IDENTIFICATION OF CELLS RESPONSIBLE FOR ORTHODONTICALLY INDUCED ROOT RESORPTION

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ABSTRACT

Root resorption is an unpredictable and an undesirable side effect of orthodontic tooth movement. The extent of the root resorption lesions varies with each orthodontic case but generally has a good prognosis for repair when the force is removed. The biological mechanism for root resorption remains poorly understood. Therefore, an animal model for inducing root resorption by orthodontic force application *in vivo* has been developed. The orthodontic appliances were calibrated from a load/deflection curve. The appliances were placed on the mandibular left first molars of 7 week old Swiss-Webster mice and remained active for various time periods. The molars and associated periodontium were prepared and stained for tartrate resistant acid phosphatase (TRAP). The TRAP positive cells were counted and correlated to the bone, periodontal ligament or root surface. Statistical analysis of these data revealed that the osteoclast-like cells associated with the bone and the putative precursor cells in the PDL behaved in a similar manner. The odontoclast-like cell population of the root surface followed a different pattern in response to orthodontic force application over time. These results strongly suggest that cells occupying the root resorption lacunae represent a different population of resorptive cells or are under different control mechanisms from those in the bone or PDL. In the future it may be possible to inhibit root resorption without decreasing the rate of bone resorption which is essential for orthodontic tooth movement.

DEDICATION

To my family, who never fail to keep my spirits up

To Gordo, a truly wonderful friend, whose kindness and support throughout the past 2 years has been exceptional and greatly appreciated

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INTRODUCTION

Root resorption occurs normally as part of the exfoliation of the primary dentition, but also may occur as an unpredictable and undesirable sequelae of pulpal or periodontal inflammation. Root resorption is particularly poorly understood in relation to orthodontic tooth movement.

A critical review of the literature shows that the studies on root resorption may be grouped under several major areas: (1) Resorption of Primary Teeth; (2) Resorption of Permanent Teeth: (i) Idiopathic Root Resorption; (ii) Traumatic Root Resorption (includes root resorption secondary to endodontic, periodontic and orthodontic etiologies). The various theories developed to explain the phenomenon of root resorption and the animal models used to test these theories are also described. The investigations on root resorption associated with orthodontic tooth movement have been divided into those using radiographic evaluation of orthodontic root resorption and those studies using light microscopy and scanning electron microscopy. A review of the ultrastructure, origin and behaviour of the odontoclast completes the state of our knowledge in root resorption to date.

RESORPTION OF PRIMARY TEETH

Physiological root resorption is a desirable phenomenon when associated with the timely exfoliation of the primary dentition (Andreasen, 1988; Jones and Boyde, 1972). Extensive research has been conducted on the primary dentition in an attempt to further understand the biological mechanisms involved in root resorption (Marks and Popoff, 1988; Ten Cate and Anderson, 1986; Takahashi et al., 1987, 1988; Sasaki et al., 1989, 1990). A study using kitten teeth at various stages of development and exfoliation confirmed that dental hard tissues were eroded by odontoclasts supported by a number of blood vessels, fibroblasts and macrophages (Ten Cate and Anderson, 1986). There was no evidence of phagocytosed, intercellular collagen in any of these cells. In addition, there was no evidence of "helper" cells to remove the organic component of the dentin and cementum, as previously suggested by Heersche (1978). The loss of periodontal ligament (PDL) during exfoliation, theoretically, would involve removal of cells and extracellular material. Two forms of fibroblast death were observed. One form was apoptotic cell death which suggests that the exfoliation of the primary dentition is a preprogrammed physiological event. The organelle integrity was maintained and no associated inflammatory changes were observed. This process is thought to be triggered by ill-defined physiological stimuli and results in focal deletion of cells during normal embryological development. Fibroblast cells containing collagen

profiles showed localized disruption of mitochondria and eventual dissolution of the cytosol was observed (Ten Cate and Anderson, 1986). This type of apoptotic cell death is very different from cell necrosis. It has been described as selective mitochondrial damage without swelling of other organelles, with maintenance of the cell membrane. This form of cell death is specific to an environment free of inflammatory change. Necrotic cell death on the other hand was not observed in association with the exfoliation of primary teeth. This form of cell death is induced by severe environmental changes and is associated with morphological changes such as marginal clumping of loosely textured nuclear chromatin, dilation of endoplasmic reticulum with mild dispersal of the ribosomes, gross swelling of the mitochondrial matrix and rupture of the nuclear, organelle and plasma membranes. Exudative inflammatory changes developed in the adjacent viable tissues. Fibroblasts, during active root resorption, were occasionally observed to have maintained a normal morphology. These various cellular responses suggest phenotypically different populations of fibroblasts in the PDL. Collagen removal occurred extracellularly and did not seem to involve an increase in phagocytotic activity by fibroblasts.

Apoptosis is one mechanism for stimulating root resorption in the primary dentition. Another possible mechanism for stimulating root resorption of the primary dentition is the pressure created by the developing secondary tooth (Obersztyn, 1963). In a study where the developing secondary tooth was

removed, a delay in the exfoliation of the primary tooth was observed (Obersztyn, 1963). These findings suggest that the pressure created on the primary tooth by the secondary tooth is a factor in the initiation of the exfoliation of the primary tooth (Obersztyn, 1963).

Cahill (1969) has shown that bone which is resorbed to create an eruption path ahead of premolars in dogs, will do so in the absence of direct pressure from an erupting tooth. It has also been suggested that this advancing line of osteoclasts creating an eruption path for the developing secondary tooth may play an aggressive and vital role in the initiation of root resorption of the primary tooth (Cahill, 1969).

In summary, although a great deal of research has focused on root resorption associated with the exfoliation of the primary dentition, conclusive evidence on the exact mechanisms responsible for this phenomenon is clearly lacking.

RESORPTION OF THE PERMANENT TEETH

It is presently accepted that mineralized tissues of the permanent teeth do not normally resorb and that root resorption observed on the surfaces of permanent teeth denotes pathology (Kvam, 1972; Rygh, 1977). Pathological root resorption is an unpredictable and undesirable sequelae of a variety of initiating events including trauma, dental treatment of various types and systemic disease conditions (Tronstad, 1988). Root resorption is, therefore, a multidisciplinary problem in dentistry .

(A) Idiopathic Root Resorption

Idiopathic root resorption is a form of pathological root resorption for which the etiology is unknown (Steadman, 1942; Henry and Weinmann, 1951; Massler and Malone, 1954). Idiopathic root resorption in human specimens free of pathology or known systemic disease, has been documented (Henry and Weinmann, 1951, Massler and Malone, 1954). A post-mortem histological investigation of fifteen individuals indicated that 90% of the teeth examined showed areas of root resorption (Henry and Weinmann, 1951). The evidence for root resorption was described as the presence of resorption lacunae and reversal lines. Active root resorption was detected in 10% of the cases (Henry and Weinmann, 1951). Most of these lacunae showed repair with new cementum present. In addition, these lesions were frequently associated with the apical region of the dental units studied. These results were further supported by the observations of Massler and Malone (1954), who found that 100% of 708 subjects had radiographic evidence of root resorption. The predominant finding was that 71% of these subjects had "slight" root resorption. This was defined as blunting of the root apex, 1 to 2mm loss of root length, interruption of the lamina dura and widening of the PDL. The study also revealed that only 10% had "obvious" root resorption, a loss of 2mm or more of apical root length. Neither investigation indicated a correlation of root

resorption with sex or age of the subjects. The mandibular incisors were most frequently resorbed while the molars were the least resorbed (Massler and Malone, 1954). Thus, the resorption potential was not constant for all teeth and also there was variability among individuals (Henry and Weinmann, 1951; Massler and Malone, 1954). There did appear to be an ill-defined resorption susceptible group (Massler and Malone, 1954). Local traumatic changes such as compression, crushing and necrosis of the PDL occurred in areas corresponding to active resorption of the alveolar bone and cementum (Henry and Weinmann, 1951). It was hypothesized that in healthy patients the primary cause of root resorption is localized microtrauma.

Therefore, every individual will have a given potential for root resorption, but only 10% will have histological evidence of root resorption (Henry and Weinmann, 1951). The individuals having clinically significant root resorption in the permanent dentition represent only a small percentage of the population.

(B) Traumatic Root Resorption

The development of a classification system of pathological root resorption has decreased the confusion over the types of resorptive lesions in this group (Tronstad, 1988). The descriptive terms used in classification very specifically define the nature of the etiology of the resorptive lesion (Tronstad, 1988). The

lesions are classified as either inflammatory or noninflammatory, and further subclassified as either transient (surface) or progressive lesions.

The most common form of pathological root resorption are transient, inflammatory resorptive lesions (Tronstad, 1988). Inflammatory resorption becomes progressive when the denuded areas of the internal or external root surfaces cannot undergo effective repair. This is mainly due to prolonged stimulation of the resorbing cells by means of increased pressure in the tissue, infection of the dentin or the root canal, or in association with certain systemic disease conditions (Tronstad, 1988).

Surface resorption or transient inflammatory resorption occurs as a result of the activity of mononuclear or multinucleated cells in the absence of an inflammatory response in soft tissue (Andreasen, 1985). This form of root resorption has been reputed to have the best prognosis for repair with newly formed cementum partially or completely restoring the root surface morphology (Kvam, 1972; Andreasen, 1985). This form of root resorption is classically associated with dental trauma, including avulsion and replantation as well as that observed in cases of idiopathic root resorption (Andreasen, 1981a; Henry and Weinmann, 1951). Progressive inflammatory root resorption, on the other hand, is accomplished by mononuclear or multinucleated cells present in inflammatory soft tissue.

The initiating events of this type of root resorption include severe trauma, of which orthodontic treatment and periodontal surgery are common initiating factors (Tronstad, 1988).

Thus, it appears that under physiological conditions the mineralized tissues of the permanent teeth are protected from resorbing cells. This protection is thought to be imparted by predentin and odontoblasts in the root canal and by precementum and cementoblasts on the external root surface (Tronstad, 1988). When these protective barriers are violated mononucleated and multinucleated cells colonize the denuded root surfaces and resorption may progress (Tronstad, 1988). This type of root resorption may be internal (on the root canal surface) or external (on the external root surface) and may be transient or progressive (Tronstad, 1988). Transient root resorption occurs frequently in traumatized teeth. This includes teeth which have undergone orthodontic and periodontal treatment, but also occurs secondary to occlusal wear (Tronstad, 1988; Henry and Weinmann, 1951).

THEORIES ON ROOT RESORPTION

Various theories have been suggested to explain the biological process of root resorption. These include the loss of a protective cell layer, calcium homeostasis, immune response and an anti-invasive factor. The most extensively investigated is the hypothesis that a protective cell layer exists which imparts

resistance to resorption to the root surface (Andreasen et al., 1978; Andreasen, 1981a,b; Boyko et al., 1981; Reitan, 1951).

The periodontal tissue appears to be able to withstand considerable injury without root resorption developing. Many experiments have been completed to identify the most critical component of the PDL imparting root resorption resistance. In a series of investigations Andreasen removed portions of PDL and \or the alveolar bone. He observed very little resorption in these monkey cuspid specimens (Andreasen, 1981a,b). This was interpreted as evidence that the resorption resistance was located more centrally. The next area of interest were Sharpey's fibers, which have since been eliminated as playing a role in limiting root resorption (Andreasen et al., 1978; Boyko et al., 1981). Sharpey's fibers when damaged in extracted teeth prior to replantation did not result in root resorption in monkeys (Andreasen et al., 1978) or in dogs, (Boyko et al., 1981). The cementum and cementoid layers were not protective against root resorption as the cementum was regenerated and root resorption limited when these structures were eliminated and histologically examined (Andreasen, 1981a, Reitan, 1951).

The innermost cellular layer including cementoblasts, fibroblasts, endothelial cells and perivascular cells were the last remaining structures to be responsible for offering protection to the root surface from resorption. The evidence for the protective effect of these cells against root resorption is provided by investigations where this cell layer is destroyed on

the root surfaces of extracted teeth by freezing or desiccation. In both instances where the cell layer was destroyed, extensive ankylosis and root resorption occurred (Andreasen, 1981b; Schwartz et al., 1988). Thus, protection of the root against resorption, based on the above observations, is apparently related to the existence of a narrow layer of vital PDL cells next to the root surface. However, which of the cells present in the mixed population residing in this layer is responsible for the anti-resorptive effect is unknown. In addition, the extent of injury required to render this layer ineffective at protecting the root from resorption is not known.

Another hypothesis is based on the role of calcium homeostasis in the susceptibility of an individual to root resorption (Engstrom, 1988; Engstrom et al., 1988; Wesselink et al., 1986). This theory was based, in part, on the studies of Engstrom (1988) who assessed the frequency of root resorption lesions in orthodontically treated rats under variable conditions of calcium homeostasis. The rats were either hypocalcemic (high bone turnover) or they were injected with bisphosphonate (low calcium turnover). The resorption lesions were decreased in the orthodontically treated rats treated with systemic injection of bisphosphonate (Engstrom, 1988). Wesselink et al.(1986), further supported these observations. A cryoprobe model was used to induce root resorption in the mandibular incisors of rats, it was observed that the rate of root resorption decreased with bisphosphonate treatment. The rate of root resorption has also

been shown to increase in rodents exposed to anti-convulsant therapy and parathyroidectomy (Robinson and Harvey, 1989). There were no detectable changes in root resorptive activity in these animals secondary to diet induced hypocalcemia (Robinson and Harvey, 1989). It is interesting to note that in association with hypocalcaemia there was a significant increase in the bone resorptive activity (Robinson et al., 1989). These results are supported by the observations of others (Midgett et al., 1981, Roberts, 1975; Goldie and King, 1984). The consensus appears to be that although parathroid hormone (PTH) increases bone resorptive activity a corresponding increase in the root resorptive activity was not observed.

The importance of the immune response in the process of root resorption has been investigated by King and Courts (1988, 1989). They have suggested two possible scenarios where root resorption may occur as a result of an immune response. One may be due to a nonspecific foreign body reaction to the damaged root tissue. Another may be due to a specific immune reaction. The evidence for the latter type of immune response is the observation that root extracts have an autoantigenic potential (King and Courts, 1989). In addition, levels of root surface antibodies follow predictable kinetics in association with traumatic root resorption and immune complexes have been found to localize on actively resorbing roots (King and Courts, 1988, 1989).

The existence of circulating autoantibodies to tooth root

antigens in both humans and dogs suggests that an immunological mechanism may play a role in root resorption but it is presently unclear if this role is active or passive (King and Courts, 1988). The difficulty in making the distinction arises because the presence of circulating autoantibodies is quite common in sera and does not necessarily denote pathology (King and Courts, 1989). It is possible that autoantibodies are suppressed by immune regulatory mechanisms. The favored mechanism for autoimmunity is that the host response system is altered by an unknown process with the resultant impairment of the individual's ability to regulate autoantibodies present in circulation (King and Courts, 1989). At present, there is no evidence linking levels of human tooth root autoantibodies to the severity of root resorption experienced by the individual. In addition, there has been no demonstration that in animal model systems, immunological modifications can change the root resorption response. However, the presence of fibrinogen and IgG in the hyaline zone of orthodontically treated teeth and the correlation of hyaline zone with root resorption tends to further support an immunological process in root resorption. Fibrinogen and IgG attract phagocytes and form a network for reorganizing the necrotic tissue in the hyalinized zone (Lindskog and Lilja, 1983).

Evidence for a role of genetics in the susceptibility of an individual to autoimmunity is available and appears to be related to the histocompatibility complex. There is evidence that root

resorption has a higher prevalence among siblings, but the mode of inheritance is undetermined (Newman, 1975). The significance of these findings is uncertain.

The presence of a factor similar to the "anti-invasive" factor isolated from cartilage tissue, has been hypothesized as playing a role in the regulation of root resorption (Lindskog and Hammerstrom, 1980; Lindskog et al., 1983). Lindskog et al. (1983) believe that PDL and cementum contain a potent collagenase inhibitor which protects the root against resorption. If severe damage to these structures occurs then the "anti-invasive" factor is, allegedly, no longer synthesized and collagenase activity is no longer controlled in the microenvironment.

The common features leading to root resorption appear to include factors such as increased local pressure in the periodontium, damage to the periodontal membrane (mechanical, chemical, thermal), increased blood supply (hyperemia associated with inflammation, infection) and individual predisposition (systemic disease, endocrine disturbance) (Rygh, 1977; Henry and Weinmann, 1951; Tronstad, 1988; Andreasen, 1988).

EXPERIMENTAL MODELS INDUCING ROOT RESORPTION

In order to further understand the process of root resorption various experimental models were developed. One of the most extensively used is the extraction-replantation model in the green Vervet monkey, introduced by Andreasen (1973). The selective desiccation of the root surface as an intermediate step

prior to replantation revealed considerable information on the response of local cell populations to the trauma of extraction, desiccation and replantation (Andreasen, 1981a,b,e). The innermost cell layer adjacent to the root surface was found not only to provide resorption protection but also repair potential (Andreasen, 1981a). A substantial injury to this cell layer allowed the "competitive healing" phenomenon to be observed. This process involved competition between two cell populations , those migrating from the neighboring bone and those cells migrating from the surrounding intact periodontal ligament and cementum. Both cell populations attempt to repair the injured root surface (Andreasen, 1981d; Rygh, 1974). The nature of the repair process was found to vary depending upon which cell population invaded the damaged root tissue first. The bone cells tended to result in ankylosis (transient or permanent) and the PDL/cementum cells tended to restore normal root and PDL morphology (Andreasen, 1981b,d,e). Other less commonly used root resorption models include the introduction of toxic agents into the pulp chamber (Cvek and Lindwall, 1985), the luxation model (Birkedal-Hausen, 1973) and the periodontal surgical injury model (Nakane and Kameyama, 1987). A freezing model has recently been developed whereby root resorption can be induced in rats by applying a cryoprobe (chilled to the temperature of liquid nitrogen) to the buccal mucosa of the mandibular incisor (Wesselink et al., 1986). Orthodontic models have been developed such that a calibrated spring applied for variable time periods

to the maxillary first molars of Wistar rats (Rygh, 1972; Williams, 1984) or the mandibular first molars of the Swiss-Webster mice (Brudvik and Rygh, unpublished, 1988; Farrell et al., 1989) will predictably and reproducibly stimulate the formation of root resorption lacunae.

The extensive research on root resorption has succeeded in elucidating histological patterns associated with the development of resorptive lesions secondary to trauma to the periodontium. However, there is insufficient evidence to conclusively support the theories presently suggested to explain the underlying mechanisms of root resorption.

ROOT RESORPTION AND ORTHODONTIC TREATMENT

Root resorption secondary to orthodontic treatment has long been recognized as an inevitable and unfortunate side effect (Rudolph, 1940; Reitan, 1947; Kvam, 1972; Rygh, 1974). At present, orthodontically induced root resorption is unpredictable and poorly understood. The original studies of root resorption induced by orthodontic treatment were limited by inconclusive results obtained from radiographic investigations. The improvement of light microscopy and the development of scanning electron microscopy (SEM) greatly contributed to our understanding of the process of orthodontic induced root resorption at the histological level. The cellular and enzymatic changes in the periodontium during orthodontic tooth movement have been described in detail. However, the factors which control the initiation and the rate of progress of root

resorption are not known. In addition, the control mechanisms which influence the prognosis for repair of these lesions remain a mystery.

(A) Radiographic Evaluation of Orthodontic Root Resorption

Ketcham was one of the first investigators to correlate root resorption with orthodontic treatment (1927). He observed 21% of 500 cases treated orthodontically had radiographic evidence of root resorption (Ketcham, 1927). These findings were further supported by those of Becks and Cowden (1932) and Hemley (1940). Rudolph (1940), during the same time period was criticized for his claim that previous reports had grossly understated the severity of root resorption associated with orthodontic treatment. The data from his study indicated an incidence of 75% radiographically detectable root resorption in patients treated orthodontically for 2 years (Rudolph, 1940). Certainly, the presumably excessive forces created by the weekly activation of the orthodontic appliances and the poorly standardized conditions under which the radiographs were evaluated contributed to the rejection of his report. Steadman (1942) concluded that up to the year of 1940 there had been essentially negligible progress in the understanding of root resorption associated with orthodontic treatment. The more recent studies on root resorption associated with orthodontic treatment tend to support 50 to 100% incidence (Massler and Malone, 1954;

DeSheilds, 1969; Hollander et al., 1980). The method of evaluating pre- and post-orthodontic treatment periapical radiographs has provided limited insight into the factors which should be considered in an attempt to assess the risk of root resorption. One observation is that maxillary incisors appear to be the teeth which are the most and the maxillary and mandibular second permanent molars the least susceptible to root resorption (Massler and Malone, 1954; Hollander et al., 1980). In addition, a variety of orthodontic appliances have been implicated as predisposing a patient to root resorption. These include intrusion arches, Class II elastics, maxillary expansion, rectangular archwires and uprighting springs (Morse, 1970; Goldson and Henrikson, 1975; Linge and Linge, 1983; DeMaraut and DeMunck, 1986; Follin et al., 1986). The root morphology, a previous history of trauma and autotransplanted teeth have all been implicated as potential factors predisposing an individual tooth to root resorption following the application of orthodontic forces (Newman, 1975; Malmgren et al., 1982; Langerstrom and Kristerson, 1986). An attempt has also been made to use radiographic assessment of the dentition as a means by which the severity of root resorption may be predicted (Levander and Malmgren, 1988). In this study a positive correlation was found between the incidence of root resorption detected within the first 9 months of orthodontic treatment and the severity of root

resorption at the end of the active phase of orthodontic treatment (Levander and Malmgren, 1988). Radiographic evaluation of post-treatment subjects revealed that the prognosis for healing of root resorption defects was good when the orthodontic forces were removed. Root surface lesions were no longer evident following termination of the active phase of orthodontic treatment, provided the retainers were not active upon insertion (Copeland and Green, 1986; Sharpe et al., 1987).

One of the most common criticisms of data generated from he studies using periapical radiographs is that the technique for obtaining the radiographs was not standardized. This results in enlargement and projection errors which limit the value of the comparison of pre-treatment and post-treatment radiographs (Goldson and Henrikson, 1974; Williams, 1984; Chapnick, 1989). In addition, only extensive root resorption lesions large enough to be detected radiographically would be assessed. Thus, root resorption evaluated on periapical radiographs focused mainly on apical "blunting" and cervical lesions which are less common and a more severe form of this phenomenon (Barber and Sims, 1981; Chapnick, 1989). Insurmountable, unexplainable problems with observer error has created even more skepticism regarding the value of radiographic assessment of root resorption. This problem was demonstrated in a recent investigation where root

resorption-like lesions were cut into the root surfaces of extracted cadaver teeth which were then radiographed and examined (Chapnick, 1989). The trained observers examining the radiographs repeatedly reported root resorption lesions which did not exist (Chapnick, 1989). Finally, interpretation and comparison of data generated from radiographic studies is complicated by various appliances used on subjects of different species, age groups, sexes and for different time periods. In spite of the list of limitations of radiographic studies, these investigations did confirm extensive root resorption to occur under a variety of orthodontic conditions.

(B) <u>Light Microscopy and SEM Evaluation of Orthodontic Root Resorption</u>

The limitations of radiographic evaluation of orthodontically induced root resorption led to a series of investigations using light and electron microscopy (Stuteville, 1937; Gottleib, 1961; Kvam, 1972; Reitan, 1947, 1951, 1974; Rygh, 1972, 1977). A classic assumption was that shallow root resorption lacunae secondary to orthodontic force application would heal readily upon removal of the stimulus leaving a scar on the root surface (Steadman, 1942). Early histology reports claimed that orthodontic root resorption, other than apical root loss, was transitory with very little, if any long term detrimental side effects on the health or function of the

periodontal tooth attachment (Henry and Weinmann,1951; Orban, 1928). Unfortunately, these claims were made without sufficient evidence.

The histological findings of Reitan (1947, 1951, 1974), Kvam (1972) and Rygh (1972, 1974, 1977) provided tangible evidence for the sequela of orthodontic induced root resorption. There is general agreement that following orthodontic force application predictable findings are evident at the level of light microscopy. The compression zone was associated with root resorption and the tension zone showed cemental apposition (Reitan, 1974; Kvam, 1972; Rygh, 1977; Follin et al., 1986). In rats, local cell free zones appear in 6 to 12 hours after the initiation of force application, while extensive hyalinization was seen at 2 to 3 days (Rygh, 1977). The PDL was compressed by 15 hours and was readily distinguished from adjacent healthy PDL which were rich in invading blood vessels (Rygh, 1977). The zone of reorganization was wider than PDL of control animals. Osteoclasts were frequently found in the adjacent alveolar bone.

Multinucleated cells were occasionally observed near the cementum surface, not adjacent to the hyalinized zone. These cells could resorb both cementum and dentin (Rygh, 1977). Resorption lacunae were often bordered by active resorbing osteoclasts and the cementum layer once penetrated was resorbed from the dentin side. This was

described as undermining root resorption and was considered to provide further evidence for the resistance of cementum to resorption (Rygh, 1977). There was an increase in blood vessels and cells. The blood vessels in the lacunae were in close contact with the adjacent odontoclasts. The light microscopy studies revealed that simultaneous with the elimination of the zone of hyalinization, resorption of cementum took place (Stuteville, 1937; Gottleib, 1961; Kvam, 1972). A clear pattern was demonstrated in that the resorptive process in cementum continued beyond the period of elimination of hyalinization (Rygh, 1977). The resorption of cementum was shown to occur on the internal surface of the cementum adjacent to the dentin (undermining). This was assumed at the time to be evidence that the deeper cemental layers were more readily resorbed than the periodontal ligament side of the cemental layer. A question still remains as to the exact mechanism of initial penetration of the cemental layer. The answer to this question may explain the difference in susceptibility of bone, which is readily remodelled and cementum, which appears to be more resistant to resorption.

The compression zone was associated with root resorption and the tension zone showed cemental deposition (Rygh, 1977; Follin et al., 1986).

A detailed time study of root resorption induced through the application of orthodontic forces to the

maxillary first molar in rats demonstrated the chronological sequence of events under these conditions (Williams, 1984). Local areas of root resorption were observed on control teeth similar to other investigations in both rodent and human species (Kvam, 1972, Henry and Weinmann, 1951). The observation of physiological movement of rat teeth was described by Sicher and Weinmann (1944), as distal tipping of the molar crowns and mesial bone resorption. This was confirmed by the mesial bone resorption in the maxillary molars of control rats (Williams, 1984). The mesial tipping of the molar crown due to the orthodontic appliance creates a reversal line indicating that there has been a change in the pattern of tooth movement (Williams, 1984).

Local PDL compression occurs by 2 to 3 days with subsequent surface resorption of the roots. Cemental apposition was shown to occur on the mesial root surface and root resorption lesions were present on the more coronal portion of the distal root. Root resorption lesions present at 2 days of force application appeared to correlate with the zone of hyalinization, as suggested by previous studies (Rygh, 1974, 1977; Reitan, 1974). The observation after 5 days of force application of zone of hyalinized tissue was eliminated and cemental apposition was evident on the apicies of the orthodontically treated molars. The cemental apposition occurring at these apices was thought to be due

to continued eruption or an extrusive component of the force system applied. Areas suggestive of partial hyalinization of the PDL on the distal root, at later stages of tooth movement suggested further tipping of the molar. A dramatic increase in the resorptive lacunae size continued up to 13 days of force application when these lesions appeared to fuse into a single resorptive surface on the distal root surface. This lesion progressed apically with continued application of a tipping force (Williams, 1984). Apposition of dentin on the pulpal wall was interpreted as an attempt to maintain root thickness, and has also been observed in humans (Reitan, 1974). A decrease in the length of the distal root and of the resorptive lesion were observed after 21 days of force application. The increase in coronal resorption by 28 days of force application was also observed. Resorption on the apex of the mesial root was considered to be evidence for a new phase in the tipping process. Resorptive activity on the mesial cemental surface after 13 and 21 days of force application suggest that root resorption can occur in association with tension (Williams, 1984). The resistance of predentin to resorption is demonstrated by the persistance of a zone of predentin after the loss of dentin and cementum after 21 days of force application (Williams, 1984).

The histological characteristics of the vascular bed in periodontal tissues in rats has been studied using a methylmethacrylate perfusion technique (Sims and Weeks, 1985). It was observed that rat molars demonstrated rich anastomoses between ligament vessels and those with the bony medulla. The interseptal region showed that terminal arterioles from within the medulla enlarged as they entered the ligament to become post-capillary venules, oriented occluso-apically (Sims and Weeks, 1985). A richer blood supply was observed in the mesial and distal aspects of the ligament. The crest of the inter-radicular septum had a very dense plexus of relatively large venous vessels, in both the alveolus and the ligament (Sims and Weeks, 1985). The venous-venous anastomoses in the ligament at the crest of the inter-radicular bone of rats were similar to the findings of Wong (1983) in mice.

The significance of the vascular arrangement has been associated with the metabolic requirements of the periodontal ligament, which are thought to be greater than for other types of connective tissue (Sodek, 1977). The vascular architecture has also been implicated to play a major role in the dissipation of occlusal forces through the peridontium (Melcher and Walker, 1976; Willes, 1976; Ng, 1981). The distribution pattern of the vascular loops mirrors patterns of both cementum formation and tooth resorption. It has therefore, been suggested that a

relationship exists between the changing patterns of vascular architecture and the resorption and repair process of bone and root and the remodelling of the PDL (Sims and Weeks, 1985). This relationship is presently unknown.

Kvam (1972) using the scanning electron microscope, did confirm that root resorption of varying degrees was an inevitable side effect of orthodontic treatment. Small root resorption lacunae were detected on all human premolars studied following 5 days of orthodontic force application. The extent of root resorption became more involved when force application was extended for up to 25 days (Kvam, 1972). The resorption lacunae were larger, more numerous and penetrated the dentin (Kvam, 1972). The resorption lesions were predictably associated with zones of hyalinization in the periodontal membrane secondary to orthodontic force application. This observation was also supported by the work of Reitan (1951), who confirmed that apical resorption started adjacent to the zone of hyalinization. The resorption lacunae have been identified adjacent to the hyalinized zone and in areas where the hyalinized tissue has been removed and the PDL has been re-established. In addition, simultaneous with removal of hyalinized tissue, resorption of the cementum took place (Reitan 1947, 1951, 1974). The fact that root resorption takes place adjacent to the zone of hyalinization lead to the hypothesis that the biological process of root

resorption may be mediated by cells from the adjacent healthy PDL and that this may be why the resorption appears to occur behind the compressed, acellular parts of the ligament (Rygh, 1977). In rats, the hyalinized zone disappeared concomitantly with the invasion of cells and blood vessels from the neighboring PDL and the removal of collagen, cellular remnants and degraded vascular elements was seen under SEM to be mediated by various forms of cellular activity (Rygh, 1974). Fibrous material was discerned in scattered areas both on the cementum and on the alveolar bone side in specimens where the invading capillaries occupied the entire space between the root and the bone (Rygh, 1974). The odontoclasts had an intimate relationship with capillaries on the non-resorbing surface. The impression was that only a thin membrane separated the lumen of the blood vessels from the cell cytoplasm (Rygh, 1977). The odontoclasts on the actively resorbing surface showed numerous mitochondria, vacuoles and a complex system of folds and clefts resembling the ruffled border of the osteoclast (Rygh, 1977). In human specimens, the breakdown of collagen fibrils occurred in front of and around the processes of invading cells. In general, the cell processes were surrounded by a light zone containing collagen. On the cementum side the projecting cytoplasmic processes of the first invading cells seemed to cut off the hyalinized

fibrils which appeared to be in a process of degeneration, leaving a naked tooth surface (Rygh, 1977).

Reitan (1951) found that root resorption was a complex process involving both resorption and repair. The pattern of repair cementum and PDL fiber processes have since been described in detail (Barber and Sims, 1981; Sims and Weeks, 1985; Langford, 1982). The PDL fibers appear to fuse and enter the organic matrix of repair cellular cementum, therefore, providing a continuous attachment during the repair process of resorption lacunae. These fibers did not have the same appearance as normal Sharpey's fiber insertions, but appeared as adhesions (Sims and Weeks, 1985; Langford, 1982). The observation that very few Sharpey fiber depressions were present in the advancing mineral front of repair cellular cementum strongly suggests that the elimination of the root resorption defect is not synonymous with reattachment of periodontal fibers (Barber and Sims, 1981). The majority of the specimens observed indicated that few principal fiber attachments had occurred and that normal root surface contours were not re-established (Barber and Sims, 1981, Langford, 1982). The possibility that this may occur at a time beyond the 9 month period of study was not ruled out. There was considerable evidence of cellular cementum deposition within the resorption defects following cessation of active resorption (Barber and Sims, 1981; Langford, 1982; Harry and Sims, 1985).

The investigations of Harry and Sims (1982) and King and Fischlschweiger (1982) have suggested a correlation of the severity of root resorption with the magnitude and duration of orthodontic force application. Harry and Sims (1982) indicated that SEM findings of resorption lacunae correlated more with duration than with magnitude of force application.

The effect of orthodontic forces is initially to compress the PDL and induce local cell perturbation. Davidovitch has attempted to quantify intracellular messengers, including cyclic AMP and cyclic GMP, which are thought to be produced secondarily to cell perturbation (Davidovitch and Montgomery, 1976; Davidovitch et al., 1984). The immunohistochemical technique applied indicated that both of these substances increased in response to orthodontic force application. The exact biological significance of these results remains uncertain. The pressure zone created by an orthodontic force eventually leads to the formation of hyalinized tissue. This process is characterized by cessation of circulation and degenerative changes in the PDL (Rygh, 1977). Local activation and differentiation of cells in the alveolar bone or cementum may also occur as a result of compressed blood vessels and circulatory changes in the microenvironment due to orthodontic force application. The associated hyperemia may increase the oxygen tension which increases osteoclast

activity. Piezo-electricity, a change in distribution and charge of electric potential, may occur due to bone bending under the influence of orthodontic force application. This process may stimulate remodelling in the periodontium. Electrical stimulation has also been shown to increase cyclic AMP and cyclic GMP production. The elimination of the hyalinized zone is achieved by foreign body reaction cells including macrophages which remove blood components and debris from the affected area (Rygh, 1977; Kvam, 1972; Reitan, 1974). These observations were further supported by enzyme assays used to evaluate further the biological process of orthodontic induced root resorption. The hyalinized zone which forms in the compression zone of the PDL following orthodontic force application was found to be devoid of specific enzyme activity, lactate dehydrogenase and acid phosphatase, indicating that when only cellular remnants and not vital cells were present, aseptic necrosis occurred (Lilja et al., 1983; Lindskog and Lilja, 1983). The necrotic tissue presumably stimulates a chemotactic response from the vital PDL at the periphery of the hyalinized zone (Lilja et al., 1983; Rygh, 1977). The migrating macrophages from vital adjacent PDL were primarily responsible for the elimination of the hyalinized zone and the enzymatic changes which were observed were assumed to be due to the influence of the immediate cellular microenvironment (Lilja et al., 1983; Lindskog and Lilja,

1983). There was an increase in prostaglandin synthetase activity, associated with an increase in the secretion of prostaglandins secondary to hyalinized zone degradation (Lilja et al.,1983; Lindskog and Lilja, 1983). The activity of arylsulfatase and aminopeptidase, which are also considered to be markers of macrophage activity, was found to increase in response to elimination of the hyalinized zone (Lilja et al., 1983; Lindskog and Lilja, 1983).

In summary, regardless of the stimulating mechanism, the primary breakthrough of the cemental layer occurs following the elimination of the hyalinization zone of the PDL (Rygh, 1977; Reitan, 1974). The adjacent mature layer of collagen also disappears with the resolution of the hyalinized zone (Rygh, 1977; Kvam, 1972; Reitan, 1974). Root resorption associated with orthodontic tooth movement which ensues following elimination of the hyalinized tissue will continue beyond the resolution period of hyalinization (Rygh, 1977). It has also been observed that root resorptive activity continues after the removal of the stimulus (Sims and Barber, 1981; Harry and Sims, 1982).

Further development of the root resorption lacunae, once formed, may follow a very different course depending on ensuing events. If the orthodontic force is continually applied then the resorption process continues with the lacunae increasing in both size and number with time (Rygh, 1977; Williams, 1984). Once established the resorption of

dentin proceeds more quickly than that of the cementum, presumably because there no longer are cellular barriers present (Rygh, 1977). If, on the other hand, the orthodontic force is removed or decreased below a threshold level then the resorption lacunae repair and fill with cementum. The exact mechanism through which this process is regulated remains unclear.

THE ODONTOCLAST- ULTRASTRUCTURE, ORIGIN AND BEHAVIOUR

(A) Ultrastructure

The odontoclast is a member of the "clast" family of hard tissue resorbing cells which also includes the osteoclast and the chondroclast (Lindskog et al., 1988). These cells are morphologically and histologically very similar and are differentiated in vivo primarily on their substrate for resorption (Lucht, 1972; Lindskog et al., 1988). The remarkable similarity of the members of the "clast" family strongly suggests a common mechanism of resorbing hard tissue in vivo. The ultrastructure of the "clast" cells will vary with the stage of resorptive activity and the proximity of the cell to the mineralized surface (Lindskog et al., 1988). The specialized ruffled border-clear zone complex is not apparent in inactive "clast" cells and the intracellular organelles are more localized near the ruffled border in the actively resorbing cells (Lucht, 1972; Miller, 1977; Lucht, 1973). Scanning electron microscopy studies of osteoclasts in vivo (Jones

and Boyde, 1972) and in vitro (Testa et al., 1981; Osdoby et al., 1982; Zambonin-Zallone et al., 1982) fail to demonstrate a relationship between cell morphology, degree of resorptive cell spreading and the stage of resorptive activity.

The inactive odontoclasts appear to have a flat profile and an unusually wide clear zone adjacent to the dentin surface (Sasaki et al., 1989). The active odontoclasts located in the resorption lacunae have well-developed ruffled borders against the dentin surfaces (Sas aki et al., 1989, 1990). Odontoclasts extend cellular processes of the ruffled border into some dentinal tubules that open into the lacunae. It has been observed that the clear zone, characteristic of the osteoclast, does not always appear in the odontoclast (Sasaki et al., 1989; Lindskog, et al., 1988; Jones and Boyde, 1972). The cytoplasm between the nuclei and the ruffled border was occupied by numerous pale endocytic vacuoles of various sizes. There was evidence that some of the vacuoles had fused to form very large ones and some of the vacuoles contained amorphous material. The cytoplasm was rich in mitochondria and rough endoplasmic reticulum (Sasaki et al., 1989). There were numerous Golgi bodies with saccules and related vesicles, and multivesicular bodies were located within the perinuclear cytoplasm (Sasaki et al., 1989). Evidence of collagen fibers were found at the surface of the resorbing dentin and

although these fibers were within the ruffles of the membranes, the odontoclasts did not appear to resorb them (Sasaki et al., 1989). The shallow resorption lacunae were not occupied by odontoclasts but rather by mononuclear fibroblasts. Apatite crystals were observed in the extracellular channels of the ruffled border and within the adjacent, pale endocytic vacuoles of the odontoclasts located in the resorption lacunae (Sasaki et al., 1989). The membrane-bound lysosomal bodies in the perinuclear cytoplasm close to the Golgi region within the odontoclasts showed both apatite crystal inclusions and dense amorphous material. The resorbing dentin facing the active odontoclast is thought to be in various stages of demineralization. This is indicated by spectral analysis of the mineral density in various areas of dentin. It is possible that the odontoclast causes a partial demineralization of dentin at the resorption surfaces which results in the release of apatite crystals from the matrix for further dissolution or resorption (Sasaki et al., 1989). Activity of the enzyme, acid trimetaphosphatase, was demonstrated along the surface of resorbing dentin and the inner walls of the dentinal tubules facing the odontoclast ruffled border. The tartrate resistant, vanadate sensitive acid adenosine triphosphatase (ATPase) has been identified in association with the resorptive cell membrane (Andersson et al., 1984, 1986; Andersson and Marks, 1989). The

appearance of reaction precipitates at extracellular sites is thought to indicate a secretion of acid phosphatases and/or proteases from the odontoclastic ruffled border onto the resorptive surface. In addition, another enzyme, tartrate resistant acid phosphatase (TRAP) has been followed as a cytochemical marker for "clast" cells (Minkin, 1982; Mostafa et al., 1982; Chappard et al., 1983). TRAP and other proteases are found to be active at a pH of 4, in vitro. If this holds true in vivo, then odontoclasts must create an acidic microenvironment. The exact mechanism is unknown but the presence of loosely arranged apatite crystals at the resorbing surface of dentin makes it probable that odontoclasts produce an acidic pH at the resorbing site. The hydrolytic enzymes are not required to dissolve the apatite crystals. The crystals, therefore, are presumably removed by secretion of acids by odontoclasts (Sasaki et al., 1989). This is a feasible hypothesis as a proton pump mechanism has been demonstrated in osteoclasts. Carbonic anhydrase isozyme II (CAII) catalyses the conversion of cellular carbon dioxide and water into bicarbonate and hydrogen (Marks and Popoff, 1988). The products of the reaction are thought to be translocated across the membrane through the membrane-bound ATPase (Baron et al., 1986; Akisaka and Gay, 1986) into the confined space beneath the ruffled border. The presumed arrangement of the CAII and the proton pump of the ruffled border is that the

CAII is on the cytoplasmic surface of the membrane (Andersson et al., 1982) and the proton pump is believed to be within the membrane itself (Baron et al., 1986; Akisaka and Gay, 1986). The osteoclast, through this mechanism, can acidify the microenvironment by the production of hydrogen ions. The isozyme CAII has been shown to be membrane bound (Gay et al., 1983; Marie and Hott, 1987). The role for CAII in bone resorption has been supported by the observation that CAII inhibitors reduce bone resorption (Minkin and Jennings, 1972), that calcitonin, a direct inhibitor of osteoclast activity, also decreases the CAII activity (Anderson et al., 1982) and that congenital absence of CAII causes a syndrome of renal tubular acidosis and osteopetrosis (a disease which results from reduced bone resorption (Sly et al., 1983). The acidic environment is also thought to facilitate demineralization and itself has been shown to stimulate cell-mediated bone resorption in vitro (Arnett and Dempster, 1986). It has been hypothesized that odontoclasts may mediate extracellular degradation of the dentin surface and subsequently resorb and dissolve liberated apatite crystals and material that accompanies them.

The ultrastructural characteristics of the odontoclast which differ from those of the other members of the "clast" family include fewer and smaller nuclei and the absence of a clear zone in some of the odontoclasts observed (Nilson,

1977; Hammerstrom and Lindskog, 1985). These observations must be interpreted in light of the possibility of artefacts secondary to the desiccating procedures required for EM specimen preparation (Hammerstrom and Lindskog, 1985). In addition, it is possible that odontoclasts observed without a clear zone may be in an early phase of activation where the clear zone has not yet formed or the odontoclast may have passed beyond the actively resorbing stage.

(B) Origin

The origin of the odontoclast has not been documented to date. However, there is considerable research on the origin of the osteoclast and this subject has been recently reviewed by Huffer (1988) and Marks and Popoff (1988). If the assumption that the osteoclast and odontoclast are the same cell is true, then these cells should also have the same pathway of origin.

An extraskeletal source of osteoclasts is presently accepted as osteoclast progenitor cells are thought to be generated from stem cells of hemopoietic origin (Chambers, 1980; Marks and Popoff, 1988, Scheven et al., 1986). These stem cells have an extensive self-maintaining capacity and are capable of giving rise to several subpopulations of progenitor cells (Vaes, 1988; Marks and Popoff, 1988; Scheven et al., 1986). The progenitors are disseminated via the blood stream. These progenitor cells can proliferate but unlike the stem cells they are not self-maintaining and

are more restricted in their differentiation (Marks and Popoff, 1988). The generation of pre-osteoclasts and osteoclast cell populations occurs peripherally in bone. The pre-osteoclast or precursor cell represents a later stage of differentiation, whereby the cell acquires some recognizable morphological and cytochemical features of the fully differentiated osteoclast cell (Marks and Popoff, 1988). Although an extraskeletal source of the osteoclast is accepted there is little agreement on the cell identity of the osteoclast progenitors (Chambers, 1980). It was first suggested that the osteoclast developed directly from the fusion of pre-existing monocytes and macrophages. However, the monocyte/macrophage cell line appear to be unlikely candidates for the direct origin of osteoclast progenitors (Vaes, 1988; Marks and Popoff, 1988; Minkin, 1986). The macrophage and the osteoclast do have histological feature in common but there is no direct evidence that the macrophage can become an osteoclast (Minkin, 1986). The macrophage does not show the characteristic ruffled border-clear zone complex of the osteoclast (Vaes, 1988). The contemporary evidence for the origin of the osteoclast suggests that osteoclast progenitors are mononuclear cells that arise in hemopoietic tissues and travel to the bone microenvironment where they fuse to form osteoclasts (Vaes, 1988; Marks and Popoff, 1988). Whether the progenitor diverges from the

monocyte/macrophage lineage at an early stage of differentiation or represents a lineage which is entirely separate from the monocyte/macrophage cells is presently unknown (Vaes, 1988; Marks and Popoff, 1988; Minkin, 1986).

The development of the pre-osteoclast has been followed using the tartrate resistant acid phosphatase (TRAP) enzyme activity as a cytochemical marker. The osteoclast progenitor cells present in hemopoietic tissues and disseminated through the blood stream to peripheral locations in the bone do not stain positive for TRAP (Scheven et al., 1986). In addition, the actively dividing osteoclast progenitors in the bone produce post-mitotic precursor cells which also do not stain positive for TRAP. It is only the immediate presursor cells, the mononuclear pre-osteoclasts, and multinucleated osteoclasts which stain positive for TRAP (Scheven et al., 1986). Thus, a TRAP negative post-mitotic mononuclear cell appears to precede the development of the TRAP positive, mononuclear, immediate precursor of the osteoclast (the pre-osteoclast). It is also of interest that the monocyte/macrophage cell line produces an acid phosphatase enzyme which is tartrate sensitive (Vaes, 1988). This has been considered further evidence that the osteoclast and its immediate precursors represent a different cell line from the monocyte/macrophage cells (Vaes, 1988).

A large number of factors, both systemic and local, have been identified to regulate the hemopoietic stem cell differentiation and influence the osteoclast development. These factors and their interactions have been reviewed by Huffer (1988); Marks and Popoff (1988) and Vaes (1988).

(C) Behaviour

Osteoclasts are capable of resorbing all types of calcified dental and skeletal tissue *in vivo* and *in vitro* (Jones and Boyde, 1988). Functionally the odontoclast and the osteoclast are considered to be the same cell (Ten Cate and Anderson, 1986; Boyde *et al.*, 1984). Demineralization occurs by proton production, with acidification of the ruffled border zone. Cysteine proteases play a role in the removal of the organic matrices. The resorption rate and the size and shape of the lacunae produced by the osteoclast reflect the composition and the structural organization of the dental or skeletal tissue (Jones and Boyde, 1988). Bone resorption is a normal phase of physiological bone remodelling, similarly, root resorption is physiological in relation to the timely exfoliation of the primary dentition (Jones and Boyde, 1988). However, clinically detectable root resorption of the permanent dentition is pathological (Massler and Malone, 1954). This observation has led to the hypothesis that root tissue resists resorption and through some unknown mechanism is protected from it.

There is no reason to believe that the odontoclast is a different cell than the osteoclast (Ten Cate and Anderson, 1986; Hammerstrom and Lindskog, 1985; Jones and Boyde, 1984). Cells identified as resorptive, lodged in Howship's lacunae in enamel, dentin or cementum have the same cytological features characteristic of osteoclasts (Yaeger and Krauncunas, 1969; Ten Cate and Anderson, 1986). Odontoclasts are polarized with respect to dental tissue and they have a ruffled border with an annular clear zone that is closely adherent to the hard tissue matrix. They are usually multinucleated and contain numerous mitochondria and lysosomes. The odontoclast can resorb enamel, dentin and cementum. Root resorption lacunae have been observed to span all three types of dental tissues and the same odontoclast may be actively resorbing different tissues simultaneously (Wesselink et al., 1986).

Odontoclasts have not been harvested in sufficient numbers to test their behaviour in vitro. Osteoclasts, however, may be removed from bone and seeded onto other substrates for cell culture (Zambonin-Zallone et al, 1984; Chambers et al., 1984; Boyde et al., 1984; Jones and Boyde, 1988). If one accepts the definition of an odontoclast as a eukaryotic cell capable of resorbing mineralized dental tissues, then osteoclasts readily become odontoclasts in vitro (Boyde et al., 1984; Jones and Boyde, 1988).

At the level of the clear zone-ruffled border complex production of acid occurs by production of protons. The migrating osteoclast leaves an acid etched trail in the surface of the mineralized tissues. This pattern is similar on dental tissues resorbed by osteoclasts *in vitro* and by odontoclasts *in vivo* (Jones and Boyde, 1988). Local variations in the degree of mineralization (which affects the depth of demineralization) the collagen orientation and packing density (affecting access to the similarly oriented crystals) determines the surface relief seen in dentin or cementum subjacent to the resorptive cell (Jones and Boyde, 1988). Peritubular dentin shows greater resistance to resorption than intertubular dentin (Lester and Boyde, 1967). Ruffled border-like extracellular phagolysosomes digests organic matrix of the tissue being resorbed (Baron, 1985). This has been further substantiated by the observations of stained tracts left behind as red stained osteoclasts perform the secretion of a material which is bound to the substrate (Boyde *et al.*, 1983).

Neutral collagenase in osteoclasts has not been demonstrated (Sakamoto and Sakamoto, 1984a,b). In addition, collagenase inhibitors do not decrease the size of root resorption lacunae formed in dentin *in vitro* (Delaisse *et al.*, 1987). Thus, if collagenase does play a role in resorption of dentin and cementum it is not of odontoclast/osteoclastic origin. Perhaps it is of

odontoblast or cementoblast origin, as has been observed in osteoblast cells (Chambers et al., 1984). Proteases and gelatinases are thought to be secreted on to the hard tissue surface (Delaisse et al., 1987; Blair et al., 1986). Inhibitors of cysteine proteases inhibit resorption and some cathepsins have optimal activity at pH levels found beneath the osteoclast and presumably the odontoclast (Etherington and Birkdhal-Hansen, 1987; Silver et al., 1988).

The possibility of "helper" cells which act to destroy demineralized organic matrix has been suggested but the narrowness of the collagen fringe which lines the resorption bay below the ruffled border of the resorbing osteoclast in vitro casts doubt on this hypothesis (Heersche, 1978; Melcher, 1989). An ultrastructure study of dentin and cementum resorption did not support the need for a "helper" cell population for the removal of collagenous material (Ten Cate and Anderson, 1986). Boyde and Jones (1985) believe that many cells observed on the surface of resorbed bone before repair represent osteocytes released during the resorption phase. Thus, it appears that odontoclasts have the ability to dissolve or denature all components of the cementum and dentin. The mechanism through which this process is achieved remains unknown..

The fundamental step in the complex process of root resorption is the initiation of this phenomenon. The degree of severity of injury required to stimulate the root

resorptive process and the controlling factors which allow resorption to continue are not known. Several levels of root surface protection have been studied in an attempt to determine which component(s) of the root surface are responsible for its resistance to resorption.

(1) Epithelial Cells

Epithelial cells may restrict access of the osteoclast to the root tissues (Spouge, 1980). The epithelial root sheath of Hertwig protects the initial layer of dentin, during dentin development. After the induction of odontoblasts and subsequent dentin formation, the epithelial cell layer degenerates enabling cementoblast differentiation adjacent to the dentin. It is hypothesized that this layer by forming an epithelial cell network, may hold off blood vessels such that the blood-borne osteoclast can not reach the vicinity of the developing dentin and later cementum (Jones and Boyde, 1988).

(2) Sharpey's Fibers

The layer of Sharpey's fibers inserting into the layer of cementoblasts has been suggested to provide a physical barrier for osteoclasts or their precursors. The percent of extrinsic fibre concentration varies with the type of cementum (Jones and Boyde, 1988). These extrinsic soft tissue fibers and their associated

noncollagenous proteins may lack recognition signals for the osteoclast. Where fibers are more widely spaced there may be an increase in tissue turnover (Jones and Boyde, 1988).

(3) Unmineralized Matrix

Unlike ameloblasts, odontoblasts and cementoblasts resemble osteoblasts in that they are separated from the mineralized tissue by a layer of unmineralized predentin or precementum. Odontoblasts form a continuous layer held together by a terminal bar apparatus at their secretory poles. Capillaries weave between the odontoblasts but do not traverse the specialized cell junctions (Boyde et al., 1978; Bishop, 1985). In order for root resorption to proceed it is hypothesized that unmineralized matrix is removed or the unmineralized tissue is eventually mineralized (Jones and Boyde, 1988). Chambers (1984) has suggested that osteoblasts secrete collagenase to remove the unmineralized portion of the bone thereby exposing the mineralized surface to attract the osteoclast and stimulate them to resorb. A role for odontoblasts and cementoblasts in the regulation of root resorptive cell activity is possible but has not yet been investigated.

The unmineralized connective tissue matrix of predentin has been demonstrated to be more resistant to resorption than bone or dentin (Emslie, 1978; Reitan,

1974; George, 1986). The sparing of predentin during apical resorption of orthodontically treated teeth (Reitan, 1974) or the resorption from the cemental aspect of buried teeth (Emslie, 1978) may be explained by the persistence of active odontoblasts in laying down predentin at the same rate at which the resorption is proceeding.

Growth factor bonding properties of heparin sulfate proteoglycans (HSPG) may be a mechanism for binding newly recruited osteoclasts on bone and of osteoblasts on vacated resorption lacunae (Jones and Boyde, 1988). The resistance of predentin, precementum or osteoid would then be explained by a lack of the needed HSPG and bound factors on the surface (Jones and Boyde, 1988). Resorption may still extend marginally into unmineralized tissues until all contact with mineralized matrix has been lost. The total, partial or negligible loss of HSPG-linked factors secondary to different mechanisms of demineralization may explain conflicting results on in vitro resorption of demineralized bone (Zoldos and Heersche, 1988) and dentin (Jones et al., 1984, 1985) matrices. Remineralization of demineralized matrix occurring both in vivo and in vitro should be considered (Linden, 1975).

(4) Cellular Network

The concept of a cellular network of osteocytes acting as strain gauges for bone, identifying regions where resorption should take place and relaying this message to osteoblasts which control resorption may apply to dental tissues. It is presently uncertain how this would occur (Lanyon, 1984).

Strain is said to be recognized in the first instance as a change in the polarization of the glycosaminoglycans (GAG's) of the bone matrix. This is transduced through an unknown system to cellular recognition. Frost (1988) proposed that the mechanical transducer could be interstitial fluid flow caused by tissue strains.

Cellular cementum is similar to bundle bone and cementocytes could operate in the same fashion as osteocytes. The vitality of cementocytes deep within the cementum is questionable but not so for those closer to the surface and for the cementoblasts. Dentin, however, does not incorporate the odontoblast cell bodies, only the long Tomes processes that extend to the cementum-dentin surface. Some of the tubules in which the terminal branches of the odontoblast process lie may achieve continuity with the canaliculi of cementocyte lacunae but continued intercellular contact has not been demonstrated (Jones and Boyde,

1988). In addition, osteoblasts and cementoblasts appear to differ in their response to systemic bone resorbing factors such as parathyroid hormone (PTH). The osteoblast responds to PTH and allows an ingress of osteoclasts by changing its cell shape and by decreased adhesion (Jones and Boyde, 1978; Rodan and Martin, 1981). Cementoblasts do not respond to PTH in this way (Lindskog et al., 1987). These data must be interpreted in light of the high potential for artefacts secondary to the drying process required to prepare the specimens for scanning electron microscopy.

(5) Organic Fractions

Another hypothesis suggests that the difference in the potential for resorption in root and bone may rest in the organic fractions of these tissues (Jones and Boyde, 1988). The mineral phases of dentin, cementum and bone are quite similar. Although the mineral content does vary between and within tissues it is not to the extent that marked inhibition of resorption would be expected as a consequence. Osteoclasts cultured on bone do not appear to select bone of greater mineral content and they do not appear to cross from osteons of different mineral density (Reid, 1986).

The collagen in mineralized root tissues is type I, as it is in normal bone, but this might differ from that of bone with respect to the associated

proteoglycans or in the crosslinking patterns. The collagen of cementum has two origins: part of it is derived from the secretory activity of the cementoblasts and part of it is from the fibroblasts of the PDL. The turnover rate of ligament collagen is high and may continue to be high up to the stage where incorporation of the extrinsic fibers into the cementum occurs (DePorter and Ten Cate, 1980). Perhaps these fibers comprise some of the fibrils which are also of the cementoblast origin. It is uncertain the extent to which the collagen of cellular cementum and of bundle bone of the cribiform plate of the tooth socket in fact differ (DePorter and Ten Cate, 1980; Jones and Boyde, 1988).

The noncollagenous proteins of radicular hard tissues are suspected of rendering these tissues resistant to resorption. They are also the fraction about which the least knowledge is presently available.

Root resorption models using extracted teeth with cavities cut into their root structure prior to replantation show that root resorption occurs in the area of the cavity and that this is often followed by ankylosis. Thus, if access to mineralized dental tissues is allowed, osteoclasts are not deterred from actively resorbing these tissues merely by the composition. Root resorption is generally superficial

and shallow and Howship's lacunae are rapidly repaired with cementum. The pattern of resorption on the root surface is generally similar to that observed on enamel or in many sites on periosteal or endosteal surfaces of bone (Jones, 1987; Reid, 1987). The observations of resorptive patterns in primary teeth, where the odontoclasts are operating at once to cut back a whole root surface has morphological features in common with resorptive fields on bone during growth remodelling (Jones and Boyde, 1988). In these situations the continued stimulation of osteoclasts by cytokines produced by cells in the microenvironment, such as macrophages or lymphocytes, should be studied to improve our understanding of the regulation of osteoclasts activity by various factors present in the local microenvironment.

The repopulation of a resorbed surface by a sheet of osteoblasts or cementoblasts excludes osteo/odontoclasts. Thus, a layer of these cells may provide protection for the root against further resorption. Jones and Boyde (1988) have suggested that perhaps it is a lack of competing cell populations which leads to idiopathic root resorption.

(6) Vasculature

The importance of the vasculature in the presence of transitory or persistent resorption of teeth is not known. Fibroblast growth factor (FGF) is in high concentration in calcified connective tissue, beta-FGF is prevalent in bone and is a potent angiogenic factor. It is also produced by endothelial cells. Capillaries which are the vehicle for the new supply of osteoclast precursors and the removal of "spoil" resulting from excavations by existing active osteoclasts are stimulated by release of FGF by resorption. Deporter and Ten Cate (1980) reported that the sac of granulation tissue at the apex of resorbing primary teeth comprises a zone high in blood vessels next to the osteoclasts. A relative abundance of blood vessels near the bone rather than the tooth side of the PDL has often been given as the reason for more rapid resorption of bone (Sims and Weeks, 1985). Rygh, (1977), also drew attention to the proximity of capillaries to odontoclasts.

(7) Unknown Substances

The possibility exists whereby undetermined substances which may modify the activity of the resorbing cell may be released from the resorbing root tissues. Released substance may be latent enzymes which can facilitate resorption or perhaps be factors

such as transforming growth factor (TGF) that may limit progress either by inhibiting resorption or by promoting formation (Mundy and Roodman, 1987). At present, this is speculation as there is no evidence that osteoclast or odontoclast activity is regulated in this way. Both bone and dentin contain bone morphogenic protein (BMP) that is activated upon demineralization of tissue (Urist, 1965). This protein has now been shown to have considerable homology with the TGF and may contribute to resorption-repair coupling mechanisms. The demineralization of dentin and cementum have been reported to increase their chemotactic effect for fibroblasts (Pitarn, 1984), perhaps by the activity of TFG. Linden, (1975) maintained that the implanted demineralized dentin or bone matrix was first partly eroded by multinucleated cells and then becomes recalcified before new bone is formed. Lindskog and Hammerstrom (1980) and Lindskog et al. (1983) have reported that cementum but not dentin has a protease inhibitory effect, comparable to the anti-invasive factor in cartilage. There is no evidence for the differential effect in the resorption potential of primary teeth. Because the cementum resorbs as readily as the dentin, Rygh (1977) assumed that undermining resorption of cementum indicated a greater resistance of cementum to odontoclast

resorption when compared to dentin. He suggested that the higher fluoride content of cementum accounted for this difference (Rygh, 1977). It is possible that this observation is coincidental and that the odontoclast may resorb dentin and cementum at the same rate but if the odontoclast happens to be near the cemento-dentin junction then undermining resorption may be present but without biological significance in relation to odontoclast behaviour.

Another extremely interesting observation is that carious dentin of primary teeth is resistant to resorption (Jones and Boyde, 1987a,b). The affected tissue remains prominent in the resorption area and is characterized by the projection of rods or tubes of intratubular mineralization. The caries crystals which are deposited within dentin tubules ahead of the main body of the lesion, are particularly resistant to resorption. It is hypothesized that leukocyte derived substances from the inflamed pulp, bacterial byproducts from the invaded carious lesion or changes in the chemistry of the mineral or protein may be contributing factors in the caries-induced resorption resistance phenomenon. This is particularly interesting in light of the resorption stimulation effect of carious lesions (chronic inflammation) on the resorptive

activity in the pulpal (internal resorption) and the periapical (apical resorption) regions of the root.

All three mineralized dental tissues can be resorbed by cultured osteoclasts (Boyde et al., 1983,1984; Jones et al., 1974). This provides an opportunity to characterize the behaviour of cultured cells on resorbable substrates and to contrast these with what we can deduce from observed physiological or pathological resorbed surfaces in vivo.

Osteoclasts given unlimited access to dental tissues or bone move in a similar way when observed on plastic (Smith et al., 1985; Jones et al., 1986a,b). There is a reproducible tendency for osteoclasts to migrate and this might explain the shallowness of sporadic resorption pits on the roots of permanent teeth (Jones and Boyde, 1988). Repair is achieved by migration of the cementoblasts over the resorbed surface, competing for the available surface and blocking out further lateral resorptive sweeps.

The movement of osteoclasts on dentin during and after active resorption in vitro has been observed and recorded using time-lapse video recording (Boyde and Jones, 1987). Although the volume of tooth or bone resorbed cannot be easily obtained the size of the resorption lacunae can be determined (Boyde and Jones, 1979). The rate of resorption of mineralized tissue by

one osteoclast can also be observed. This system allows measurement and comparison of the rates at which resorbing cells can remove different tissues under standard conditions. Sperm whale dentin was selected as a substrate for studying the behaviour of cultured osteoclasts because it is available in large quantities, it is homogeneous when compared to bone (avascular and low rate of tissue turnover), it has more uniform mineralization than bone, it has optical properties which are superior to those of bone and its surface consistency was uniform (Boyde and Jones, 1979). Kanehisa and Heersche (1988) report osteocyte and blood vessel lacunae exposed at the surface of a bone slice appeared to be preferred sites for cultured osteoclasts to start resorbing. Resorption of bone versus dentin revealed that the average depths and volumes (but not the area) were significantly larger in human lamellar bone than in the sperm whale dentin (Jones and Boyde, 1988). The difference in resorption rates of these two tissues may be related to their different mineral content, the bone being less mineralized and containing less fluoride than the dentin. The shape of the pits did not differ significantly, however, when assessed in three dimensions (Jones and Boyde, 1988).

The effect of fluoride in the incubation medium is to reduce the rate at which dentin and cementum resorb. The shape and size of resorption pits were altered significantly by the addition of fluoride (Jones and Boyde, 1988). Enlargement of the big lacunae appeared to be more dependent on an increase of plan area than an increase in the depth, as compared to the small lacunae (Jones and Boyde, 1988).

The stereophotographic analysis of the pattern of resorption in vitro is consistent with the scanning electron microscopy observations of in vivo resorption of both bone and dental tissues where the resorptive episode is transitory (Jones and Boyde, 1988). A restriction in the depth of the resorptive pits may be caused by an inherent impulse for the osteoclast for translocatory movement over a surface or by the osteoclast rendering the resorbed surface unacceptable or inhibitory to demineralization by the nature of its secretory products or by release of factors stored within the tissue (Jones and Boyde, 1988). Such patterns occuring in cultures of disaggregated bone (and marrow) cells suggests that a defined close spatial relationship between the different cell types is not of prime importance for this movement during and between resorptive episodes (although cytokines released into the medium may play a regulatory role).

It is essential that the surface undergoing the resorption be free of adherent cells ahead of the resorptive wave. Competition for the surface of bone or dental tissue by an increased number of osteoclasts, active simultaneously, and unrestricted by "blastic" cells would encourage deeply eroding resorption and restrict lateral migration of an individual cell.

An increase in the area of resorption has been shown to occur in cultures of osteoclasts on enamel (Jones and Boyde, 1988). Cultured osteoclasts also have been shown to resorb a greater surface area, presumably due to a greater tendency to migrate, when resorbing calcite crystals or calcium carbonate containing shells (Jones et al., 1985, 1986a). The demineralized collagenous matrix does not appear to inhibit the tendency for osteoclasts to migrate during active resorption (Kanehisa and Heersche, 1988). It has, therefore, been hypothesized that the stimulus for translocation of the osteoclast during active resorption is the increase in local calcium ion concentration, as a direct result of resorption, in the ruffled border-clear zone area (Jones and Boyde, 1988).

The most controversial aspect of resorption is the method by which the degradation of the organic matrix of tooth and skeletal tissues occurs following

demineralization. The in vitro resorption assay has been used to observe the effect of specific inhibitors of collagenase and cysteine proteases on resorption (Delaisse et al., 1987). Cysteine proteases of osteoclastic origin have an important role in the formation of resorptive pits in calcified connective tissue (Jones and Boyde, 1988).

The depth of the collagen fringe lining the resorption pit in dentin, cementum or lamellar bone is greater in vitro than in vivo. It is hypothesized that this difference is due to an imbalance between the rate of pumping of protons and the rate of production and secretion of proteases by cultured osteoclasts. This has been deemed as evidence that neutral proteases of osteoblastic (and presumably odontoblast and cementoblast) origin are essential for breakdown of the organic matrix. However, degradation of the matrix does occur directly below the ruffled border of the osteoclast/odontoclast as evidenced by the fibrillar structure. The thicker collagen fringe is still present in cultures of mixed bone and marrow cell (and even where osteoblasts and PTH have been added to the culture medium).

The possibility of procollagenase being incorporated into the dentin during dentinogenesis has also been ruled out or at least if it is there the

procollagenase is not released as a self-destructive mechanism upon osteoclastic demineralization of the tissue. In vitro resorption of dentin and cementum allows the observation of the degradation of unmineralized regions of the matrix such as interglobular dentin and unmineralized Sharpey's fiber cores. These unmineralized matrix regions are never left behind in resorption lacunae. This strongly suggests that the osteoclast is capable of resorbing the organic matrix. Because the osteoclasts used in these studies are from neonate animals, their activity may differ from osteoclast behaviour from adult animals. In addition, the physical properties of resorption pits, such as depths of demineralized matrix below the osteoclast resorbing woven or lamellar bone, may also vary in vivo (Jones and Boyde, 1988).

Bone marrow cultures give rise to osteoclasts in vitro when the cells are cultured on plastic (Roodman et al., 1985) or bone or dentin (Jones et al., 1986c; Takahashi et al., 1988). Thus, the presence of calcified tissue is not essential for osteoclast differentiation. Clearly, on calcified tissue substrates the formation of classical resorption pits is unequivocal proof of the identity of the multinucleated or resorptive mononucleated cells produced in such cultures (Suda et al., 1988).

In summary, root resorption both in physiological and pathological scenarios remains poorly understood. Root resorption induced by orthodontic tooth movement, in particular, is an enigma. The initial resorption must break through the outer layer of cementum. Clearly, the cementum is more resistant to resorption than bone, that is why orthodontic forces can move teeth and not resorb the root surfaces entirely. The ultrastructural properties of cementum have been hypothesized as imparting resistance of cementum to resorption.

The high fluoride content of the outermost layers of cementum have been thought to decrease the extent of root resorption. Also, the vascularity of bone as compared to cementum will increase the potential for bone to resorb (as the "clast" progenitor cells are delivered directly to bone). Alveolar bone is continually remodelled under physiological conditions and osteoclasts on the bone surface are a normal part of the bone environment (Marks and Popoff, 1988, Vaes, 1988). The rate of bone turnover is high and therefore the bone tissues are newer than cemental tissues. Cementum does not normally have odontoclasts present as part of the cell environment and cementum turnover rate is very low. Cemental tissues tend to be more

mature and possibly more resistant to local chemical changes than bone (Rygh, 1977; Selvig, 1968).

The unmineralized precementum or cementoid layer, 3 to 5 microns thick in acellular cementum (and thicker in cellular cementum) is continually being deposited. Thus, an uncalcified precementum layer will always be present on the root surface (Rygh, 1977). Reitan has demonstrated that the presence of a cementoid or predentin layer on the root surface delays the resorption process (Reitan, 1974). The observation that odontoclasts do not readily resorb unmineralized matrix is consistent with well documented findings of the same behaviour in osteoclasts in vitro and in vivo (Boyde et al., 1984; Chambers et al., 1984).

The exposed root has been thought to be analogous to exposed mineralized matrix of bone, a known potent stimulator of bone resorption (Kahn and Malone, 1988; Jones and Boyde, 1988). Thus, it is possible that the less protected root surface is more susceptible to resorbing cells in the microenvironment and/or the exposed dentin may stimulate odontoclast formation in the microenviroment. The hypothesis, therefore, on the development of local root resorption in association with hyalinization is that barriers on the root surface are eliminated after hyalinization. The initial resorption lesion penetrating the cementum may

easily take place in the resorption promoting environment around the hyalinized zone. A small breakthrough of the barrier layers may be all that is required to start the resorption process.

The theories on the initiation and propagation of root resorption lesions remain speculative as there is no direct evidence in support of these theories to date.

CHAPTER II

STATEMENT OF THE PROBLEM

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The research on root resorption which has focused on the exfoliation of the primary dentition (Cahill, 1969; Ten Cate and Anderson, 1986; Sasaki et al., 1989, 1990) and severe trauma to the permanent dentition (Andreasen, 1978, 1985, 1988 a,b,d,e,; Lindskog, et al., 1988) has greatly contributed to advance our understanding of the process of root resorption. The root resorption which occurs in association with orthodontic tooth movement is thought to represent a form of mechanical trauma to the root surface (Andreasen, 1988; Tronstad, 1988; Reitan, 1951, 1974; Kvam, 1972; Rygh, 1977). There is little dispute at present that root resorption is an inevitable side effect of orthodontic treatment. The variability occurs only in the extent to which root resorption lesions develop and subsequently repair (Massler and Malone, 1954; Rygh, 1977; Barber and Sims, 1981). The individual variability coupled with many other factors including appliance system used, force magnitude and duration, root morphology, age and sex of the patient may all play a role but the importance of each factor has yet to be determined.

The investigations using the light and scanning electron microscopy (SEM) have confirmed that root resorption routinely occurs as a side effect of orthodontic treatment (Kvam, 1972; Rygh, 1972, 1977). There is convincing evidence that a histological difference exists in the resorption potential in the root as compared to the bone. This difference in resorption

potential is thought to be responsible for the ability of the orthodontist to move teeth through bone without the exfoliation of the permanent dentition. At the cellular level, there are no doubt many factors which determine the extent of root resorptive activity. The hypothesis that cementum provides a protective barrier for the root surface remains a viable possibility. The histological studies of orthodontically induced root resorption suggest that a barrier does exist such that when this barries is breached by microtrauma the resorptive cells have access to the root surface (Reitan, 1951; Kvam, 1972; Rygh 1972, 1977). The mechanism through which this protection is imparted and the degree of injury to the cementum layer which is required to allow root resorption to proceed remain unknown.

The odontoclast cell responsible for resorbing the root structure is histologically and functionally very similar to the osteoclast (Sasaki, 1989 1990; Ten Cate and Anderson, 1986; Boyde and Jones, 1972). It has been suggested that the osteoclast and odontoclast are identical cells and they differ only in the substrate on which they act (Ten Cate and Anderson, 1986; Boyde and Jones 1972; Jones and Boyde, 1988). The similarity of the osteoclast and odontoclast at the cellular level provides a basis for improving our understanding of the cell involved in orthodontically induced root resorption. The advances already achieved in basic bone biology, in particular the study of bone resorption, may be applied to the study of the resorptive cells on the surface of teeth.

There have been many difficulties in studying orthodontically induced root resorption. One problem has been minimizing the differences in individual susceptibility by decreasing the genetic variability of the experimental subjects (animals). This problem is best resolved by the development of an animal model in which root resorption can be induced in a known species (rats or mice). In this way the genetic variability among experimental animals is minimized. A rodent study model has the additional advantages of being readily available at a reasonable cost and ethical dilemmas associated with the research are considerably reduced. The orthodontic appliance must be calibrated such that force levels delivered to the molar may be quantified. The use of a mouse model also has the advantage of yielding an explant small enough to be viable in organ culture. Once the viability of the resorptive cells of the explant in organ culture has been established then the effect of various bone resorbing factors on the behavior of root resorbing cells can then be tested in vitro. The use of the tartrate resistant acid phosphatase (TRAP) stain has been used to identify osteoclast-like cells, odontoclast-like cells and putative precursor cells. The development of these cells was followed over a specific time period and the changes in the absolute number and distribution of these cells was assessed.

The objectives of this study were:

1) To develop an animal model in which orthodontic root resorption could be predictably and reproducibly induced (Chapter III)
2) To calibrate an appliance such that the force levels applied to the mouse molars could be determined (Appendix I)
3) To test the viability of the mouse molar explant in organ culture by ^{3}H-proline labelling (Chapter III)
4) Conduct a pilot study to assess the effect of prostaglandin E_1 on the resorptive cell activity (Chapter III)
5) To determine whether orthodontically induced odontoclast-like cells would stain similarly to osteoclast-like cells for TRAP (Chapter IV)
6) To employ the TRAP stain to follow changes in the osteoclast-like and odontoclast-like cells over time and to correlate this with the length of bone and root surface as well as the area of the periodontal ligament (Chapter IV)
7) To assess the absolute changes in the cell numbers and changes in the distribution of these cells at different time periods of <u>in vivo</u> orthodontic force application up to 10 days (Chapter IV)

CHAPTER III

DEVELOPMENT OF AN ANIMAL MODEL FOR INDUCING ORTHODONTIC ROOT RESORPTION IN VIVO

CHAPTER III - DEVELOPMENT OF ANIMAL MODEL FOR INDUCING ORTHODONTIC ROOT RESORPTION

SUMMARY

The development of root resorption lesions in response to *in vivo* orthodontic force application on the mandibular left first molars of 7 week old, Swiss-Webster, male mice was studied using Hematoxylin-Eosin (H & E) staining. The viability of the molar explants was determined using ^{3}H-Proline labelling in organ culture. The purpose of the study was to determine whether the orthodontic appliance system developed would predictably and reproducibly stimulate the formation of root resorption lacunae in the mouse model. Once the reliability of the root resorption system was established then this model was used to study the viability of the resorptive cells in organ culture. A pilot study of the effect of prostaglandin E_1 on the bone and root resorption, in organ culture was completed. Examination of the H&E stained sections of the orthodontically treated mouse molars confirmed that reproducible root resorptive lesions could be induced by the orthodontic appliance system as early as 2 days following *in vivo* force application. The resorptive lacunae were generally localized in the furcation area. The number and size of root resorption lacunae increased up to 14 days of force application and these resorptive lesions involved a more extensive root surface area in the furcation of the treated molar. The ^{3}H-proline labelled explants verified the viability of the odontoclast-like cells present in root resorption lacunae

under the conditions of the organ culture system described. The preliminary results of the in vitro effects of prostaglandin E_1 (PGE_1) on bone and root resorption activity in molar explants stressed orthodontically for 5 and 9 days in vivo suggests PGE_1 has a stimulatory effect on the resorptive cell populations.

INTRODUCTION

Root resorption has long been recognized as an unpredictable and undesirable side effect of orthodontic tooth movement. Investigations using light microscopy or scanning electron microscopyy greatly improved the ability to detect resorptive lesions on the roots of orthodontically treated teeth. The most striking conclusion from these investigations was that root resorption was an inevitable side effect of orthodontic tooth movement. The root resorption lesions were consistently associated with the cementum adjacent to the hyalinized zones and were found to occur following the elimination of the hyalinized zone (Reitan, 1951; Rygh, 1972, 1977; Kvam, 1972; Barber and Sims, 1981). Root resorptive lesions in different stages of resorption and repair were commonly observed (Reitan, 1951; Rygh, 1977; Williams, 1984).

In addition, root resorption lacunae were often found to persist beyond the period of initiation of root resorption (Rygh, 1977; Barber and Sims, 1981 and Harry and Sims, 1982). The hypothesis developed that the external layers of the root in some way provide a protective barrier for the root surface. The exact mechanism through which this protection occurs as well as the

nature and degree of injury required to allow root resorption to proceed are not known. There is considerable research required to understand the process of root resorption at the cellular level. The development of an *in vivo* animal model for inducing root resorption in response to orthodontic tooth movement will allow investigations on the specific cells responsible for root resorption. The resorptive cells induced by orthodontic forces applied to the mouse molars *in vivo* may then be placed in organ culture for further investigation. A viable molar explant in organ culture will provide a means of testing the *in vitro* response of these root resorptive cells to known bone resorbing factors.

MATERIALS AND METHODS

(A) Orthodontic Appliance Insertion

Seven week old Swiss-Webster, male mice were anaesthetized using an intramuscular injection (0.05ml/50g) of ketamine: xylazine:water (ratio 10:1:1.6, respectively) into the left gluteus muscle. The orthodontic appliance, (Fig.3.1) was inserted by first ligating the elastic O-ring to the mandibular left first molar using a .007" stainless steel ligature. The elastic O-ring was then secured to the mandibular left incisor using a .007" stainless steel ligature. The mandibular incisors were then ligated together using a .007" stainless steel ligature. The elastic O-rings were calibrated for load/deflection ratios (see Appendix I). The load/deflection curve is presented in

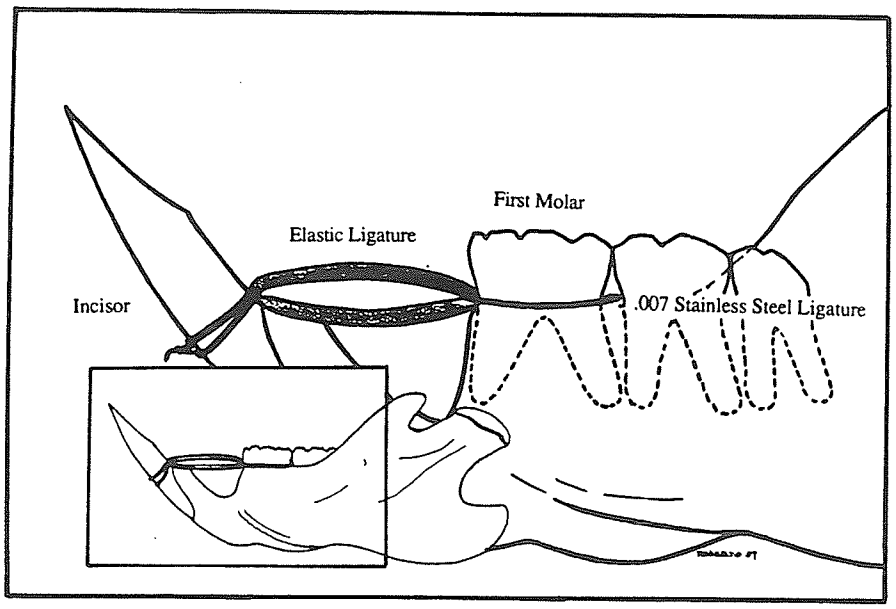
Fig.3.2a and b. The mesial tipping force applied to the molar was on average 101g. The twenty-five orthodontic appliances inserted remained active for 0, 3, 5, 7, 9, 10 and 14 days. The three experimental mice for each time period were prepared; one explant would be placed into organ culture and three specimens would be placed directly into Bouin's fixative for 48 hours, (2 experimental and 1 control). The contralateral molars served as controls. An additional four appliances were placed, two for 5 days and two for 9 days. All of these specimens were placed in organ culture following the in vivo orthodontic force application.

(B) **Dissection and Culture System**

The mice were euthanized by cervical dislocation and the two mandibles removed. All soft tissue was freed from the specimen and discarded. The first, second and third molars and their associated periodontium were then separated from the remainder of the mandible by a cut inferior to the root apices and another cut distal to the third molar. This dissection was performed with the mandible completely submersed in oxygenated organ culture medium, warmed to 37°C. This is illustrated in Fig. 3.3.

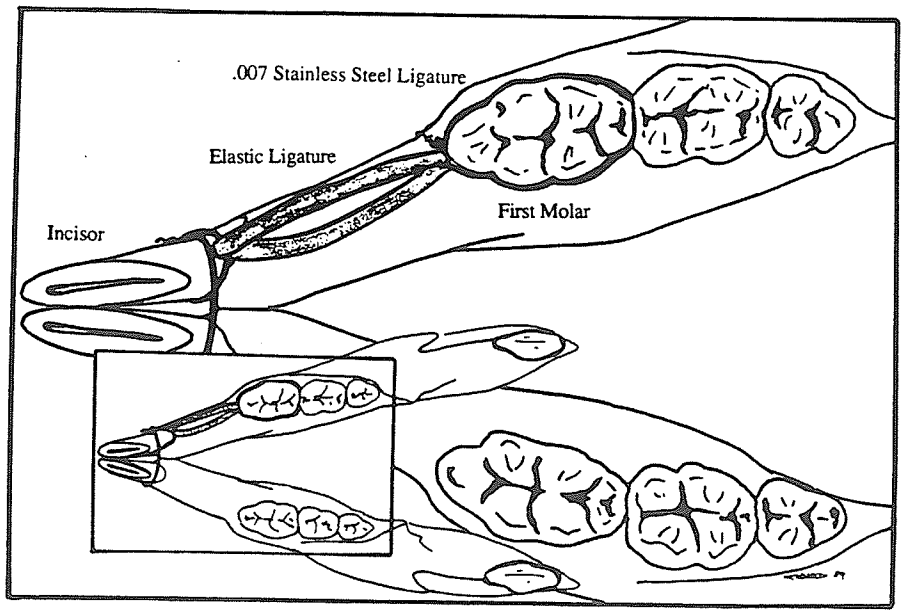
Fig. 3.1

In Vivo Mouse Model



Sagittal View

In Vivo Mouse Model



Occlusal View

Fig. 3.2a

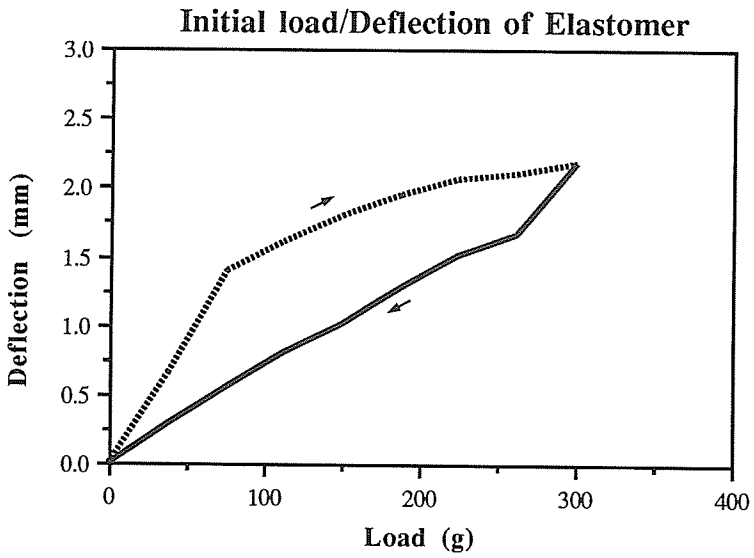


Fig. 3.2b

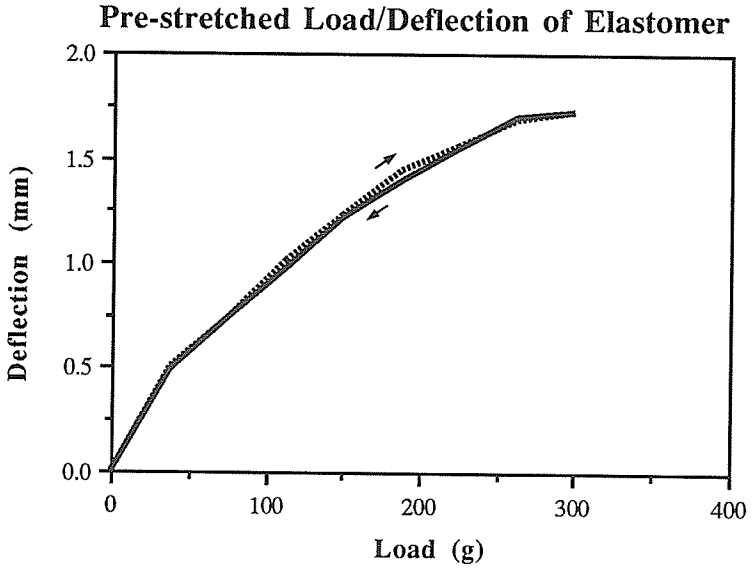
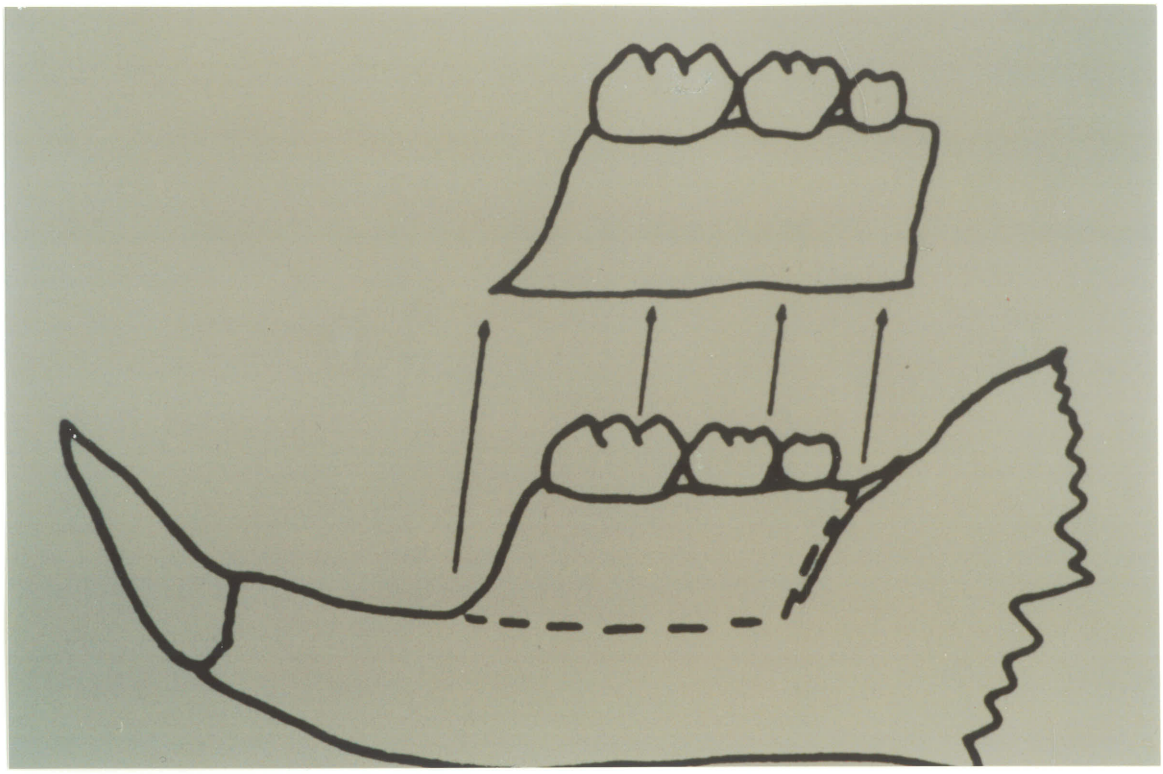


Fig. 3.3 Diagram of periodontal organ explant comprised of three mandibular molars and their supporting alveolar tissues.



The explants were cultured in Trowell-type (Trowell, 1959) organ culture dishes (60 x 15mm, Falcon #3037, Becton, Dickinson and Co., Cockeysville, Maryland) in which the tissue rested on a stainless steel grid (Falcon #3014, Becton, Dickinson and Co., Oxnard, California), suspended over the centre well of the culture dish. The culture medium (1000μl) was introduced into the centre well such that the suspended explant rested at the gas:medium interface. The external portion of the culture dish was filled with distilled water. The dishes were covered and incubated for 6 hours at 37°C.

(C) <u>Medium, Gas, Prostaglandin E_1 and Isotope</u>

The medium used was Waymouth's 752/1 (Grand Island Biological Co., Grand Island, New York). The medium was supplemented with 300μg/ml ascorbic acid (Fisher Scientific, Fair Lawn, New Jersey) and antibiotic-antimycotic mixture (1.5ml/100ml medium) consisting of 10,000 U/ml, penicillin; 25μg/ml, amphotericin B and 10,000μg/ml, streptomycin (Grand Island Biological Co., Grand Island, New York).

The incubator was continuously infused with a mixture of humidified 95% O_2 and 5% CO_2.

Ten μCi/ml ^{3}H-Proline (NET 323, L-proline 2,3-3H(N), New England Nuclear, Boston, Mass.) with a specific activity of 23.7 Ci/mmol was added to each organ culture dish 4 hours prior to termination of the culture.

The additional organ culture explants for 5 day and 9 day <u>in vivo</u> force application had 1μl of 0.01ug/ul

prostaglandin E_1 added to the organ culture medium just prior to the incubation of the explant.

(D) H & E Staining and Radioautography

The mouse molar explants, at the end of the organ culture period, were placed in Bouin's fixative for 48 hours. All specimens were then placed in 70% alcohol for 48 hours, decalcified in 12% EDTA (pH 7.2) for 3 weeks and embedded in paraffin. The paraffin embedded specimens were cut into 5 micron sections, mounted on glass slides and every third slide was stained with H & E. The intervening slides were prepared for radioautography by dipping in Kodak NTB-2 nuclear tracking emulsion (Eastman Kodak Co., Rochester, New York), stored in the dark at 4°C for 2 weeks, developed in Dektol developer and fixer (Eastman Kodak Co.) and stained through the emulsion with H & E.

RESULTS

Observation of the stained sections under the light microscope confirmed that control mice did not show any root resorption over the time periods studied (Fig.3.4 and 3.5). Resorptive lacunae were apparent in the furcation area of the orthodontically treated mouse molars after 5 days of *in vivo* force application. The number and size of the resorptive lacunae increased with an increase in the duration of *in vivo* force application (Fig.3.6 to 3.10). The resorption lacunae appeared to fuse and extend apically along the mesial surface of the

distal root following 14 days of in vivo force application. The bone resorptive activity appeared to increase in response to the orthodontic forces applied. The addition of prostaglandin E_1 to the organ culture medium appeared to stimulate bone resorptive activity in comparison to those specimens where orthodontic force alone was applied for the same time period in vivo (Fig. 3.11 to 3.13).

The radioautographs showed labelling throughout all ^3H-proline treated explants. The labelling was apparent over the odontoclast-like cells occupying the resorption lacunae and over the osteoclast-like cells present in the bone (Fig. 3.13 to 3.17).

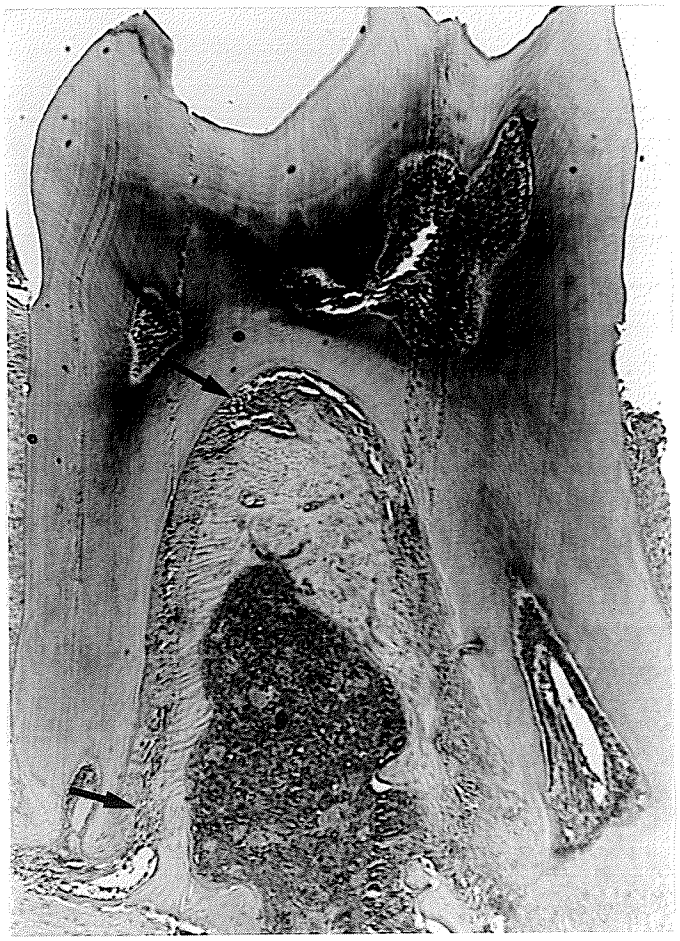


Fig. 3.4 Control mouse mandibular right first molar. H & E. 13X

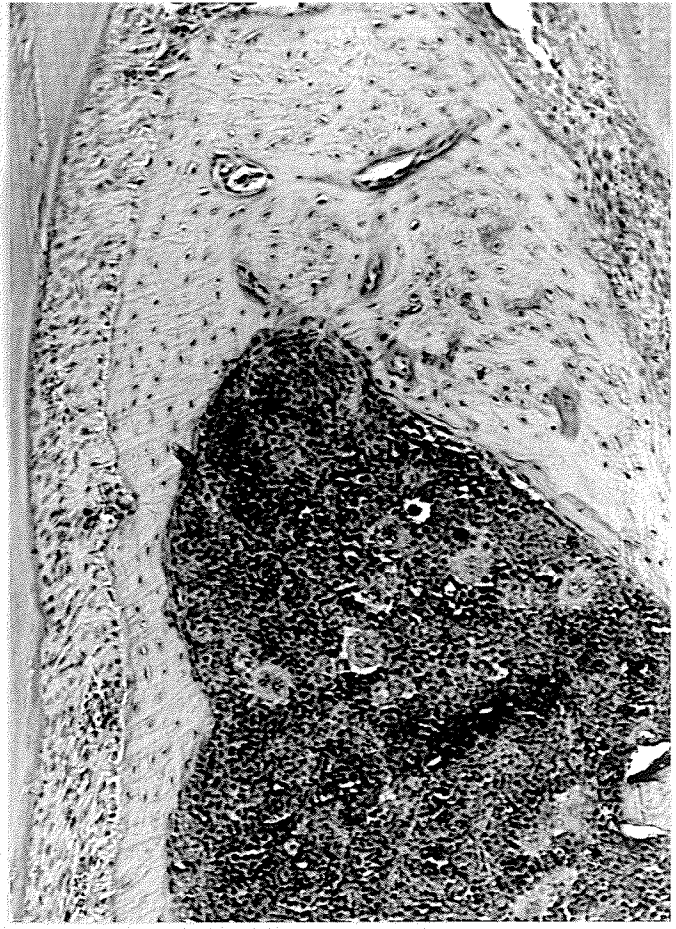


Fig. 3.5 Magnification of the area indicated by arrows in Fig.3.4. Note the continuous cementum layer and the absence of root resorption lacunae. H & E. 82X.

Fig. 3.6 Mouse molar periodontium orthodontically stressed for 7 days <u>in vivo</u>. Note the bone and root resorption lesions in the furcation area. H & E. 32X

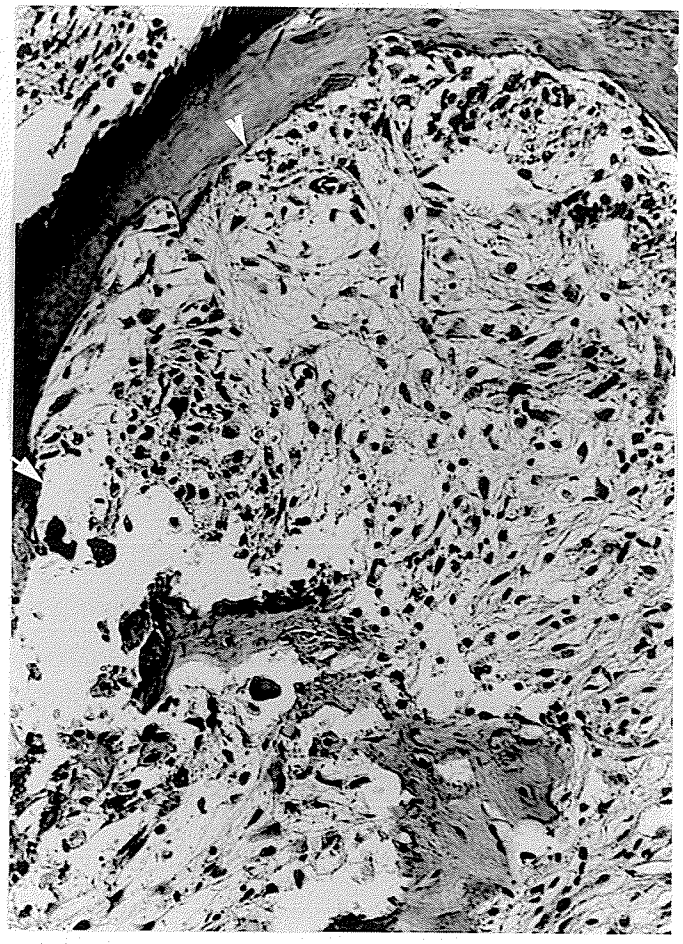


Fig. 3.7 Magnification of the area indicated by the arrows in Fig. 3.6. H & E. 82X

Fig. 3.8 Magnification of the cells in the resorption lacunae in Fig. 3.7. H & E. 205X

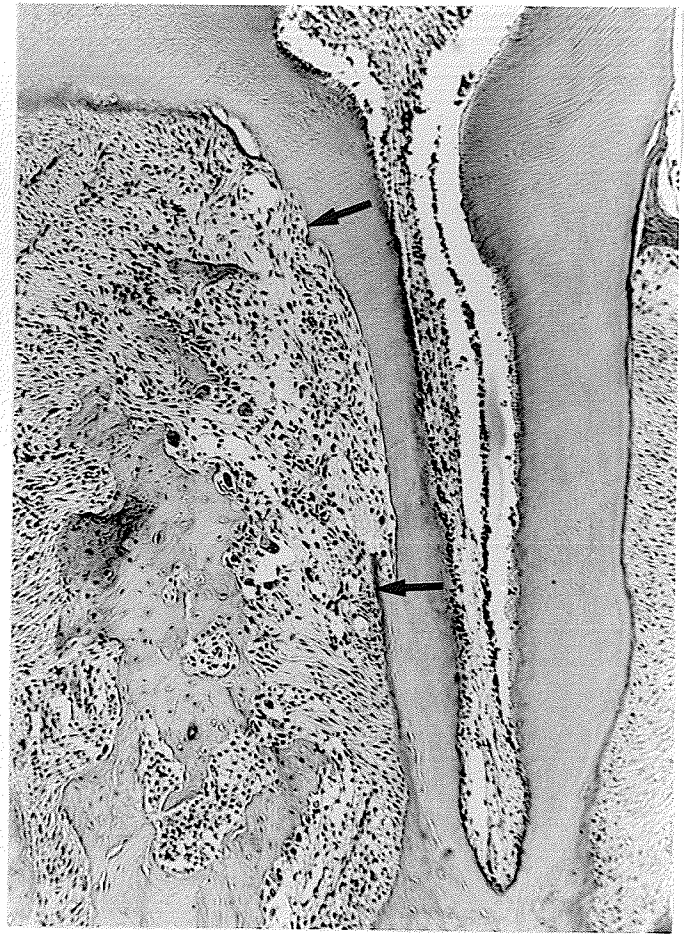


Fig. 3.9 Mouse molar periodontium orthodontically stressed for 14 days *in vivo*. Note the extensive resorptive lesion in the furcation area along the distal root. H & E. 13 X.

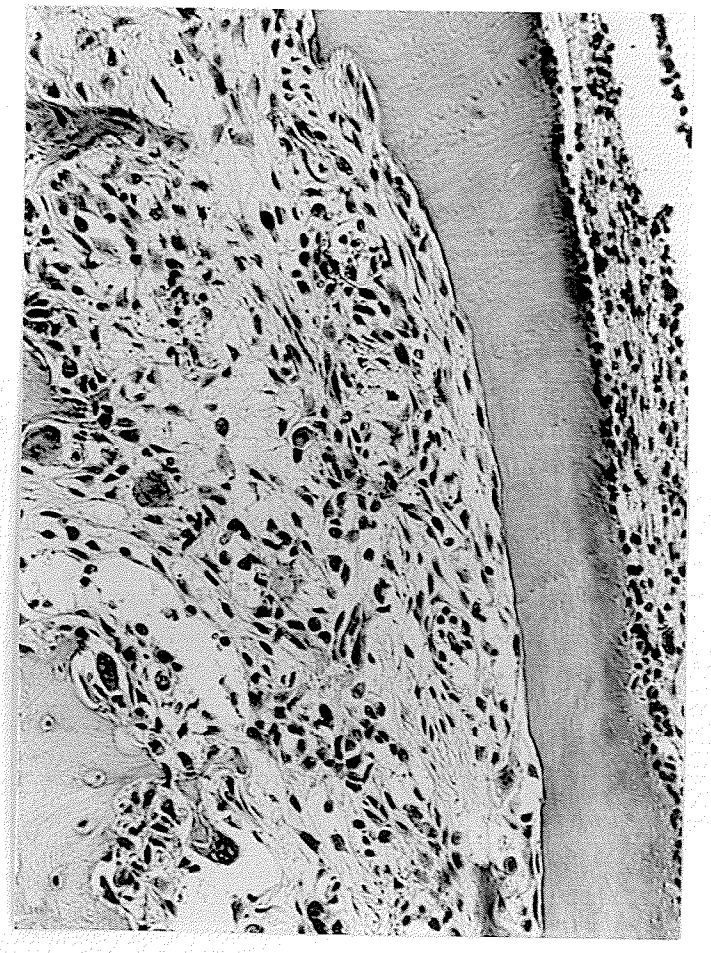


Fig. 3.10 Magnification of the resorptive lesion indicated by arrows in Fig. 3.9. H & E. 82X

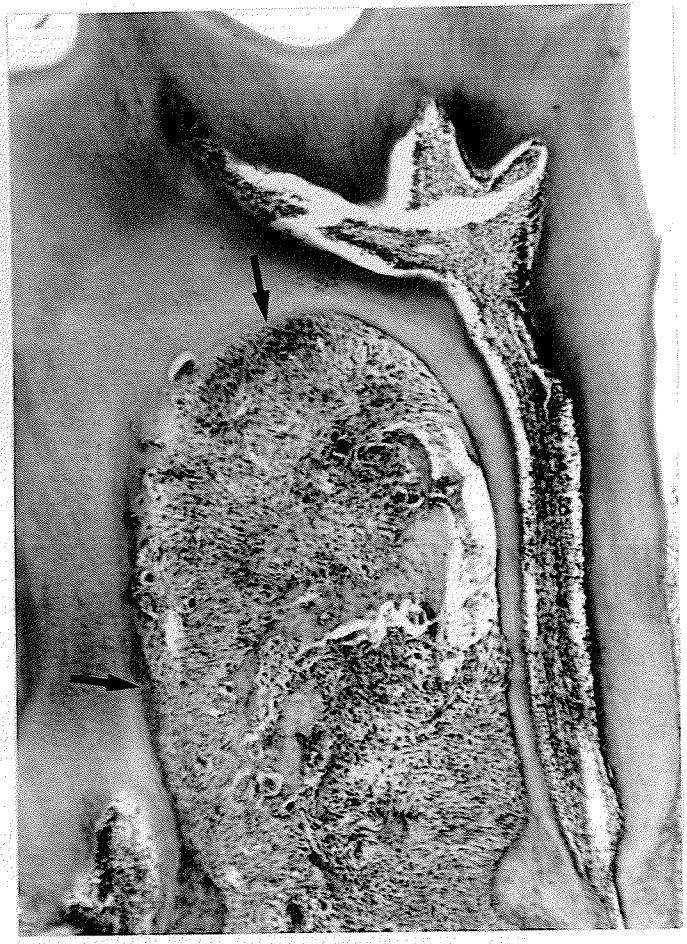


Fig. 3.11 Mouse molar periodontium orthodontically stressed for 9 days *in vivo* and incubated with 0.1μg/ml of PGE_1 for 6 hours *in vitro*. Note the large number of resorptive lesions on the root surface and the dramatic bone resorptive activity. H & E. 13X.

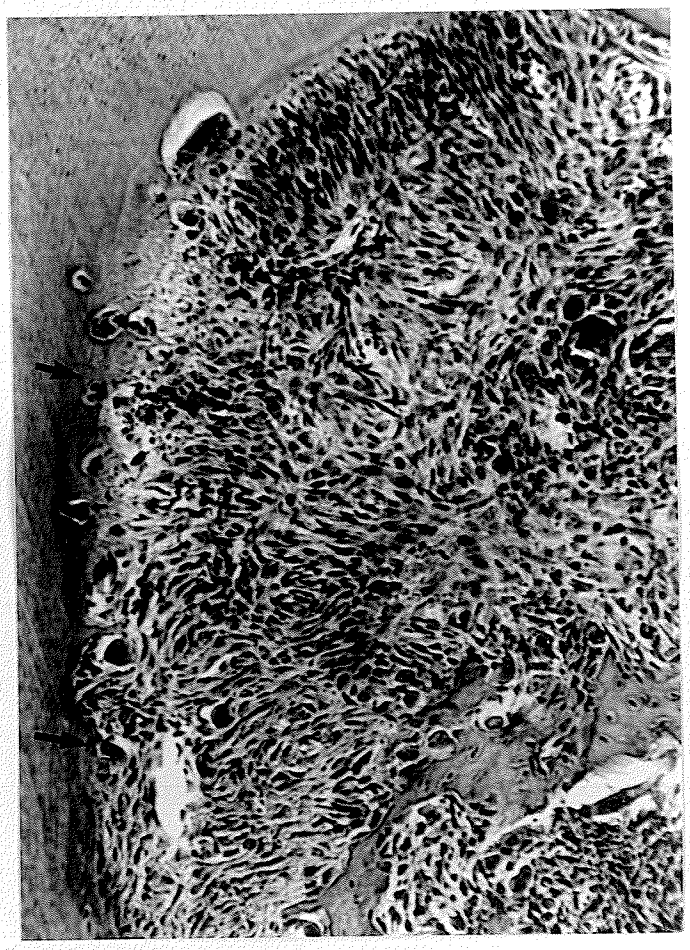


Fig. 3.12 Magnification of the area indicated by the arrows in Fig. 3.11. H & E. 82X.

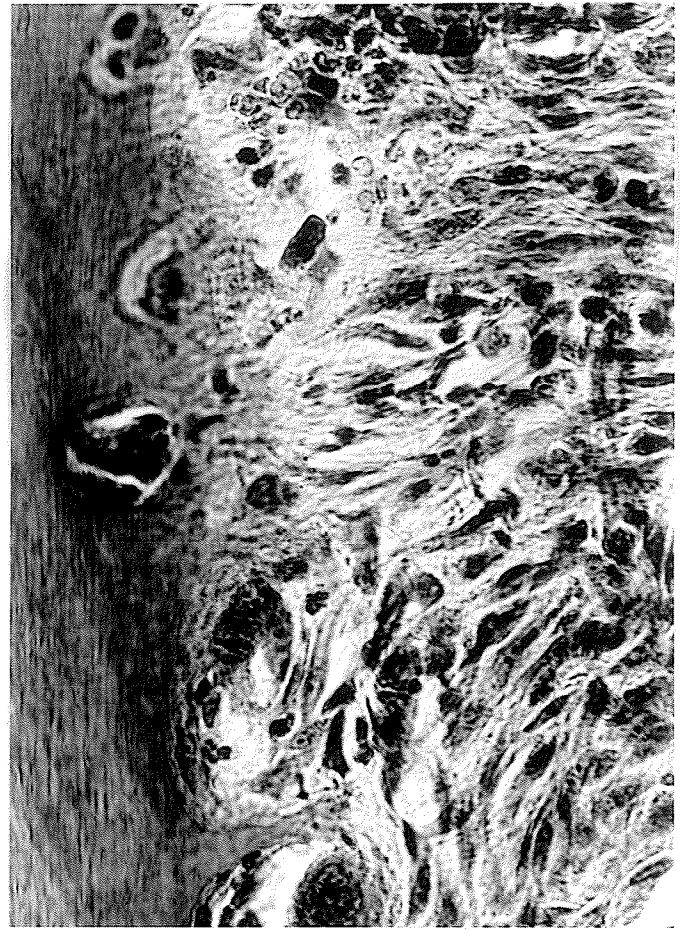


Fig. 3.13 Magnification of the area indicated by the arrows in Fig. 3.12. Note the numerous resorptive lacunae on the root surface.
H & E. 205X

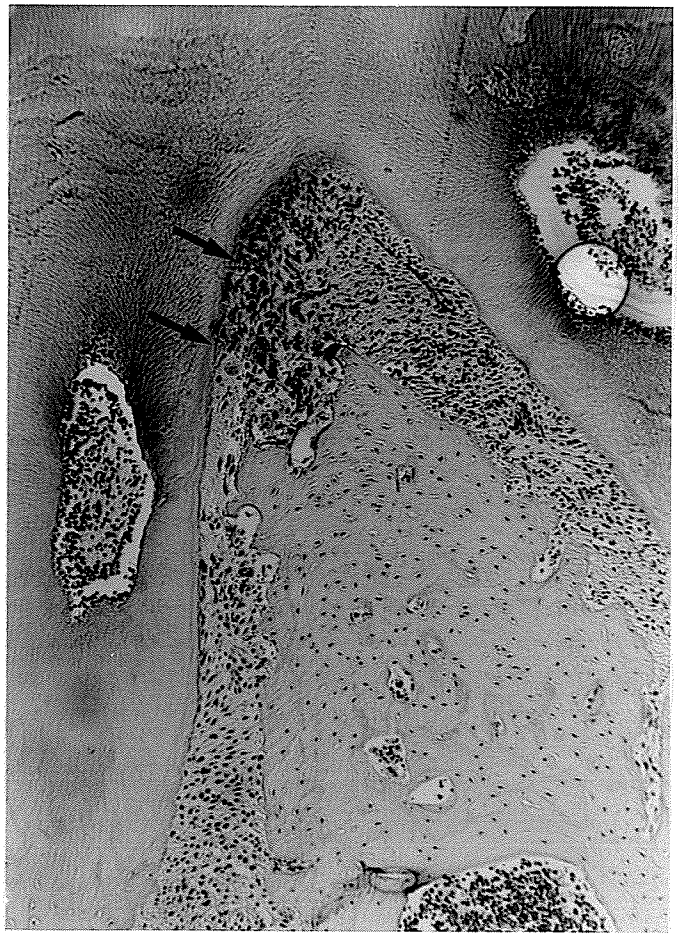


Fig. 3.14 Mouse molar periodontium orthodontically stressed for 5 days *in vivo* and labelled with ^{3}H-proline *in vitro*. Note the extensive resorptive activity in the bone and on the root surface.
H & E. 32X.

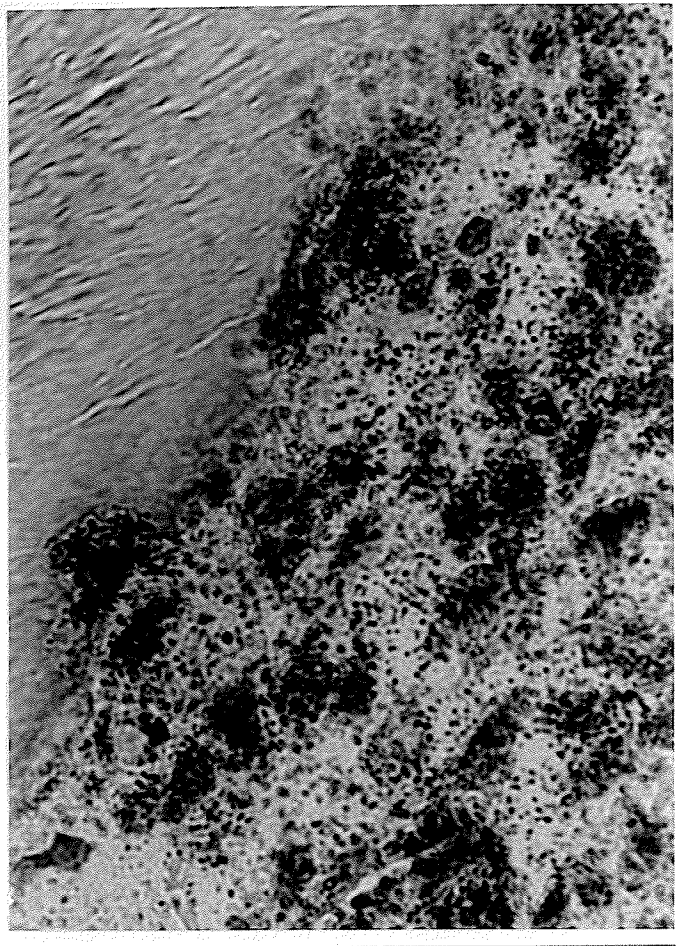


Fig. 3.15 Magnification of the area indicated by the arrows in Fig. 3.14. Note the extensive root resorption lacunae. H & E. 82X

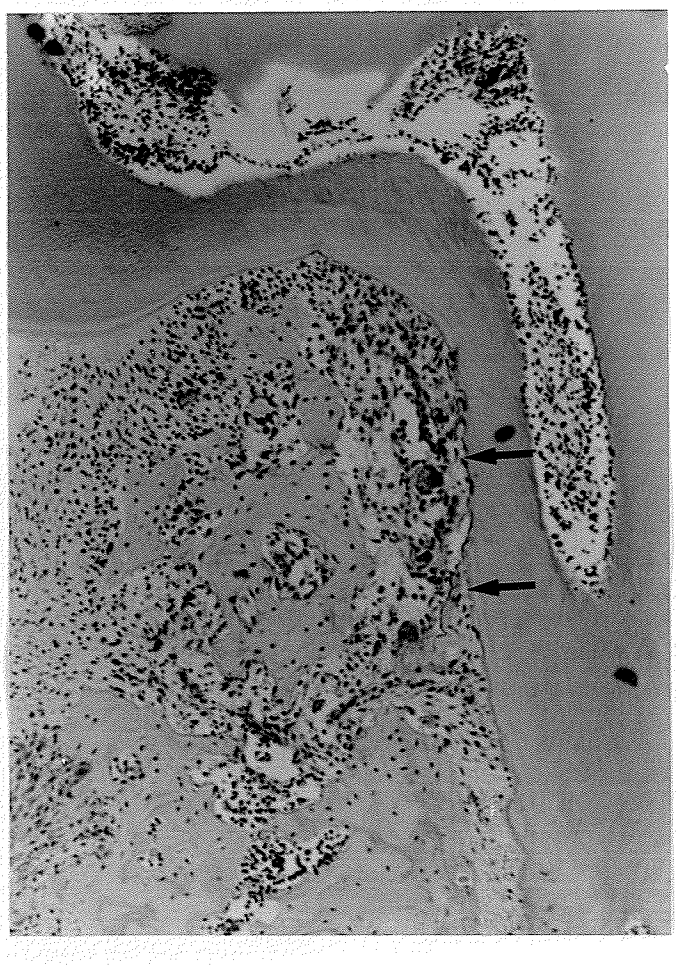


Fig. 3.16 Mouse molar periodontium orthodontically stressed for 9 days <u>in vivo</u> and labelled with ^{3}H-proline <u>in vitro</u>. Note the extensive resorptive activity in the bone and on the root surface. H & E. 13X.

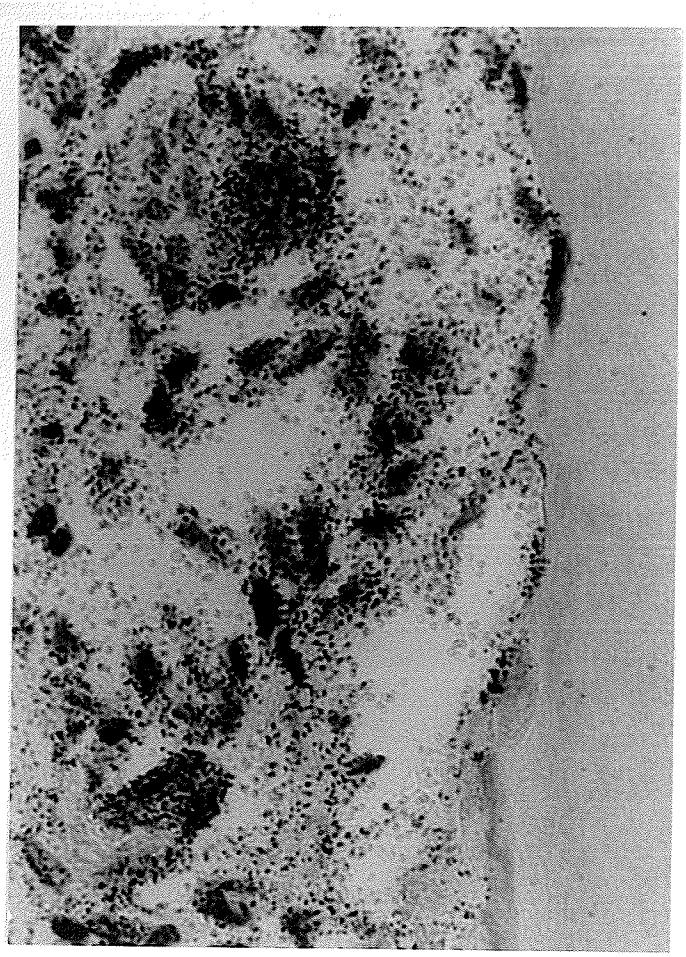


Fig. 3.17 Magnification of the area indicated by the arrows in Fig.3.16. Note the extensive root resorption lacunae.
H & E. 205X

DISCUSSION

The root resorption lesions induced by the orthodontic appliance over different periods of time appear to follow the patterns previously reported by Rygh (1977) and Williams (1984). The first resorptive lesions were observed in the furcation area of the root after 5 days of *in vivo* force application. The development of root resorption lesions at this time has been shown to correlate with the resolution of the zone of hyalinization (Rygh, 1977; Williams, 1984). The cells responsible for this phase of orthodontic tooth movement are thought to migrate from the vital PDL adjacent to the area of the compression and resultant hyalinization. Resorptive lesions were

also detected on the external surfaces of the root in areas adjacent to the zone of compression. The resorptive lesions occasionally appeared in association with zones of tension. It is not certain why this occurs. One possibility is that the force system applied, in three dimensions, does not result in pure mesial tipping of the molar. There is a mesiobuccal moment created due to the buccolingual position of the appliance as well as an extrusive movement as the molar tips mesially. The zones of compression and tension traditionally have only considered orthodontic tooth movement in 2 dimensions. Thus, the unusual locations of resorptive lacunae on the root may be a reflection of the areas of compression occurring in response to the 3 dimensional force system applied. The extrusive component of the force system has been implicated as a stimulus for the hypercementosis frequently on the apices of the orthodontically treated rat molars (Williams, 1984).

The organ culture system appears to be able to maintain the molar explants tested. The incorporation of ^{3}H-proline labelling into odontoclast cells present in the root resorption lacunae during the organ culture period is convincing evidence that active protein synthesis has occurred in the labelled odontoclasts during the period of incubation. The isotope labelling provides an objective assessment of the cellular viability under the organ culture conditions studied. The investigation of Duncan et al. (1982) has ruled out non-specific binding of ^{3}H-proline as a means by which the a non-

labelled explant might appear to have incorporated the isotope. The size of the explant and the oxygen tension are important factors in the viability of an explant in organ culture (MacDougall and Coupland, 1967; Yen and Melcher, 1978).

The role of prostaglandin E_1 has not been conclusively established in bone biology (Vaes, 1988). It has been suggested that this substance has a stimulatory effect on bone resorption (Vaes, 1988).

If we assume that osteoclasts and odontoclast are the same cell then PGE_1 should also stimulate root resorption. The precise mechanism by which PGE_1 stimulates bone resorption has not been established but it has been suggested that PGE_1 effects the differentiation and recruitment of osteoclast progenitor cells and also increases osteoclast activity (Vaes, 1988). The concentration of PGE in the bone microenvironment is of great importance to the regulation of bone remodelling. It must be emphasized that PGE_1 is only one of many local and systemic factors, such as the cytokines and parathyroid hormone (Vaes, 1988). The interactions of bone resorbing factors are complex and are presently not well understood.

The preliminary data from the organ culture incubation with PGE_1 strongly suggests that there is a stimulatory effect of PGE_1 on bone and root resorbing cells initially stimulated by orthodontic forces applied *in vivo*.

CONCLUSIONS

1) The animal model modified from Brudvik and Rygh (1988) has been shown to induce root resorption in the mandibular left first molar of Swiss-Webster mice.
2) The resorption lacunae were initially located in the furcation area of the orthodontically treated molar.
3) The resorption lacunae increase in size and number beginning at 2 days of *in vivo* force application
4) Resorptive lesions covered a greater area of the furcation after 14 days of *in vivo* force application.
5) The odontoclasts present in the root resorption lacunae were viable in organ culture for the 6 hour incubation period studied.
6) The preliminary data suggest that PGE_1 has a stimulatory effect on the resorptive cells initially induced by *in vivo* orthodontic force application.

CHAPTER IV

THE IDENTIFICATION OF ORTHODONTICALLY INDUCED RESORPTIVE CELLS USING THE TRAP STAIN

CHAPTER IV - THE IDENTIFICATION OF ORTHODONTICALLLY INDUCED RESORPTIVE CELLS USING THE TRAP STAIN

SUMMARY

The staining technique specific for tartrate resistant acid phosphatase (TRAP), a cytochemical marker for osteoclast cells and their immediate precursors, was used to identify cells responsible for orthodontically induced root resorption. Thirty, 7 week old Swiss-Webster mice had their mandibular first left molars orthodontically stressed for various time periods. Three experimental mice were sacrificed for each time period and their molars with the associated periodontium were removed and prepared for histological staining. The resorptive cells on the bone and root and the putative precursor cells in the PDL all stained positive for TRAP. The number of TRAP stained osteoclast-like cells associated with the bone and the putative precursor cells present in the periodontal ligament both reached a maximum after 5 days of *in vivo* orthodontic force application. The number of odontoclast-like cells stained positive for TRAP did not appear to reach a maximum during the time intervals studied. These data support previous observations that bone resorption occurs more readily than root resorption in response to *in vivo* orthodontic stress. The possibility of revealing separate control mechanisms for regulating bone and root resorption offers the potential for clinical inhibition of root resorption without compromising the bone resorptive activity.

INTRODUCTION

Root resorption associated with orthodontic tooth movement has been extensively studied (Steadman, 1942; Reitan, 1951; Massler and Malone, 1954; Kvam, 1972; Rygh, 1977; Brown, 1982; Williams, 1984). The histological changes which occur during various stages of orthodontic tooth movement have been well documented (Reitan, 1951; Rygh, 1972, 1977; Williams, 1984). Investigations have shown that the root surface is normally more resistant to resorptive cell activity than is bone, as bone is observed to readily resorb under physiological conditions and in response to orthodontic force application while the roots in comparison exhibits minimal resorption (Kvam, 1972; Rygh, 1977; Williams, 1984; Massler and Malone, 1954; Vaes, 1988; Marks and Popoff, 1988). The reason for the difference in susceptibility of bone and root to resorptive cell activity is presently unknown.

The osteoclast is the cell primarily responsible for bone resorption (Vaes, 1988; Marks and Popoff, 1988). The odontoclast cells which occupy the root resorption lacunae are morphologically and functionally very similar to the osteoclast (Ten Cate and Anderson, 1986; Sasaki *et al.*, 1989, 1990). The resorption of dentin *in vitro* by osteoclast cells implies that the odontoclast and the osteoclast may even be the same cell (Boyde *et al.*, 1984). The cells of the "clast" family are known to contain high concentrations of specific lysosomal enzymes, one of which is tartrate resistant acid phosphatase (TRAP). TRAP is

is one of many isozymes in a broad category of acid phosphatase enzymes (Hammerstrom et al., 1971). These isozymes vary in molecular form as well as cellular localization, substrate specificity and sensitivity to inhibitors and fixatives (Hammerstrom et al., 1971). The presence of TRAP within a cell membrane is considered to be a cytochemical marker for osteoclasts (Minkin, 1982; Cole and Walters, 1987). The ultrustructural similarity between the osteoclast and odontoclast implies that TRAP may also be a cytochemical marker for odontoclasts.

In this study, in order to locate and quantify the osteoclast-like and odontoclast-like cells as well as their immediate precursors, the TRAP stain was used. The cells stained positive for TRAP were quantified in the bone, the periodontal ligament and on the root surface. The TRAP stained cells were divided by the area of bone, periodontal ligament or length of external bone or root surface. These ratios were compared over different time intervals of orthodontic force application in vivo. The resorptive cells and their putative precursor cells stained positive for TRAP. The resorptive TRAP stained cells of the bone and periodontal ligament behaved in a similar manner over time. The resorptive cell population on the root behaved differently suggesting that separate control mechanisms exist for these cells. An understanding of the control mechanisms involved in root resorption may eventually allow the inhibition of the

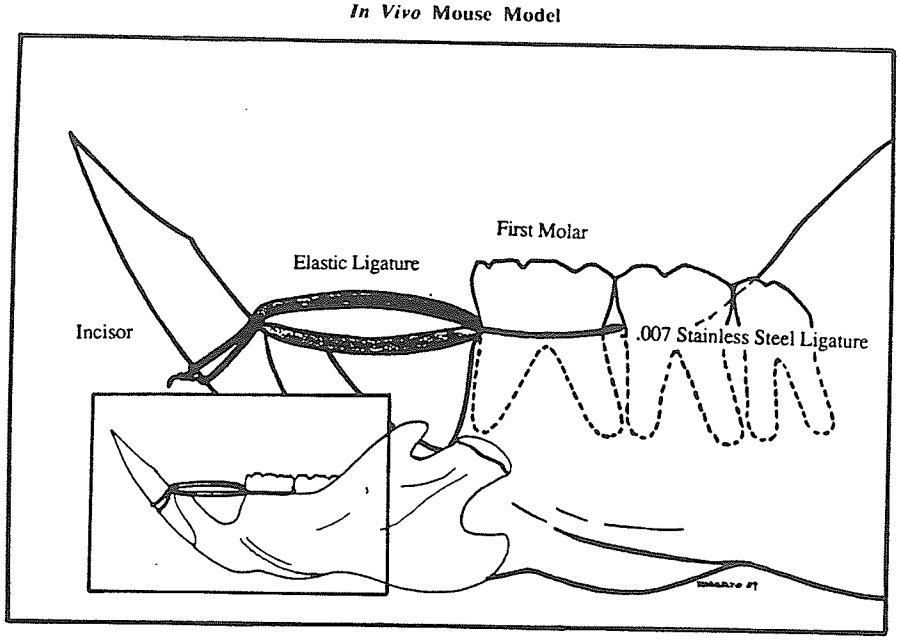
activity of the root resorptive cell population while promoting the bone resorption required for orthodontic treatment.

MATERIALS AND METHODS

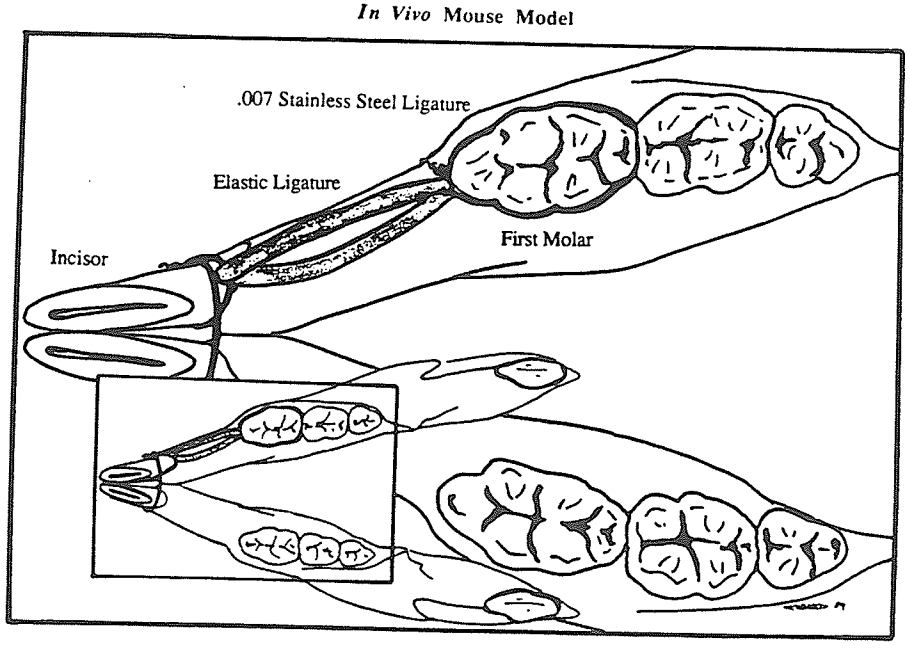
(A) Orthodontic Appliance Insertion and Dissection

The 7 week old Swiss-Webster, male mice were anaesthetized using an intramuscular injection (0.05ml/50g) of ketamine: xylazine:water (ratio 10:1:1.6 by volume, respectively) into the left gluteus muscle. The orthodontic appliance, shown in Fig.4.1, was inserted by first ligating the elastic O-ring to the mandibular left first molar using a .007" stainless steel ligature. The elastic O-ring was then secured to the mandibular left incisor using a .007" stainless steel ligature. The mandibular incisors were then ligated together using a .007" stainless steel ligature. The elastic O-rings were calibrated for load/deflection ratios (see Appendix I). The load/deflection curve is presented in Fig.4.2a and b. The mesial tipping force applied to the molar was on average 101g. A total of thirty orthodontic appliances were inserted and remained active for various time periods. Three experimental mice were euthanized by cervical dislocation for each time period (6, 12, 24, 36, 48 hours, 3, 5, 6, 9 and 10 days) and the mandibles were removed. The contralateral molars served as controls.

Fig. 4.1



Sagittal View



Occlusal View

Fig. 4.2a

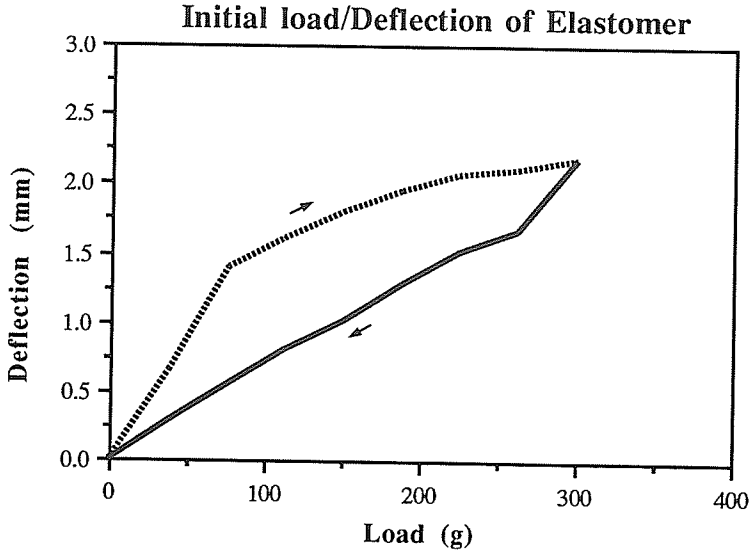
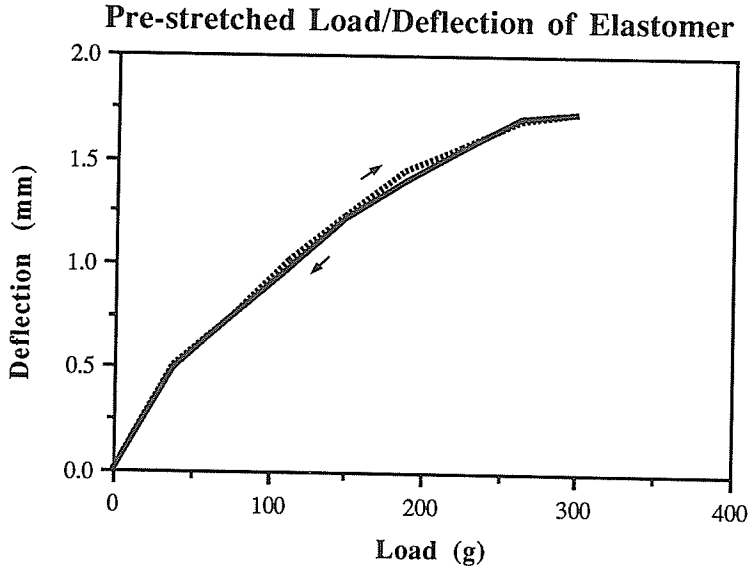


Fig. 4.2b



All soft tissue was freed from the specimens and discarded. The first, second and third molars and their associated periodontium were then separated from the remainder of the mandible by a cut inferior to the root apices and another cut distal to the third molar. This dissection was performed with the mandible completely submersed in chilled (4°C) buffered phosphate solution (pH 7.4). The specimen was immediately fixed in 3.7% formalin in phosphate buffer (pH 7.4) with 7% sucrose for 4 hours. The specimens were rinsed three times and stored 17 - 19 hours in phosphate buffer (pH 7.4) and decalcified in 10% EDTA in 0.2M sucrose (pH 7.2) for 3 weeks at 4°C, dehydrated and embedded in paraffin. The 5μm serial sections were dewaxed and stained for TRAP.

(B) <u>TRAP Stain</u>

Burstone's complete medium was prepared by dissolving 4mg naphthol AS-BI phosphate substrate (Sigma, St. Louis, MO) in 0.25ml of N,N-dimethylformamide, followed by the addition of 25ml of 0.2M acetate buffer (pH 5.0), 35mg of Fast Red Violet LB (Sigma, St. Louis, MO) as a coupling agent and 2 drops of 10% $MgCl_2$. The media was filtered into acid washed Coplin jars. A control was also prepared by omitting the substrate. In addition, sodium tartrate, disodium salt was added to a final concentration of 50mM and the medium was prewarmed to 37°C. Tissue sections stored at

4°C were allowed to come to room temperature and incubated for 90 minutes. The slides were washed for 30 minutes, air dried and counterstained with 1% fast green for approximately 15 minutes (Cole and Walters, 1987). All slides of the molar sections were stained and every third section was included in the total count of TRAP positive cells per specimen. The decision to count TRAP positive resorptive cells in every third section was based on the assumption that the average osteoclast was no more than 15μm in diameter. The elimination of two thirds of the sections would reduce the possibility of counting the same resorptive cell twice.

(C) <u>MOP-Videoplan Image Analysis</u>

The sections which were included in the TRAP stained cell counts were then evaluated for the area of periodontal ligament and bone, the length of external bone and the length of the root surface associated with the experimental molars. The task of measuring the above lengths and areas was performed by using the MOP-Videoplan (Kontron Electronic Group). The "Image Analysis" computer is designed for data acquisition and computation of geometric characteristics by tracing structures of images placed on a measuring table. The measuring program allows the operator to measure numerous geometric parameters. The parameters chosen for this investigation were area and length. The slide on which the section(s) to be traced was placed on a microscope

stage. The microscope was connected to computer monitor such that the section to be traced was displayed as a clear image on the monitor. The image was traced using a stylus on a digitizer tablet. The movement of the stylus was co-ordinated with the movement of the cursor on the monitor over the desired anatomic structures. This data was then stored in the computer memory. The number of resorptive cells and putative precursors stained positive for TRAP and the parameters measured by the MOP-Videoplan were combined to give a ratio of resorptive cells per unit of external length of bone or root length and per area of PDL or bone for each molar specimen. The numerical values for the ratios were extremely small and were therefore multiplied by 100,000 such that the ratios could be easily presented in graphical form. The changes in these ratios were statisitcally analyzed for significant differences relative to one another and relative to time (Kleinbaum and Kupper, 1978). One-way ANOVA using Tukey's correction for multiple comparison was used to determine whether there was a significant difference over time within a functional system (eg: odontoclast-like cells on the root). After normalizing the data, a Two-way ANOVA with Bonferroni's correction for multiple comparison tests was used to determine if there was an effect of time on (1) osteoclast-like cell counts per unit of external bone (ExBone) surface length versus the odontoclast-like cell counts per unit root

surface length (2) osteoclast-like cell counts per unit bone area versus putative precursor cell counts per unit area of periodontal ligament (PDL). The multiple comparison tests determined which time points showed a significant difference ($p>0.005$) between functional systems (eg: osteoclast-like cells per unit ExBone surface versus odontoclast-like cells per unit root surface).

RESULTS

Qualitative Observations

The slides incubated in Burstone's control medium, without the substrate added, did not show any TRAP positive cells. The control mouse molars which did not have orthodontic forces applied *in vivo*, showed very little resorptive cell activity as shown by the presence of very few TRAP positive cells (Fig.4.3). The resorption lacunae on the roots were observed as early as two days following orthodontic force application and were primarily located in the furcation area of the mandibular left first molar. The osteoclast-like cells in bone and the odontoclast-like cells in the root resorption lacunae stained positive for TRAP, as did the putative precursor cells located in the PDL (Fig.4.4 to 4.9). The TRAP stained odontoclast-like cells were also detected on the external surface of the apical and cervical thirds of the mouse molar root surface in putative zones of compression.

Fig. 4.3 Control mouse mandibular left first molar stained for TRAP. Note the absence of root resorption (RR) lacunae and associated odontoclast-like (OD) cells and the presence of few osteoclast-like (OC) cells. 63X

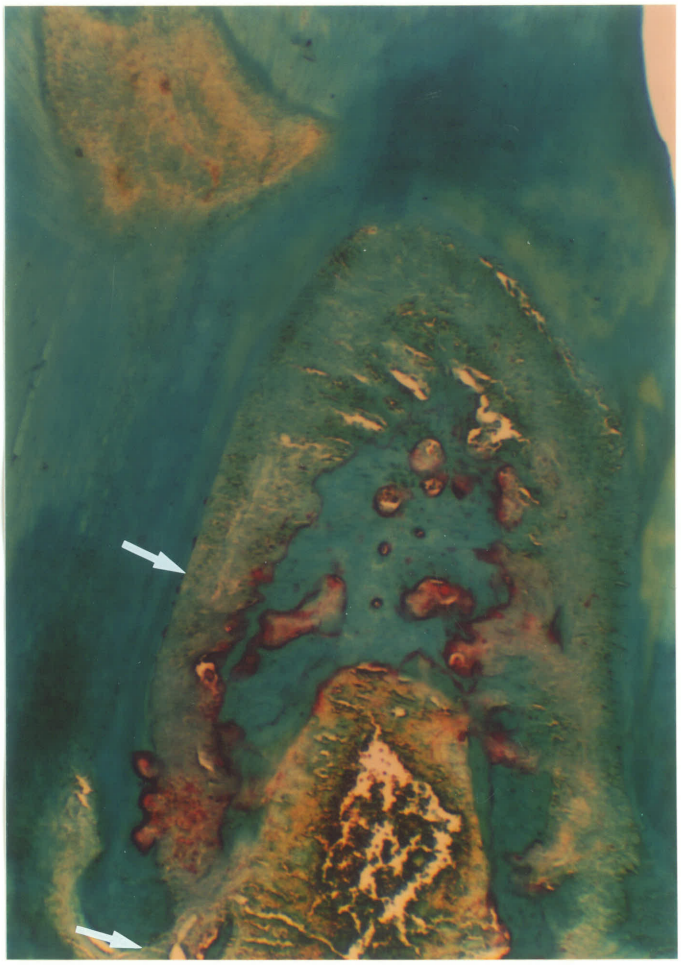


Fig. 4.4 Mouse molar periodontium orthodontically stressed for 9 days *in vivo* and stained for TRAP. Note TRAP positive OC and OD show active bone and root resorption. 63X

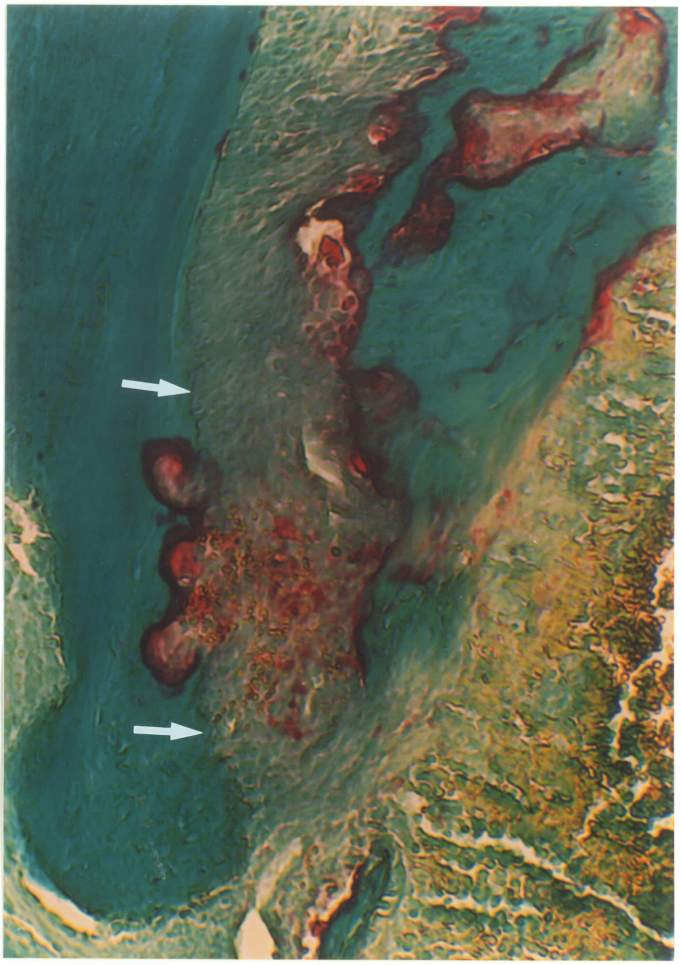


Fig. 4.5 Magnification of the area indicated by arrows in Fig.4.4
Note the TRAP positive OD in resorption lacunae and OC on the adjacent bone surface. 400X

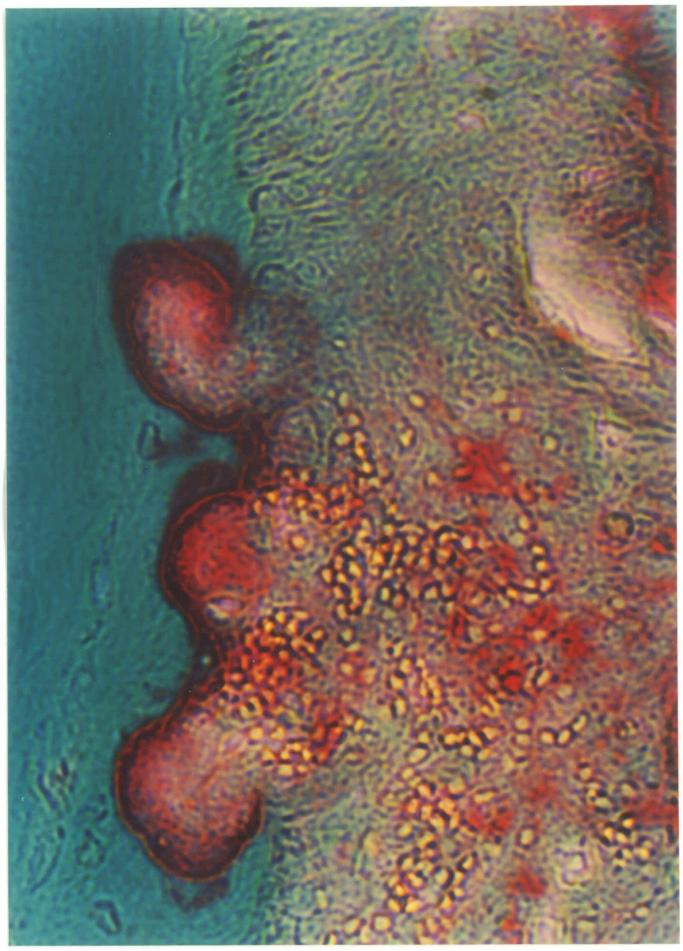


Fig. 4.6 Magnification of the area indicated by the arrows in Fig.4.5. Note TRAP stained putative precursor cells in the PDL. 1000X

Fig. 4.7 Mouse molar periodontium orthodontically stressed for 9 days *in vivo* and stained for TRAP. Note numerous TRAP stained OC and OD in the putative zones of compression. 63x

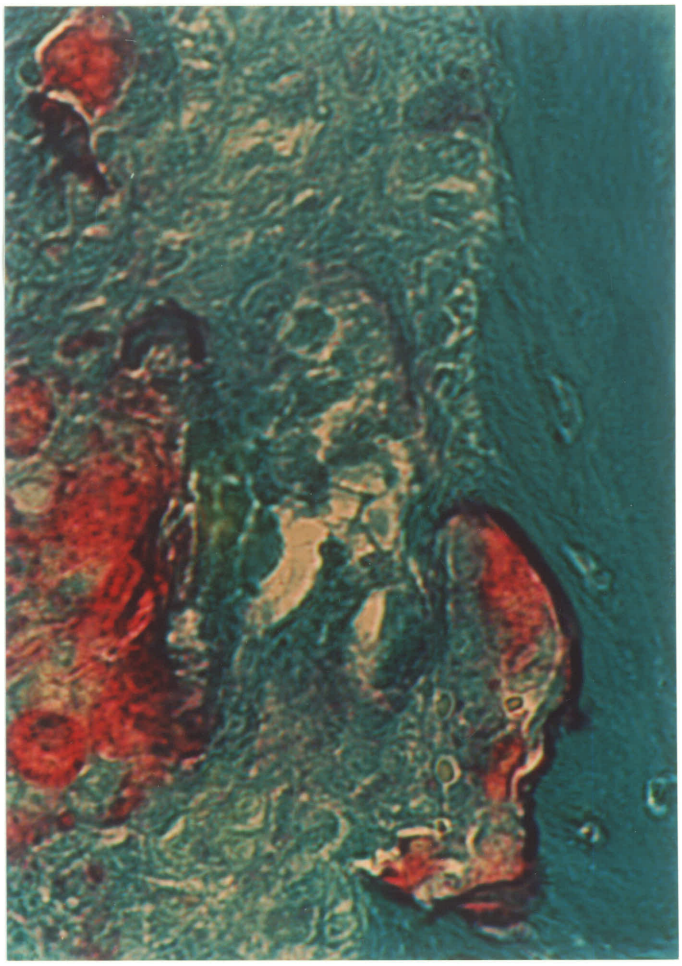


Fig. 4.8 Magnification of the area indicated by arrows in Fig.4.7.
Note the OD in the root resorptive lacunae. 1000X

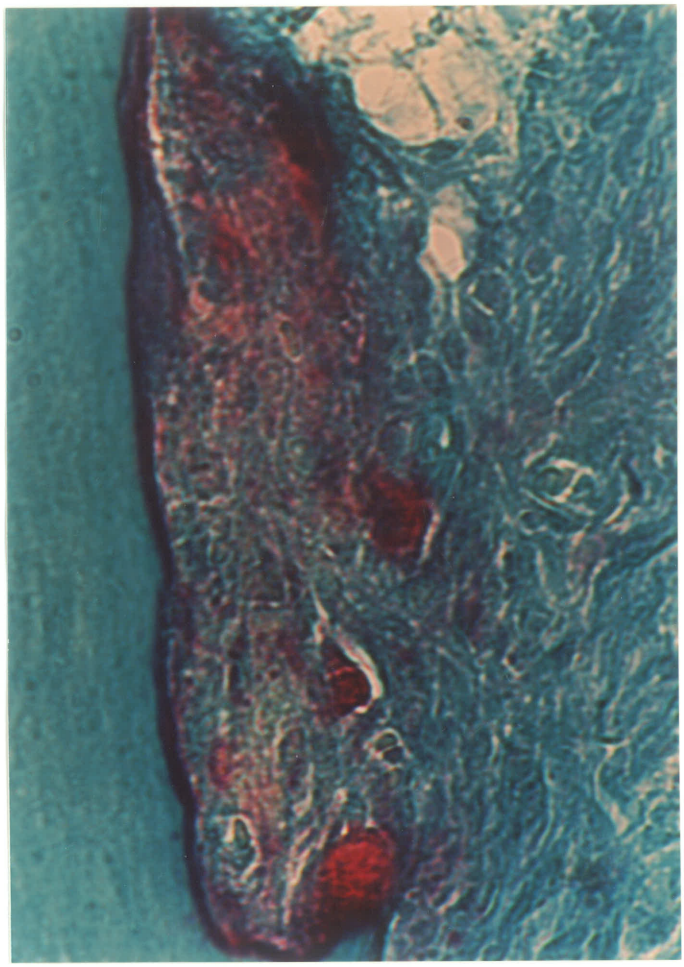


Fig. 4.9 Magnification of the area indicated by the double arrows in Fig. 4.7.
Note the TRAP positive cells on the root surface and in the PDL. 1000X

Quantitative Observations

The ratios of the number of TRAP stain cells to the areas of bone and PDL as well as to the lengths of the external bone surface and the root surface measured were calculated and are recorded in Table 4.1. The results are summarized in the graphs of Fig. 4.10 and Fig. 4.11.

The number of osteoclast-like cells and precursors (TRAP positive cells associated with bone) per unit area and per unit length of external bone surfaceperked at 5-6 days of orthodontic stress (Table 4.1). A similar pattern was found in the number of putative osteoclast-precursors in the periodontal ligament area. In contract, the members of the osteoclast-like cells and precursors only start to increase at five days and continue to increase even by 10 days of stress.

Table 4.1 TRAP Stain Cell Counts per Unit Area or Length

Time (days)	Bone (area)	PDL (area)	Root (length)	ExBone (length)
0.25	183.8±66.4	14.7±4.8	83.6±83.6	502.3±32.6
0.50	109.0±47.9	4.6±2.6	19.9±19.9	597.2±190.7
1.00	143.6±7.3	20.3±5.4	23.7±23.7	1107.2±166.9
1.50	138.1±29.8	18.8±2.6	31.6±26.9	1235.2±244.7
2.00	148.6±77.5	13.8±9.4	14.6±14.6	1020.2±471.3
3.00	280.8±85.2	62.1±37.2	0.00±0.00	1808.1±181.0
5.00	818.5±205.8	476.7±102.8	853.3±426.9	5208.8±1177
6.00	656.8±72.8	491.6±76.2	877.3±308.3	4135.1±216.3
9.00	570.9±242.2	428.9±156.2	1468.2±651.0	4000.4±1520
10.00	574.1±202.2	356.7±171.9	2226.2±1372	2572.9±624.3

* Each value represents an average of n=3±S.E.

Fig. 4.10 Trap stained cell counts per 100,000 unit area of Bone and PDL vs. Time

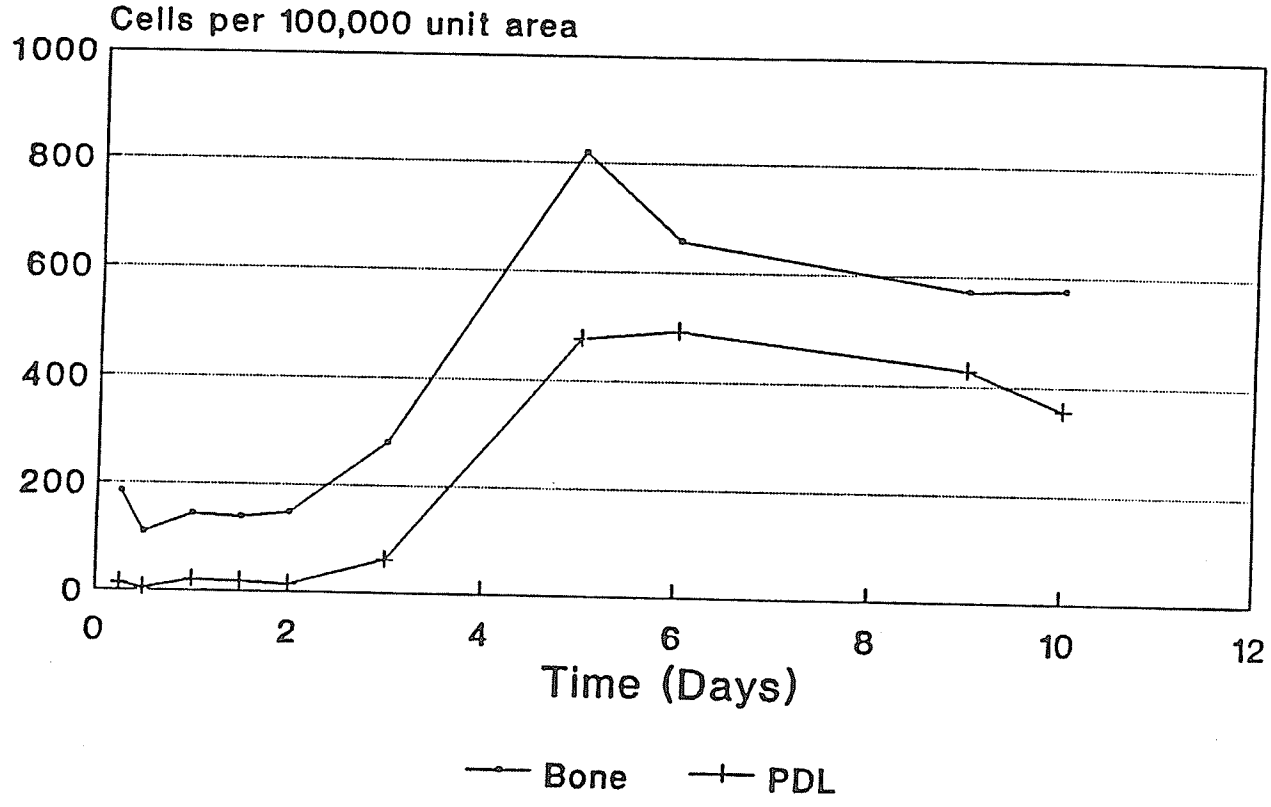
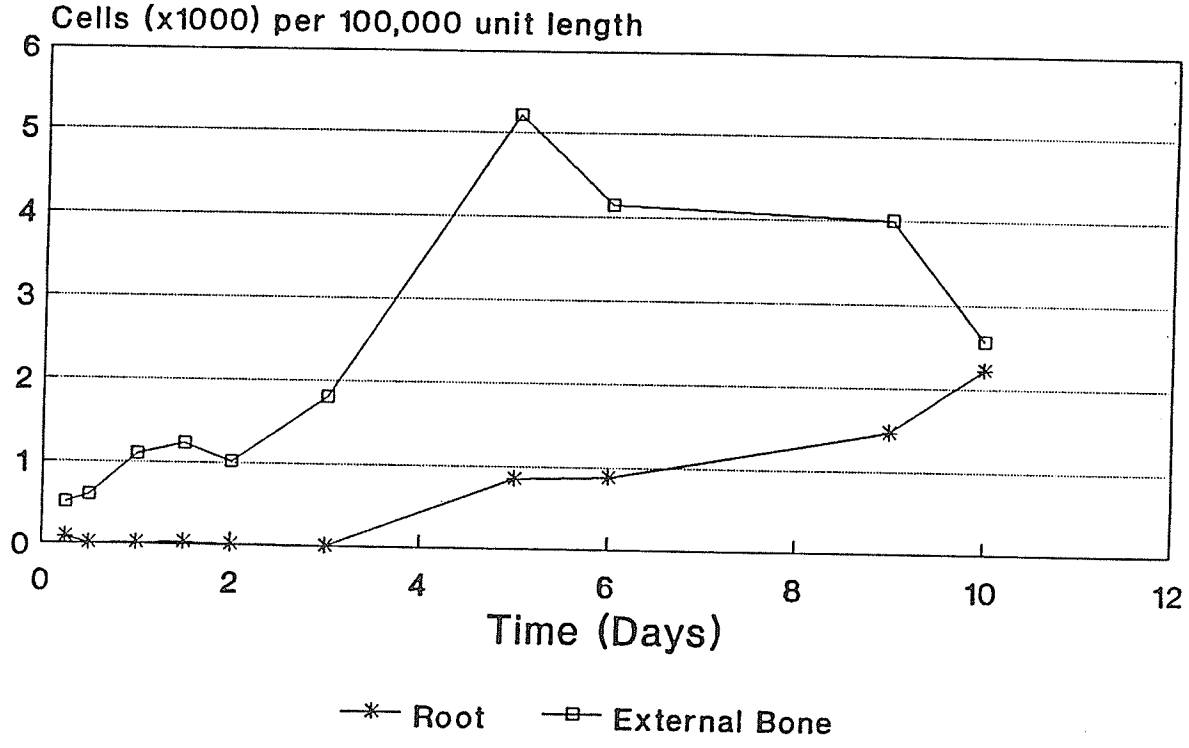


Fig. 4.11 Trap stained cells on External Bone and Root per 100,000 unit length versus Time



DISCUSSION

The lack of cells staining positive for TRAP when incubated in medium without substrate verifies the specifity of the chemical reaction. The addition of tartrate to the incubation eliminates the possibility of confusion of osteoclast-like cells with histologically very similar macrophages. The cells of the macrophage lineage contain acid phosphatase but it is tartrate sensitive (Vaes, 1988).

The general impression from inspecting the molar sections is that an increase in the number of TRAP-positive resorptive cells occurs with an increase in the duration of orthodontic force application *in vivo*. This was obvious by the increase in the area occupied by resorptive cells stained bright red for TRAP. The first odontoclast-like cells were detected after 2 days of force application *in vivo*.

The quantitative data presented verify the general impression. The One-way ANOVA with Tukey's correction showed a significant peak in the number of resorptive cells in the ExBone surface at 5 days. This value was significantly higher than all times before this form 6 hours to 3 days of *in vivo* force application. Similarily, a peak was found for resorptive cells per unit bone area at 5 days which was significantly higher than 6 hours to 3 days as well as 9 and 19 days of *in vivo* orthodontic force application. The periodontal ligament showed a peak in the ratio at 6 days. The ratio at 5 and 6 days were significantly different from all other time intervals. The odontoclast-like

cells associated with the root surface did not show a peak value during the time period studied. There was a trend of an increase in the resorptive cell number up to 10 days of *in vivo* orthodontic force application. The Two-way ANOVA of the data confirms that the number of osteoclast-like cells which dramatically increase with 5 days of orthodontic stress *in vivo* are significantly different ($p<0.01$) from the numbers present in shorter periods of orthodontic stress. The putative precursor cells in the PDL follow the same pattern but to a smaller extent, by reaching their maximum number by 6 days of force application *in vivo*. The resorptive cells on the root surface behaved completely differently from those of the bone or PDL.

The parallel apprearance of the changes in the TRAP stain cell counts in the bone area and PDL area indicates that these two cell populations behave in a similar manner. The 2-way ANOVA confirms that the is no interaction between the resorptive cell populations of the bone and PDL. This suggests that the resorptive cells in the bone and PDL area represent related or identical cell populations. The curve describing the resorptive cell populations of the external bone and root surfaces show a divergent or non-parallel relationship which suggests that there is an interaction between these groups of cells with respect to time. This interaction was also confirmed by the 2-way ANOVA of the TRAP stained cell counts on the external bone surface and the root surface. This strongly suggests that the resorptive cell population of external bone surface behave differently from those

cells which resorb the root. Bone resorptive activity has an earlier onset and peaking of resorptive cell numbers when compared to the root resorptive activity. This may explain why clinically orthodontists, generally, achieve the bone resorption necessary for orthodontic tooth movement with considerably less root resorption. This also suggests that separate control mechanisms may exist for bone resorption and root resorption. Those individuals suffering from extensive root resorption are considered to represent population of individuals at high risk for further for root resorption. This may be due to a breakdown or change in the control mechanisms specific for the root resorption cell population. Should root resorption be controlled by separate mechanisms than bone resorption it may be possible in the future to introduce clinical or therapeutic inhibitors of root resorption without jepordizing the bone resorption necessary for clinical orthodontic tooth movement.

The experimental specimens within each time interval showed a considerable degree of variation in the number of TRAP stained cells. This was somewhat surprising as the orthodontic appliances were active for the same period of time. A potential variability in activation exists due to inaccuracies resulting from small variations in the extent of deflection of the elastic during insertion. This is translated as variability in the loads applied to the molars. The range of load applied to any given molar has been calculated as 90 to 170g (see Appendix 1). The force decay was also not assessed. According to Ash and Nikolai

(1978), there is rapid decay of the initial forces generated by elastomers within the first 24 hours post-insertion. This rate of decay is dependent on the conditions under which the elastomer is tested and the extent of initial deflection of the elastomer on insertion. Therefore, the variations in the activation and subsequent behavior of each elastomer may account for some of the variability in TRAP stained cells counted. If there exist a threshold of stress which must be generated in the periodontium in order to stimulate bone resorption then it is possible that the load applied to the mouse molar was inappropriate to generate the required stress. This also becomes significant when considering the rate of decay of the elastic force. If the minimum force was applied (eg:90g) when the appliance was inserted then by 24 hours the force level could be as low as 24g.

The Swiss-Webster mice used are a genetically controlled strain of mice which implies that variability in the response of the resorptive cell population should not be related to genetic differences in the mice. It is possible that the variability observed within the time intervals studied may in part be due to biological variability.

There was also variability observed in the TRAP staining, occasionally, large multinucleated cells were present in the sections but did not stain positive for TRAP. It is possible that these cells were aging nonvital cells which did not contain TRAP. In addition, these cells may represent the population of cells from the monocyte/macrophage lineage which are high in acid

phosphatase but it is the TRAP sensitive isozyme and would therefore not react positive for the TRAP stain.

A considerable amount of research remains to be done before the exact mechanism of initiation and control of orthodontic ally induced root resorption is elucidated. The investigations completed for this project provide a foundation for continuing research. The mouse model may be applied to test the effects of bone resorbing factors in vitro on orthodontically induced root resorptive cell populations. These studies may further define the behavior of odontoclasts and contribute to our understanding of the control mechanisms involved.

The mouse model may also be used to study the effects of various force levels on the root resorptive cell populations. This may help to define an optimal force level for inducing the stress level required for bone resorption but non-optimal to stimulate the root resorbing cell population.

The TRAP stain should be used to follow the resorptive cell population beyond the 10 day in vivo force application used in this study. This would provide a more complete profile of the behavior of the different resorptive cell populations induced in response to orthodontic force application, especially the root resorptive response which was still increasing at the last time period of study. In addition, a more detailed study focusing on the 3 to 6 day period of in vivo force application may provide more repesentative information on the behavior of the resorptive

cell populations during the critical period in which the bone and PDL TRAP stained cells reach their maximum.

The presence of tartrate resistant, vanadate sensitive adenosine triphosphatase (ATPase) has also been shown to be a reliable marker for identifying osteoclasts and their immediate precursors. An even more precise means of identifying the resorptive cell populations should be attempted by raising antibodies to osteoclast/odontoclast cell populations. The immunohistochemical staining in addition to the presence of TRAP staining would be a more sensitive means of tracking and quantitating these cells in histological sections.

The behaviour of the internal bone resorptive cell population is another area which should be investigated. Preliminary data indicates that this cell population follows a similar pattern of increase as the external cell population. Further quantitaion and statistical analysis are required to complete this area of study.

CONCLUSIONS

1) Osteoclast-like cells and odontoclast-like cells stained similarly for TRAP.

2) The TRAP positive osteoclast-like cells appeared to reach a peak by 5 days of *in vivo* orthodontic force application in the overall bone area and the external bone surface.

3) TRAP positive cells in the PDL reached a maximum after 5 days of orthodontic force application.

4) Root resorption cells followed a delayed response in increase which was significantly different ($p<0.01$) from the resorptive cell pattern shown by resorptive cells on the external bone surface and in the periodontal ligament. This suggests that different control mechanisms may exist for root resorption as compared to bone resorption in response to orthodontic force application. The root resorbing cell population was still increasing after 10 days of *in vivo* force application.

CHAPTER V

DISCUSSION AND CONCLUSIONS

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DISCUSSION

There are several factors which are thought to influence the development of orthodontic root resorption. These include individual susceptibility (Massler and Malone, 1954); root morphology (Ketcham, 1929); force magnitude and duration (Harry and Sims, 1982; King ang Fischlschweiger, 1982) as well as the type of orthodontic tooth movement (Follin *et al.*, 1986). The role of each of these factors in the process of orthodontically induced root resorption is poorly understood. The controlling mechanisms at the cellular level are not known.

The development of an animal model in which root resorption can be predictably induced by orthodontic force application *in vivo* will enable more rigorous investigation of this phenomenon. The mouse model for orthodontic force application *in vivo* provides a means through which numerous root resorption specimens may be generated for study. The gene pool is homogenous and individual variation in susceptibility should therefore be reduced. The mouse molar has another advantage of yeilding an explant small enough to be viable in organ culture. This allows the study of the effect of bone resorbing factors *in vitro* on resorptive cells induced by orthodontic force application *in vivo*. The preliminary data from the pilot study are suggestive that PGE_1 *in vitro* has a stimulatory effect on the resorptive cell populations induced by orthodontic force application *in vivo*. Robinson and Harvey (1989) have also shown

that an increase in systemic parathyroid hormone increases the rate of bone and root resorption in rats. The observation that the osteoclast and the odontoclast appear to respond in the same manner to bone resorbing factors strongly suggests that the osteoclast and the odontoclast represent the same cell population. SEM has provided convincing evidence that these two cells are morphologically identical and differ only in the substrate which they resorb in vivo, dental hard tissue for the odontoclast or bone for the osteoclast (Ten Cate and Anderson, 1986; Sasaki et al., 1989).

The close relationship of the osteoclast and odontoclast is further supported by the studies in vitro which have shown that osteoclasts will readily resorb sperm whale dentin (Jones and Boyde, 1988). The positive staining of the odontoclast for the cytochemical marker of the osteoclast, TRAP, further substantiates the extent of the similarity of the osteoclast and odontoclast. The question remains as to why such a dramatic difference in the rate and extent of bone resorption in comparison to root resorption has been repeatedly observed (Rygh, 1977; Williams, 1984; Farrell et al., 1989). The cemental layer has been reported to offer a protective barrier against root resorbing cells (Rygh, 1977; Andreasen, 1988). The exact mechanism of how this is achieved is presently unknown. It is uncertain whether the resistance to root resorption is provided by the physical presence of the cementum,in particular, the thin layer of cells immediately adjacent to the root surface

(Andreasen, 1988). The cementum has been shown to contain an anti-invasive factor, collagenase inhibitor, which is thought to contribute to the resistance of the root surface to resorptive cell activity (Lindskog et al., 1984). The degree of injury to the root which is necessary for the initiation of root resorption is another unanswered question. Perhaps, a much greater extent of microtrauma is required to elicit a resorptive response on the root surface.

Histologically, a comparison between bone and root reveals that the root is an avascular structure while the bone is rich in blood supply. This may play a role in the efficiency of delivery of resorptive cell progenitors to their ultimate site. This could explain, in part, the observed lag in the root resorption as compared to bone resorption. The bone is immediately accessible to the osteoclasts whereas the root is more remote. The chemotactic signals may be firing to elicit migration of the resorptive cells to the root surface but because of the relative distance from the progenitors the root surface does not resorb as readily. Dormant osteoclasts are routinely observed as a normal member of the bone cell population (Vaes, 1988). This does not hold true for the cementum layer of the root surface and this may also be a factor.

The bone tissue is also rich in osteoblasts. The exact role of osteoblasts in regulating bone resorption is not yet completely understood. There is evidence to suggest that the osteoblast acts at various levels in regulating osteoclast

resorption. The osteoblast has been shown to release collagenase in response to bone resorbing factors. The collagenase is thought to play a role in the degradation of a protective layer of organinc material which cover the surface of mineralized bone (Heath et al., 1985). The end result is exposed bone which is accessible to osteocolast activity. It has also been shown that the osteoblast releases a soluble factor into the local microenvironment which is required for osteoclastic bone resorption (Chambers, 1980). The surface of the osteoblast has receptors for bone resorbing hormones further suggesting that it plays a vital role in regulating osteoclast activity. (Chambers et al., 1984). Exactly how the mechanism of osteoblast control of osteoclasts may apply to odontoclasts is uncertain. It may be that the nature of the cells which occupy the root surface layers may be sufficiently different such that they do not react to bone resorbing factors in exactly the same way or at the same rate as osteoblasts.

The results presented in Chapter IV verify that the cell populations in the bone and PDL significantly differ from those resorptive cells occupying the root resorptive lacunae in their response to orthodontic force application in vivo. Therefore, regardless of the evidence of the morphological and cytological similarity of the osteoclast and the odontoclast there is now sufficient evidence to suggest that based on the response of these cells to orthodontic force application they may represent two separate resorptive cell populations or they may be under the

influence of separate control mechanisms. It is possible that these cells may differ in a manner which is not yet understood. The control mechanisms regulating bone resorption are extremely complex and are far from well understood. The multitude of known factors which interact to effect stimulation of bone resorption may only represent a fraction of the total scheme if there are unknown factors operational in the control of the resorptive process.

Therefore, the reason for the difference in resorption potential between bone and tooth structure is presumably very complex. It is very possible that on the root surface, just as in bone, there are many levels at which resorption is regulated. The control mechanisms are not known but it appears as though the activity of the cell population responsible for root resorption is considerably more tightly regulated than that responsible for resorption of bone. This regulation may be achieved by the physical properties of the root and/or the sensitivity of the root resorbing cell population to bone resorbing factors.

CONCLUSIONS

1) Orthodontically stressing mouse molars *in vivo* for various time periods has been shown to predictably induce root resorption.

2) The pilot study using ^{3}H-proline labelling of the mouse molar explants *in vitro* verified the viability of orthdontically induced root resorptive cells in organ culture. In addition, preliminary data suggest that PGE_1 may have a stimulatory effect on the root and bone resorptive cell activity.

3) The odontoclast-like cells stained positive for TRAP indicating that these cells are cytochemically similar to the osteoclast.

4) The number of TRAP stained resorptive cells reached a maximum in the both the bone and the periodontal ligament after 5 days of orthodontic stressing *in vivo*. The number of TRAP stained resorptive cells on the root surface began to increase after 3 days and continued to increase up to the tenth day of orthodontic stressing *in vivo*.

5) The study suggests that although the resorptive cells induced by orthodontic stressing *in vivo* (eg: the osteoclast and odontoclast) are morphologically, functionally and cytochemically very similar they behave differently in response to orthodontic stress. The reason for this is unknown.

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APPENDIX 1

LOAD/DEFLECTION CURVE FOR IN VIVO ORTHODONTIC APPLIANCE

APPENDIX 1 - LOAD/DEFLECTION CURVE FOR IN VIVO ORTHODONTIC APPLIANCE

(A) INTRODUCTION

Root resorption which occurs in response to orthodontic tooth movement is affected by a number of factors of which the force level applied is one. The magnitude and duration of the orthodontic forces applied (Harry and Sims, 1984) the type of appliance used and the type of tooth movement (Follin et al., 1986) and the particular tooth involved all play a role in the tendency for root resorption to develop. In addition, other factors such as the nutriture of the patient (Steadman, 1942); endocrine disorders (Steadman, 1942); calcium homeostasis (Engstrom, 1988; Engstrom et al., 1988); immunological factors (King and Courts, 1988, 1989) and root morphology (Rudolph, 1940) have all been implicated in affecting the rate of formation and the extent of root resorptive lesions. The susceptibility of an individual to develop root resorption in response to orthodontic treatment has also been suggested as an important factor in the process of root resorption (Henry and Weinmann, 1951; Massler and Malone, 1954). Root resorption in response to orthodontic tooth movement is presumably a result of complex interactions between many or all of these factors.

The development of an orthodontic appliance system which would predictably induce root resorption in experimental animals was first reported by Rygh (1972, 1977). This appliance was

designed to deliver orthodontic forces to tip the maxillary first molar of Wistar rats in a buccal direction. This model has since been modified to orthodontically induce root resorption in the mandibular first molar of Swiss-Webster mice (Brudvik and Rygh, unpublished, 1988; Farrell et al., 1989). The load/deflection curve for the appliance system to be used in the mouse model was completed such that the force levels applied during activation of the appliance could be approximated. The applied load is an important parameter as it provides a basis for comparison of observations made in previous investigations. An understanding of the force levels delivered by the appliance is also necessary in order to assess whether the appliance is comparable to those used clinically on human orthodontic patients. The load/deflection curve will also provide baseline data from which future investigations may be completed on the level of root resorption which occurs in response to a number of different force levels.

(B) MATERIALS AND METHODS

Elastic ligatures were suspended from a hook by a 0.007" stainless steel ligature. An increasing load was then applied and the change in length of the elastic O-ring was then measured to the nearest .01mm. The elastics tested include Kwik-stik Alastic ligatures; Ormco powerchain and Generation II powerchain. Two elastics from each group were tested. Each elastic was assessed over three cycles of loading and unloading. The distance from the mesial of the mandibular left first molar to the distal

of mandibular left incisor was measured to the nearest .25mm on 15 different mice. The external diameters of 15 Kwik-stik Alastic ligatures were also measured.

(C) RESULTS

The deflection of the Kwik-stik Alastic for a given load was measured and the data from one trial of 3 loading-unloading cycles is summarized in Table.A1.1. The load/deflection curve was then plotted (Fig.A1.1,A1.2). The first loading-unloading cycle has been plotted separate from the third cycle. The distance from the molar to the incisor on 15 mice was measured, the external diameters of 15 Kwik-Stik Alastic O-rings were also measured, the differences between these values were calculated and are recorded in Table.A1.2. The average deflection was calculated by subtracting the average external diameter of the O-rings from the average length of the space between the molar and the incisor. This value was then used to interpolate the average load applied to the mouse molars.

Table.A1.1 Load/deflection data. Trial One Kwik-stik Alastic

Load (g)	Deflection (mm)					
	Loaded	Unloaded	Loaded	Unloaded	Loaded	Unloaded
0	0.00	0.00	0.00	0.00	0.00	0.00
37.5	0.28	0.65	0.51	0.81	0.49	0.51
75	0.56	1.39	0.75	1.06	0.73	0.74
112.5	0.80	1.61	0.98	1.31	0.98	1.01
150	1.02	1.79	1.20	1.50	1.21	1.23
187.5	1.28	1.94	1.40	1.62	1.39	1.44
225	1.51	2.06	1.57	1.74	1.54	1.56
262.5	1.67	2.11	1.71	1.87	1.69	1.68
300	2.17	2.17	1.90	1.90	1.72	1.72

Fig. A1.1a

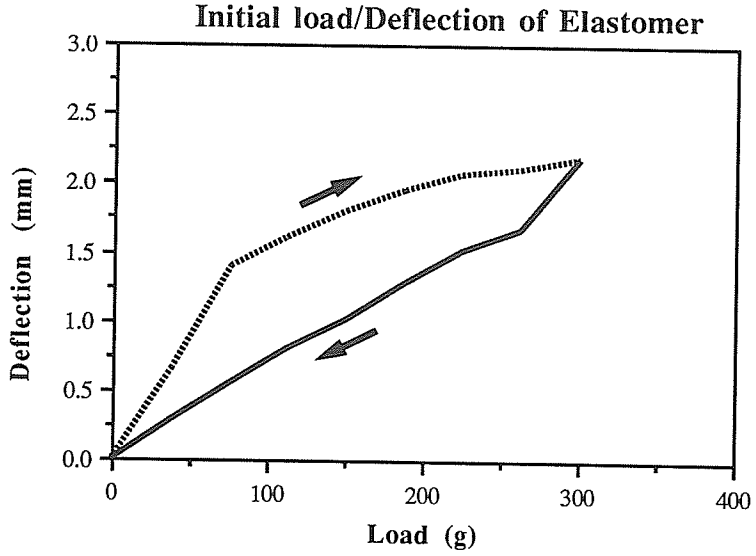

Fig. A1.1b

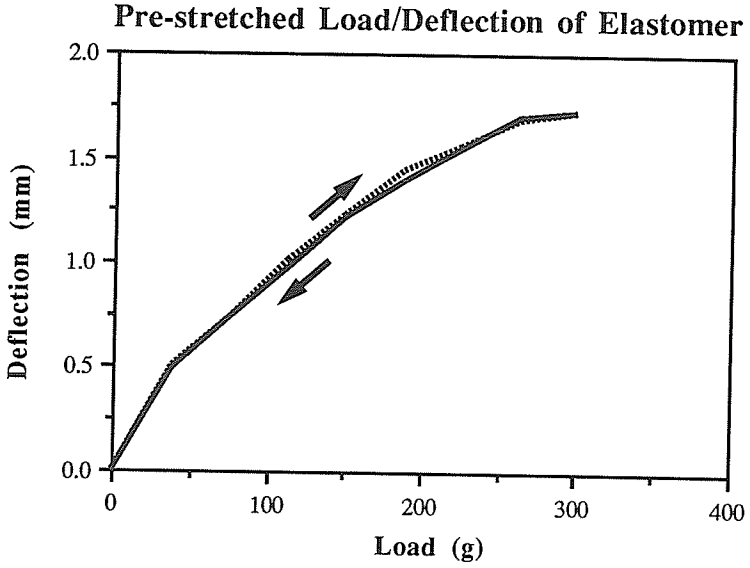

Table.A1.2 Determination of range and average load applied using Kwik-stik Alastic to orthodontically stress mouse molars.

	Distance (mm)	Distance (mm)	Deflection (mm)	Load (g)
	3.50	2.50	1.00	110
	3.75	2.50	1.25	150
	3.50	2.50	1.00	110
	3.50	2.50	1.00	110
	3.40	2.50	0.90	100
	3.60	2.75	0.85	95
	3.50	2.75	0.75	75
	3.50	2.50	1.00	110
	3.60	2.50	1.10	120
	3.50	2.75	0.75	75
	3.50	2.75	0.75	75
	3.40	2.50	0.90	100
	3.50	2.50	1.00	110
	3.50	2.75	0.75	75
	3.60	2.75	0.85	95
Total	**52.85**	**39.25**	**13.85**	**1510**
Average	**3.50**	**2.62**	**0.92**	**101**

The difference between the distance from the mesial of the molar to the distal of the incisor was calculated. This value represents the change in the external diameter of the O-ring. The change in diameter, or the deflection allows the interpolation of the load applied to the molar. The average deflection was 0.92mm which resulted in an average load of 101g. The range of deflection was from 1.25 to 0.75mm, the range of applied load was 75 to 150g.

(D) DISCUSSION

The load/deflection curves of the Kwik-stik Alastic example indicates that after the first loading/unloading cycle the hysteresis is significantly reduced such that it cannot easily be detected. The changes observed in the elastic behaviour following the first loading/unloading cycle reflect plastic deformation. This explains why the elastic then behaves in a more predictable manner for the subsequent loading cycles. This observation confirmed the importance of pre-stretching the elastics prior to their insertion (Brantley et al., 1979). Each elastic O-ring was, therefore, pre-stretched for each appliance by increasing the external diameter by 4mm.

The 101g of mesial tipping force would be expected to decay over the time period studied as the molar moves mesially and the degree of deflection of the elastomer is decreased. The rate of decay was not measured in this project. The behaviour of

elastomers has been investigated and it is generally agreed that 50-70% of the initial force is dissipated after the first 24 hours post-insertion of the appliance (Killany and duplessis, 1985; DeGenova et al., 1985; Kuster et al., 1986; Rock et al., 1986). This is followed by a period of more gradual force decay (Killany and Duplessis, 1985; Kuster et al.,1986). The difficulties in measuring the force decay of elastomers, particularly under intraoral conditions of 100% humidity at 37°C, prohibit the finite determination of the loading of the mouse molar. The values therefore represent approximations. The rate of decrease in the forces applied is a function of the type of elastomer used and the conditions under which the force decay is studied (Ash and Nikoli, 1978; Rock et al., 1986; Rock et al.,1985). The forces appear to decrease more rapidly under intraoral conditions (Ash and Nikoli, 1978; Rock et al., 1986). The rate of decay is also affected by the amount of extension of the elastomer (Andreason and Bishara, 1970; Bretl and Droschl, 1986).

(E) CONCLUSION

The load/deflection curve for the elastic O-rings, Kwik-stik Alastic, was completed. The force level applied in vivo was also determined based on the average deflection of the elastic. This force level was found to be 101g. This load/deflection curve may be used for future studies where the deflection of the elastic is varied to produce different force levels.