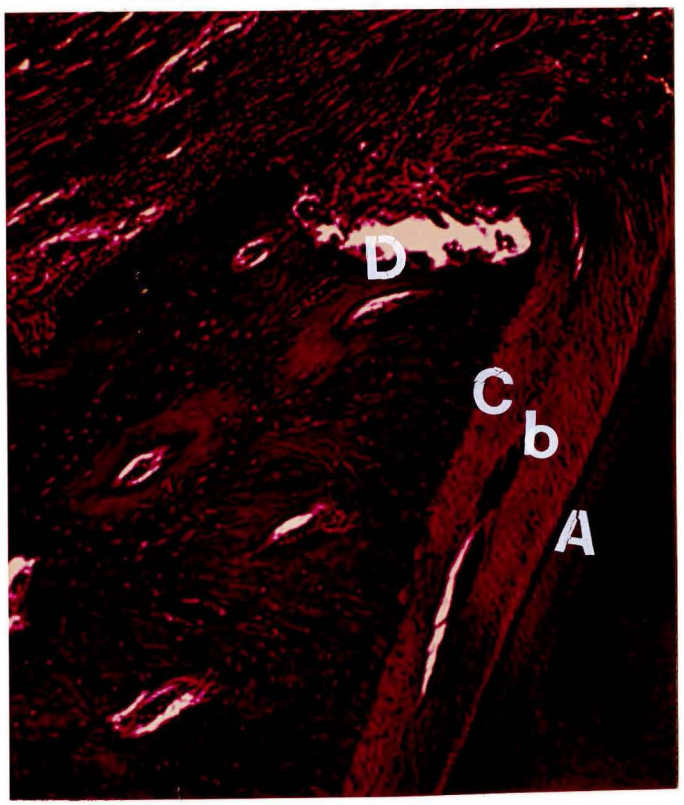
B. PDL - increase in number of cellular elements

C. Frontal bone resorption with osteoclasts

FIGURE 3

Pilot Study

Pressure side of premolar moved with 90 gram force.



A. Root surface

B. Compressed cell free fibers of the PDL.
   Hemosidirin is present.

C. Border line between bone and hyalinized tissue

D. Undermining bone resorption

CHAPTER IV

THE EXPERIMENT

Methods and Materials

Animals

Fourteen young male common domestic cats were used as the experimental animals in this study. The domestic cat was chosen because of the convenient size and accessibility of its teeth, because of the relative ease with which it is handled and housed, and because of its prior use in other orthodontic research. (Davidovitch: 1975, 1980,1984; Chambly: 1981; Debbane: 1958) Interference with orthodontic tooth movement that are normally present because of occlusion are negligible in the cat because of the lack of interdigitation of the opposing teeth. The maxillary dentition overlaps the mandibular dentition.

The exact ages of the animals were not determined. All cats were males because the female is known to have sex hormone changes which might influence PGE_2 level (Elattar:1976). The animals weighed between 7 to 9 pounds at the beginning of the study, and no signifi-

cant weight changes occurred during the experimental duration. The animals were fed a soft diet of Quake Oat Puss N Boot Fish flavor.

The maxillary first premolars were extracted five days prior to the beginning of the experiment. In a pilot study we found out that the extraction sockets were filled with soft tissue after five days. All treatments were done under general anesthesia with an intramuscular injection of ketamine (Ketaset, 30 mg/kg, Bristol, Syracuse, N.Y.)

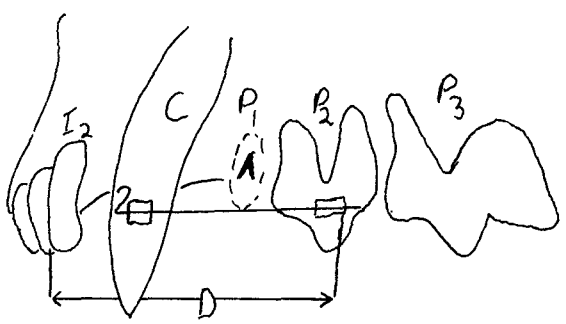
Method of Tooth Movement

Figures 4 and 5 show the orthodontic mechanism of mesial movement of the maxillary second premolars. .022 molar tubes (American Orthodontics No 611 Pad 069) were bonded on canines and second premolars with concise (3M Orthodontic bonding system). .020 S.S. straight round sectional wires were placed between the canines and the second premolars. These wires had a loop to hold the elastics in place. The orthodontic forces were delivered to the teeth by means of stretched elastics (Ormco Quail Elastics 2B(3/16")202), which delivered 30 gram force. The force was measured by

Correx Swiss made gauge ± 5 grams. The amount of force was determined by a pilot study. In order that the magnitude of force delivered by the appliance could be more uniform during the duration of the entire experiment, the elastics to be used were stretched to a distance of 15 mm. and were soaked in water for 24 hours immediately preceeding placement of the appliance.

FIGURE 4

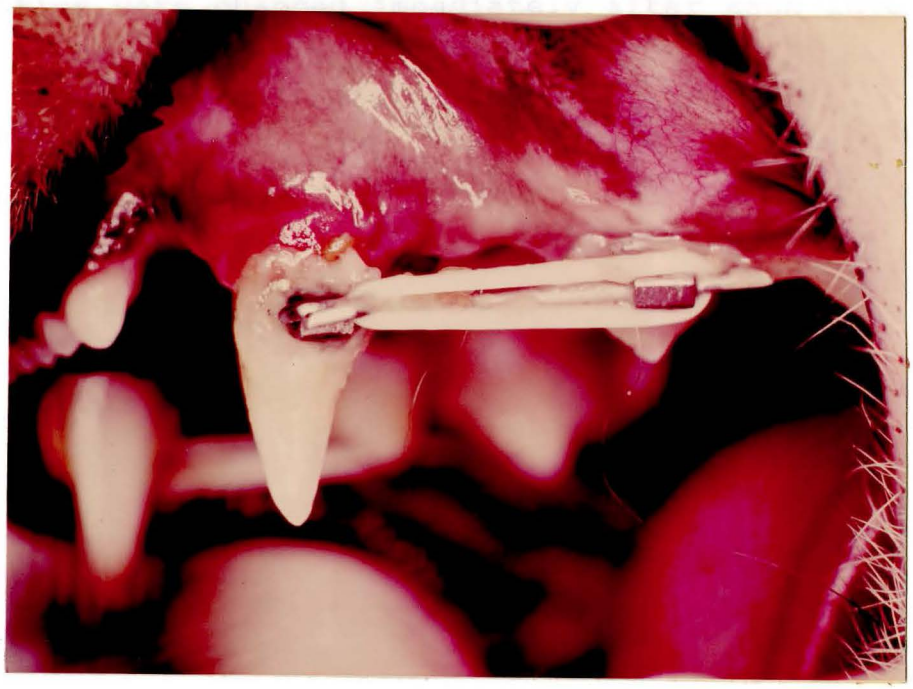
An orthodontic tooth movement mechanism of the maxillary second premolar mesial movement.



I_2 - lateral incisor: C - Canine: P_1 - first premolar was extracted: P_2 - second premolar: P_3 - third premolar: D - the distance between the mesial of lateral incisor and the distal of second premolar tube. Arrow indicates the injection area of PGE_2 or saline.

FIGURE 5

Orthodontic appliance set in a cat.



Prostaglandin E_2 (Sigma Chemical Co., St. Louis) was dissolved in saline at a concentration of 800 micro g/ml. 0.05 ml of PGE_2 (40 micro g) (Yamasaki: 1982) was injected locally into the extraction site of the right first premolar. The left side served as a control (saline injection only). Injections were made with a 1 - ml syringe with 26 gauge half - inch needle. Injections were done at zero four and eight

days after orthodontic treatment. Tooth movement was measured with Helios Caliper Fowlor Germany ± 0.05 mm. at each injection time and also on the twelfth day. Elastics were changed immediately after each injection. The actual and residual force of the new and old elastics were measured. The actual force of the new elastics was 30 g ± 5, and the residual force of the old elastics was 25 g ± 5.

Histologic Procedure

On the twelfth day all the nine cats were sacrificed by Pentoborbiton Butanchia B-special Burns Omaha Nebraska. The maxillae were cut with a Gigli saw into two halves at the mid palatine suture. Then the canine and the premolar were separated by a bone disc in both halves. The premolars segments were fixed in 10 percent neutral buffered formaline solution for three weeks followed by demineralization in a solution of twenty percent sodium citrate in 45 percent formic acid for about three months. The decalcified premolar segments were embedded in paraffin wax and sectioned at 8 microns paralleled (Mesial-distal) to the long axis of the roots and stained with Hematoxylin and Eosin. A comparison between control and experimental segments

was studied.

Histological examination to determine the influence of PGE_2 focused on the alveolar bone proper adjacent to the mesial surface of the second premolar. For the purpose of estimating the degree of bone resorption, the number of osteoclasts lacumae were counted along the surface of the bone starting from the alveolar crest on the mesial side of the second premolar (Table II) and ending on apical portion of the bone.

Forty eight sections were examined composing an overall thickness of 400 microns from each premolar. The sections were cut mesio distally starting from the buccul surface of the premolars. The first 50 sections were discarded. Sections were taken just after the crown of the tooth started to be showing out of the alveolar bone, and stopped to be taken when the crown disappeared inside the bone. The reason for this is to examine the middle surface of the root where the osteoclastic activity is believed to be higher. At the time of examining the slides I was not informed of which group was being graded.

CHAPTER V

RESULTS

Nine of the original twelve animals successfully completed the experimental phase without complications. Each animal maintained a good appetite and there was no apparant sign of the appliance interfering with their eating. The cats remained friendly during the duration of the experiment, despite the repeated injections.

Distance and Rate of Tooth Movement

Linear measurements of the distance of tooth movement were determined for the nine animals on the zero, fourth, eighth, and twelfth day. Table I shows the rate of tooth movement over a period of 12 days. Four days after treatment, in the experimental side, the average tooth movement was 0.27 mm. whereas, in the control side the average tooth movement was 0.16 mm.. Four days later the experimental side showed about the same rate of tooth movement as the first four days, an average of 0.25mm.. However, in the control side, tooth movement was measured at 0.08 mm. showing a decrease in

the movement to half. At the twelfth day the experimental side showed an increase of tooth movement to an average of 0.32 mm. The control side showed an average tooth movement to be 0.18 mm., double the rate of the previous fourth day.

The total tooth movement of all nine cats during the twelve days in the experimental side was 7.65 mm., an average of .85 mm per cat. The total tooth movement in the control side was 3.95 mm., an average of .43 mm per cat.

Figure 6 shows the PGE_2 slope is greater and the rate of change is more continuous than the control plot. The last segment of the PGE_2 plot shows the most rapid tooth movement.

In contrast to this, the segment of 4-8 days in the control side shows the slowest rate of tooth movement. A comparison of the beginning and last intervals of the control and PGE_2 groups shows that the slope of the PGE_2 group is two times greater than the control slope, or, the PGE_2 group shows double the rate of tooth movement than the control side.

Table I

The Effect of PGE_2 Injections on the Rate of Tooth Movement of Second Premolars

Cat	Tooth Movement (mm.) 4th Day		8th Day		12th Day		Total		PGE_2 (ratio)
Number	PGE_2	Control	PGE_2	Control	PGE_2	Control	PGE_2	Control	Control
2614	0.20	0.05	0.25	0.10	0.20	0.10	0.65	0.25	2.60
2615	0.35	0.20	0.25	0	0.15	0.20	0.75	0.40	1.87
2616	0.15	0.30	0.15	0	0.20	0.20	0.50	0.50	1.00
2617	0.45	0,30	0.05	0.10	0.60	0	1.10	0.40	2.75
2618	0.50	0.25	0.15	0.10	0.40	0.40	1.05	0.75	1.40
2619	0.20	0.10	0.80	0.20	0.35	0.35	1.35	0.65	2.07
2620	0.30	0.25	0.45	0.10	0.50	0.20	1.25	0.55	2.27
2621	0.25	0.05	0.10	0.05	0.15	0.10	0.50	0.20	2.50
2574	0.05	0	0.10	0.10	0.35	0.15	0.50	0.25	2.00
Average	0.27	0.16	0.25	0.08	0.32	0.18	0.85	0.43	2.05 ± .05

Average Ratio

Mean + S.E.

Analysis of Variance

Repeated Measures - Time and Tooth Position

Dependent Variance - Time - 1 Time - 2 Time - 3

Source	Mean Square	Degree of Freedom	F	P
Mean	35327.0143	1	7609.9400	0.6000
Treatment	3.6225	1	0.7800	0.3901
Error (1)	4.6422	16		
Time	1.3340	3	67.0900	0.0000
Treatment time*	0.1492	3	7.5100	0.0003
Error (2)	0.0198	48		

Testing for effects of treatment

f (1,16) = 0.78 P = 0.39 no significant

Testing effects of time

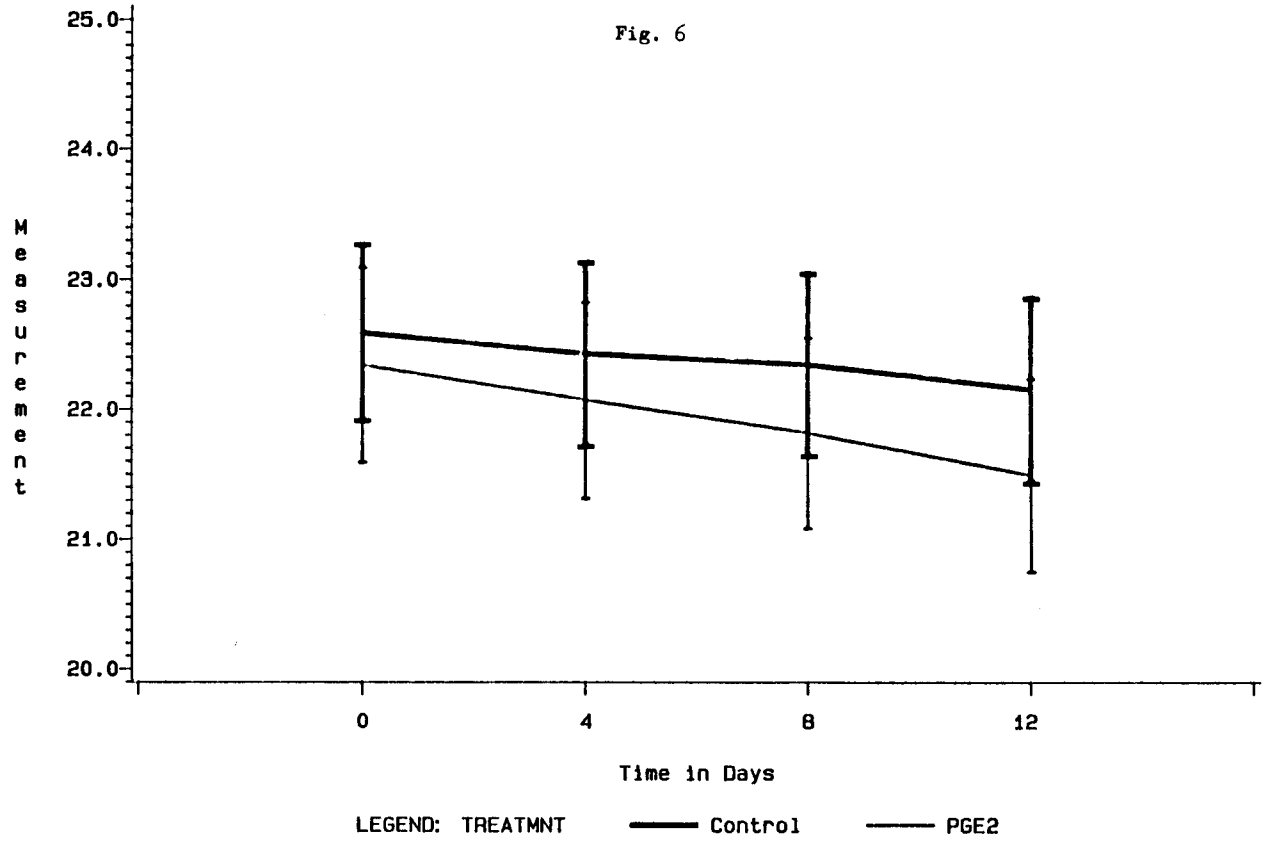
f (3,48) = 67.09 P = 0.0000 very highly significant difference exists over time.

However, while testing to determine the existance of an interaction between the effect of time and treatment protocol it was found that it does exist.

f (3,48) = 7.51 P = 0.0003 very highly significant

Tooth Movement (mm)
- across Treatment Protocol -

Fig. 6



Intervals represent the MEAN ± 2 SE

Effects of Local Injection of PGE_2 on Alveolar Bone Tissue

Two osteoclast counts were conducted independently on the same sections. Averages of the counts were taken and the ratio of the averages was calculated (Table II). Table II and Figure 7 show that administration of PGE_2 increases the number of osteoclasts in the experimental side. One specimen, number 2621, did not show an increase in the number of osteoclasts with an injection of PGE_2. It showed a 6% decrease in the number of osteoclasts in the experimental side.

Figures 8 - 13 demonstrate the histological changes of alveolar bone and PDL by applying orthodontic force via elastics and the injection of PGE_2 in the experimental side vs. saline in the control side, on the histological changes of the alveolar bone and the PDL.

Table II

Osteoclastic Activity (Number of Lacunae)

Alveolar Crest to Apex of Alveolar Bone Proper

Cat Number	Experimental Right Side			Control Left Side			
	Investigator I	Investigator II	Average	Investigator I	Investigator II	Average	Ratio of Averag
2571	160	160	160	83	105	94	1.70
2614	65	88	76.50	52	48	50	1.53
2615	110	90	100	92	61	76.50	1.31
2616	162	134	148	100	75	87.50	1.69
2617	148	101	124.50	90	16	53	2.34
2618	217	121	169	152	91	121.50	2.78
2619	278	296	287	185	189	187	1.53
2620	179	156	167.80	69	73	71	2.36
2621	114	127	120.50	128	126	127	0.94
							1.79 average Ratio Mean

Osteoclastic Activity

Analysis of Variance

Dependent variable - Investigator I Experiment, Control

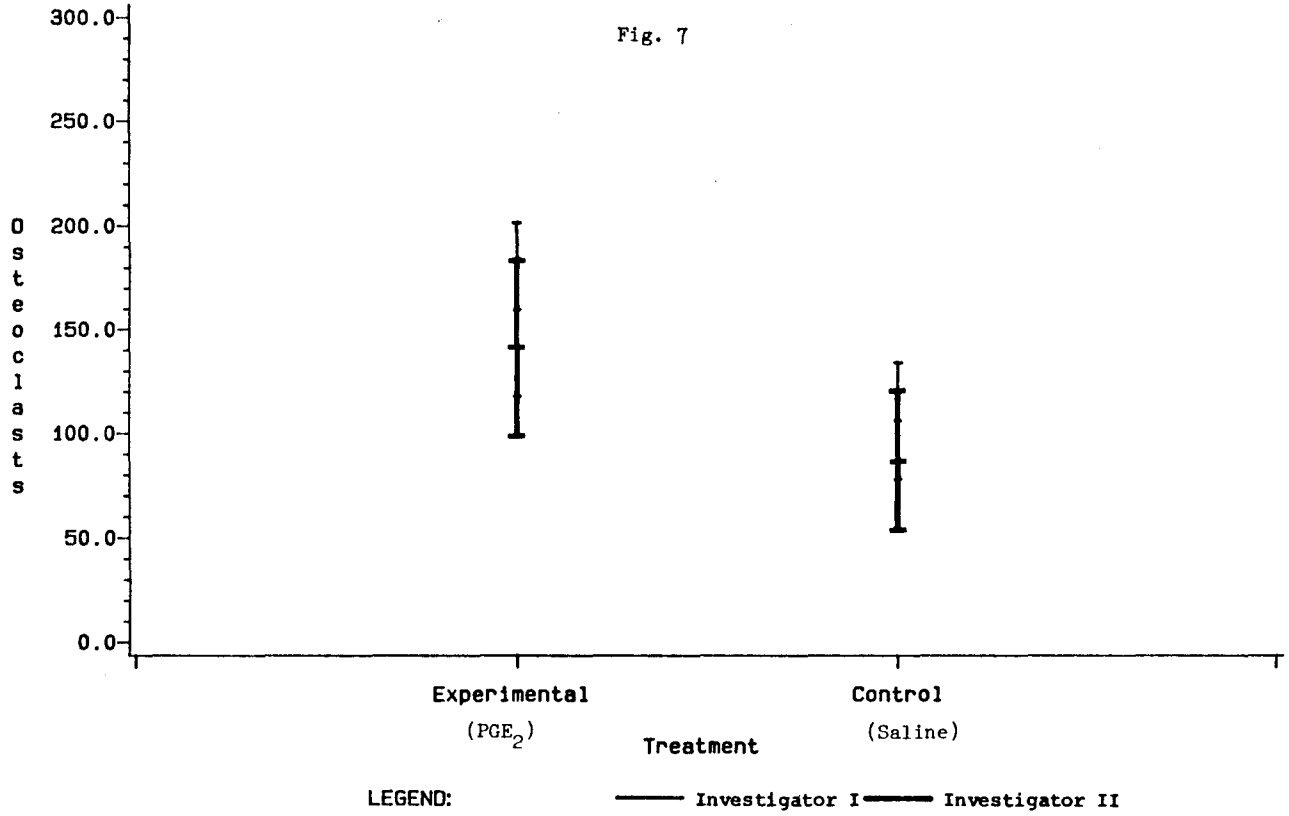
- Investigator II Experiment, Control

Source	Mean Square	Degree of Freedom	F	P
Mean	548389.6132	1	56.0800	0.0001
Error	9778.9427	8		
Investigator	2973.8841	1	2.7500	0.1359
Error	1081.5345	8		
Treatment	26276.4103	1	21.3300	0.0017
Error	1232.1600	8		
Investigator - Treatment interaction	1.5212	1	0.0100	0.9197
Error	140.5211	8		

1. Testing for the effects due to the investigator
   f (1,8) = 2.75 P = 0.1400
   there were not any significant differences.
2. Testing for the effects due to treatment
   f (1,8) = 21.3300 P = 0.0017
   very high significant.
3. Examining the existance of an interaction between the investigators and treatment.
   f (1,8) = 0.01 P = 0.9197
   No interaction can be demonstrated

Osteoclastic Activity
- Across Treatments -

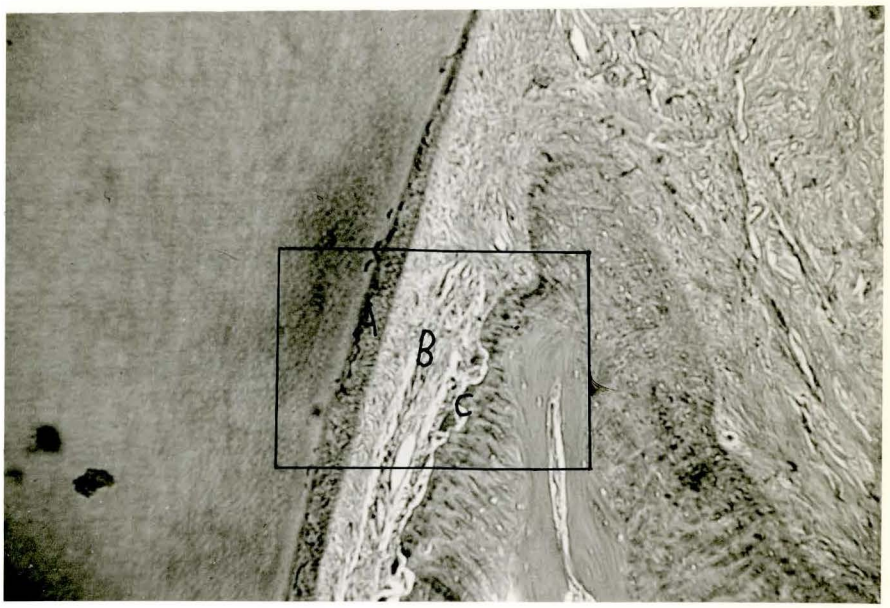
Fig. 7



Intervals represent the MEAN ± 2 SE

FIGURE 8

Pressure side of the control premolar.



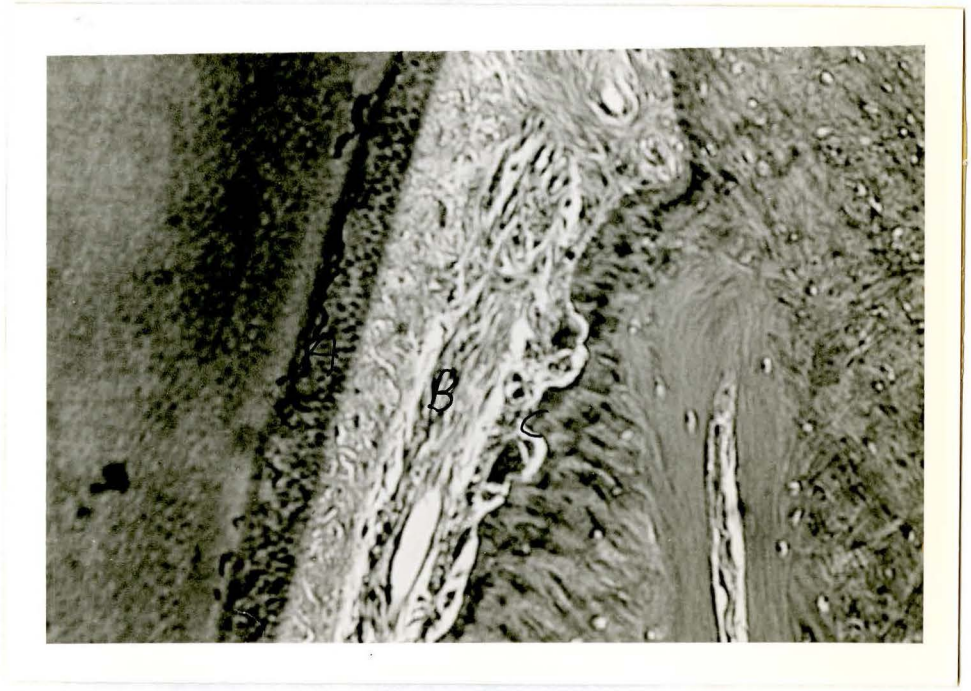
A. Root surface

B. Compressed PDL

C. Frontal bone resorption with few osteoclasts (X100)

FIGURE 9

Pressure area corresponding to the bordered area of Figure 8.



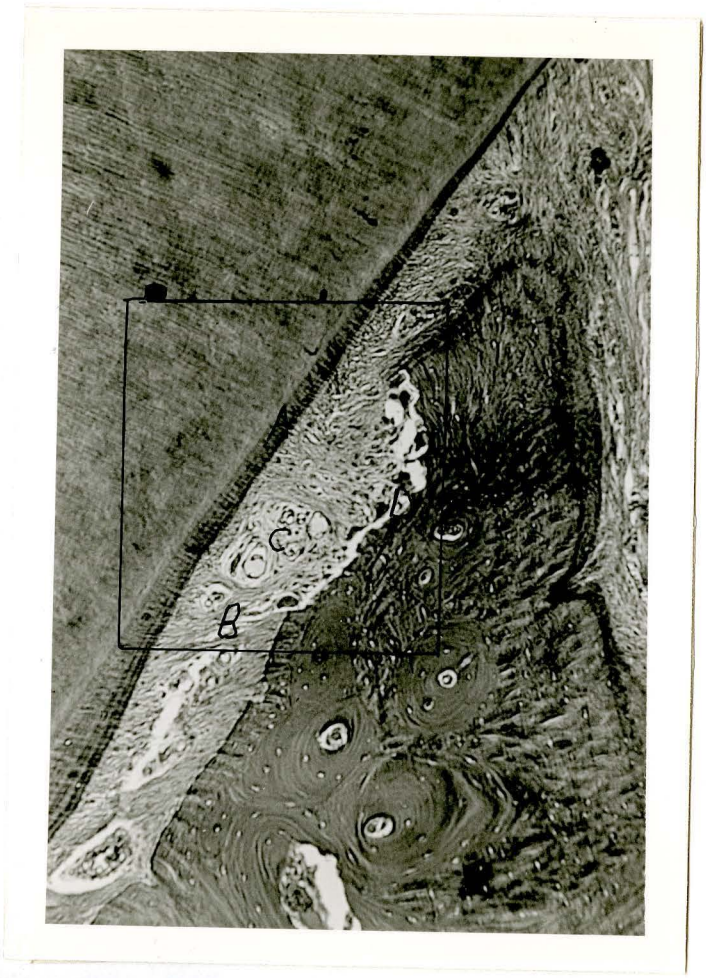
A. Root surface

B. Compressed PDL with relatively normal appearance, no hyalinized zone is present.

C. Frontal bone resorption with few osteoclasts.(X 200)

FIGURE 10

Pressure side of the experimental - PGE_2 Premolar



A. Root surface

B. Compressed PDL.

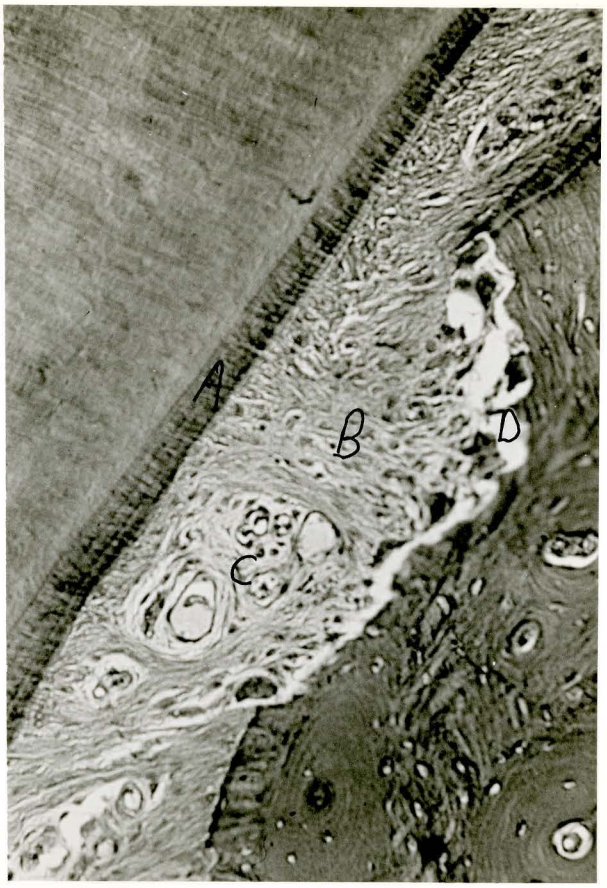
C. Blood vessels intact

D. Frontal bone resorption with many osteoclasts.

(X 100)

FIGURE 11

Pressure area corresponding to the bordered area of Figure 10.



A. Root surface

B. Compressed PDL with increase cellularity, without hyalinized zones

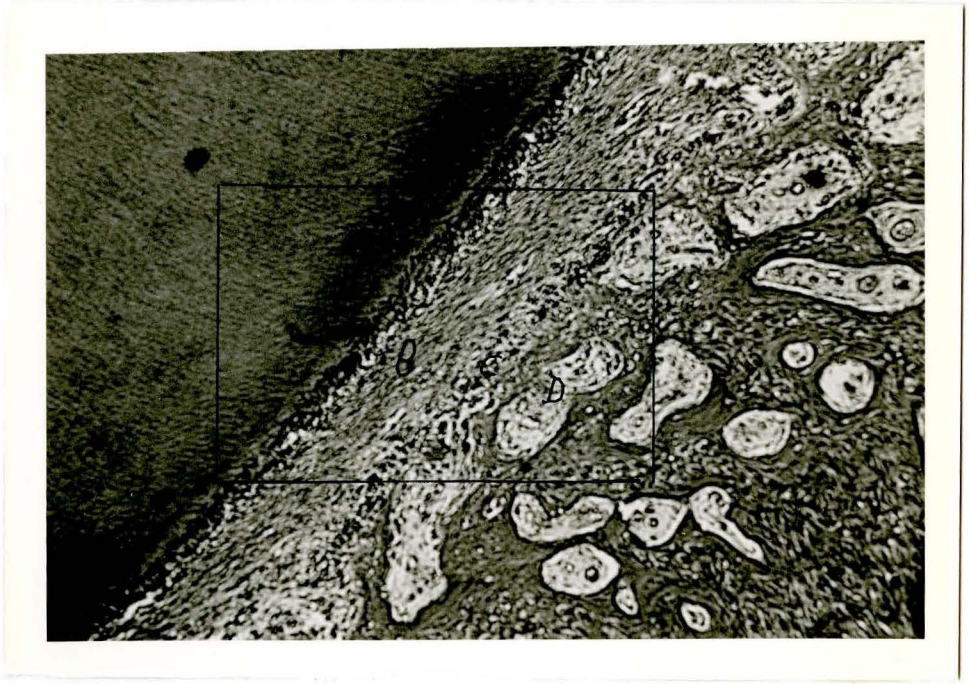
C. Group of cappillaries sectioned horizontally

D. Frontal bone resorption with many osteoclasts.

(X 200)

FIGURE 12

Tension side of experimental premolar.



A. Root surface with layer of cementoblast.

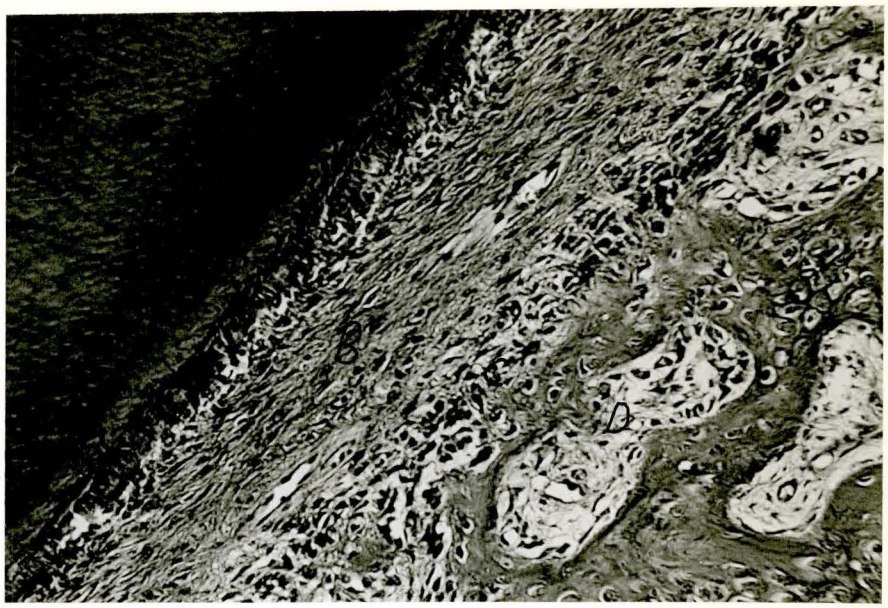
B. Stretched PDL.

C. A layer of osteoblast.

D. Bone formation within the marrow space.

FIGURE 13

Tension side corresponding to the bordered area of Figure 12.



A. Root surface, cementoblastic activity is present with cementoblasts forming a new cementum.

B. Stretched PDL with increased cellularity.

C. Bone apposition with many osteoblasts.

D. Bone apposition within the marrow space.

CHAPTER VI

DISCUSSION

The young common domestic cat proved to be an excellent research animal for investigations related to experimental orthodontic tooth movement. The convenient size, shape, lack of occlusal interference, and accessibility of the teeth were factors which made it possible and facilitated bonding and placement of the orthodontic appliances.

The experimental results reported in this study suggest that local administration of PGE_2, combined with mechanical tooth movement, accelerates the rate of tooth movement in cats (Table I , Figure 6). The ratio of PGE_2-treated:control is over 2:1. These results agree with data obtained in experiments on monkeys and humans by Yamasaki (1982, 1984).

Evidence that local PGs stimulated bone resorption in vivo has been reported by Goodson et al.(1974), Yamasaki et al.(1980), and Arendorf (1981).

Davidovitch and Shanfeld (1980) have shown an increase in the PGE_2 level in compression sites of orthodontically induced tooth movement in cats.

The pattern of the orthodontically induced tooth movement in the control side is slightly different in this experiment than what was reported in previous experiments by Reitan (1960, 1964), and Yamasaki (1982). The results in this study show no plateau stage in the tooth movement of the control side. A difference in rate decrease was shown between the 0 to four day time interval and the 4 to 8 day interval. During the first time period, a 0.18 mm distance was found where as in the 4 to 8 day period a 0.08 mm distance was recorded. The reasons for the differences between the present study and the previous studies are:

(1) The orthodontic movement attained in this study was primarily translation or bodily movement while in previous studies tooth movement was primarily tipping.

(2) Light force levels were continuously applied.

(3) Upon histologic examination no areas of hyalinization of periodontal membrane or undermining resorption of adjacent bone were found.

The experimental side treated with PGE_2 shows continuous tooth movement with significant increase in the rate of movement in the 8 to 12 day interval. The findings of continuous tooth movement in the PGE_2 side are in agreement with those of Yamasaki (1982).

Reitan (1964) reported hyalinization zones in the periodontal membrane. He stated this cellular response is a normal occurrence in bodily movement performed with continuous forces. Story (1973) stated the development of the inflammatory process in the periodontal tissues occurs when continuous loads in excess of 35 g are applied to the guinea pig incisor tooth. Azuma (1970), Rygh (1973) and Yamasaki et.al. (1980) used a tipping mechanics in rats and found evidence of hyalinization zones and an inflammatory process in the periodontal tissue on the pressure side.

In the present study the histologic results show that the periodontal tissue on the tension and pressure side of all the sections, appeared to be within normal limits. No inflammatory changes or hyalinized zones in the periodontal tissue were detected. These findings disagree with previous studies (Reitan:1964; Azuma:1970; Rygh:1973; and Yamasalo:1980).

Differences in the findings are attributable to several factors. Force levels used in the present study did not exceed the plastic deformation of the collagen fibers of the periodontal tissue. However, future studies to evaluate force levels in relation to collagen mechanical properties will be needed for

verification. A second reason is that the orthodontic appliance used in this study resulted in primarily bodily movement with minimal tipping. Finally the use of cats as the experimental animal may have had an influence on the results. Cats have a different bone structure than the animals used in the other experiments such as rats and guinea pigs. Davidovitch (1984) used cats and found an inflammatory response occurring in the PDL soon after force application to the teeth. However, he used a much heavier force than those used in the present study.

In small animals, such as cats and monkeys, in which forces similar to those used in man have been tested, there is little difference recorded between arbitrarily designated heavy and light forces, as all such forces used could be regarded as being heavy in these animals, particularly in view of the smaller root area of teeth moved. In the present study, a 30 g force that was determined in the pilot study, proved to be a physiological light force. The design of the orthodontic appliance proved to be a simple effective appliance which allowed the application of well defined orthodontics forces, and induced a primarily bodily tooth movement.

The mechanism of acceleration of the rate of tooth movement in PGE_2 treated cats may be related to evidence that local PG's stimulate bone resorption in vivo (Goodson et. al.:1974, Yamasaki:1980, Arendorf: 1981). PG's cause a significant increase in the content of cyclic AMP (Yu:1976). Furthermore Yu (1976) showed intracellular calcium to be important in the mechanism of bone resorption in vitro. Somjen et. al. (1980) reported mechanical stretching of isolated calvarial bone cells caused a significant elevation in cAMP and PGE_2 synthesis. These reports suggest that the effects of mechanical force on tooth movement involve cyclic nucleotides and prostaglandins as second messengers and/or modulators. Throughout life the human teeth drift normally in the jaws. Bone remodeling is constantly occurring when teeth are in function. PG's are probably released and are involved in this bone remodeling process which most likely does not involve inflammation. Based on this assumption and on the results of the present study one can postulate that the light orthodontic force could induce frontal bone resorption without inflammation, and the injection of PGE_2 could increase the rate of physiological tooth movement.

While PG's had this effect, one must know that

PG's are one of the cellular products of cell membrane deformation in response to extracellular signal such as orthodontic force. There are some other products such as cAMP, protein kinase C, Ca^{2+} influx and inositol phospholipids (Nishizuka:1984) that might play a role in the mechanism of bone resorption. Further studies are needed to varify this.

It is possible that PG's may be useful in the future in clinical orthodontic treatment because PG's are rapidly inactivated on circulating through the lungs (Ferreira and Vane:1967, Piper et. al. 1970). Furthermore, PG's have a short biological half life of the order of a few minutes. Thus the systemic effects should be minimal if any. Further studies are needed to enable clinical use of PG's.

CHAPTER VII

SUMMARY AND CONCLUSION

The effect of PGE_2 on orthodontically induced tooth movement was studied in nine young domestic cats. The second maxillary premolars teeth were orthodontically moved in a mesial direction toward an extraction site of the first maxillary premolar. The orthodontic mechanical force was delivered to the teeth by means of stretched elastics which delivered 30 g force. The teeth were moved along a .020 inch stainless steel round sectional wire.

PGE_2 (40 mg/site) was injected locally into the extraction site of the right first premolar. The left side served as a control (saline injection only). Injections were done at zero, four, and eight days after the initiation orthodontic treatment. The distance and rate of tooth movement were determined.

On the twelfth day, the cats were sacrificed. The premolar segments were prepared for histological study. A comparison between control and experimental segments was studied.

(1) Local administration of PGE_2, combined with mechanical tooth movement double the rate of tooth movement compared to the saline injected side.

(2) Using analysis of variance statistical test, one can see a highly statistically significant existance of an interaction between the effect of time and treatment protocol $P = 0.0003$.

(3) Injection of PGE_2 is associated with an increased number of osteoclasts on the experimental side by 79% more than on the control side. The observation is highly statistically significant ($P = 0.0017$).

(4) On the experimental side the pattern of tooth movement was constant while the control side showed variable tooth movement rates.

(5) Both groups showed primarily bodily tooth movement.

(6) The pressure side of the periodontal membrane shows osteoclastic activity without inflammation or hyalinization zones. A normal cellular architecture remained throughout tooth movement.

(7) The tension side shows osteoblastic and cementoblastic activity without inflamation.

(8) The experimental design used in this study was effective in evaluating in vivo tooth movement.

(9) This study is the first report of physiological tooth movement without inflammation and hyalinization zones present.

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11/22/95
Date

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