CHANGES IN INTRAOSSEOUS FIBERS OF THE PERIODONTIUM PRODUCED BY ORTHODONTIC TOOTH MOVEMENT

THESIS

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WINNIPEG, MANITOBA

BY

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CHANGES IN INTRAOSSEOUS FIBERS OF THE PERIODONTIUM PRODUCED BY ORTHODONTIC TOOTH MOVEMENT

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ABSTRACT

This study examined the effects of tooth movement on patterns of mineralization, protein synthesis and glycosaminoglycan distribution of extrinsic (intraosseous) and intrinsic fibers of the interdental septum of the rat. Calibrated springs produced a continuous separating force (25 g) to the first and second maxillary molar teeth of rats, aged six weeks. The untreated side served as control. Animals were killed 1, 2, 3, 4, 5 days after spring placement for scanning electron microscopy (SEM) to study changes in patterns of mineralization, and light microscopy (LM) to study changes in glycosaminoglycan distribution. A radioautographic (RAG) study was designed to study changes in collagen synthesis. A single injection of [^{3}H]-proline was administered at the time of spring placement, and animals were killed 1, 2, 3, 7, 10, 14 days after injection. Both qualitative and quantitative analyses were done.

The results indicate that extrinsic (intraosseous) fibers maintain or increase their mineral content, maintain a collagen synthesis rate that is higher than that of intrinsic fibers and maintain a low glycosaminoglycan content. The intrinsic fibers decrease their mineral content as a result of the force, do not change their collagen synthesis rate significantly, and decrease their glycosaminoglycan content.

The findings suggest that intraosseous fibers are not largely affected by orthodontic forces and possibly serve as a framework

for the remodelling of intrinsic fibers, which were greatly influenced by the force.

To Dr. Matilde Leon

for her invaluable support throughout my education

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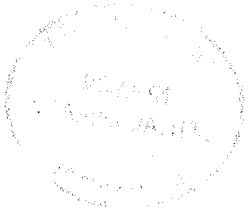
INTRODUCTION

The concern of orthodontists for efficient mechanics of tooth movement and a stable final tooth position, has encouraged investigators to study the histological changes resulting from orthodontic tooth movement. For years researchers have been concerned about specific events in the periodontium such as identification of cellular events induced by tooth movement, mechanisms that transmit mechanical influences into cellular reactions and variables that influence these cellular reactions. The high quality of the research developed in this area has allowed us to understand many factors that contribute to tooth position relapse. In spite of our increased knowledge, we have not been able to completely control tooth movement and stability.

Some types of orthodontic position relapse have been attributed to periodontal ligament fibers, in particular to transseptal fibers. Changes in the periodontal ligament and the alveolar wall have been studied in detail. Since the beginning of the last decade, it has been established that cementoalveolar fibers penetrate deeply into alveolar bone. This situation somewhat contradicts earlier observations which concluded that these fibers were anchored close to the bone surface. The remodelling of these deeply embedded fibers, the "intraosseous fibers" incident to orthodontic tooth movement, has not been studied. Understanding the changes taking place in these fibers will contribute to our knowledge of alveolar bone and periodontal ligament remodelling resulting from orthodontic tooth movement.

This project was designed to evaluate the changes in mineralization, protein turnover and glycosaminoglycan distribution in bone matrix and intraosseous fibers resulting from orthodontic tooth movement.

THE MODEL



THE ANIMAL

Thirty-two young female albino rats, six weeks of age, bred in the Faculty of Dentistry Animal House were used for the experiments. They were maintained on Wayne Laboratory, Blox F-9 specialty food, given water ad libitum, and kept at a temperature of 20 ± 5°C. The rats were anaesthetized with ether, placed on an operating table and a spring (Fig. 1) was placed between their left first and second maxillary molar teeth (Fig. 2). The right side was not treated and was used as a control. The weight of each animal was measured before spring placement and before sacrifice, allowing for assessment of normal growth during the experimental period. A group of 30 control rats of the same age maintained under the same conditions, were weighed at the same time intervals as the experimental animals. A comparison between the mean weight increase of control and experimental animals can be seen in Fig. 4.

Anaesthetized animals were killed by creation of an haemothorax and the maxilla was removed immediately by blunt dissection. Tissue was either fixed for 3 hours in Karnovsky's fixative (Karnovsky, 1965) for light (LM) and scanning electron microscopy (SEM), or for 24 hours in Bouin's fixative (Beersten and Tonino, 1975) for radioautography (RAG).

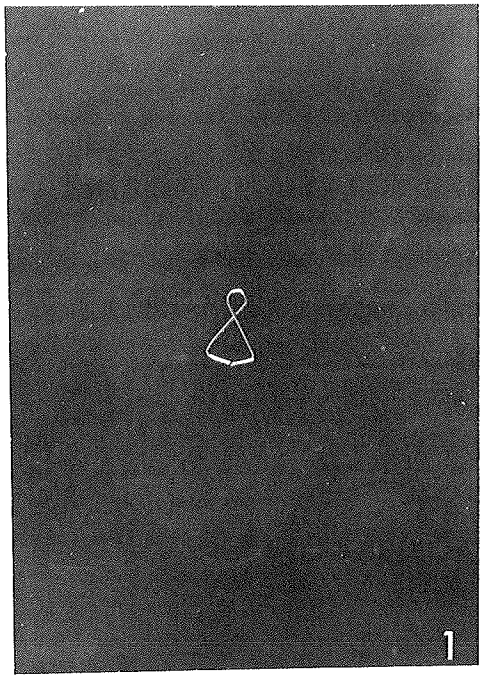
SPRING

The spring used in the experiments to create orthodontic tooth movement has been described by Hadji-Salem (1971). Stainless steel orthodontic wire, 0.012 inches in diameter, was bent as shown in Figure 1. The dimension of the spring was such that a deflection of 1 mm from its active portion produced a force of 25 gms. After approximately five days, the spring was loose, indicating that maximal separation had been accomplished.

Fig. 1 Spring of 0.012 stainless steel orthodontic wire.

Fig. 2 Spring placed between first and second maxillary molars.

Fig. 3 Force diagram. Arrows indicate movement of the molars as a result of force applied. The nature of the movement will create pressure on the apical third of the interdental septum, tension on the occlusal third and probably a neutral force at the middle third.
S - Spring between first and second molars.
B - Alveolar bone.



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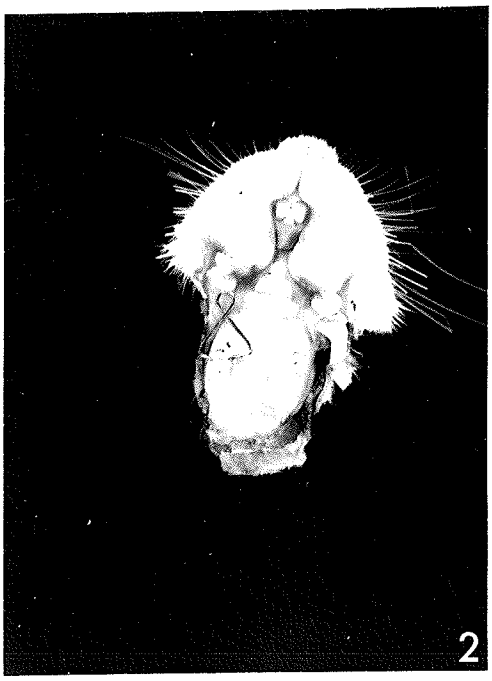

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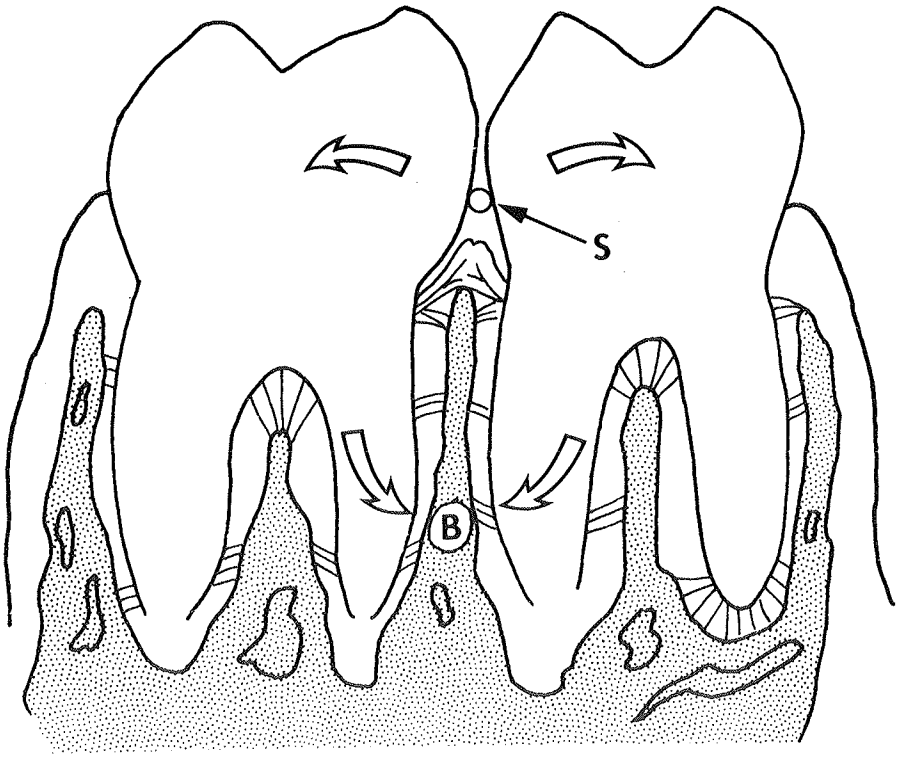

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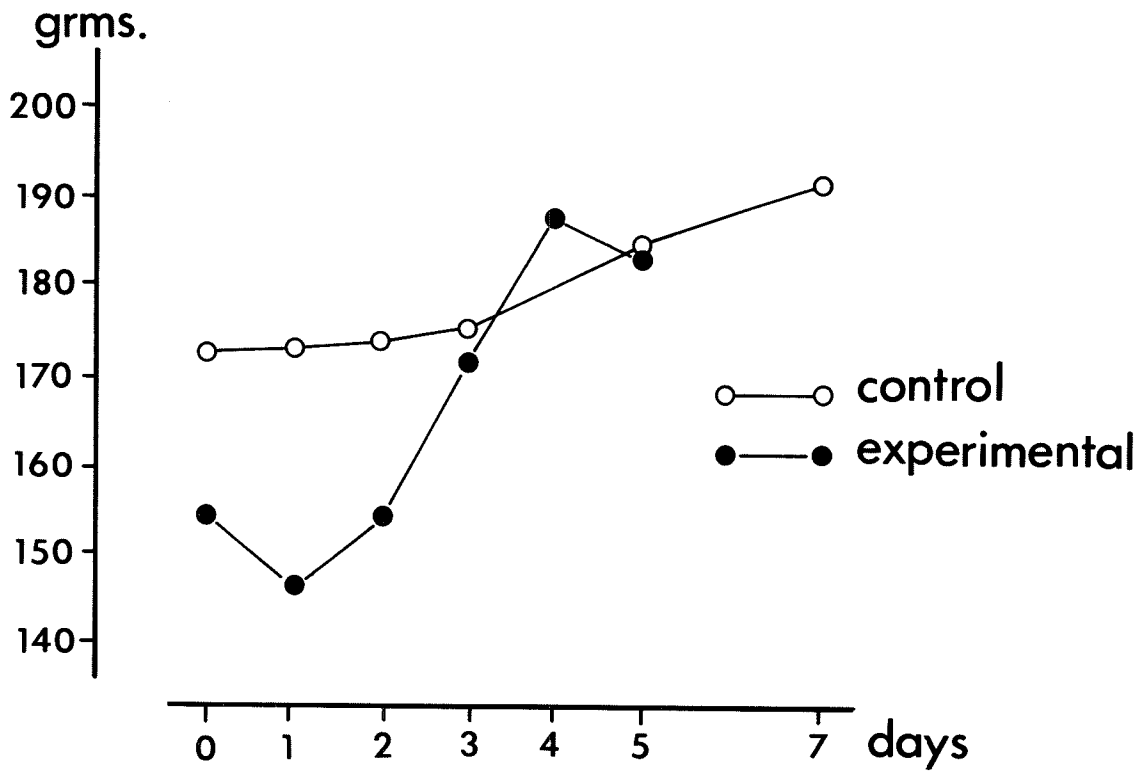
Fig. 4 Graph illustrates mean changes in weight of animals used in the different experiments of this study (experimental) (32 animals in total for the three different studies). A control untreated group (control) indicates the normal weight increase in albino female rats kept under identical conditions (30 animals).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days
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Fig. 4

INTRODUCTION

There is much information in the literature concerning the remodelling of the periodontium during orthodontic tooth movement. The interest in this field has developed mainly from the concerns about relapse of orthodontically corrected teeth. Much time has been spent trying to clarify the changes taking place in the periodontal ligament as a result of tooth movement. Some of the fiber groups of the periodontal ligament (i.e. transseptal fibers) have been found to play an important role in the relapse of tooth movement (Thompson, 1958, 1959; Reitan, 1959, 1960; Edwards, 1968). The changes occurring in Sharpey's fibers during tooth movement have been studied (Reitan, 1960; Rygh, 1973; Kurihara and Enlow, 1980a; Rygh et al., 1982). Some of these fibers are deeply embedded in the alveolar bone. These fibers have been named "intraosseous fibers" and their response to tooth movement is unknown. This chapter will report on scanning electron microscopic (SEM) observations of changes in these fibers produced by orthodontic tooth movement.

LITERATURE REVIEW

Several studies of alveolar bone have demonstrated that fibers of the periodontal ligament were embedded deeply within alveolar bone. These fibers have been named "Sharpey's", "intraosseous" or "transalveolar" fibers. The term "intraosseous" fiber will be used herein. Intraosseous fibers have been studied in light microscopy (LM) by Quigley (1970), Cohn (1970, 1972a,b, 1975), Johnson (1981, 1983), Johnson and Low (1981), Bernick et al. (1974, 1977). Sharpey's Fibers at the margin of the alveolar septum were studied by Shackleford (1971a,b, 1973) in TEM, Boyde and Jones (1968), Jones and Boyde (1974), Svejda and Skach (1973) in SEM. Intraosseous fibers have been studied in electron microscopy by Johnson and Low (1982, 1983) in high voltage electron microscope (HVEM), and SEM, Johnson (1983) in HVEM and SEM, and Johnson and Highison (1983) in SEM.

Reports on Intraosseous Fibers Embedded Deeply in the Alveolar Bone

In this chapter reference will be made only to electron microscopic studies. Several studies described the development of intraosseous fibers [Johnson and Low (1981, 1983) LM, Bernick (1960, 1974) LM]. Johnson and Low (1982) studied developing intraosseous fibers of the mouse in the high voltage electron microscope (HVEM). Intraosseous fibers were described as early as

day 14 in the mouse, prior to the eruption of the first mandibular molar. These fibers were composed of unit collagen fibrils. In cross section, they displayed electron dense areas which subdivided the fiber bundle. Following the eruption of the second molar, the fibers increased in diameter.

The maintainence of intraosseous fibers at the bone margin has been described by Deporter and Ten Cate (1980) and by Garant and Cho (1979). However, little attention has been given to their maintenance in bone. Johnson and Low (1982) did not observe fibroblasts adjacent to the intraosseous fibers. They suggested that since the collagen of the bone matrix was produced by osteocytes, the intraosseous fiber collagen could also be produced by osteocytes. This was based, in part, on their observations of the proximity of osteocytes and intraosseous fibers and the lack of a lacunar wall between the osteocytes and the fibers. Johnson and Low (1983) studied intraosseous fibers in SEM. The study revealed that they were composed of a bundle of smaller fibers. This fiber bundle passed through channels in bone and was attached to the bone by anchoring fibers. Some fibers branched within bone. Johnson and Highison (1983) developed a technique that allowed for the selective removal of bone matrix and osteocytes exposing intraosseous fibers for observation by SEM. These fibers passed through bone without interruption at all levels. Johnson (1983) described morphological differences between intraosseous fibers of different levels of the interdental septum. They became less mineralized as they passed from the wall of the tooth socket to the midline of the interdental septum in all areas except in the cres-

tal third of the septum. On fractured surfaces of anorganic bone of the superior crestal third, intraosseous fibers were recessed from the surrounding bone matrix. They exhibited a network of fissures on their surfaces which subdivided the fiber into fascicles. As anorganic specimen preparation removes non-mineralized tissue, these fissures were likely sites of unmineralized components of the fiber. Surrounding each fiber was an elevation of bone, termed a "sheath". In the middle and inferior crestal thirds of the interdental septum, anorganic SEM preparations revealed that intraosseous fibers were of smaller diameter and of more circular outline than those of the superior crestal third. In the middle and apical thirds, intraosseous fibers were of smaller diameter than in the crestal third. These fibers had large, central uncalcified cores. Each fiber was completely surrounded by a sheath and was recessed from the bony surface. Intraosseous fibers at the surface of the socket (Sharpey's fibers) either projected from or were recessed from the bone surface. The results of this study indicated that intraosseous fibers of the alveolar crest were more heavily mineralized than in bone of more apical levels. HVEM observation suggested that the uncalcified cores observed in SEM were of similar distribution as electron-dense areas observed in HVEM preparations (Johnson, 1983). Thus, these areas might represent unmineralized cores. This finding is in agreement with Selvig (1965), who reported that Sharpey's fibers near the alveolar wall contained an uncalcified core of collagen fibrils.

Intraosseous Fibers near the Tooth Socket Wall

Shackleford (1973), in electron microscopy studies, described intraosseous fibers of anorganic root sockets from mongrel dogs. Unmineralized cores were present within these fibers. They were larger and more regular in distribution near the alveolar crest than in other areas. These findings agree with Selvig (1965), Boyde and Jones (1968), and Jones and Boyde (1974). Selvig (1965) described intraosseous fibers of human bone at the alveolar wall. They contained an uncalcified core of collagen fibrils, surrounded by a dense, crystalline peripheral layer. Boyde and Jones (1968) suggested that the presence of an unmineralized central core in these fibers was related to a perfectly mineralized external periphery that prevented the diffusion of mineral ions to the centre and, thus, acted as a block to its central mineralization. Irregularities of the calcified portions of intraosseous fibers (trabeculae and orifice boundaries) appear to constitute, according to Shackleford (1973), elevations or protuberances directed along force lines. The calcified irregularities would point in the same direction as the force vector, along the fiber's central axis. It is possible that this arrangement of calcified matrix, in combination with unmineralized collagenous fibrils, would allow for a degree of flexibility and, at the same time, impart maximum strength to the fiber bundle. Jones and Boyde (1974), in a SEM study of intraosseous fibers, concluded that they were very constant in size among various species and at various sites of the socket wall. Those fibers at the alveolar wall differed little

from those of cemental Sharpey's fibers (Jones and Boyde, 1974).

In reference to SEM observations of calcified tissues, Boyde (1972) described some of the characteristics of the anorganic bone surface. He recommended the use of 5-10% solution of sodium hypochlorite (NaClO) to expose the mineral components of a hard tissue. According to Boyde, with the SEM, it is possible to identify the forming, resting and resorptive areas in a particular part of the bone structure by using simple morphological criteria. Resorbing surfaces show excavated pits, Howship's lacunae, and collagen fibers whose orientation varies in a random fashion over small areas. Forming and resting surfaces are characterized by the presence of large areas of uniform collagen fiber orientation. New bone deposition is marked by the presence of clusters of calcospherites which were recently formed within the new matrix. These mineral deposits are also described by Frasca (1981) in SEM studies of human fetal bone. The author studied tibias and femurs of human fetuses 4 months old. The bone samples revealed spherical structures approximately 0.1 μm in diameter. He suggests that these "bone nodules" coalesce into coarse linear structures and become increasingly scarce as the primitive bone fibrils develop into mature bone fibrils. Calcospherites are thought to form from an aggregation of the $CaPO_4$ particles which originate, in part, from matrix vesicles.

An interesting finding of Boyde (1972) is the description of changes of intraosseous fibers at the alveolar wall in relation to bone matrix fibers. In anorganic preparations the intraosseous fibers appear as holes in the mineral surface in areas where new

intrinsic matrix has recently been formed. Conversely, the intraosseous fibers are mineralized to the level of the surrounding matrix surface, and commonly beyond that level, so that they appear to project from the surface in resting areas. Resorption of Sharpey fiber bone appears to present no remarkable characteristics, except that there is a tendency for the intrinsic matrix to be resorbed slightly faster than the extrinsic bundles.

Svejda and Skach (1973) studied the periodontal fiber arrangement in human bone by SEM. They concluded that the bundles of fibers are inserted similarly into the bone and cementum, but they are anchored more firmly in the cementum than in bone. This conclusion was based on the fact that, in several places, the surface layer of the bone was detached from the base, and although this was probably a preparation artifact, it was not seen in cementum. The authors recognized that they were unable to find the depth to which the collagen fibers entered the bone, therefore making their conclusions about fiber anchorage in bone questionable.

Deporter and Ten Cate (1980) in EM observations of periodontal ligament fibers suggested that they were penetrated by numerous processes of ligament fibroblasts containing phagocytosed collagen fibrils. These processes often reached the hard connective tissue surface and were thought to sever ligament fibrils at their point of insertion into the bone for subsequent phagocytosis and degradation. Deporter and Ten Cate (1980) concluded that ligament fibers anchored to alveolar bone or cementum are not as inert as previously thought and probably can undergo remodelling in a simi-

lar manner for fibers in more central portions of the periodontal ligament.

Kurihara and Enlow (1980a) studied the periodontal ligament attachment to bone in rats by transmission electron microscopy. Three types of fiber attachments were described for remodelling surfaces of the bony alveolar wall. The adhesive type of attachment was a common fiber anchorage to resorptive bone surfaces during active tooth movement. The continuous and intermediate type of attachments were commonly found at appositional bone fronts. The adhesive attachment consists of a layer of proteoglycans produced by fibroblast-like cells on the naked surface of recently reabsorbed bone. Newly synthesized collagen and the dissociated ends of collagenous fibrils became embedded in the accumulating proteoglycan. These embedded fibrils were joined to intact collagen fibers and blended with the remainder of the periodontium. Adhesive attachments continuously form and re-form as bone resorption proceeds. In the continuous attachment, some of the bone matrix fibrils survive the resorptive process. These fibers become incorporated into the principal fibers of the periodontal ligament, providing continuity between the bone matrix and the fibers of the adjacent periodontal membrane.

Rygh (1972), in a SEM study, described ultrastructural changes in pressure zones of the human periodontium as a result of tooth movement. Details of the process of degeneration and necrosis of cells and blood vessels in the hyalinized zone were given. Although unit collagen fibrils disintegrated after the application of forces similar to those forces used clinically, the majority of

the fibrils retained cross striations.

Roberts and Chamberlain (1978) studied the cellular elements of the rat periodontal ligament by SEM. Sinusoids, capillaries and arterioles were located almost exclusively near the bone and were usually oriented parallel to the bone surface. The tissue preparation allowed the identification of four types of cells. Irregular oblong shaped cells were oriented along principal PDL fibers. A type of stellate and fibroblast-like cell with multiple cellular processes was observed in lacunar spaces among the principal PDL fibers. A third group of cells of nodular or spheroid shape was often seen in perivascular areas. Occasionally, a fourth type of cell was observed, which was of an elongated, stellate shape. This type was usually oriented along principal PDL fibers and had numerous pseudopodic-like cellular processes. Shackleford (1971a,b) and Svejda and Skach (1973), in SEM studies, described the fibrous elements of the PDL, but their specimens were prepared by sawing through a formalin-fixed tooth and periodontium with a diamond wheel. The resulting surface was criticized by others as being unsatisfactory for studying PDL cells. Roberts and Chamberlain (1978) fractured their specimens manually in a longitudinal plane, allowing for better preservation of the cellular elements.

The development of intraosseous fibers and their morphological characteristics have been studies in detail by various authors. Many investigators have also described the ultrastructural changes in the periodontal ligament resulting from orthodontic tooth movement. There is no reference in the literature, how-

ever, to a study of intraosseous fibers during orthodontic tooth movement. The tension and pressure forces transmitted to the periodontal ligament during orthodontic tooth movement have been described in detail. This study attempts to examine the extent to which intraosseous fibers are altered by orthodontic forces. Changes in mineralization of these fibers and of the surrounding bone matrix is a method to assess how some septal components are altered by the force. These changes in mineralization patterns will be studied in anorganic SEM specimens.

MATERIALS AND METHODS

Animals were killed 1, 2, 3, 4, 5 days after spring placement. A total of 10 animals were used for the study. The maxilla was removed, divided into halves, and fixed in Karnovsky's fixative (Karnovsky, 1965) for three hours. Each specimen (control and experimental) was cut with a sharp razor blade in a coronal plane through the midline of the interdental septum between first and second molar teeth and then rendered anorganic by immersion in 5.25% sodium hypochlorite (Johnson, 1983). Sodium hypochlorite is an effective agent for removal of unmineralized components of bone (Wink, 1982). The specimens were then dehydrated in graded series of acetones, dried, mounted on stubs with silver paste, coated with 150-200 nm of palladium-gold (60:40), and examined in a JEOL-35C scanning electron microscope utilizing 20 KV accelerating voltage.

RESULTS

Controls

A coronal section of the interdental septum (Fig. 5) demonstrated numerous intraosseous fibers in cross-section. In all instances these fibers were highly mineralized (Figs. 6, 7, 8) and presented irregular unmineralized fissures within the fiber bundle (Fig. 6). Appositional, resorptive and intermediate fronts could be identified according to Boyde's (1972) morphological criteria for anorganic bone surfaces. Near the resorptive front of the control septa (Fig. 6), the fibers had numerous unmineralized fissures, subdividing the fiber bundle. The adjacent bone matrix was dense and the intraosseous fibers projected above the matrix surface. The relation of fiber and matrix indicates a lower mineral content of the matrix compared to the intraosseous fiber (Fig. 6). Near the appositional front (Fig. 8) the intraosseous fibers did not project from the bone matrix. Unfused calcospherites were abundant within the bone matrix. Intraosseous fibers did not have as many fissures as fibers at the resorptive front. Near the midline of the septum, intraosseous fibers appeared at the same level as the surrounding bone matrix (Fig. 7). A prominent sheath partially surrounded the fibers. The bone matrix was not as dense as near the resorptive front. An unmineralized area surrounding the fiber was clearly identifiable.

Experimental

After two days of force application (Fig. 9), the bone matrix was less dense than in control specimens. The intraosseous fibers had less unmineralized fissures and appeared to project above the surface of the bone matrix. After five days of force application (Figs. 10, 11), the intraosseous fibers clearly projected from the matrix surface after removal of organic substance. The bone matrix was loosely arranged (Fig. 11). The sheath surrounding the fibers was hypermineralized in relation to the rest of the bone matrix, as it can be seen surrounding the intraosseous fiber at a higher level than the matrix surface (Fig. 10).

To compare the findings in the sectioned septum with findings described in the alveolar wall by previous studies (Shackleford, 1971, 1973) Figs. 12, 13, and 14 illustrate the alveolar wall and intraosseous fibers of control specimens. The concavity of the alveolar wall is evident in Fig. 12. Intraosseous fibers of the middle third of control septa (Fig. 13) were densely mineralized and presented few unmineralized fissures within the fibers and were surrounded by an unmineralized area. Calcospherites were present and were distributed evenly over fibers and bone matrix. The intraosseous fiber projected above the surface of the matrix. In a higher magnification of the cervical third alveolar wall (Fig. 14), an even distribution of calcospherites is evident. The fissures within the fiber and the unmineralized area around the fiber was also clearly demonstrated. A mineralized area surrounding the fibers was not evident. The alveolar wall of experimental

specimens is seen in Figure 15. The concavity of the wall serves as evidence of the area studied. In a higher magnification of the middle third, intraosseous fibers (Fig. 16) were at the same level as the surrounding bone matrix, showing evidence of even mineralization of both structures. The bone matrix was dense. In the apical third of the alveolar wall (Fig. 17) the intraosseous fibers had more unmineralized fissures within the fiber than fibers of the middle third. The matrix was densely arranged and did not indicate obvious effects of resorption.

DISCUSSION

This study confirmed the presence of deeply embedded fibers in the interdental septum as reported previously by Quigley (1970), Cohn (1970, 1972a,b, 1975), Johnson (1981, 1983), Johnson and Low (1981) and Bernick et al. (1974, 1977). These fibers were highly mineralized and had unmineralized fissures as described by Selvig (1965), Boyde and Jones (1968) and Jones and Boyde (1974) within intraosseous fibers. These authors described an unmineralized central core in Sharpey's fibers. This study suggests that a central unmineralized core is present only near the alveolar wall. Irregular unmineralized fissures were representative of more deeply embedded intraosseous fibers. The presence of a channel surrounding the intraosseous fibers as described by Johnson and Low (1983) was confirmed in this study. This observation confirms that the fiber passes through a channel in bone. The anchoring system described by Johnson and Low (1983) could not be studied in this experiment, due to the coronal orientation of the sections which only allowed for a cross-section visualization of the fibers. For this same reason the continuity of intraosseous fibers from the ligament of one tooth to that of the neighbouring tooth could not be confirmed.

A prominent sheath surrounding the intraosseous fibers confirm the findings of Shackleford (1973) and Johnson (1983). This sheath was present in both control and experimental specimens. No obvious differences between the size of the fibers at different septal levels was observed in my study, differing from Johnson

(1983). My observations of intraosseous fibers of the alveolar socket wall confirm findings of Boyde (1974) that the fibers are constant in size throughout the socket wall and have an unmineralized central core (Selvig, 1965; Boyde and Jones, 1968; Jones and Boyde, 1974). Shackleford's (1973) relation of the size of the central core to the area of the socket wall studied could not be assessed. The control intraosseous fibers studied here could be subdivided depending on the phase of calcification or resorption that the plane of section exposed. In sections near the appositional front of the control septum, the intraosseous fibers appeared to be depressed from the surface of the bone matrix, indicating a comparatively higher mineral content of the matrix. The description confirms Boyde's (1972) observations of socket walls. The presence of unfused calcospherites suggested recent bone apposition, as reported by Boyde (1972) and Frasca (1981). The larger accumulations of calcospherites in the bone matrix compared to the extrinsic fibers observed in this study taken together with the faster rate of demineralization of the bone matrix in resorptive areas confirms Boyde's (1972) findings of a tendency of the intrinsic fibers of the bone to be resorbed or deposited faster than the extrinsic fibers. Study of the resting surfaces of the interdental septum suggested that the intraosseous fibers and the surrounding matrix changed their mineral content at an even rate, since the intraosseous fiber appeared at the same level with the surrounding matrix. At physiologic appositional and resorptive fronts the bone matrix loses or takes up mineral at a faster rate than the intraosseous fiber as shown by changes in

fiber to matrix topographical relation of unorganic specimens. The sheath around the intraosseous fiber appeared to change at the same rate as the rest of the intrinsic matrix in control specimens. A differentiation in the mineralization of the intraosseous fiber according to the septum level (as in Johnson, 1983) was not found.

Experimental

In experimental specimens the deeply embedded intraosseous fibers had less mineralized fissures than controls. The fiber demonstrated increased mineral content while the matrix had a comparatively smaller mineral content. This was represented in anorganic specimens by intraosseous fibers projecting from the bone matrix surface as experimental time increased. The increase in mineral content of the fiber is suggested by a decrease in unmineralized fissures. The decrease of mineral content of the matrix is suggested by a disorganization of its mineralized fibers, and change in topographical relation of fiber and matrix. The intraosseous fibers appeared to project from the surrounding matrix. The disorganized arrangement of the remaining mineralized collagenous fibers suggest that resorption is occurring, as described by Boyde (1972) in socket walls. The treatment of the specimen with 5.25% sodium hypochlorite removes all the organic material present on the cut surface (Johnson, 1983). Therefore, one can only speculate about the type of tissue and cells present

in these unmineralized areas of the bone matrix. The presence of cellular elements can only be determined by study of demineralized specimens submitted to the same experimental conditions. The sheath surrounding the fiber seemed to lose minerals at a slower rate than the rest of the matrix, suggesting that the former has a higher mineral content, or its mineral content is increased as force is applied, a situation similar to that of the intraosseous fiber.

The force system implemented herein produced pressure on the middle and apical thirds of the interdental septum, a product of the approximation of the first and second molar roots (Fig. 3). The findings of this experiment can then be attributed to a pressure force. The resorption of the occlusal third of the septum in several specimens, a result of the presence of the spring under the interproximal content, does not allow for study of this area. The force system would induce tension if the septum were intact. The absence of appositional fronts in the experimental specimens confirms that only pressure, and therefore only resorption, was present in the septum during the experimental period. The short duration of the experiment, and the constant action of the spring during this time (up to five days) probably did not allow initiation of bone apposition. The findings described above for experimental orthodontic forces demonstrate that minerals of deeply embedded intraosseous fibers are not removed at the same rate as the rest of the surrounding bone. This slow change in the fiber, and its possible hypermineralization suggest that the pressure transmitted to the intraosseous fiber creates alterations in its

mineral content. Pressure transferred to deeply embedded fibers induces a mineralization of unmineralized fissures of the fiber. The fiber hypermineralization can be produced by uptake of the calcium ions left in the interstitial tissue as a result of bone matrix demineralization. The matrix reacts differently, probably because of the ability of the matrix to change its mineral content at a faster rate. The sheath surrounding the fiber may be influenced by forces occurring in both intraosseous fiber and matrix, or may only reflect a relative mineral density of this sheath.

The alveolar wall was not affected in the same way by experimental force. The intraosseous fibers and the alveolar wall seem to be very densely arranged, and no dramatic change in the fiber, in the matrix or in the relation of intraosseous fiber to bone matrix, other than normal evidence of bone resorption, was found. This finding suggests that the degree of mineralization at the surface of the alveolar wall is not affected as much as bone in deeper portions of the septum. In spite of the more direct force impact on the alveolar wall, this presented less evidence of differential mineral content changes. The high mineral content and density of the alveolar cortical bone, and highly mineralized Sharpey's fibers may explain why these structures would be less affected by the pressure force. Obliteration of blood vessels and the consequent hyalinization of the periodontal ligament can delay any remodelling of cortical alveolar bone. Deeply embedded fibers and bone matrix also have a rich blood supply and forces affect them more indirectly. This factor, in addition to the fact that deeply localized structures of the septum are not as densely min-

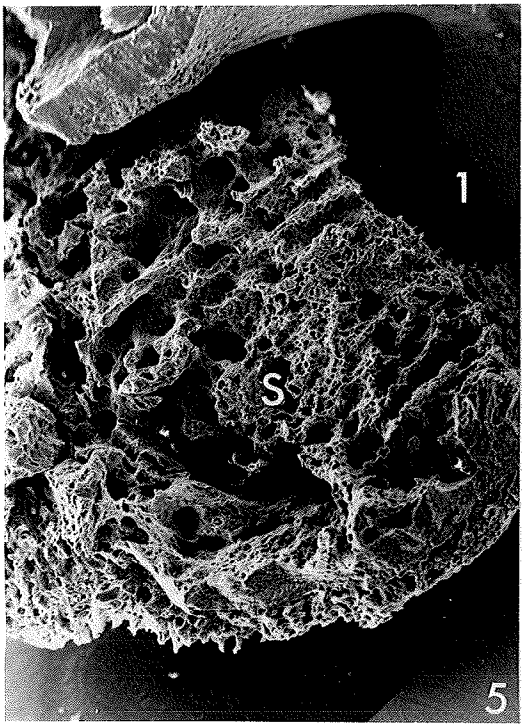
eralized as surface structures, may explain why the effects of force are seen more readily inside the septum.

Fig. 5 Interdental septum, experimental, five days. This specimen demonstrates a coronal section through interdental bone (S), between the maxillary first (1) and second molar teeth. (44 X)

Fig. 6 Interdental septum, middle third near resorptive surface, one day control. Intraosseous fibers (I) project from the surrounding matrix (M). Intraosseous fibers exhibit more unmineralized fissues (arrow) than fibers at the midline or appositional surface. Bone matrix is densely arranged. (1600 X)

Fig. 7 Interdental septum, middle third near midline, one day control. Intraosseous fibers (I) do not project from the bone matrix (M). A prominent sheath partially surrounds fibers. Fibers demonstrate unmineralized fissures (arrow). An unmineralized area surrounds the fiber (U). (1800 X)

Fig. 8 Interdental septum, middle third, near appositional surface, one day control. Intraosseous fibers (I) appear recessed from the surrounding bone matrix (M). Calcospherites are abundant (arrow) in the matrix but not within the fiber bundle. (2000 X)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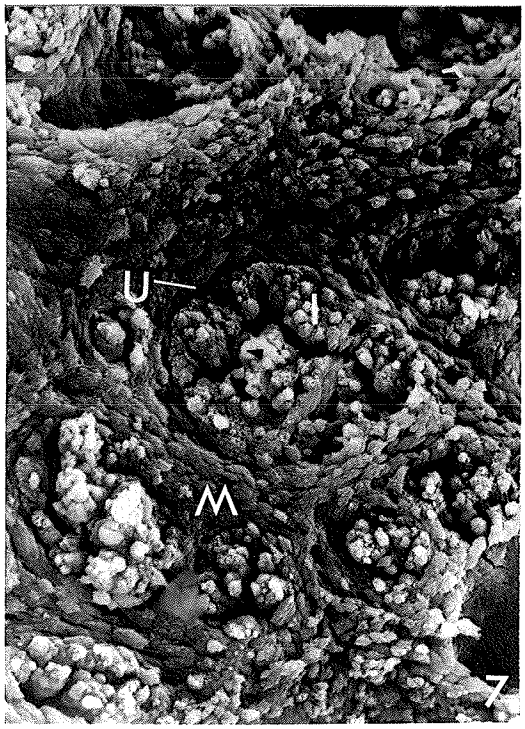
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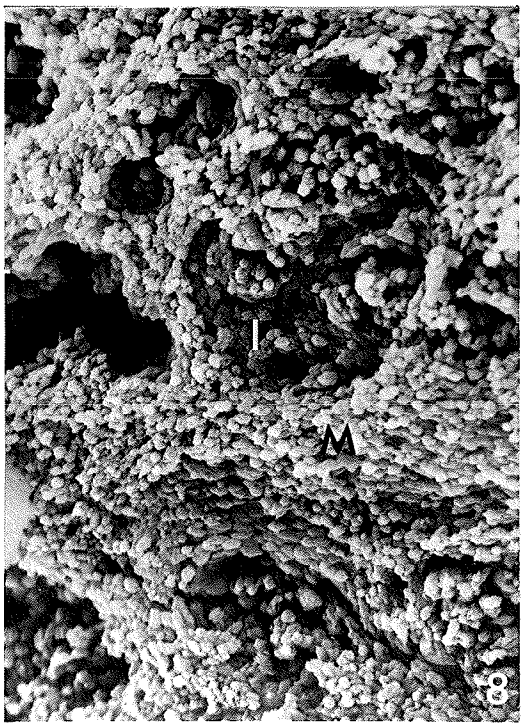
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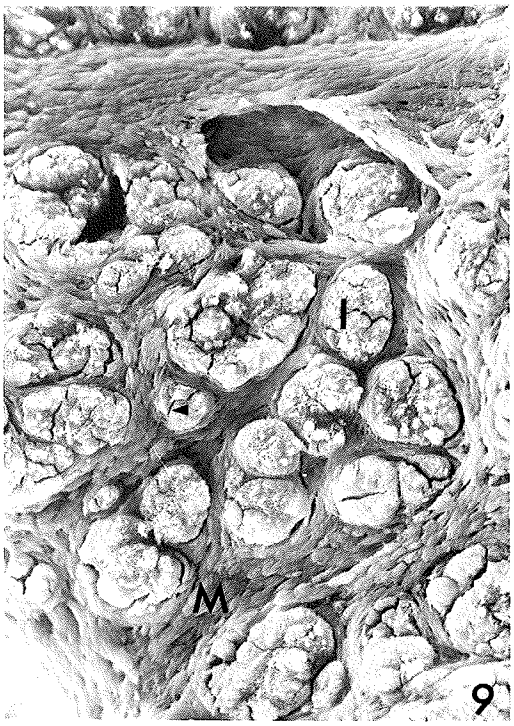
Fig. 9 Interdental septum, middle third, experimental, two days. Intraosseous fibers (I) exhibit a decreased number of unmineralized fissures (arrow) and project from the surrounding bone matrix (M). Matrix appears less dense than controls. (1100 X)

Fig. 10 Interdental septum, middle third, experimental, five days. Intraosseous fibers (I) project from bone matrix (M) which exhibits extensive resorption. Sheaths (arrow) surround fibers. (1600 X)

Fig. 11 Interdental septum, middle third, experimental, five days. Intraosseous fibers (I) project from the surrounding bone matrix (M). The bone matrix shows evidence of extensive resorption. Sheaths (arrow) do not lose minerals at the same rate as the matrix. (2400 X)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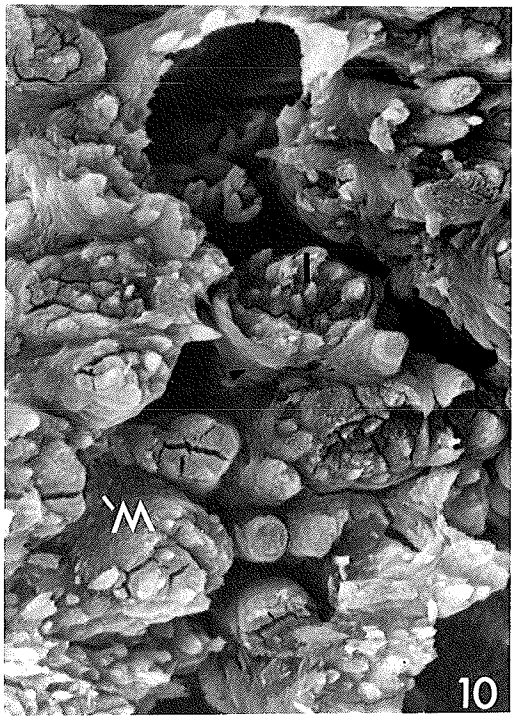
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M
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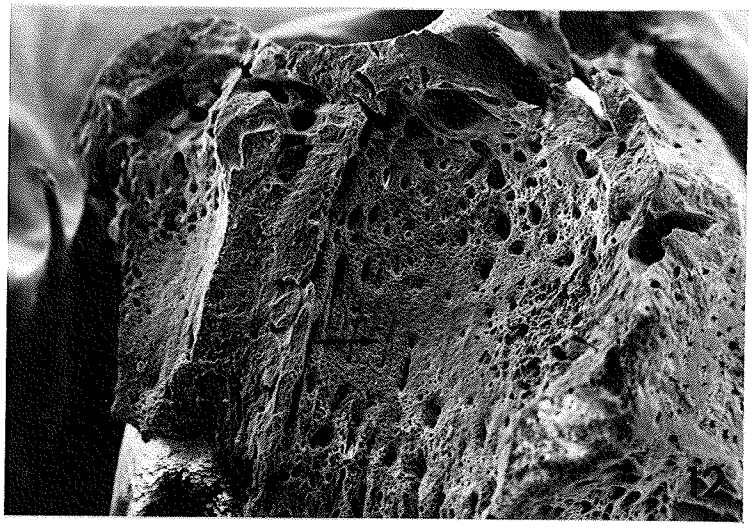
Fig. 12 Alveolar wall, control. For comparison with section through interdental septum. The concavity of the alveolar wall is evident (arrow). (36 X)

Fig. 13 Alveolar wall fibers (F), middle third, control. The bone matrix (M) is densely organized. The fiber (F) is highly calcified (940 X).

Fig. 14 Alveolar wall fibers cervical third, control. Fibers (F) and matrix (M) are densely calcified. Calcospherites (arrow) are scattered over matrix and fibers. An unmineralized area is evident around the fiber (U). (2000 X)



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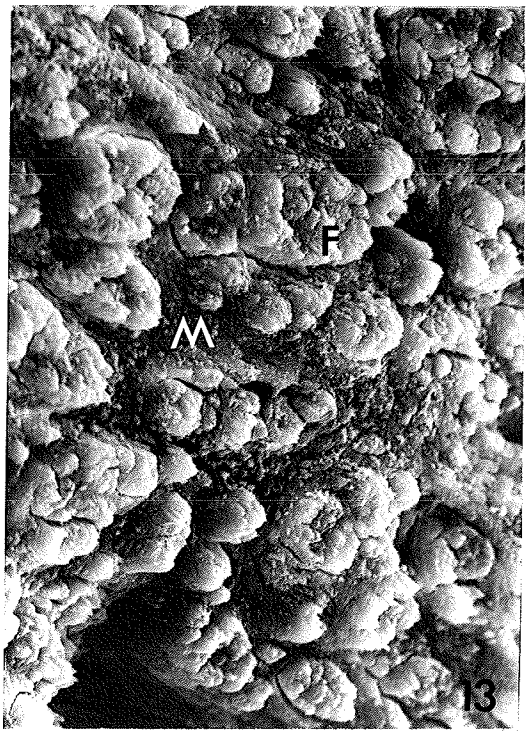

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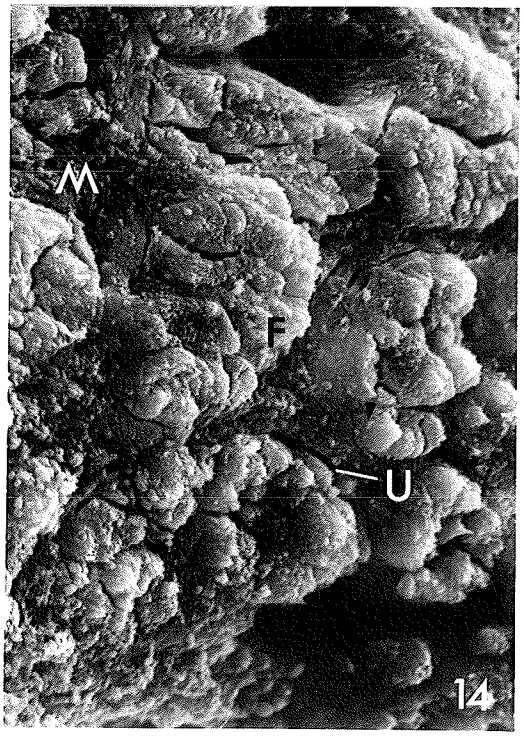

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Fig. 15 Alveolar wall, middle third, experimental, two days. Fibers (F) are arranged in rows. The matrix (M) is well organized and is at the same level as the fibers. (360 X)

Fig. 16 Alveolar wall, middle third, experimental two days. The matrix (M) is densely arranged. Intraosseous fibers are at the same level as the surrounding matrix. (3200 X)

Fig. 17 Alveolar wall, apical third, experimental, two days. Intraosseous fibers (F) are less mineralized than fibers of the crestal third. The matrix is dense and recedes slightly from the fibers. The fibers project from the matrix here.

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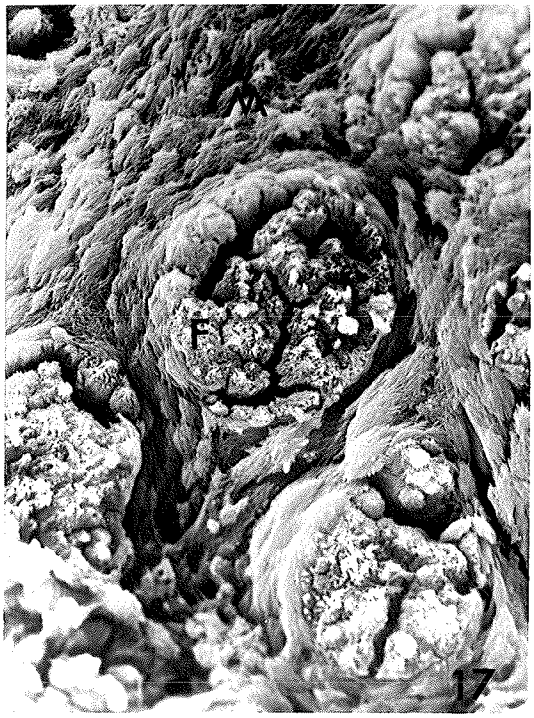

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CHAPTER II

RADIOAUTOGRAPHIC STUDY

LITERATURE REVIEW

Connective tissue "remodelling" is a physiologic process which results in a change in shape and/or the structure of a tissue without any simultaneous impairment in function (Lapiere, 1967). As an organism develops and grows, for example, there must be an extensive, continuous remodelling of soft and hard connective tissues to meet changing functional demands.

In contrast "turnover" involves breakdown and replacement of macromolecules, including collagen, without any apparent alteration in tissue structure; that is, it represents the mechanism whereby wornout macromolecules of mature connective tissue are continuously being replaced so that a net balance between synthesis and degradation is maintained (Melcher and Bowen, 1969). In practice both mechanisms overlap considerably, so that in any given tissue, it is not possible to assess their respective quantitative effects (Melcher and Bowen, 1969).

Glycine and proline are the most abundant amino acid residues in collagen (Bowes and Kenten, 1948). These two amino acids are utilized for the synthesis of collagen and are suitable for the detection of the sites of synthesis and migration of collagen by means of radioautography (Carneiro and Leblond, 1959). To measure collagen turnover directly, radioactively labelled amino acids are used to label the collagen. If non-specific amino acids such as glycine are chosen, the collagen must be purified for assay. Alternatively, if radioactively labelled proline is used, labelled hydroxyproline, which is found only in collagen, can be measured.

The turnover of collagen can then be followed by observing changes of specific radioactivity with time. The most popular method for collagen turnover studies has been to prelabel the collagen and then follow the loss of collagen specific radioactivity over extended periods of time.

Technique

There are a series of considerations for accuracy of "in vivo" radioautographic procedures:

1). The tissue is subject to the effects of growth (Sodek, 1976).

2). Recycling of proline label. Jackson and Heininger (1974) demonstrated in an radioautographic study using $^{18}O_2$ labelling, that protein fragments could be translocated and reused for tissue repair or restructuring without proteolysis to free amino acids and resynthesis. The efficiency of this recycling process was shown by the high specific activity of amino acids transferred to granulation tissue.

3). Effects of fixation and demineralization. During fixation, proteins are made somewhat insoluble. If fixation is incomplete, proteins and other substances may be extracted during demineralization and subsequent preparative procedures (Merriam, 1958; Schneider and Schneider, 1967). Beersten and Tonino (1975) tested different fixative solutions and determined that with Bouin's fixative (picric acid, formalin and glacial acetic acid), higher grain densities were obtained

than with formalin or Carnoy's fluid. A greater loss of radioactivity occurred during the demineralization of the jaws fixed with Carnoy's fluid, particularly when this was performed in a formic acid and sodium citrate mixture. Biochemical analysis of the dialysates of the formic acid and sodium citrate mixtures from these tissues showed that part of the radioactivity lost was bound to collagen.

4). Variables in the radioautographic process that can introduce errors in the final radioautographic countings.

a). The size, density and sensitivity of the crystals in the emulsion are in general out of the hand of the experimenter, who relies on the supplier for a reasonably standardized product (Rogers, 1979).

b). Probability is high that the emulsion layer will vary in thickness from one place to another, with consequent variability in emulsion response (Rogers, 1979).

c). The conditions of drying and exposure can critically affect the emulsion's performance. An incompletely dry emulsion exposed in the presence of oxidizing agents will have very low efficiency. Even within one batch of slides prepared for light microscopic radioautography, variations in drying can easily occur, with the slides furthest from the fan or those in an exposure box with less drying agent, showing significantly lower efficiencies. On the same slides, areas where the emulsion is thicker than usual may fail to dry adequately (Rogers, 1979).

d). Without a specially designed developing tank, it is difficult to get reproducible conditions of developing (Rogers, 1979).

e). Variation in section thickness will unavoidably contribute to variability of measurements (Rogers, 1979).

f). Differences in the size and shape of the source can account for variability in the counting (Rogers, 1979), but is not a significant factor in the present study.

5). Statistical analysis: Working with low total counts of silver grains, corrections for radioactive background assume far greater importance, and the level of radioactivity in the source, estimated from the mean grain density, becomes less accurate than with larger numbers of observed events (Rogers, 1979).

6). Background: In every radioautograph, silver grains appear in the developed emulsion which are not due to the experimental source, but to other causes, which will be shortly enumerated. As the temperature, duration or strength of development is progressively increased, more and more silver grains will be developed, regardless of the degree of exposure of the emulsion to radiation. All of the emulsions for radioautography show increased background with increasing exposure to light. Appropriate humidity conditions are recommended. Emulsions are also sensitive to pressure. Scratches, fingerprints and other gross insults should be avoided. Gelatin (Subbing solution) contacts on drying, and, if this process is carried out too fast or taken too far, background grains

will be produced. Where the emulsion is in direct contact with the glass slide, the emulsion may be subjected to sliding, lateral stresses as it shrinks, producing background grains (Rogers, 1979). Pressure artifact will also occur over the tissue section. The upper profile of a tissue section is usually very irregular. If the section was cut at a thickness of 5 μm, it may be 5 μm thick only in places. Over openings in the tissue, a blood vessel, for instance, section thickness drops abruptly to zero. These spaces fill with emulsion and are very vulnerable to stress artifacts (Rogers, 1979).

Chemography: Emulsions come into contact with biological material of some sort during exposure. Many reactive groups, particularly those that are reducing agents, are capable of producing a latent image in silver halide crystals by direct chemical action. Tissue that has been through the processes of fixation, dehydration, embedding in paraffin wax, sectioning and subsequent dewaxing are less likely to give rise to this type of artifact than fresh tissue sectioned on a cryostat (Rogers, 1979).

Contamination of the emulsion: Glass, certain plastics and high grade stainless steel are the only materials that should be allowed to come into contact with nuclear emulsion and they should all be scrupulously clean (Rogers, 1979).

Environmental radiation: X-ray machines and laboratories using gamma-emitting isotopes, are the most dramatic source of background.

Spontaneous background: The nuclear emulsions used in radioautography are very highly sensitized products. In all of them an occasional silver halide crystal will develop a latent image speck spontaneously.

Background eradication: The simplest method of background eradication is to expose the radioautographs in air at a fairly high relative humidity. Latent image fading proceeds together with the formation of new latent images by the radiation from the specimen (Rogers, 1979). The last and obvious factor to consider is the human error introduced during grain counting. It is recommended that only one person does the counting, and that the criteria for silver grain definition, location of the countings, area of field selected for the grain counting, etc. is defined prior to initiation of the counting.

Periodontal Ligament Remodelling

1). Rate

One of the first studies of the changes in cell replication and protein synthesis in the periodontal ligament induced by tooth movement was done by Baumrind (1970). He reported a significant decline in the protein synthetic activity in periodontal ligament of teeth submitted to orthodontic forces. His study covers only 72 hours subsequent to placement of the force. The reduction in collagen synthesis as an initial response to tooth movement is in agreement with Crumley (1964) and Ross and Benditt (1962). In

experimental animals the activity on the pressure side was significantly lower than on the tension side. Sodek (1976) in a study of the rate of [^{3}H]-proline incorporation into newly synthesized collagen and mature collagen fractions over short periods of time, demonstrated that collagen turnover in adult periodontal tissue is rapid and a much more efficient maturation process than it is in skin collagen. [^{3}H]-proline appears to enter the vascular system in a pulse form with a peak between 20-30 minutes after injection. Minkoff and Engstrom (1979) reported the highest labelled collagen counts 4 hours post injection in periodontal ligament of mice. According to Sodek (1976), the label is incorporated into periodontal ligament at a rate five times faster than that of alveolar bone. Many studies have been performed to determine the half-life of collagen in different areas of the periodontium. Rippin (1976) reported half-life of collagen turnover from 2.4 days in apical areas of the periodontal ligament, and of 6.4 days in the crestal areas for young rats. This high turnover occurred throughout the whole width of the ligament and allowed the tissue to remodel as the tooth moves. Sodek (1977) reported half-life of collagen turnover in the rat periodontal ligament of 1 day, 6 days in the alveolar bone and 15 days in skin corium. Minkoff and Engstrom (1979) found half-life of 5-7 days in the cementoalveolar region, 8.4 days in the transseptal region and 2.5 days in the dentogingival region of the periodontal ligament of mice. The difference between collagen turnover rate in developing periodontal ligament and in mature periodontal ligament was reported by Minkoff, Stevens and Karon (1981). The half-life of collagen of the devel-

oping PDL fibers was much shorter than that of the mature periodontium.

Duncan et al. (1984) developed an organ culture system capable of receiving orthodontic forces, and the effect of such forces upon collagen synthesis was evaluated. They reported a significant increase in type III collagen synthesis after three and five days of force application.

2). Reliability

Rossman, Rosenbloom and Robinson (1975) and Orlowski (1976) reported a substantial incorporation of [^{3}H]-proline into non-collagenous protein. Sodek (1977) demonstrated that in an in vitro system, 90% of the [^{3}H]-proline reaching alveolar bone was incorporated into collagenous protein. His findings confirm the validity of [^{3}]-proline as a label for collagen turnover in alveolar bone. In ligament and alveolar bone the incorporation of proline into non-collagenous protein reached a basal level after 2 hours and then remained constant. Carneiro (1965) in an earlier study stated that about 95% of the protein content of bone is collagen. The amount of collagen present and the orientation of the cells that secrete it, makes this tissue an excellent model to study collagen synthesis by radioautography. He found, however, that inaccuracy in turnover time could conceivably occur because of varying dilution of the isotope administered to experimental animals. He found considerable variation in the level of circulating proline, which if not compensated for would produce signif-

icant error in resulting hydroxyproline specific activity.

3). Distribution

McCulloch and Melcher (1983a) in a tritiated thymidine labelling study reported that cell division in the periodontal ligament occurs predominantly in a paravascular location. McCulloch and Melcher (1983b) in another [^{3}H]-Tdr study reported that the labelling index of cells adjacent to bone increased eight times within one day after injection, indicating that labelled cells had migrated to the bone surface. The turnover of collagen fibers in the periodontal ligament apparently occurs in all areas of the periodontal ligament, although perhaps at different rates, depending upon functional stresses. Carneiro and Fava de Moraes (1965) reported a more pronounced incorporation of tritiated proline in the upper and lower regions of the periodontal ligament in adolescent mice. Skougaard et al. (1970) reported no such distribution in the periodontal ligament of marmosets. Stallard (1963), Crumley (1964), Anderson (1967) and Diaz (1978) have reported an increased incorporation of tritiated-proline in the periodontal ligament near the alveolar bone relative to the area near cementum. Skougaard et al. (1970) and Rippin (1976) reported no evidence for such distribution.

Alveolar Bone Remodelling

There are few radioautographic studies of collagen turnover in alveolar bone. Stutzmann and Petrovic (1983) in a study of osteoblastic activity and bone mineralization rate determined that alveolar bone turnover is higher when orthodontic forces are light. In addition, the biological effectiveness of the light force is amplified when it is applied intermittently. Carneiro and Fava de Moraes (1965), in a time sequence study of collagen formation in the periodontal structures, using [^{3}H]-proline labelling, found that alveolar bone showed labelled osteoblasts 30 minutes after injection. In bone the number of radioactive areas decreases with time, but the number of silver grains per unit area in areas of persistent radioactivity decrease only slightly or not at all. While there was variation in the size of the sites and intensity of amino acid uptake, the authors found that in any given alveolus, maxima were present at the crest and near the root apex. These results are compatible with morphological observations that in alveolar bone there is a slow but continuous renewal of the bone tissue (Glickman, 1962). Carneiro (1965) also reported that [^{3}H]-proline labelling of alveolar bone presented radioactivity in the cells first, half an hour after labelling. The radioactivity soon decreased in cells while increasing in osteoid (4 hours), appearing later in calcified parts of the tissue (8 hours). After treatment with collagenase, he reported that 50% of the label present in osteoblasts was removed after 20 minutes. After 4 and 48 hours, there was a pronounced decrease in

the amount of radioactivity present in the cells. However, the label that appeared in osteoid at 4 hours, and in bone at 48 hours, was completely removed by collagenase. The radioactivity present in cells known not to secrete collagen (liver cells, epithelial cells) was not removed at all by collagenase confirming the specificity of the enzyme. [^{3}H]-proline is a convenient precursor, since it is incorporated into collagen as proline or hydroxyproline and taken together these two amino acids constitute 23% of the residues in collagen (Carneiro, 1965). This study together with Sodek (1977) clearly explains why [^{3}H]-proline is the radioactive label chosen for a study where the alveolar bone collagen synthesis rate is going to be assessed.

Garant and Cho (1979) in a multiple [^{3}H]-proline injection over a period of ten days in mice, found that appositional surfaces of periodontal alveolar bone not only contained parallel appositional bands, but also a second series of bands perpendicular to appositional bands. These perpendicular bands were continuous with Sharpey's fibers of the adjacent periodontal ligament. This pattern of [^{3}H]-proline labelling suggests that new Sharpey's fibers are secreted simultaneously with new bone deposition. Tonna (1975) studied the rates of appositional bone growth in mice. In his report no mention is made of labelling over Sharpey's fibers. Difference in label dose, sacrifice intervals, and mode of [^{3}H]-proline administration may account for the different findings.

Roberts and Chase (1981) using radioautography, studied the cell kinetics of rat molar periodontal ligament during orthodonti-

cally induced osteogenesis. According to their findings, orthodontically induced osteoblasts are derived solely from the local periodontal ligament cells. Preosteoblasts are present in the periodontal ligament even along resorbing surfaces. These cells can differentiate to osteoblasts without synthetizing DNA. Heersche, Tam and Jones (1981) labelled rats simultaneously with [^{3}H]-proline and tetracycline. They found that the bone apposition rate measured by tetracycline labelling is the same as the rate of collagen matrix apposition. The lag between organic matrix deposition and subsequent mineralization is approximately 24 hours in the normal rat and is increased in vitamin D deficient animals.

The changes in collagen turnover in the periodontal ligament, resulting from orthodontic tooth movement and its relation to treatment stability, have been studied extensively. The literature indicates that no conclusive findings are available. The slower collagen turnover in some groups of periodontal fibers has been related to the relapse of orthodontically moved teeth. There is no report in the literature of altered collagen synthesis in the interdental septum resulting from orthodontic tooth movement. This study attempts to evaluate the collagen synthesis in the untreated interdental septum and to compare it to synthesis in the septum between orthodontically moved teeth. The effects of pressure and tension on collagen synthetic activity of alveolar bone will be evaluated. The degree to which synthesis of the collagen of intraosseous fibers and bone matrix is affected by orthodontic forces will provide insight into the role played by these struc-

tures in the remodelling and stability of the periodontium during tooth movement.

MATERIALS AND METHODS

Twelve six-week old female albino rats were weighed, anaesthetized with ether and springs placed between maxillary left first and second molars. Five microcuries per gram body weight of [^{3}H]-proline (New England Nuclear [L-2,3-^{3}H]-proline), specific activity 33 Ci/mmol, were injected intraperitoneally immediately after spring placement. Two animals were killed 1, 2, 3, 7, 10 and 14 days after force application. Maxillae were dissected, divided in halves (right side used as control and the left as experimental), fixed in Bouin's fixative for 24 hours (Beersteen and Tonino, 1975) and demineralized in 4.13% EDTA, pH 7.0 (Warshawsky and Moore, 1967). Blocks containing the three molars and surrounding bone were dehydrated in graded series of alcohols, embedded in paraffin wax and then serially sectioned at 6 μm in a sagittal plane. Sections were mounted on previously prepared gelatin subbed slides. The slides were then hydrated and taken to the darkroom. Under a safelight, with temperature at 18-20°C and humidity at 40-50%, the slides were dipped in NTB-2 (Eastman-Kodak) nuclear track emulsion. The NTB-2 emulsion was previously placed in 43°C waterbath for 30 minutes. The emulsion was then poured into the dipping jar, and again placed into the waterbath. The slides were dipped and placed on plastic stands for one hour in an incubator at 80°F and 78% humidity. They were then transferred to drying boxes containing a drying agent ($CaSO_4$ in small gauze packages) and exposed for 2 weeks at 0-4°C. Slides were then developed with D-19 Kodak developer at 21°C for 5 min-

utes and fixed in Kodak fixing bath for 8 minutes. Afterwards, slides were stained by the van Gieson method (Luna, 1968). Grain counts were made on every sixth section of the serial sectioned tissues. The presence of the interdental septum between first and second molar determined the limits in buccolingual direction of sections selection. The sections were projected on a wall with a Nikon Micropan Microprojector at a standardized distance. A grid was designed so that counts were made on an area equivalent to 100 μm^2. Sections of unlabelled tissues were placed in every slide batch as control for latent image, chemography or fading. Three cervical counts (one on the mesial, one on the middle and one on the distal aspect of the septum), three middle and three apical counts, distributed in the same manner were made.

The mesial and distal location of the grid was standardized by positioning one of the vertical sides of the grid against the wall of the septum. In each field the counts within the intraosseous fiber and the counts in the bone matrix were plotted separately. The data was analyzed by a mixed analysis of variance.

RESULTS

(1) Observations

(a) Controls

The grain distribution after one day showed a clear pattern, which is in accordance with the distal drift and passive eruption of the rat molars. The highest concentration of grains was seen at the distal wall of the interdental septum and at the alveolar crest. The mesial wall of the septum was resorptive and did not demonstrate a clear grain band as seen in the distal wall and on the crest (Fig. 18). The density of the grains was higher near the septal wall than in the middle. With time the apposition of new bone could be seen on the distal and crestal aspects of the septum, giving the image of an apical migration of the grain band. This sequence can be appreciated in Figures 18, 20, 22, 23 and 24. As bone apposition continued, thin grain bands could be seen migrating perpendicular to the thicker grain band described above, following the Sharpey's fibers to the septal wall (Fig. 22).

(b) Experimentals

The grain distribution pattern after one day of force application was similar to that seen in the control (Fig. 19). After two days, the pattern of grain distribution was altered (Fig. 21). An appositional band on the distal wall of the septum was no longer evident. The grain concentration was higher at the

crest of the septum. Within the remainder of the septum, the grains were evenly distributed within the tissue (Figs. 24, 26). Most of the experimental specimens showed resorption of the occlusal third of the septum. Therefore, the grain counts of the occlusal third were omitted in the statistical analysis, both in control and experimental septa. In both control and experimental tissues, grains were evident within the intraosseous fibers and within bone matrix, evidence that synthesis occurs in both the fiber and the matrix (Figs. 27, 28).

(2) Data Analysis

A mixed analysis of variance was designed to evaluate the difference between the nine areas of the septum where grain counts were made. The difference in grain counts between control and experimental septa in the various areas was also evaluated statistically. In addition, differences in grain counts were also evaluated at various experimental time intervals. The analysis of data is illustrated in Tables 1 to 6.

After a mixed analysis of variance, the following findings can be described. A separate comparison of the mean grain counts for fiber and matrix was made (Fig. 29). The experimental grain counts, both for the fiber and the matrix, peaked at day three, while control grain counts peaked at day one after injection. Experimental septa had higher grain counts at their peak as compared to controls. There was not a significant difference between

the total grain counts of control versus experimental tissues for either fibers, matrix or a combination of the two (Tables 4, 5, 6).

When the experimental and control counts are combined, there was a significant difference in the mean number of grains per field as a function of time ($p < 0.001$) (Fig. 30). The difference was also significant when the counts of the fiber and of the matrix were analyzed separately ($p < 0.001$). The fiber had higher counts than the matrix after day one. This trend persisted during the experimental time (Fig. 31).

The difference between counts of the middle and apical thirds was significant between the controls and experimentals ($p < 0.05$) (Fig. 32).

In the bone matrix the mean counts between the middle and apical thirds of the septum are significantly different (control and experimentals together) ($p < 0.001$) (Fig. 33).

The mean number of counts per field in the mesial, middle and distal thirds of the septum (control and experimental pooled together) were significantly different for the fiber, for the matrix, and for both of them combined ($p < 0.001$) (Figs. 34, 35).

The variation in the mean number of counts per field was significantly different between mesial, middle and distal thirds of the septum at the different time intervals (control and experimentals pooled together) ($p < 0.001$) (Fig. 36).

Within the fiber, the differences between mesial, middle and distal thirds of the septum as a function of time was significant ($p < 0.01$) (Fig. 37).

The significant findings in grain count analysis are synthesized in Tables 1, 2, 3. The data analysis of significant and non-significant findings are illustrated in Tables 4, 5, 6.

DISCUSSION

There are a number of factors that must be considered when analyzing radioautographic results. To keep an order in this section, the technical considerations will be discussed first, followed by a discussion of the microscopic observations, and finally a discussion of the data findings.

(1) Technical Considerations

In the Literature Review some of the more significant factors influencing the results of a radioautographic study were reviewed. In this study, all the technical recommendations were followed to avoid an unnecessary introduction of errors. The specificity of the radioactive label was constant, since all the [^{3}H]-proline used in the study belonged to a same batch. The circulating level of isotope was not calculated. It is evident that reutilization of the proline label occurred (as shown by Jackson and Heninger, 1974). Since the half-life of mature collagen turnover in alveolar bone is approximately 6 days (Sodek, 1977), it is possible that the data obtained in days 10 and 14 is influenced by reutilization. However, I expect that if any change in synthesis of collagen took place as a consequence of tooth movement, the collagen reutilization would be proportional to this change. Therefore, I expect the grain counts to reflect the real changes in collagen synthesis, even after reutilization has taken place.

The technical procedures used and the use of the same developer batch made possible that the background counts were negligible. Background was checked and the number of background grains was of non-uniform distribution and was less than 1 grain per 100 μm^2 so that subtraction of background grains was not attempted. It can be assumed that proline incorporation represented collagen turnover accurately (Carneiro, 1965; Sodek, 1977).

(2) Discussion of Observations

The pattern of grain distribution of control specimens was different from experimental specimens. The controls had a band of grains at the appositional wall of the septum and at the alveolar crest. This band, indicative of the initial high dose of labelled proline injected into the animal, migrated apically as new bone was deposited in these areas. A different pattern of grain distribution in experimental tissues, marked by a high apposition at the alveolar crest, was observed. There was not a definite band of grains on the distal wall of the septum demonstrable and an even distribution of grains was evident within the septum. This appearance is probably a result of pressure exerted by the spring which disrupted the physiologic distal migration of the molars. The apices of the first and second molars tipped towards each other, while the crowns tipped apart (as shown in diagram of force system, Fig. 3). This induced a different remodelling pattern within the septum. In cases where the cervical third of the sep-

tum was preserved in experimental sites, a high grain density was observed (Figs. 24, 26). The force system would create tension at the level of the cervical third of the septum, therefore, an increase in bone synthesis would be expected. The findings of Garant and Cho (1979) of grain bands perpendicular to appositional bands in the physiologically remodelling septum were confirmed here. In the control specimens (Figs. 22, 25), this pattern was also found.

The resorption of the occlusal third of the septum in some of the experimental sites was probably created by impingement of the subgingival tissue by the spring, creating pressure on the alveolar crest and subsequent resorption.

(3) Data Analysis

Baumrind (1970), Crumley (1964) and Ross and Benditt (1962) reported a decrease in the synthetic activity of the periodontal membrane as a result of orthodontic forces. In this study, no significant difference in synthesis of alveolar bone proteins was detected between control septa and septa submitted to orthodontic forces (Tables 4, 5, 6). The rapid incorporation of labelled proline in the periodontal ligament, described by Sodek (1977), and Minkoff and Engstrom (1979), was not studied herein. In my study, a rapid incorporation was noted in alveolar bone one day after injection. After 14 days the level of radioactive proline had decreased considerably. In alveolar bone Stutzmann and Petrovic

(1983) showed an increase in alveolar bone turnover rate, as determined by mineralization rate and osteoblastic activity, when light forces were used in comparison to heavy forces. No significant difference in collagen synthesis was noted in my experiment between controls and experimentals. Probably the force used here was heavy enough to inhibit synthesis of collagen.

The fact that the spring was active only for approximately five days according to experimental observations, may explain why differences between control and experimental counts was not significant. During the five day period, force on the septum would likely progressively decrease as spreading occurred. The cessation of force allowed for septal reorganization and probably a return to a normal situation. When the data was combined, counts after day five may be similar for controls and experimental. This could balance out any significant difference between controls and experimental counts that took place during the first five experimental days. Carneiro and Fava de Moraes (1965) reported that in alveolar bone the higher grain counts were at the crest and near the root apex. In the present study the data collected from the occlusal third was not used, since some of the experimental septa had significant crestal resorption. Comparisons between middle and apical thirds of the septum showed a higher synthetic rate in the middle third than in the apical third (Fig. 33). There was a significant difference in the ratio of middle-to-apical counts between controls and experimentals, largely produced by a decrease in apical counts in the experimental septa (Fig. 32). If one considers that the nature of the force applied created an approxima-

tion of the root apices of first and second molars, this pressure in the apical area could decrease the amount of blood circulation in this area and therefore decrease the collagen synthesis rate. It must be clarified, however, that an adequate blood supply likely existed in this area, as there is a rich plexus of blood vessels supplying the periodontal ligament and the interdental septum. The lack of degenerative changes in the apical third of the periodontal ligament support this fact.

The lack of significant difference in total grain counts between control and experimentals (Tables 1, 2, 3) suggest that the orthodontic force did not alter the blood circulation in the septum to the extent that would cause a significant alteration in the collagen synthetic rate. The richer blood supply near the alveolar walls due to the vicinity to the periodontal ligament vasculature may explain why the grain distribution was in all instances higher on the mesial and distal thirds of the septum than in the middle third. This increased synthetic rate on the walls of the septum can also be produced by the more direct relation of these walls to the tipped roots. The forces in deeper structures of the septum can be significantly dissipated. When control and experimental counts were combined, counts in the distal third were always higher than on the mesial, both in matrix and fiber. This was produced by the distal drift of the molars that induced higher activity on the distal wall.

As described before, the only significant difference between control and experimental grain counts was found in the ratio of middle to apical grain counts (Fig. 32). The pooling of the con-

trol and experimental counts allowed for further analysis: (1) the pattern of grain distribution in the septum, and (2) the grain distribution in relation to experimental time. These findings will be described herein.

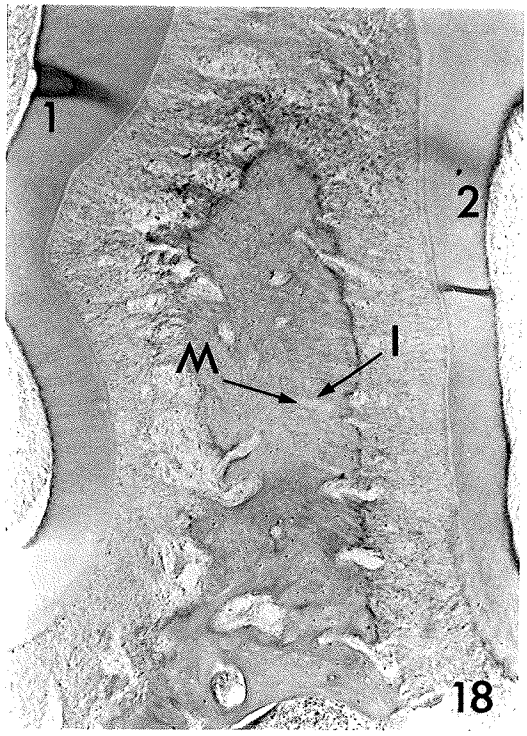
The difference in counts as a function of time demonstrated an initial high incorporation of the label, followed by a progressive decline (Figs. 29, 30, 31). A decline in grain counts began at day two. The significance of this decline was not analyzed, but this finding is probably associated with the significant mean weight loss of the animals during the first two experimental days (Fig. 4). At day three the incorporation reached a peak when control and experimental counts were combined and then declined progressively as degradation of the labelled collagen occurred. No significant differences were found between intraosseous fiber and matrix counts as a function of time, suggesting that the collagen synthesis was approximately even for the fiber and the matrix for each determined area. The intraosseous fiber seems, however, to have a slightly higher synthetic rate than the matrix from day two to day 14. This would allow for a faster remodelling of the fiber in response to orthodontic force in comparison to the matrix. The higher synthetic rate on the alveolar wall could account for the necessary remodelling of the septum and fibers. In that case the deeply embedded intraosseous fiber would not be influenced considerably by the forces applied. The lower synthetic rate in the middle third might also reflect the dissipation of the force within deeper parts of the septum.

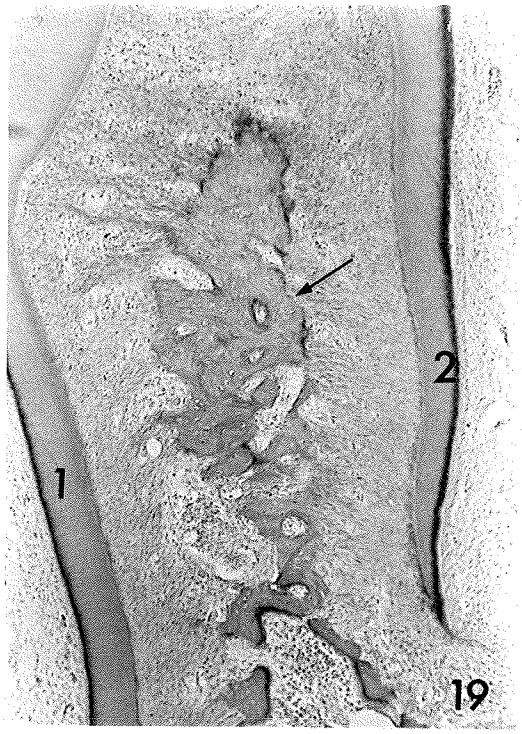
Fig. 18 Van Gieson method, control, one day (40 X). First molar (1), second molar (2) and interdental septum. A band of grains is present one day after injection at the alveolar crest and the distal wall of the septum. Intraosseous fibers (I) and surrounding bone matrix (M) are demonstrable.

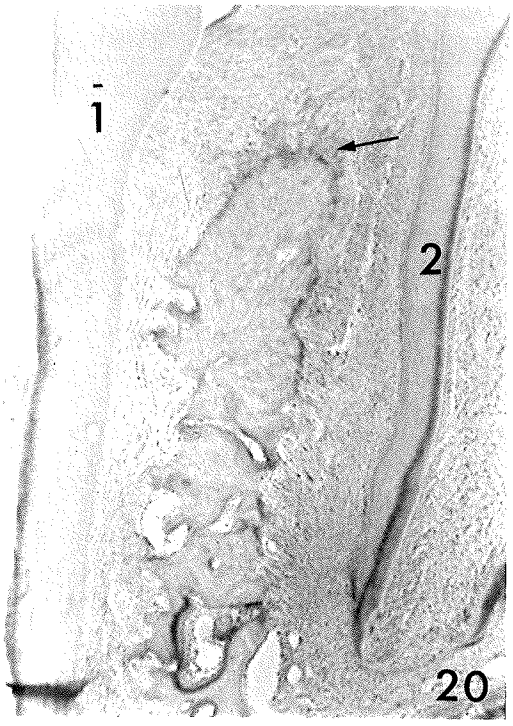
Fig. 19 Van Gieson method, experimental, one day (40 X). The continuous grain band seen in controls is interrupted as a result of the force (arrow). Bone remodelling occurs both at the septum wall and within the septum.

Fig. 20 Van Gieson method, control, two days (40 X). New osteoid has been deposited (arrow) at the alveolar crest.

Fig. 21 Van Gieson method, experimental, two days (40 X). A dense concentration of grains is localized to the alveolar crest (arrow). The band of grains on the distal wall of the septum is interrupted.


1
2
M
I
18


2
1
19


1
2
20

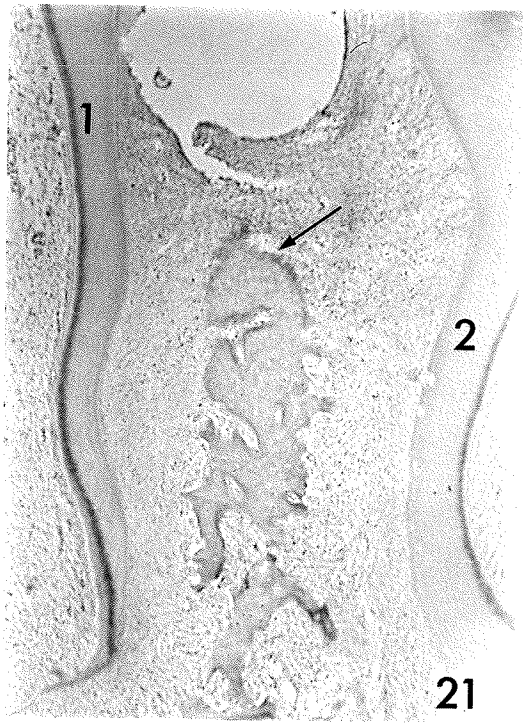
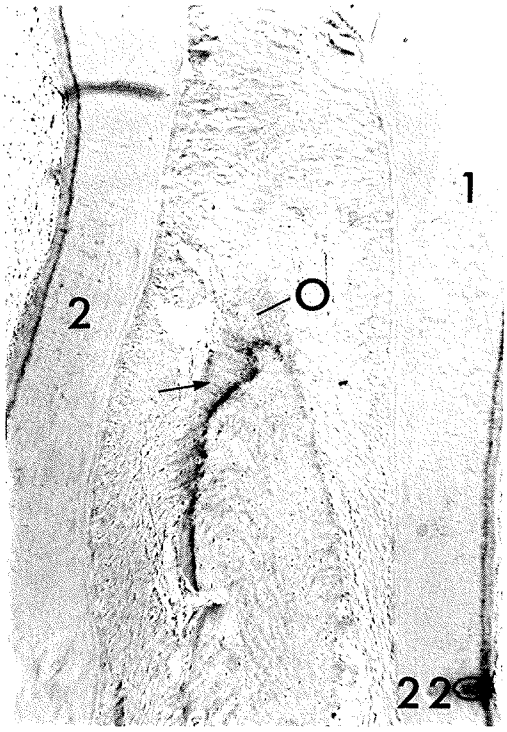

1
2
21

Fig. 22 Van Gieson method, control, seven days (40 X). Further apposition of new osteoid (O) is demonstrated. Grains marking the insertion of Sharpey's fibers within bone can be identified (arrows). First molar (1), second molar (2).

Fig. 23 Van Gieson method, control, ten days (40 X). The grain band appears to have migrated further apically (arrow) as a result of bone apposition at the alveolar crest.

Fig. 24 Van Gieson method, experimental, ten days (40 X). There is no grain band at the appositional wall. The alveolar crest is remodelled by the spring impingement (arrow). Internal bone remodelling is evident inside the septum. Root resorption on the first molar has occurred (R).


1
2
O
22


1
2
23

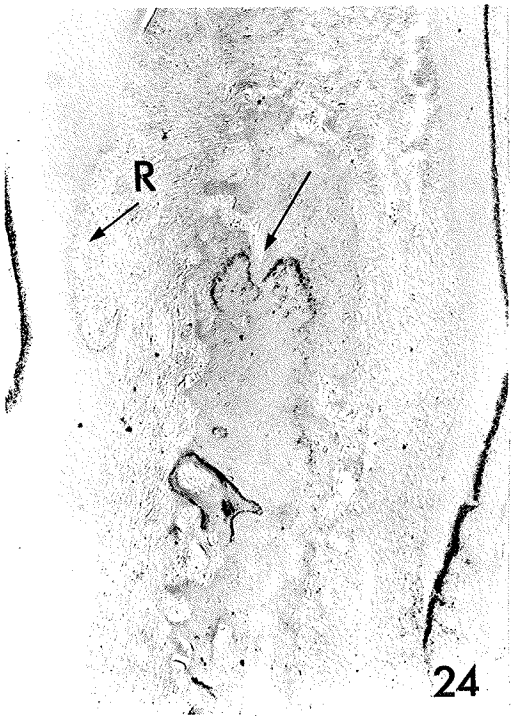
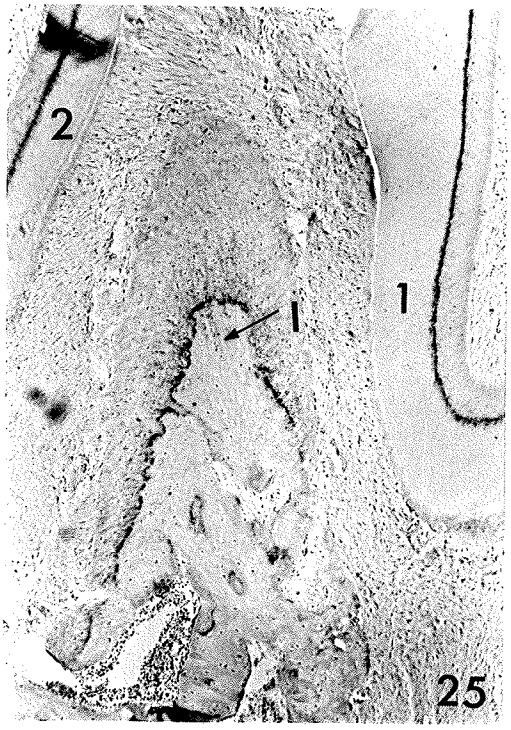

R
24

Fig. 25 Van Gieson method, control, 14 days (40 X). Two weeks following isotope injection, the grain band is still evident. Intraosseous fibers (1), first molar (1), second molar (2).

Fig. 26 Van Gieson method, experimental, 14 days (40 X). A high concentration of grains at the alveolar crest (arrow) is evident.


2
1
1
25

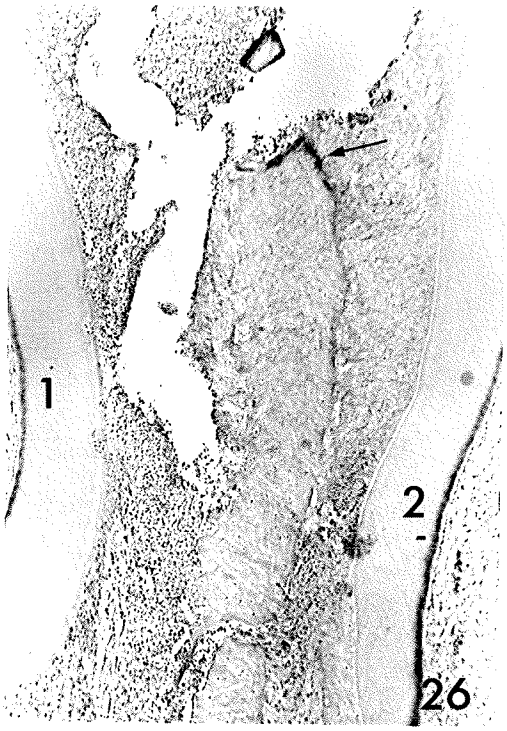
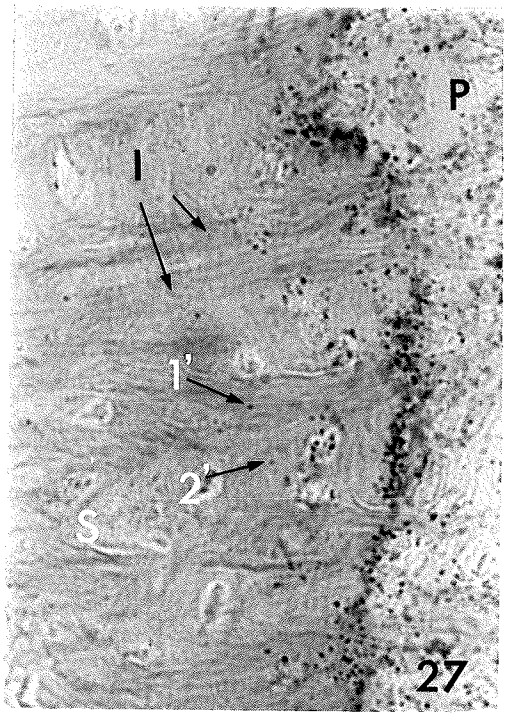
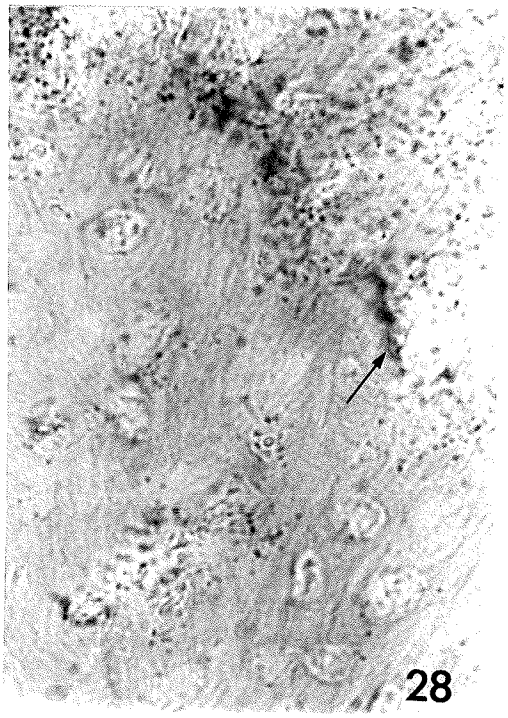

1
2
26

Fig. 27 Van Gieson method, control, one day (100 X). Interdental septum (S) and periodontal ligament (P). Intraosseous fibers (1). Grains are evident within the fiber (1') and on the surrounding bone matrix (2').

Fig. 28 Van Gieson method, experimental, one day (100 X). The highest grain concentration is found at the alveolar wall (arrow).



27



28

Fig. 29 Mean grain counts in control and experimental septa at different experimental times.

(c/f) - fiber control
(c/m) - matrix control
(e/f) - fiber experimental
(e/m) - matrix experimental

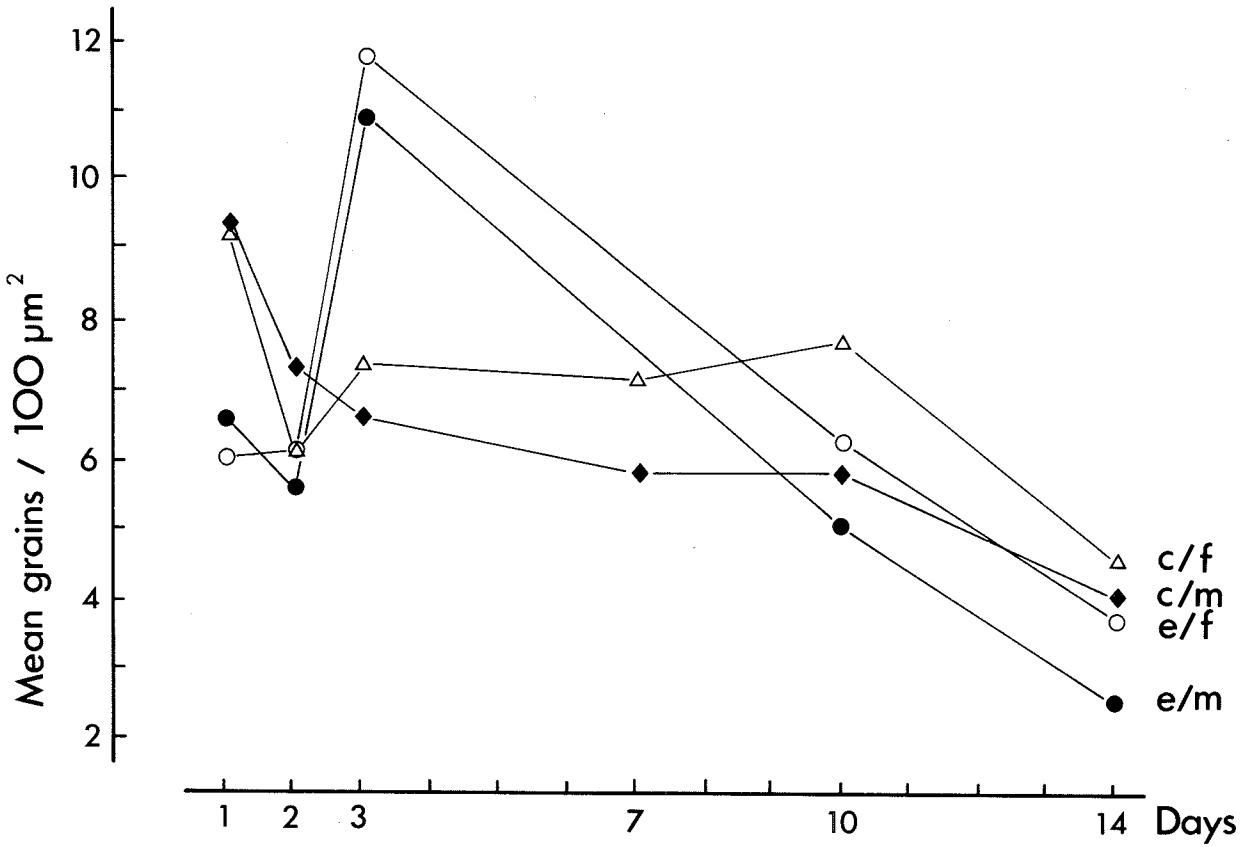


Fig. 29

Fig. 30 Mean number of grains over fiber and matrix per 100 μm^2 at different time intervals. Control and experimental counts are combined. The data indicates significant differences in the number of grains per unit area as a function of time ($p < 0.001$).

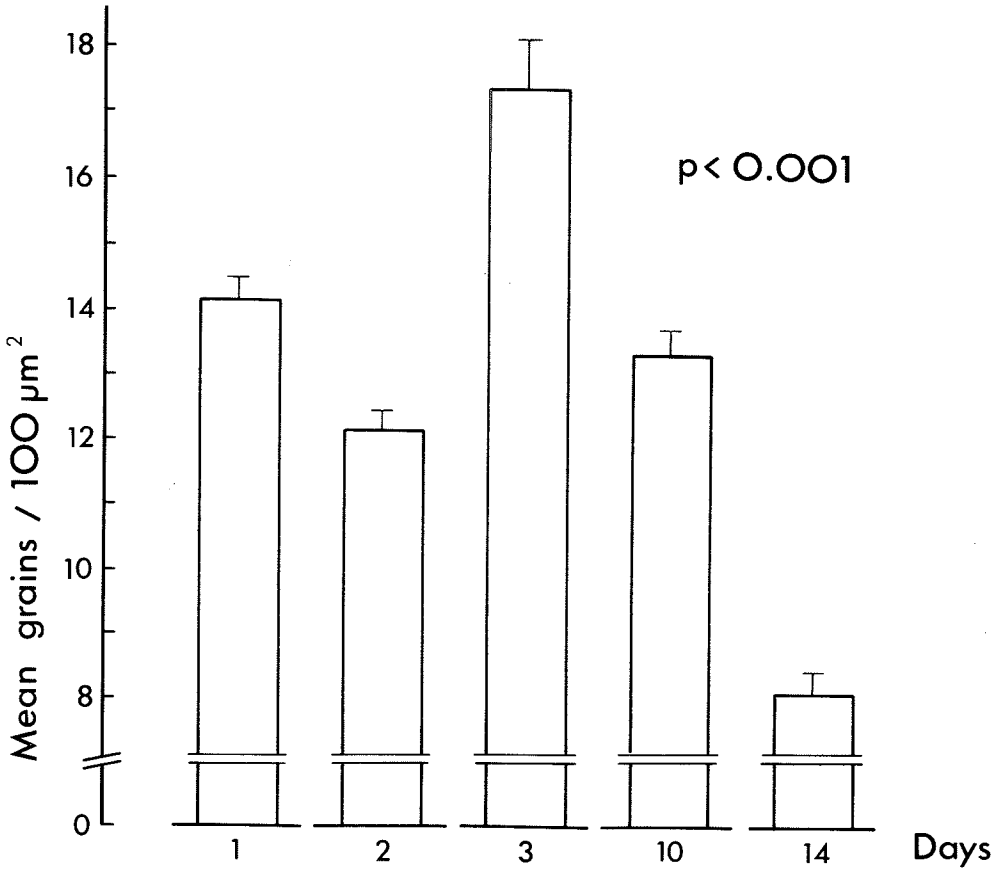


Fig. 30

Fig. 31 Mean grain counts per 100 μm^2 within intraosseous fibers, and in bone matrix at different time intervals. Control and experimental counts are combined. There is a significant difference in the number of grains per unit area as a function of time ($p < 0.001$) and in the number of fiber grains compared to the number of matrix grains as a function of time ($p < 0.001$).

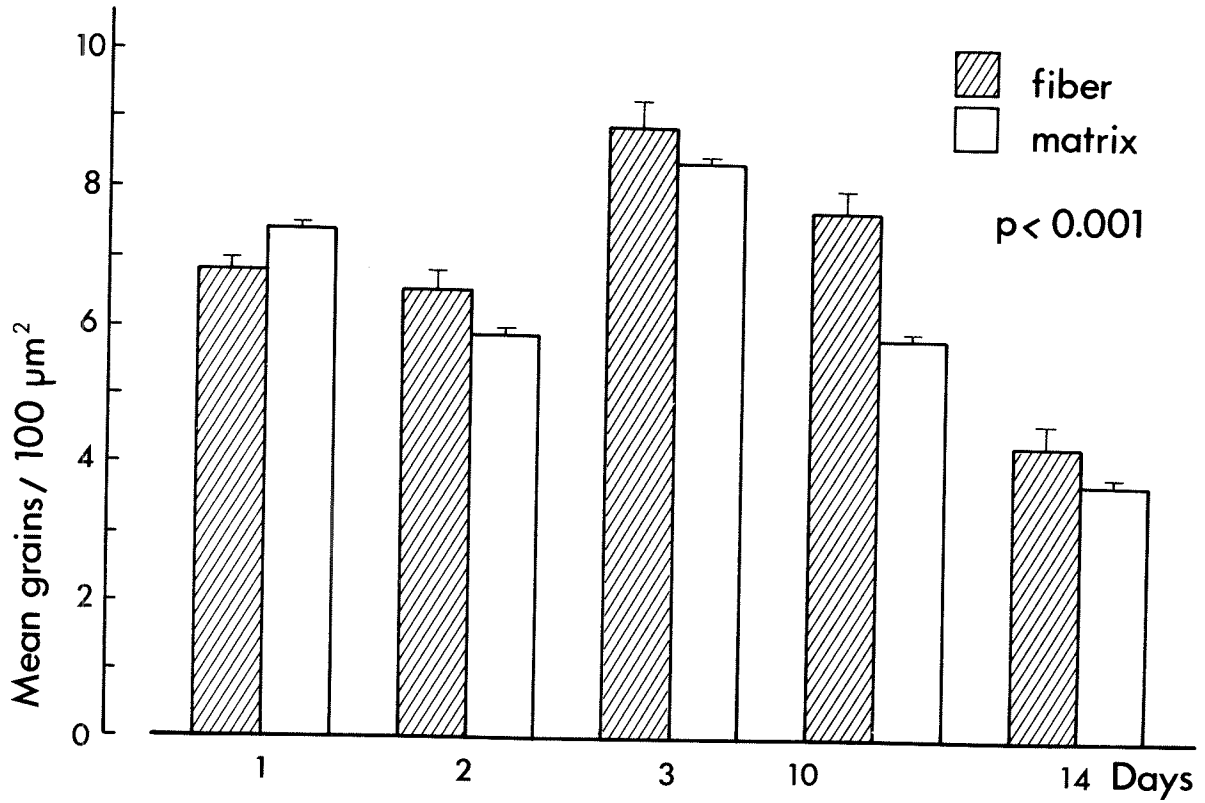

10
8
6
4
2
0
Mean grains / 100 μm²
fiber
matrix
p< 0.001
1
2
3
10
14 Days

Fig. 31

Fig. 32 Relation of middle versus apical mean grain counts of control and experimental septa ($p < 0.05$). Data reflects the mean of all grain counts made at each studied time interval. There is a significant difference in the middle to apical third counts ratio between control and experimental ($p < 0.05$). The middle third counts increased, while the apical third counts decreased as a result of the force.

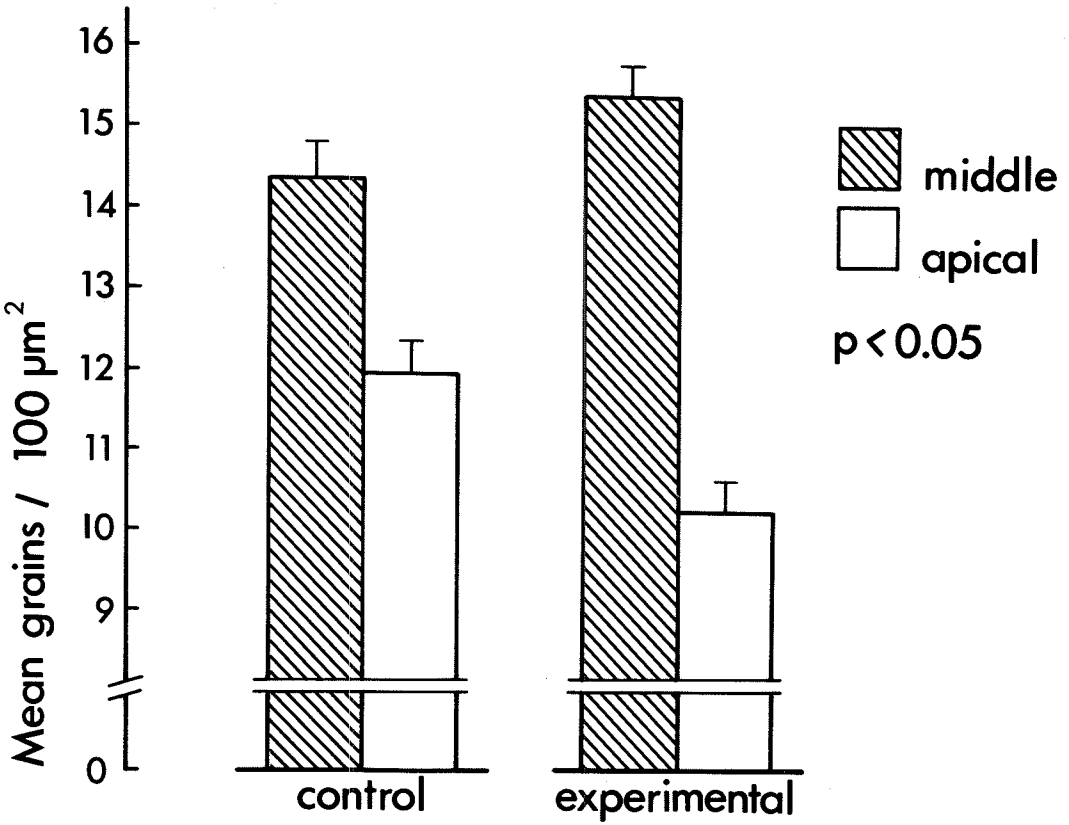
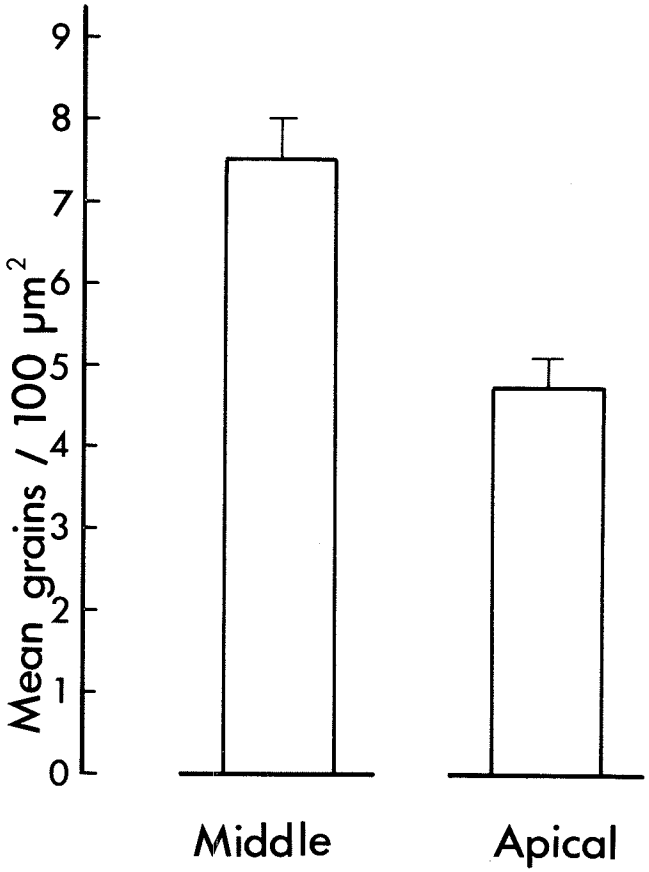

Mean grains / 100 μm²
16
15
14
13
12
11
10
9
0
control
experimental
middle
apical
p < 0.05

Fig. 32



p<0.001

Fig.33

Fig. 34 Mean grain counts per 100 μm^2 comparing mesial, middle and distal thirds of the septum. Data reflects the mean of all grain counts made in experimental and control animals at each selected time interval. Fiber and matrix counts individually. Control and experimental counts are combined. The distal third of the septum has the highest grain counts in fiber and matrix. The lower grain counts are found in the middle third of the septum for both fiber and matrix. There is a significant difference in counts between mesial, middle and distal thirds of the septum ($p < 0.001$).

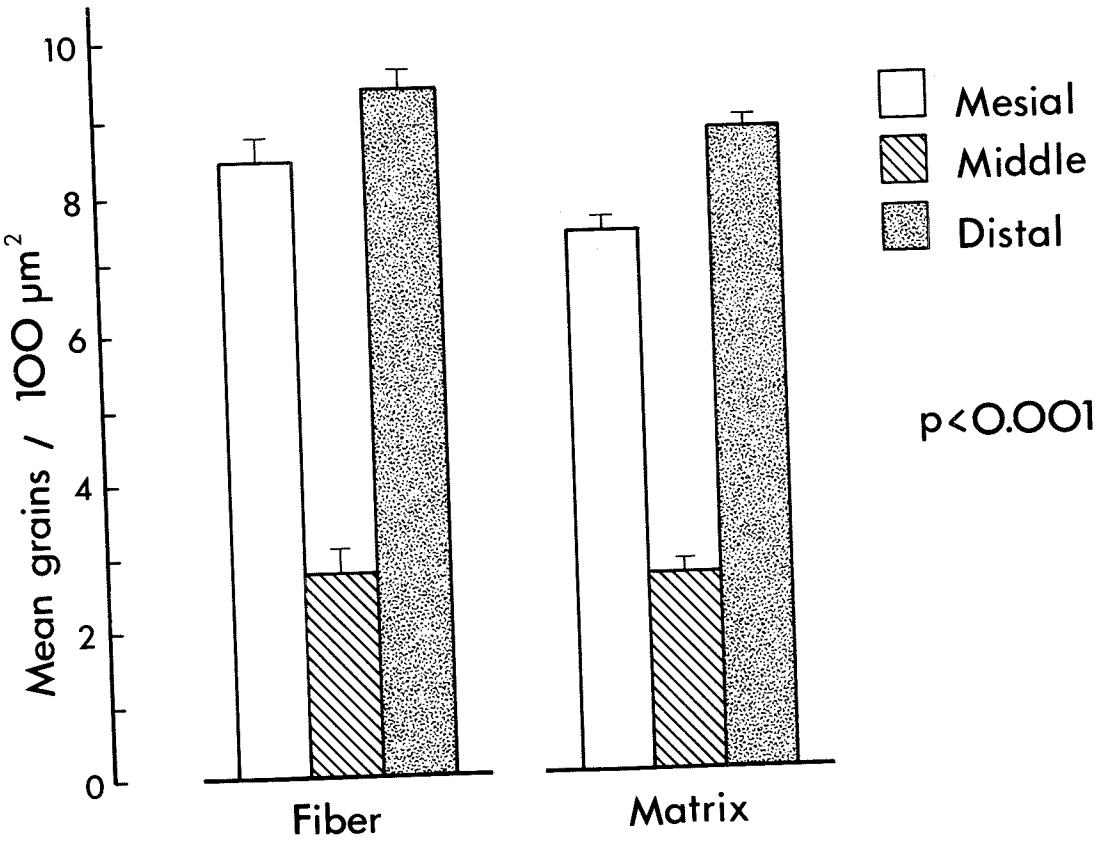
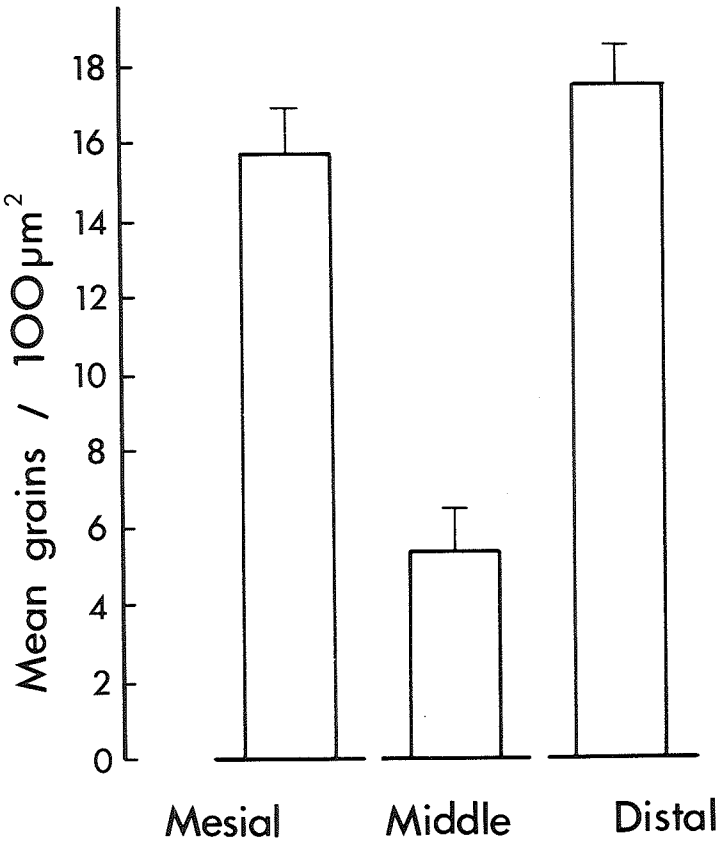

Mean grains / 100 μm²
10
8
6
4
2
0
Fiber
Matrix
Mesial
Middle
Distal
p<0.001

Fig. 34

Fig. 35 Mean grain counts per 100 μm^2 comparing mesial, middle and distal thirds of the septum. Data reflects the mean of all grain counts made in experimental and control animals at each selected time interval. Control and experimental counts combined. Fiber and matrix counts are combined. There is a significant difference in grain counts between mesial middle and distal thirds of the septum ($p < 0.001$).



$p<0.001$

Fig. 35

Fig. 36 Comparison of mean grain counts in the mesial, middle and distal thirds of the septum at different time intervals. (Intervals 1, 2 and 3 correspond to days 1, 2 and 3, interval 4 corresponds to 10 days and interval 5 to 14 days). Control and experimental counts combined. There is a significant difference between mesial, middle and distal third counts at every time interval ($p < 0.001$).

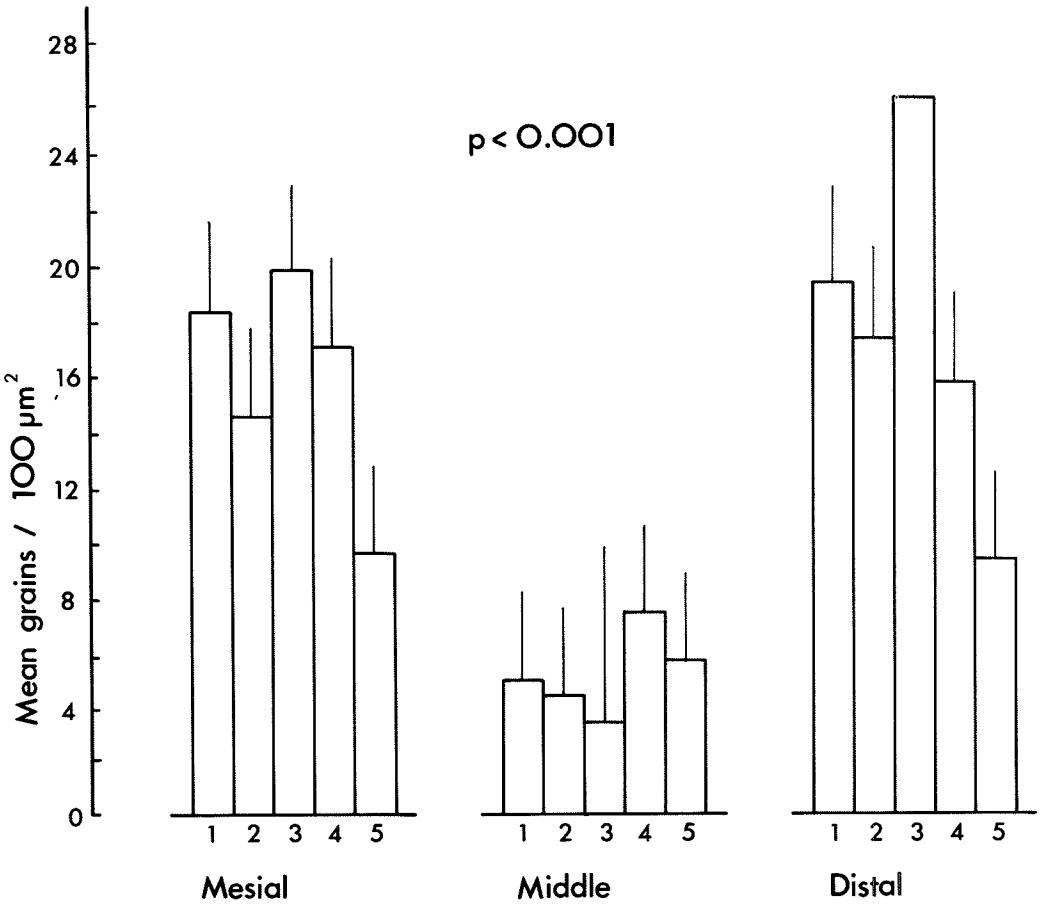

28
24
20
16
12
8
4
0
Mean grains / 100 µm²
p < 0.001
1 2 3 4 5
Mesial
1 2 3 4 5
Middle
1 2 3 4 5
Distal

Fig. 36

Fig. 37

Table 1 An analysis of variance of the grain counts in the intraosseous fibers of the interproximal septum. The significant values are listed here. The source of variation, degrees of freedom, sum of the squares (S.S.), mean of the squares (M.S.), f-value (F), and p-value (P) are listed. The analysis containing significant and non-significant values are contained in Table 4.

TABLE 1: FIBER

SOURCE OF VARIATION	DEGREES OF FREEDOM	S.S.	M.S.	F.	P.
DAY	4	233.7225	58.4306	8.323	P<0.001
CONTROL vs. EXPERIMENTAL	1	2.5617	2.5617	0.117	NON SIGNIFICANT
DAY/CONTROL vs. EXPERIMENTAL	4	12.9514	30.2379	1.377	NON SIGNIFICANT
MESIAL vs. MIDDLE vs. DISTAL	2	896.4541	448.2271	36.271	P<0.001
DAY/MESIAL vs. MIDDLE vs. DISTAL	8	275.5142	34.4393	2.787	P<0.01
TOTAL	107	2457.8394			

Table 2 Analysis of variance of the bone matrix grain counts of the interproximal septum. The significant values of the analysis are listed in this table. The degrees of freedom, sum of the squares (S.S.), mean of the squares (M.S.), f-value (F) and p-value (P) are listed. The complete analysis containing significant and non-significant values is contained in Table 5.

TABLE 2: MATRIX

SOURCE OF VARIATION	DEGREES OF FREEDOM	S.S.	M.S.	F.	P.
DAY	4	250.7860	62.6965	37.602	P<0.001
CONTROL vs. EXPERIMENTAL	1	0.5880	0.5880	0.039	NON SIGNIFICANT
DAY/CONTROL vs. EXPERIMENTAL	4	175.9548	43.9887	2.901	NON SIGNIFICANT
MESIAL vs. MIDDLE vs. DISTAL	2	777.2603	388.6301	79.458	P<0.001
DAY/MESIAL vs. MIDDLE vs. DISTAL	8	226.7036	28.3379	5.794	P<0.001
MIDDLE vs. APICAL/MESIAL vs. MIDDLE vs. DISTAL	2	75.0468	37.5234	5.365	P<0.001
TOTAL	107	2387.8494			

Table 3 Analysis of variance of fiber and matrix grain counts in the interdental septum. The significant values of the analysis are listed in this table. The degrees of freedom, sum of the squares (S.S.), mean of the squares (M.S.), f-value (F) and p-value (P) are listed. The complete analysis containing significant and non-significant values can be seen in Table 6.

TABLE 3: FIBER AND MATRIX COMBINED

SOURCE OF VARIATION	DEGREE OF FREEDOM	S.S.	M.S.	F.	P.
DAY	4	921.7273	230.4318	12.881	P<0.001
CONTROL vs. EXPERIMENTAL	1	2.7193	2.7193	0.037	NON SIGNIFICANT
DAY/CONTROL vs. EXPERIMENTAL	4	579.3022	144.8256	1.984	NON SIGNIFICANT
CONTROL vs. EXPERIMENTAL/MIDDLE vs. APICAL	1	49.2970	49.2970	4.244	P<0.05
MESIAL vs. MIDDLE vs. DISTAL	2	3271.4871	1635.7434	63.092	P<0.001
DAY/MESIAL vs. MIDDLE vs. DISTAL	8	940.8206	117.6026	4.536	P<0.001
TOTAL	107	8707.8477			

Table 4 Analysis of variance of the grain counts in the intraosseous fiber of the interproximal septum. The sources of variation are abbreviated as follows:

DAY = Day
CVE = Control vs. Experimental
MVA = Middle vs. Apical
MMD = Mesial vs. Middle vs. Distal

The degrees of freedom (D.F.), sum of the squares (S.S.), mean of the squares (M.S.) and f-value (F) are listed. The significant values are listed in Table 1.

TABLE 4: ANALYSIS OF VARIANCE FIBER

SOURCE OF VARIATION	DF	S.S.	M.S.	F.
DAY	4	233.7225	58.4306	8.323
CVE	1	2.5617	2.5617	0.117
DAY CVE	4	120.9514	30.2379	1.377
MVA	1	35.1303	35.1303	1.198
DAY MVA	4	94.4618	23.6154	0.805
CVE MVA	1	8.1476	8.1476	0.752
DAY CVE MVA	4	38.4064	9.6016	0.886
MMD	2	896.4541	448.2271	36.271
DAY MMD	8	275.5142	34.4393	2.787
CVE MMD	2	64.6998	32.3499	2.186
DAY CVE MMD	8	175.4300	21.9287	1.481
MVA MMD	2	7.7594	3.8797	0.461
DAY MVA MMD	8	45.4879	5.6860	0.676
CVE MVA MMD	2	9.1024	4.5512	0.976
DAY CVE MVA MMD	8	40.6811	5.0851	1.090
TOTAL	107	2457.8394		

Table 5 Analysis of variance of grain counts in the bone matrix of the interdental septum. The sources of variation are abbreviated as follows:

DAY = Day
CVE = Control vs. Experimental
MVA = Middle vs. Apical
MMD = Mesial vs. Middle vs. Distal

The degrees of freedom (D.F.), sum of the squares (S.S.), mean of the squares (M.S.) and f-value (F) are listed. The significant values are listed in Table 2.

TABLE 5: ANALYSIS OF VARIANCE MATRIX

SOURCE OF VARIATION	DF	S.S.	M.S.	F.
DAY	4	250.7860	62.6965	37.602
CVE	1	0.5880	0.5880	0.039
DAY CVE	4	175.9548	43.9887	2.901
MVA	1	201.8424	201.8424	7.883
DAY MVA	4	48.6856	12.1714	0.475
CVE MVA	1	11.9698	11.9698	1.272
DAY CVE MVA	4	32.2776	8.0694	0.857
MMD	2	777.2603	388.6301	79.458
DAY MMD	8	226.7036	28.3379	5.794
CVE MMD	2	15.8678	7.9339	0.542
DAY CVE MMD	8	218.9542	27.3693	1.871
MVA MMD	2	75.0468	37.5234	5.365
DAY MVA MMD	8	45.8431	5.7304	0.819
CVE MVA MMD	2	9.8974	4.9487	1.047
DAY CVE MVA MMD	8	27.1415	3.3927	0.718
TOTAL	107	2387.8494		

Table 6 Analysis of variance of fiber and matrix grain counts of the interdental septum combined. The different sources of variation are abbreviated as follows:

DAY = Day
CVE = Control vs. Experimental
MVA = Middle vs. Apical
MMD = Mesial vs. Middle vs. Distal

The degrees of freedom (D.F.), sum of the squares (S.S.), mean of the squares (M.S.) and f-value (F) are listed. The significant values are listed in Table 3.

TABLE 6: ANALYSIS OF VARIANCE FIBER AND MATRIX COMBINED

SOURCE OF VARIATION	DF	S.S.	M.S.	F.
DAY	4	921.7273	230.4318	12.881
CVE	1	2.7193	2.7193	0.037
DAY CVE	4	579.3022	144.8256	1.984
MVA	1	380.0347	380.0347	3.650
DAY MVA	4	188.1796	47.0449	0.452
CVE MVA	1	49.2970	49.2970	4.244
DAY CVE MVA	4	149.4794	37.3699	3.217
MMD	2	3271.4871	1635.7434	63.092
DAY MMD	8	940.8206	117.6026	4.536
CVE MMD	2	126.3187	63.1593	1.130
DAY CVE MMD	8	760.0264	95.0033	1.700
MVA MMD	2	95.3697	47.6849	1.602
DAY MVA MMD	8	74.4893	9.3112	0.313
CVE MVA MMD	2	38.8143	19.4071	1.862
DAY CVE MVA MMD	8	102.4939	12.8117	1.229
TOTAL	107	8707.8477		

CHAPTER III

HISTOCHEMICAL STUDY

LITERATURE REVIEW

This chapter deals with light microscopic observations of alveolar bone remodelling during orthodontic tooth movement. The attention will be focused on intraosseous fiber remodelling, changes in the proteoglycan content of the different structures of the interdental septum, and its relation with time. The observations of the periodontal ligament have been extensively studied by others and will not be reviewed in detail. The remodelling of alveolar bone during physiologic and orthodontic tooth movement, and the proteoglycan distribution of developing and mature alveolar bone are surveyed.

Development of the Periodontium

Of interest in this study is the development of the alveolar bone and of the principal periodontal fiber groups. The prenatal tooth and periodontium development of the mouse was described in detail by Cohn (1957). Odontogenesis starts at day 12 prenatal in the mouse. The upper and lower first molar buds are present at day 14, and the second molar buds at day 16 prenatal. Root formation and eruption of the first molar tooth begins at day 10 postnatal. Connective tissue from the dental sac invades the epithelial root sheath at day 11 and 12. This constitutes the initial formation of periodontal ligament fibers. At day 16 to 17 postnatal the first molar erupts in the mouse (Cohn, 1957). This event

occurs at day 21 in the rat (Bernick, 1960; Trott, 1962). The fibers of the periodontal ligament become enclosed in acellular cementum at day 21 to 22 in the mouse (Cohn, 1957), while in the rat the first principal fibers are present seen in the occlusal third by day 25 (Bernick, 1960; Trott, 1962). Below the occlusal third the fibers are directed occlusally and obliquely from cementum to bone. The formation and organization of the attachment fibers begin as early as the first appearance of cementum on the root. Fine collagen fibers pass from the bone between the osteoblasts, and form non-oriented fibers within the periodontal ligament space. The change in orientation of the principle fibers from a steep incline is related to the stage of eruption and their relative height to the alveolar bone (Bernick, 1960; Trott, 1962; Grant et al., 1972). With the onset of clinical occlusion and function, the fiber bundles become thicker and present an appearance of continuity from bone to cementum. Grant et al. (1972) recognize the presence of an intermediate plexus in the erupting secondary teeth of squirrel monkeys. The cells in the central zone, by their secretion of precursors of collagen and proteoglycan complexes, may participate in the lengthening and thickening of the principal fibers. An "intermediate plexus" is not demonstrable in the functioning tooth. Eccles (1964) described the development of periodontal membrane in rat incisors, and stated that it is fundamentally similar to that in the rat molar. Eccles states that the incisor periodontal ligament has a clearly defined intermediate plexus while that of the molar has not. This difference can reasonably be explained by the much greater rate of erup-

tion and growth of the incisor.

Johnson and Low (1981, 1982) indicated that intraosseous fibers are present prior to tooth eruption. Johnson (1981) and Johnson and Low (1981) reported that intraosseous fibers are first represented as reticular fibers that undergo transformation into collagen fibers. Wilder's reticular stain revealed intraosseous fibers in the crestal alveolar bone at day 14 postnatal, and throughout the interdental septum at day 17 postnatal in mice. Van Gieson's method for collagenous fibers revealed intraosseous fibers in the crestal alveolar bone at day 19 and throughout the interdental septum at day 25 (Johnson, 1981). In the present study, orthodontic tooth movement is studied in rat molar, a tooth that is not preceded by primary teeth. Clear differences in development of the periodontium were reported by Grant et al. (1972). In their study of marmosets the teeth with predecessors had only a few organized periodontal fiber groups at eruption (dentogingival, alveolocrest and horizontal fibers), while teeth without predecessors had continuous principal fibers which were well developed. When teeth with predecessors came to occlusal contact, fibers of the apical third were still forming and an intermediate plexus was still demonstrable. Teeth without predecessors have, at the time of first occlusal contact, a continuous completed periodontal fiber system. The present study is performed on teeth without predecessors, and at the time of experiment initiation (six week old rats), the periodontium is fully developed.

Transalveolar Fibers

Cohn (1970) and Quigley (1970), in independent studies, reported for the first time that cemento-alveolar fibers of the periodontal ligament could pass without interruption through the full thickness of the entire surrounding alveolus of the mouse and rat. Cohn (1972a) in light microscopic study of alveolar bone in the mouse reported that Sharpey's fibers passed without interruption through the entire thickness of the surrounding alveolus and were not anchored at the bony margin. In the same year, Cohn (1972b) examined the transalveolar fibers in primates to confirm that these fibers passed from the periodontal ligament through the entire thickness of the alveolar bone, and were not anchored as Sharpey's fibers at the bony margin. Bernick et al. (1974, 1977), in studies of the development of the "transosseous" alveolar fibers in the marmorset, reported that during the formation of the septum, mesial and distal fibers from the ligament arborize from bone in the midcrestal region to form an intertwining network of collagenous fibers. According to Bernick et al., at no time was there morphological evidence of fibers passing through bone as intact groups to insert in cementum of adjoining teeth. Cohn (1974, 1975) described transalveolar fibers in human periodontium. Many electron microscopic studies have been done to study the morphological characteristics of the intraosseous fibers and their surrounding tissues (Selvig, 1965; Boyde and Jones, 1968; Quigley, 1970; Shackleford, 1973). These and other studies are reported in detail in another chapter (SEM study).

Alveolar Bone Remodelling Produced by Orthodontic Forces

When a horizontal orthodontic force is applied to the crowns of a tooth, pressure and tension zones can be distinguished. The diagram (Fig. 3) illustrates the type of forces applied in the experimental model used by this study. The spring in the interproximal space creates pressure in the apical third of the septum and tension in the crestal third.

Once the tooth has assumed the new position, a series of cellular events occurs (Rygh et al., 1982). On the tension side, an increase in connective tissue cells is observed. For young humans, incipient cell proliferation is seen after 30-40 hours of force application particularly near the socket wall. Shortly after, osteoid will be deposited on the wall (Rygh et al., 1982). According to Reitan (1950), where the periodontal fiber bundles are thick, new bone appears to be deposited along them. If the bundles are thin, a more uniform layer is deposited along the root surface (Reitan, 1950). There are different hypothesis as to how the fibers of the PDL are embedded in the alveolar bone when physiologic remodelling takes place. Kraw and Enlow (1967) suggested that the principal fibers became passively entrapped by the advancing front of new bone formation to form Sharpey's fibers. Garant and Cho (1979) on the other hand suggested that new Sharpey's fibers are secreted at the same time as new bone is deposited. Rygh et al. (1982) reported that, with orthodontic loads, there is both an incorporation of pre-existing collagen fibers into new osteoid and a considerable production of new col-

lagen fibers near the advancing front of the tension zone. On the pressure side the crest of the alveolar bone is slightly deformed (Picton, 1965; Picton and Davies, 1967). Certain cell activities will occur within the periodontal ligament and at the alveolar bone surface. These changes can be categorized broadly into "direct resorption" where the pressure is relatively light and "hyalinization" where the pressure is large enough to produce degenerative changes (Rygh et al., 1982). The cellular events occuring in areas of direct resorption and of hyalinization are described in detail by Rygh et al. (1982). Within both the tension and pressure zone of the alveolar bone, deposition and resorption occur on both the endosteal and the periosteal aspects. The alveolar wall adjacent to a hyalinized area of periodontal ligament is resorbed from the endosteal side. However, where the bone is of a more compact nature, it is resorbed directly on its external surface. At the same time osteoclasts differentiate from the relatively normal periodontal ligament tissue at the periphery of the hyalinized zone (Reitan, 1950; Rygh, 1973). Such osteoclast resorb alveolar bone and allow for relocation of the tooth.

The advantages of a bodily translation of a tooth over a tipping force were described by Reitan (1950), where he showed histological evidence of decreased bone and root resorption in teeth translated bodily. A bodily movement with light forces seem to imply an even tension within the periodontal fibers at all areas of the root surface. Pressure areas are not created to the same degree as in tipping of a tooth. Reitan (1964) also emphasizes the importance of light forces. In his experiments root resorp-

tion was more frequent and extensive when higher forces were applied.

The effect of tooth loading on blood circulation has been examined by Rygh (1972) and others. Gaengler and Knut (1983) studied the functional effects of tooth loading on blood circulation in the arterioles, capillaries, venules as well as in the arteries and veins of the periodontal ligament. By the application of a continuous force, the blood flow characteristics are different in the areas of tension and compression. After a continuous force of 100 mN for 30 minutes in Wistar rats, the pressure induces irreversible changes (thrombosis of some venules and capillaries, being the compression zone the most affected). This irreversible impairment can be detected histologically in terms of necrosis (Rygh, 1972). The direct relation between carotid arterial pressure and the relative displacement of the alveolar socket was demonstrated by Zengo et al. (1974), indicating a direct relationship between hemodynamic force and alveolar wall displacement.

Picton (1965) and Picton and Davies (1967) studied the displacement of alveolar bone after application of forces to teeth in monkeys. Bone displacement started in response to forces appreciably less than 100 gms. Horizontal forces of more than about 50 gms tended to cause the labial and lingual alveolar plates to be displaced in the same direction as roots. Bone distortion was less, but usually in the same direction as the movement of the root. This implies increasing tissue compression on the side to which the tooth was moved and decreased pressure on the other side

with the elastic deformation of the socket. The average change in width of the membrane was approximately 20 μm with 500 gms of force.

Kardos and Simpson (1980) proposed that periodontal ligament collagen presents in its labile or partially polymerized form, and therefore, the collagenous matrix exhibits the properties (deformation or flow of matter) of a thixotropic gel. In general a thixotropic system presents an isothermical change in viscosity brought about by pressure alone. When the pressure is removed, the system undergoes a time dependent recovery and maintains its contour. Kardos and Simpson assume that the periodontal membrane has the properties of a thixotropic gel, therefore, hyalinization does not represent areas of degeneration within collagenous matrix, but a change in consistency. The alteration in viscosity and, hence, in physical character of the collagenous matrix would permit rapid cell movement away from the area of compression. The gel, in time, would re-reform and re-equilibrate with the forces applied to the tooth and the balance of all of the force acting upon the masticatory system. Unfortunately, no experimental evidence of any kind is presented by the authors.

Although much time has been spent in studying histologic events in the alveolar wall produced by orthodontic tooth movement, no studies are available describing histologic changes inside the septum. Events taking place within the deeply embedded fibers of bone, as well as in the bone matrix itself, are unknown. The remodelling of the septum and deeply embedded fibers may be of significance in the understanding of tooth movement and relapse.

It seems important to clarify if the deeply embedded fibers and septal bone matrix remodel with orthodontic forces in the same manner as the walls of the septum, which have already been studied.

Proteoglycans and their Relation with Alveolar Bone and PDL

Bone matrix does not become mineralized immediately after it is formed by osteoblasts. A certain amount of time elapses before recently formed matrix begins to mineralize, and as a result, a band of unmineralized osteoid is seen at the sites of new bone formation. Loe (1959) and Tonna (1959) developed the concept that osteoid must undergo certain chemical changes, not as yet clearly defined, before it becomes mineralizable. Howell (1963) and Logan (1935) among others, stated that loss of protein polysaccharides favors the initiation of mineralization. Glimcher (1960) speculated that protein polysaccharides function either negatively by masking nucleation sites on collagen or positively by acting as a template for nucleation during mineralization (Campo, 1970; Smith, 1970). Most histochemical studies suggest that the concentration of polysaccharides is higher in sites of new bone formation than in mature bone matrix (Loe, 1959; Tonna, 1959). There is general agreement that proteoglycans consist of chains of glycosaminoglycans (GAG) connected to a protein core by a oligosaccharide linkage (Silbert, 1978). Proteoglycans have been demonstrated in many tissues by LM techniques utilizing Alcian Blue (Johnson and Low,

1983). This dye is known to demonstrate the acid glycosaminoglycans components of proteoglycans (Scott et al., 1964). Acid glycosaminoglycans are negatively charged either because of the presence of sulfate ester groups or because of carboxyl groups. As a consequence they bind cationic dyes. Alcian Blue at pH 2.5 demonstrates both carboxyl and ester sulfate groups whereas Alcian Blue at pH 1.0 or less demonstrates only ester sulfate groups (Scott et al., 1964). There is general agreement that sulphated glycosaminoglycans possess a regulatory function in mineralization. Chondroitin-4-sulphate, chondroitin-6-sulphate and dermatan sulphate strongly bind calcium so that the ions are not available to form crystals (Glimcher, 1960; Sauk et al., 1976). Initiation of calcification is not considered possible without calcium ions. Removal of sulphated glycosaminoglycans allows release of calcium ions into the tissue space allowing calcification to proceed. Baylink et al. (1972) tested the validity of Alcian Blue staining as a means to determining distribution of protein polysaccharides. Demineralized sections were incubated in hyaluronidase, which hydrolyzes chondroitin sulphate and hyaluronate. After 1 hour of incubation with hyaluronidase (60 U/ml), no stainable material was subsequently found in either osteoid or perilacunar regions. These results confirms that Alcian Blue and Toulidine Blue stains protein polysaccharides. The same authors tested the possibility that protein polysaccharides were present in unstained sites such as young bone matrix. Since some protein polysaccharides are known to be soluble in aqueous solutions, it is possible that polyanions were lost from young bone matrix dur-

ing demineralization. To evaluate this, bones were fixed in formalin-CPC and ground sections from these bones were prepared by grinding in 1% CPC rather than in tap water. These sections were then stained with Alcian Blue dissolved in 3% acetic acid. Young bone matrix that had been demineralized did not stain with Alcian Blue. These results suggest that protein polysaccharides are not present in young bone matrix. Thus, even though it is probable that some protein polysaccharides are lost during demineralization, it would appear that this loss affects the intensity of staining more than the distribution of protein polysaccharides in bone matrix. Another possible cause of false negative staining is blocking of staining reactions between the basic dye and the tissue polyanions by basic proteins. Evidence that basic proteins can inhibit staining was given by French and Benditt (1953). The addition of neutral salts enhances staining, but does not significantly alter the distribution of protein polysaccharide staining in bone (Baylink et al., 1972). To determine whether the decline in protein polysaccharide staining occurred precisely at the demineralization front or beyond the mineralizing front in young bone matrix, the same authors used two labelling procedures: (1) Lead deposition at the mineralizing front and (2) fluorescent porcion which is taken up in osteoid but not in mineralized bone. The results indicated that protein polysaccharide staining of osteoid extended up to, but not beyond the mineralizing front. Since the mechanism of loss of protein polysaccharide is most likely enzymatic, the authors conducted experiments to determine the types of enzyme capable of hydrolyzing protein polysaccharides in osteoid.

Two proteolytic enzymes, trypsin and papain and two saccharidases, hyaluronidase and beta-glucuronidase, substantially reduce or completely remove all Alcian Blue staining material from unfixed osteoid. These results indicate that both proteases and saccharidases are capable of hydrolyzing the protein polysaccharides present in osteoid. According to previous studies of Baylink et al. (1970), the width of new matrix added per day at the periosteum is about 10 μm and since periosteal osteoid width is 5-10 μm, matrix protein polysaccharides are normally turned over in 12-24 hours. Mathews (1965) suggests that the protein polysaccharide function is to orient unit collagen fibrils once they are formed near osteoblasts. Howell (1963) proposes that since protein polysaccharides bind calcium, the hydrolysis of these macromolecules could cause a local boost in calcium ion concentration and thereby promote calcium phosphate precipitation at the mineralizing front. Another possible function of protein polysaccharides in the process of mineralization of bone is that protein polysaccharides act as a template in the nucleation process at the mineralizing front (Schubert and Pras, 1968). Borcherding et al. (1975) stated that a decrease in organization of collagen fibers in the cornea was accompanied by a large fall in total proteoglycan concentration and the appearance of detectable amounts of dermatan sulphate. Lorber (1951) and Johnson and Low (1983) described channels for intraosseous fibers in the lamina dura of the alveolar bone. The walls and channels for the intraosseous fibers have reaction for lipid and acid mucopolysaccharides as well as calcium ions and phosphate ions.

Baumhammers and Stallard (1968) observed the utilization, turnover and retention of S^{35}-sulfate labelled mucopolysaccharide in the periodontal membrane, cementum, alveolar bone and dentin in adult mice. The fibroblasts, cementoblasts, osteoblasts and odontoblasts appeared to take an active part in the synthesis of the sulfated mucopolysaccharides of their respective tissues. There was no turnover of the S^{35}-labelled sulfated fractions of the ground substance of the mineralized cementum, alveolar bone and dentin. Johnson and Low (1983) studied the relationship of proteoglycans to developing intraosseous fibers in the alveolar bone of the mouse. Johnson and Low (1982) postulate that proteoglycan complexes may be essential in the maintenance of the collagen of intraosseous fibers. Johnson and Low (1983) described the progressive decrease in the concentration of proteoglycans of maturing bone. Although, in general, proteoglycans are lost from the interdental septum during mineralization, there seems to be appreciable concentration of them in areas adjacent to intraosseous fibers. Johnson and Low (1982) report that transalveolar fibers are closely associated with osteocytes and osteocytic processes. The ability of osteoblasts to synthetize the collagen of the bone matrix is well established (Carneiro and Leblond, 1959). The immobility of osteocytes suggest that a carrier system may function in the transport of tropocollagen from outside the osteocyte to the intraosseous fiber. Johnson and Low (1983) suggest that the proteoglycan carrier system described by Hay (1978) and others may function in the transport of tropocollagen from outside the osteocyte to the intraosseous fiber. Kurihara and Enlow, after a

description of the three different types of intraosseous fiber attachments happening on the resorptive and appositional wall of physiological remodelling of alveolar bone (1980a), studied these attachments histochemically (1980b). They concluded that in the "adhesive attachments" (described in detail in the SEM chapter), one or more types of osteoclast companion cells appear to function in the secretion of components of proteoglycans (probably glycosaminoglycans) and tropocollagen. These proteoglycans appear to serve as a principal component of the adhesive substance secreted on the bone surface and also as a binding agent for the linking and progressive re-linking of new and old collagenous fiber to each other and to the resorbed bone surface.

A study of the changes in proteoglycan concentration in the interdental septum and in the channels surrounding intraosseous fibers during orthodontic tooth movement is not available. The increased rate of bone remodelling necessary to maintain the septal integrity during orthodontic tooth movement may cause changes in the level of calcification of the septum. The changes induced in intraosseous fibers may create changes in the proteoglycan content of the cores surrounding intraosseous fibers, and in the proteoglycan carrier system proposed by Johnson and Low (1983).

MATERIALS AND METHODS

Springs were placed between the left maxillary first and second molars of ten albino rats, six weeks of age. Animals were killed 1, 2, 3, 4 and 5 days after spring placement. The maxillae were dissected and divided into a right and left half (control and experimental). The segments were fixed in Karnovsky's fixative (Karnovsky, 1965) for three hours and decalcified in 4.13% EDTA at pH 7.0 (Warshawsky and Moore, 1967). Following decalcification the tissue was washed in running water, dehydrated in graded series of ethanols and embedded in paraffin. Serial sections, 6 μm in thickness, were mounted on glass slides and stained with Alcian Blue at pH 1.0 and pH 2.5. Both control and experimental sections were stained with Alcian Blue pH 1.0 and pH 2.5. Sections were studied and photographed in a Zeiss photomicroscope. The areas that stained positive for Alcian Blue appeared dark blue in the tissue and were classified as Alcian Blue positive (AB+). The areas that appeared clear in the finished tissue section were named Alcian Blue negative (AB-).

RESULTS

Controls

Alcian Blue demonstrated, in control specimens, the arrangement of intraosseous fibers of the interdental septum. The fibers, which stained AB- can be clearly distinguished in the septum. The bone matrix, in constrast, is AB+ (Fig. 38). In the septal wall, Alcian Blue positive bands are distinguishable, especially at the alveolar crest. On the appositional wall the band is not uniform, and on the resorptive wall, some Alcian Blue positive segments are distinguishable. At day two (Fig. 40), the matrix is AB+ and the AB- fibers can be clearly distinguished from the surrounding bone matrix. The intensity of the stain appears decreased in comparison with day one. An AB+ band can be detected at the appositional front of the septum. On the resorptive front the AB+ areas are less evident than at day one. As experimental time increases (day 3 and 4) (Figs. 42, 44), the delineation of the boundary of intraosseous fibers and the AB+ matrix is more difficult. The stain intensity of the whole septum decreased with time. There is no evidence of a thickening of the AB+ band on the appositional side.

Experimental

At day one after force application (Fig. 39) the bone matrix was AB+, the intraosseous fibers AB-, and the boundary between them is clearly distinguishable. The septum stains less intensely than the one day control septum (Fig. 38) and the septal walls do not demonstrate AB+ bands. The apical third of the septum stains less intensely indicating a lower concentration of sulphated glycosaminoglycans. After two days of experimental force (Fig. 41), the intraosseous fibers AB- and the matrix AB+ are clearly delineated. The crestal third of the septum stains more intensely than apical areas. The pressure of the spring on the alveolar crest has created a concave resorption pattern, probably produced by impingement of the gingiva by the spring (Fig. 41). No AB+ bands are evident on the mesial or distal walls of the septum. After three days of force application, the bone matrix stains less intensely. The intraosseous fibers are well delimited by AB+ bands. The intensity of Alcian Blue stain in the three day experimental (Fig. 43) septum appears less than in three day control specimens (Fig. 42). After four days a similar staining reaction in the septum is seen. The identification of intraosseous fibers is more difficult. Some AB+ bands can be identified near the septum walls. A similar pattern is seen after four days of experimental force (Figs. 45, 47). Intraosseous fibers and bone matrix demonstrate similar AB staining characteristics. The boundary between matrix and fiber is clearly delineated by an AB+ band adjacent to the fiber. The only distinguishable difference

between the control and experimental septa is found in the septal walls and the AB+ bands surrounding the intraosseous fibers. Control specimens have AB+ bands on both mesial and distal walls of the septum. This is not observed in experimental specimens. Bone matrix around osteocytic lacunar walls stained AB+ consistently during the experimental time in both control and experimental specimens. Observations of the periodontal ligament of experimental specimens indicate an increase in sulphated-GAG as experimental time was increased (Figs. 39, 41, 45, 47). The changes in AB staining characteristics in control specimens is not as evident. Sulphated and carboxylic GAGs demonstrated with AB pH 2.5 show only a slight change during the experimental period (Figs. 38, 40, 46). An increase in sulphated GAGs in the periodontal ligament (AB pH 1.0) is detected between days 3 and 4 (Figs. 42, 44). The difference in AB pH 1.0 uptake of the periodontal ligament between controls and experimentals can be seen comparing Fig. 44 and 45.

DISCUSSION

A number of publications have dealt with the role played by sulphated glycosaminoglycans in calcification. Several studies giving some evidence of this relation were summarized by Campo (1970). The observations of less sulphated polysaccharides in bone than in cartilage, the loss of polysaccharides in aging human rib associated with increased mineralization, the *in vitro* observations of chondroitin sulphate binding calcium and phosphate, and *in vitro* observation of toulidine blue, protamine, leutocobaltic chloride and chlorpromazine (all reactants or precipitants of chondroitin sulphate) inhibiting calcification, suggest a close relation of sulphated glycosaminoglycans with calcification.

The present study correlates the changes in proteoglycan distribution in the interdental septum with orthodontic tooth movement. This experiment deals with mature periodontium. The distribution of proteoglycans similar to that reported by Johnson and Low (1983) for mature interdental septum was found. The sulphated glycosamioglycan content decreased as experimental time increased. The AB+ areas of the septum, that is the bone matrix surrounding the intraosseous fibers and the alveolar wall, showed a decrease in Alcian Blue uptake with time in control specimens. This phenomenon could be associated with the change in normal diet and, therefore, in animal weight during the first experimental days. Dehydration of the studied animals could produce a decrease in GAGs. Battelheim and Brady (1979) reported the relation of hyaluronate and water uptake, and from sulphated glycosaminoglycans and

water retention in skin. If dehydration occurred, a decrease in GAGs could then be related to this event. The methods used for measuring the sorption and retention of water have been criticized for not giving physiological relevant values (Comper and Laurent, 1978). Bone matrix around osteocytic lacunar walls stained consistently during the experimental time, suggesting that GAG synthesis was constant during the experiment. A clear AB+ band on the appositional front of the septum, related with new osteoid was observed. On the resorptive side of the septum, several AB+ fragments were seen. This observation can be related to glycosaminoglycan deposits occurring during the reattachment of the intraosseous fibers. This was described on resorptive walls of the interdental septum by Kurihara and Enlow (1980b) as "adhesive attachments".

Experimental

The AB+ band on the appositional wall and on part of the resorptive wall are not clearly identifiable. This may be related to an interruption of the physiological drift of the molars, created by the applied force. The AB+ bone matrix was distinguishable through all the experimental time. The stain became less intense with time. This situation can be produced by a decrease in synthesis or an increased repletion of GAGs in the bone matrix. The bone matrix around osteocytic lacunae stained AB+ consistently through the experiment. This suggests that GAG synthesis was not

interrupted. A decrease in rate of GAGs could, however, have taken place without being perceived by my experiment. After five days of force application the AB uptake of matrix and fiber was very similar. An increase in mineralization of the bone matrix would produce a decrease in GAG content. That did not happen, as shown clearly in SEM study of mineralization of bone matrix after force application. The intraosseous fibers were delineated by the AB+ surrounding the intraosseous fiber. This band was consistent during the experimental time. The AB+ uptake around osteocytes and in channels surrounding the intraosseous fibers are consistent with Johnson and Low (1983). These authors relate high concentrations of GAG around osteocytes and areas adjacent to intraosseous fibers with a proteoglycan carrier system that may function in the transport of tropocollagen from outside the osteocyte to the fiber.

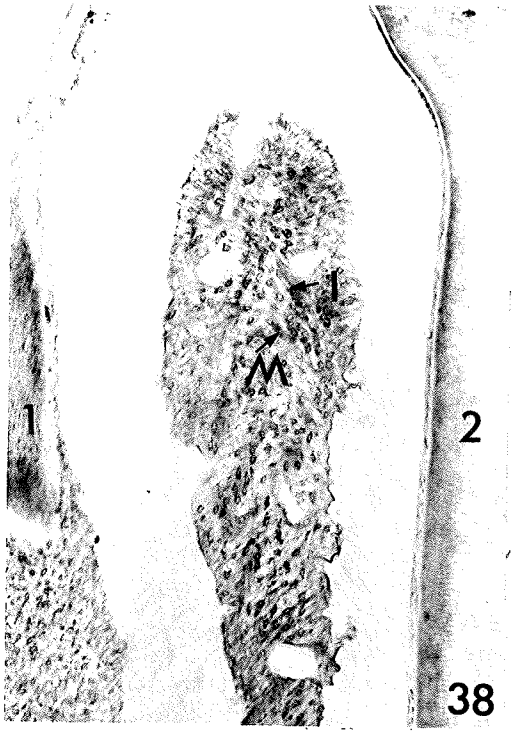
The general loss of GAGs can also be related to a dietary deficiency and the weight loss observed in the animals during the first experimental days, as related earlier with Battelheim and Brady (1979).

Fig. 38 Alcian Blue stain, pH 2.5, one day, control (40 X). Interdental septum between first (1) and second maxillary molar teeth (2). Intraosseous fibers (I), stain AB- and surrounding bone matrix (M) stains AB+. Lacunar walls (arrow) stain AB+.

Fig. 39 Alcian Blue stain, pH 1.0, one day experimental (40 X). Apical half of the septum (arrow) are AB- in comparison to the cervical half. Intraosseous fibers (I), are AB- and bone matrix (M) AB+. First molar (1), second molar (2).

Fig. 40 Alcian Blue, pH 2.5, control, two days (40 X). Interdental septum. The distal wall of the septum presents AB+ band (arrow). First molar (1) and second molar (2).

Fig. 41 Alcian Blue, experimental, pH 1.0, two days (100 X). Interdental septum. The crest of the septum has remodelled to the spring pressure (arrow). There is no evidence of AB+ band on distal wall of the septum.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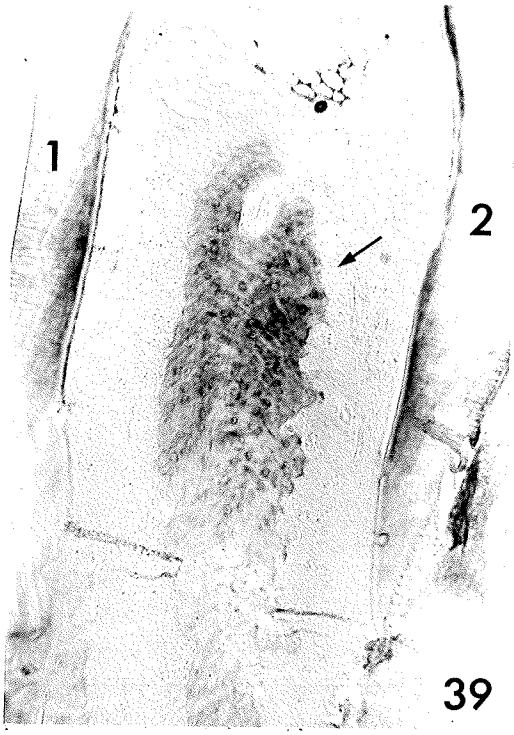
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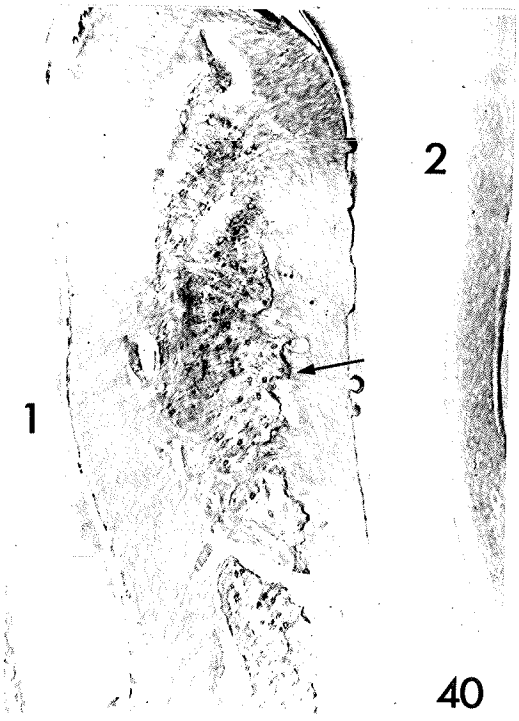
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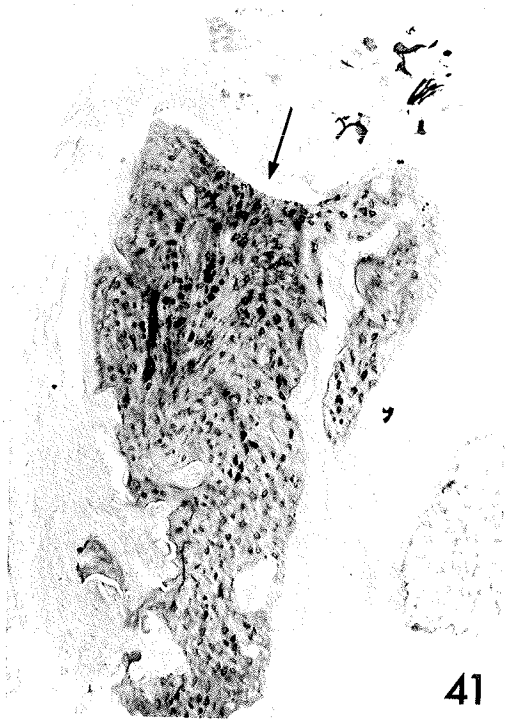
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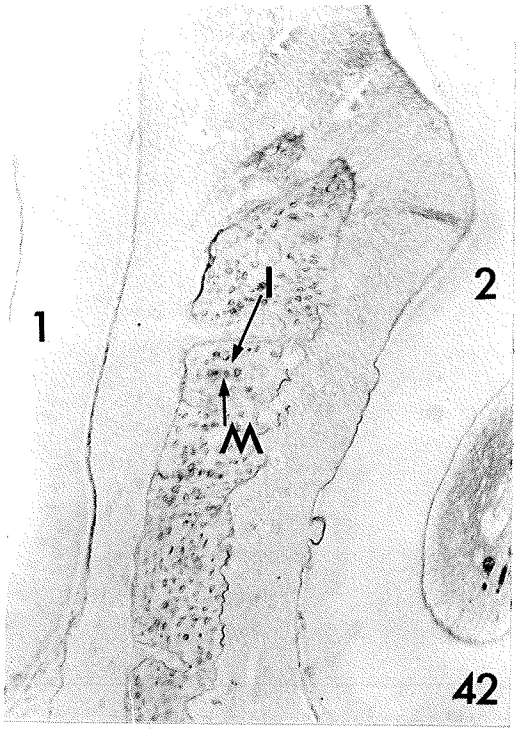
Fig. 42 Alcian Blue, pH 1.0, control, three days (40 X). Interdental septum between first (1) and second maxillary molar (2). Intraosseous fibers (I) and bone matrix (M) can be distinguished. A loss in staining intensity is evident.

Fig. 43 Alcian Blue, pH 2.5, experimental, three days (40 X). The interdental septum stains less intensely. Intraosseous fibers (I) are AB- and matrix (M) is slightly AB+.

Fig. 44 Alcian Blue, control, pH 1.0, four days (40 X). The intraosseous fibers and the bone matrix are AB- and are difficult to distinguish.

Fig. 45 Alcian Blue, experimental, pH 1.0, four days (40 X). Matrix (M) stains with similar intensity as intraosseous fibers (I). Intraosseous fibers are delimited by AB+ stain of surrounding channels.



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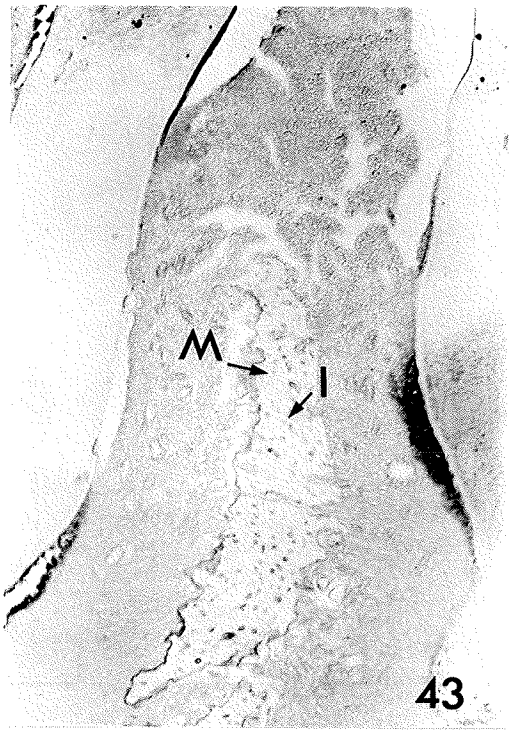

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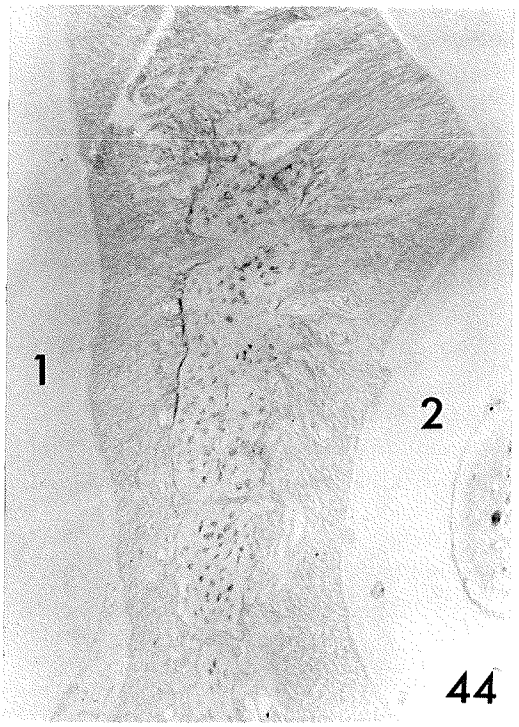

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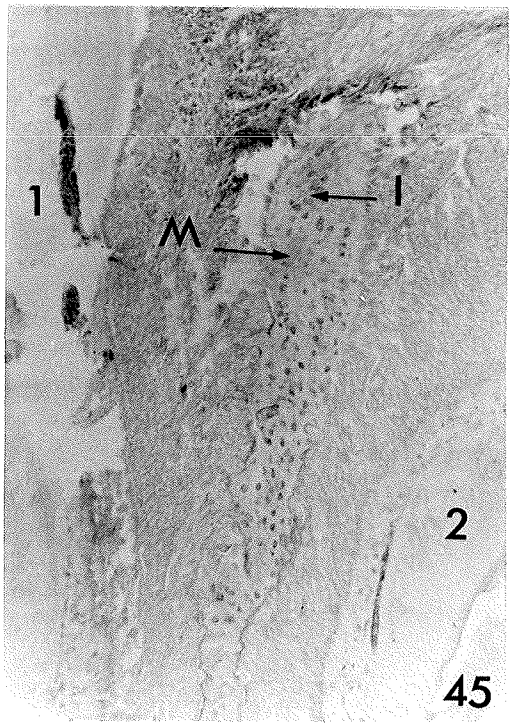

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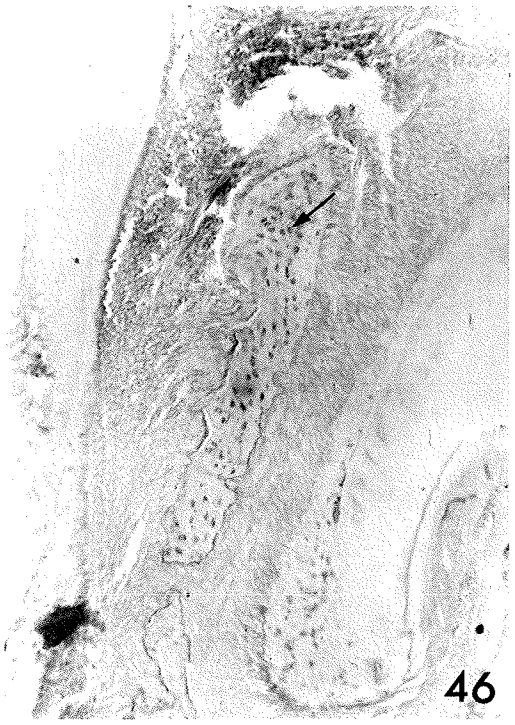
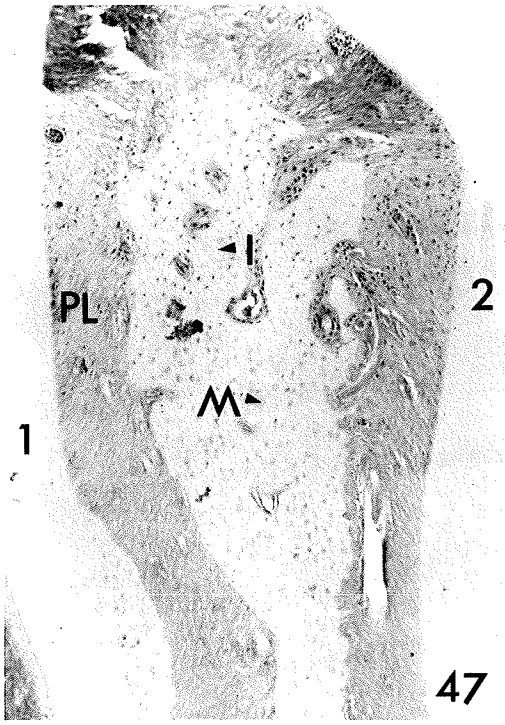
Fig. 46 Alcian Blue stain, pH 2.5, control, five days (40 X). Lacunar walls (arrow) stain AB+. The intraosseous fiber and matrix stain are AB-.

Fig. 47 Alcian Blue, pH 1.0, experimental, 4 days (40 X). The staining intensity of intraosseous fibers (I) and bone matrix (M) is very similar. The channels surrounding the fiber stain AB+. Periodontal ligament (PL) is AB+. First (1) and second molar (2) roots.



46



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CHAPTER IV

DISCUSSION

The Model

The spring used during this experiment produced orthodontic tipping of the first and second molars. This was evident by the alteration in bone apposition on the walls of the interdental septum. This situation can be demonstrated by comparison of the histologic appearance of control and experimental specimens (Figs. 20, 21). The spring impinged on the papilla and exerted pressure on the crestal portion of the septum. This situation is demonstrated by the fact that, in the SEM specimens, the crestal third of the septum was reabsorbed (Fig. 5). As a result, in the RAG study, grain counts were not made of the crestal third. A similar observation was made in Alcian Blue stained specimens, where hyalinization of the transseptal ligament and recession of the crestal third of the septum was observed (Fig. 41). Impingement of the crestal 1/3 by the spring altered the force system and created pressure in the crestal 1/3 rather than tension. The effects of tension on the septal bone and intraosseous fibers could be analyzed only in small areas of the middle 1/3. The impingement of the spring on the gingiva might have produced alterations in the blood supply to the alveolar crest. However, collateral circulation from the apical portion of the septum and periodontal ligament can satisfactorily maintain an adequate supply for the complete septum. This is supported by the observation of numerous osteoclasts at the crest of the septum. Their internal location within the septum suggests that the crestal resorption occurred as a result of the pressure exerted by the spring, rather than by

necrosis. The force exerted by the spring was approximately 25 to 30 gms. It must be considered, however, that the force was not directly applied to one individual tooth, but that force was distributed to the first and second molars. The reaction of the second molar should also be diminished by the resistance opposed by the third molar. Therefore, it can be assumed that the force applied to the first or the second molar teeth was less than 25-30 gms. This method of tooth separation produced a separating force for about five days. Lilja et al. (1983), in a study of the enzymes associated with bone resorption, identified a force of the magnitude delivered by my spring as a high force. High forces induce an increase in acid phosphatase and lactic dehydrogenase and create a zone lacking these two lysosomal enzymes in most compressed areas of the periodontal ligament (Lilja et al., 1983). In some of my studied specimens the periodontal ligament showed some signs of hyalinization, suggesting high forces.

The initial mean weight loss of the animals after spring placement must be taken into consideration. The mean weight decreased during the first day and reached normal values between days three and four. A decrease in metabolic activity might explain the apparent progressive loss of glycosaminoglycans observed in the experiment. This was marked by a decrease in Alcian Blue staining with time. The total grain counts also showed an initial increase followed by a decrease at day two and a peak at day three. This possibly reflects the initial weight loss and metabolic alteration (Figs. 30, 31).

Controls

The presence of deeply embedded fibers in the interdental septum was confirmed in this study. These fibers can be observed, in SEM (Figs. 6, 7, 8), in light microscopy (Figs. 27, 28). These fibers possessed a high mineral content, as revealed by anorganic SEM observations (Figs. 6, 7, 8). Absence of Alcian Blue staining of intraosseous fibers (Figs. 38) also suggests that these fibers are highly mineralized. An unmineralized central core, as suggested by Boyde and Jones (1968), Johnson (1983), was confirmed in intraosseous fibers near the bone surface, but not in more deeply embedded fibers. Irregular, unmineralized fissures were commonly observed in intraosseous fibers. Intraosseous fibers were composed of a bundle of smaller fibers which passed through channels in bone. Each fiber was partially surrounded by an elevation of bone. This description confirms this of Johnson (1983). Intraosseous fibers were a continuation of Sharpey's fibers (Figs. 18, 27) and were deeply embedded in the septum. The magnification used for RAG and AB studies did not allow for a detailed observation of these fibers. In LM the interdental septum gave evidence of distal drift with bone apposition on the distal wall of the septum and resorption on the mesial wall. There was also evidence of occlusal tooth movement evidenced by bone apposition on the alveolar crest. Both situations were marked by characterized collagen remodelling (Figs. 20, 22) and sulphated glycosaminoglycan distribution (Figs. 40). The radioautographic counts confirm a higher protein labelling on the distal wall of the septum than on

the mesial (Fig. 35), suggesting higher turnover. In SEM appositional and resorptive walls were evident. The presence of unfused calcospherites in anorganic SEM images suggest recent bone matrix deposition (Fig. 8), while the resorptive front demonstrates a dense matrix and intraosseous fibers with several unmineralized fissures, projecting from resorbing bone matrix (Fig.6). The middle portion of the septum remodelled at a slower rate (Figs. 34, 35). The fibers and the matrix at this level remodelled more slowly than that of mesial and distal walls of the septum.

Experimental

The experimental specimens, submitted to pressure forces, demonstrated signs of resorption. The SEM study suggested a selective demineralization: the bone matrix being less mineralized than the intraosseous fibers. The intraosseous fibers demonstrated signs of hypermineralization as they had fewer unmineralized fissures. It is possible that free mineral ions present in the matrix, a product of the resorbing matrix were absorbed by the fibers, producing hypermineralization. Hypermineralization could also be the result of increased occlusal trauma or hyperfunction produced by the presence of the spring. The RAG study indicated a higher protein synthesis rate in the fiber than in the matrix (control and experimental) (Fig. 19 RAG). This may also explain in part the maintainance of the intraosseous fiber and the faster resorption rate of the surrounding matrix as seen in the SEM

study. The higher synthetic rate in the fiber allowed for a faster remodelling of collagen fibers, while in the bone matrix the slower synthetic rate permitted resorption. The tipping force possibly produced higher pressures in the apical third than in the middle third of the septum. If this increased pressure decreased blood flow to this area, a decline in synthetic activity, as hypothesized by Baumrind (1970) could have occurred. This could be confirmed by findings in Fig. 32 (RAG). The ratio of middle to apical third counts in control is significantly different from that in experimentals ($p < 0.05$). A decrease in sulphated glycosaminoglycans was evident in experimental animals compared to controls during the first experimental days. The decrease in GAGs in the bone matrix can be related to a decreased collagen synthesis in the matrix relative to the fiber. GAGs are present in bone as proteoglycans. A decrease in protein synthetic rate can be reflected in a decrease in synthetized proteoglycan. Obviously the GAGs decrease in bone matrix as a result of orthodontic forces cannot be related to increased mineralization of the matrix, considering the evidence of decreased mineral content produced by the force (SEM). The GAG synthetic activity was probably constant during the experiment as shown by bone matrix around osteocytic lacunar walls consistently staining AB+. A decrease in synthesis of GAG can, however, have occurred without being perceived in this experiment. An increased degradation of GAGs can also have occurred. The interruption of a normal distal drift pattern as a result of force application can be seen by changes in the labelled protein distribution (Figs. 19, 21, 24) and by changes in the

sulphated GAGs distribution pattern (Figs. 39, 41). The collagen and glycosaminoglycans deposition on the appositional wall appears to be interrupted. The radioactive label is distributed more evenly through the septum. There is no significant difference in the intraosseous fiber or matrix turnover between control and experimental (Tables 4, 5, 6). Collagen is being synthesized within both fiber and matrix as shown in Figures 27 and 28. The band of glycosaminoglycans on the appositional wall is interrupted as well by the orthodontic force. Fragmented bands on mesial and distal walls, as seen in Figure 45, might represent adhesive attachment of periodontal ligament fibers as described by Kurihara and Enlow (1980b). The intraosseous fibers were AB- during the experimental period, suggesting a high content of mineralized collagen (as seen in SEM) and no significant change in its mineral content. The bone matrix was AB+, indicating a higher GAG content. This observation might explain why the matrix is resorbed more rapidly by pressure forces, as seen in SEM.

CHAPTER V

SUMMARY AND CONCLUSIONS

The changes in intraosseous fibers induced by orthodontic tooth movement were studied. The changes in fiber mineralization in protein turnover and in glycosaminoglycan distribution were analyzed.

The changes in mineralization patterns were studied in anorganic SEM specimens. In comparison to controls, experimental intraosseous fibers were hypermineralized. The surrounding bone matrix became increasingly unmineralized during the experiment. The sheath, surrounding the intraosseous fibers in the control specimens, lost minerals at a slower rate than the rest of the bone matrix. The changes in collagen remodelling induced by the orthodontic force were assessed by radioautography by a single injection of [^{3}H]-proline followed by killing the animals at different time intervals. The results suggest a temporary interruption in the physiologic distal drift of the molars. There was a significant decrease in protein synthesis in the apical third of the septum, creating a significant difference in the middle-to-apical third counts ratio between controls and experimentals. This reflects the higher pressure on the apical third resulting from the force applied. There was no significant difference in the total protein synthesis rate between control and experimental specimens. In a mesio-distal direction, the highest synthetic rate was consistently on the distal wall of the septum, followed by the mesial wall. The lowest counts were always in the middle third, suggesting a slower collagen turnover of intraosseous fiber and matrix in this area of the septum. There were no significant differences in counts between controls and experimentals.

The glycosaminoglycan content of control and experimental specimens was assessed in demineralized sagittal sections stained with Alcian Blue stain. The intraosseous fibers were Alcian Blue negative (AB-) in both control and experimental animals, reflecting their low glycosaminoglycan content. The bone matrix stained AB+ indicating higher GAGs content than the fiber. The experimental bone matrix decreased its staining properties in the apical third during first experimental days, suggesting decreased GAGs synthesis or increased depletion rate. Control and experimental tissues demonstrated decreased GAG levels as a function of time. This situation was related to the initial weight loss of the animals which probably reflects altered diet or metabolism. The findings of the experiment suggest that orthodontic pressure forces induce an increase in mineral uptake of intraosseous fibers. The intraosseous fiber synthesized collagen at a higher rate than the bone matrix. This pattern is not affected by force application. The GAG content of the fiber was low and remained so when orthodontic forces are applied. The fiber seems to be affected by orthodontic force in a different manner than the surrounding bone matrix, which showed decreased mineralization, maintainance of a lower collagen synthetic rate and a decrease in GAG content. Two possible explanations can be drawn for these observations; as a result of pressure forces, remodelling of intraosseous fiber could occur mainly close to the alveolar walls, and deeply embedded fibers would not be significantly influenced by the force. Another possibility is that there is deformation of deeply embedded fibers creating tensions which are released slowly

by the slow remodelling of the intraosseous fiber near the midline of the septum. This second alternative is analogous to observations of the slow remodelling of transseptal fibers after tooth movement, and their relation to relapse (Thompson, 1958, 1959; Reitan, 1959, 1960; Edwards, 1968). The intraosseous fibers appear to serve as a framework for remodelling for the interdental septum. The surrounding bone matrix appears to be affected more by the force, showing signs of extensive remodelling around very stable intraosseous fibers.

Future Research Recommendations

The following projects would further clarify the intraosseous fiber role in orthodontic tooth movement:

(1) Development of a tooth moving device which allows for the study of intraosseous fibers under tension, preferably rotational movements.

(2) Study of the intraosseous fiber changes with forces applied to a more extended period of time, delivering lighter forces, and during relapse to original position.

(3) Study of the organic component, if any, filling the spaces left by receding bone matrix after force application. The cellular components located in this unmineralized spaces might help explain the events observed in this study.

(4) Comparison of the synthetic rate of glycosaminoglycans in interdental septum, between orthodontically moved teeth and controls.

REFERENCES

REFERENCES

Anderson, A.A.: The protein matrixes of the teeth and periodontium in hamsters: A triated proline study. J. Dent. Res. 46:67-78, 1967.

Battelheim, F.A. and Brady, E.: Hydration and proteoglycan content of rat skin. IN "Glycoconjugates", R. Schamer (Ed.), Stuttgard, George Thieme, pp. 662-664, 1979.

Baumhammers, A. and Stallard, R.E.: S^{35}-sulfate utilization and turnover by the connective tissues of the periodontium. J. Periodont. Res. 3:187-193, 1968.

Baumrind, S. and Buck, D.: Rate changes in cell replication and protein synthesis in the periodontal ligament incident to tooth movement. Am. J. Orthod. 57:109-131, 1970.

Baylink, D., Wergedal, J., Starffner, R. and Rich, C.: Fluoride in Medicine. T.L. Vischer (Ed.), Hans Huber, Bern, pp. 37-69, 1970.

Baylink, D., Wergedal, J. and Thompson, E.: Loss of protein polysaccharides at sites where bone mineralization is initiated. J. Histochem. Cytochem. 20:279-292, 1972.

Beersten, W. and Tonino, G.J.M.: Effects of fixation and demineralization on the intensity of radioautographic labelling over the periodontal ligament of the mouse incisor after adminis-

tration of [^{3}H]-proline. Archs Oral Biol. 20:189-193, 1975.

Bernick, S.: The organization of the periodontal membrane fibers of the developing molars of rats. Arch Oral Biol. 2:57-63, 1960.

Bernick, S.D., Grant, D., Levy, B.M. and Dreizen, S.: The formation and organization of so-called transosseous fibers. J. Dent. Res. 53:97 (Abs. 171), 1974.

Bernick, S., Levy, B.M., Dreizen, S. and Grant, D.A.: The intraosseous orientation of the alveolar component of marmoset alveolodental fibers. J. Dent. Res. 56:1409-1416, 1977.

Borcherding, M.S., Blacïk, L.J., Sittig, R.A., Bizzell, J.W., Breen, M. and Weinstein, H.G.: Proteoglycans and collagen fiber organization in human corneoscleral tissue. Exp. Eye Res. 21:59-70, 1975.

Bowes, J.H. and Kenten, R.H.: The amino-acid composition and titration curve of collagen. Biochem. J. 43:358-365, 1948.

Boyde, A.: Scanning electron microscope studies of bone. IN "The Biochemistry and Physiology of Bone", Vol. 1, 2nd edition, G.H. Bourne (Ed.), Academic Press, New York, pp. 259-309, 1972.

Boyde, A. and Jones, S.J.: Scanning electron microscopy of cementum and Sharpey fiber bone. Zeitschr. Zellforsch. 92:536-548, 1968.

Campo, R.D.: Protein-polysaccharides of cartilage and bone in health and disease. Clin. Orthop. 68:182-209, 1970.

Carneiro, P.: Synthesis and turnover of collagen in periodontal tissues. IN "The Use of Radioautography in Investigating Protein Synthesis", Symposia of the International Society for Cell Biology, Vol. 4, C.P. Leblond and K.B. Warren (Eds.), Academic Press, New York and London, pp. 247-257, 1965.

Carneiro, J. and Fava de Moraes, F.: Radioautographic visualization of collagen metabolism in the periodontal tissues of the mouse. Archs Oral Biol. 10:833-848, 1965.

Carneiro, J. and Leblond, C.P.: Role of osteoblasts and odontoblasts in secreting the collagen of bone and dentin, as shown by radioautography in mice given tritium-labelled glycine. Exp. Cell Res. 18:291-300, 1959.

Cohn, S.A.: Development of the molar teeth in the albino mouse. Am. J. Anat. 101:295-320, 1957.

Cohn, S.A.: A new look at the orientation of cementoalveolar fibers of the mouse periodontium. Anat. Rec. 166:292, 1970.

Cohn, S.A.: A re-examination of Sharpey's fibers in alveolar bone of the mouse. Archs Oral Biol. 17:255-260, 1972a.

Cohn, S.A.: A re-examination of Sharpey's fibers in alveolar bone of the marmoset. Archs Oral Biol. 17:261-269, 1972b.

Cohn, S.A.: Transalveolar fibers in the human dentition. IADR

Abstracts, J. Dent. Res. 53:97 (Abs. 171), 1974.

Cohn, S.A.: Transalveolar fibers in the human periodontium. Archs Oral Biol. 20:257-259, 1975.

Comper, W.D. and Laurent, T.C.: Physiological function of connective tissue polysaccharides. Physiol. Rev. 58:255-315, 1978.

Crumley, P.J.: Collagen formation in the normal and stressed periodontium. Periodontics 2:53-61, 1964.

Deporter, D.A. and Ten Cate, A.R.: Collagen resorption by periodontal ligament fibroblasts at the hard tissue-ligament interfaces of the mouse periodontium. J. Periodontol. 51:429-432, 1980.

Diaz, E.A.: Periodontal ligament collagen response to tooth movement: histochemical and radioautographic reactions. Am. J. Orthod. 73:443-458, 1978.

Duncan, G.W., Yen, E.H.K., Pritchard, E.T. and Suga, D.: Collagen and prostaglandin synthesis in force-stressed periodontal ligament in vitro. J. Dent. Res. 63:665-669, 1984.

Eccles, J.D.: The development of the periodontal membrane in the rat incisor. Arch Oral Biol. 9:127-133, 1964.

Edwards, J.: A study of the periodontium during orthodontic rotation of teeth. Am. J. Orthod. 54:441-460, 1968.

Frasca, P.: SEM studies of human fetal bone. IN "The Chemistry and Biology of Mineralized Connective Tissues", A. Veis (Ed.), Elsevier/North Holland Inc., New York, pp. 543-546, 1981.

French, J.E. and Benditt, E.P.: The histochemistry of connective tissue: II. The effect of proteins on the selective staining of mucopolysaccharides by basic dyes. J. Histochem. Cytochem. 1:321-325, 1953.

Gaengler, P. and Knut, M.: Effects of force application on periodontal blood circulation - a vital microscopic study in rats. J. Periodont. Res. 18:86-92, 1983.

Garant, P.R. and Cho, M.-I.: Autographic evidence of the coordination of the genesis of Sharpey's fibers with new bone formation in the periodontium of the mouse. J. Periodont. Res. 14:107-114, 1979.

Glickman, I.: Clinical Periodontology. 3rd ed., pp. 46-53, W.B. Saunders Co., Philadelphia, 1962.

Glimcher, M.J.: Specificity of the molecular structure of organic matrices in mineralization. IN "Calcification in Biological Systems". American Association for Advancement in Science, Washington, D.C., p. 421-487, 1960.

Grant, D., Bernick, S., Levy, B., Dreizen, S.: A comparative study of periodontal ligament development in teeth with and without predecessors in marmosets. J. Periodontol.

43:162-169, 1972.

Hadj-Salem, H.M.: Method for continuous controlled application of separating force to rat molar teeth. Angle Orthod. 41:76-79, 1971.

Hay, E.D.: Fine structure of embryonic matrices and their relation to the cell surface in ruthenium red-fixed tissues. Growth 42:399-423, 1978.

Heersche, J.N.M., Tam, C.S. and Jones, G.: Measurement of the apposition rate of mineralized bone matrix and osteoid in normal, D-restricted and D-repeleted rats using simultaneous labelling with [^{3}H]-proline and tetracycline. IN "The Chemistry and Biology of Mineralized Connective Tissues", A. Veis (Ed.), Elsevier/North Holland Inc., New York, pp. 313-315, 1981.

Howell, D.S.: Concepts of calcification. Arthritis Rheum. 6:736-742, 1963.

Jackson, S.H. and Heininger, J.A.: A study of collagen reutilization using $^{18}O_2$ labelling technique. Clin. Chim. Acta 51:163-171, 1974.

Johnson, R.B.: Development of transalveolar fibers in the mouse periodontium. PhD. Dissertation, University of North Dakota, Grand Forks, 1981.

Johnson, R.B.: A new look at the mineralized and unmineralized

components of intraosseous fibers of the interdental bone of the mouse. Anat. Rec. 206:1-9, 1983.

Johnson, R.B. and Highison, G.J.: A new technique for exposure of osteocytes and transalveolar fibers of the interdental septum in the mouse for scanning electron microscopy. Archs Oral Biol. 28:273-277, 1983.

Johnson, R.B. and Low, F.N.: Development of transalveolar fibers in alveolar bone of the mouse. Archs Oral Biol. 26:971-976, 1981.

Johnson, R.B. and Low, F.N.: Development of intraosseous fibers of the mouse periodontium. A high-voltage electron microscopic study. Am. J. Anat. 163:327-335, 1982.

Johnson, R.B. and Low, F.N.: The relationship of proteoglycans to developing transalveolar fibers in the alveolar bone of the mouse. Anat. Anz. 153:107-118, 1983.

Jones, S.J. and Boyde, A.: The organization and gross mineralization patterns of the collagen fibers in Sharpey fiber bone. Cell Tiss. Res. 148:83-96, 1974.

Kardos, T. and Simpson, L.: A new periodontal membrane biology based upon thixotropic concepts. Am. J. Orthod. 77:508-515, 1980.

Karnovsky, M.J.: A formaldehyde-glutaraldehyde fixate of high osmolality for use in electron microscopy. J. Cell. Biol.

27:137A-138A, 1965.

Kraw, A. and Enlow, D.: Continuous attachment of the periodontal membrane. Am. J. Anat. 120:133-148, 1967.

Kurihara, S. and Enlow, D.: An electron microscopic study of attachments between periodontal fibers and bone during alveolar remodelling. Am. J. Orthod. 77:517-531, 1980a.

Kurihara, S. and Enlow, D.: A histochemical and electron microscopic study of an adhesive type of collagen attachment on resorptive surfaces of alveolar bone. Am. J. Orthod. 77:532-546, 1980b.

Lapiere, C.M.: Mechanism of collagen fiber remodelling. IN "The Mechanisms of Tooth Support", A Symposium, John Wright and Sons Ltd., Bristol, pp. 20-24, 1967.

Lilja, E., Lindskog, S. and Hammarstrom, L.: Histochemistry of enzymes associated with tissue degradation incident to orthodontic tooth movement. Am. J. Orthod. 83:62-75, 1983.

Loe, H.: Bone tissue formation. A morphological and histochemical study. Acta Odontol. Scand. 17:311-407, 1959.

Logan, M.A.: Composition of cartilage, bone, dentin and enamel. J. Biol. Chem. 110:375-389, 1935.

Lorber, M.: A study of the histochemical reactions of the dental cementum and alveolar bone. Anat. Rec., 3:129-144, 1951.

Luna, L.G.: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edition, L.G. Luna (Ed.), McGraw-Hill Book Co., New York, 1968.

Mathews, M.B.: The interaction of collagen and acid mucopolysaccharides. Biochem. J. 96:710-716, 1965.

McCulloch, C.A.G. and Melcher, A.H.: Continuous labelling of the periodontal ligament of mice. J. Periodont. Res. 18:231-241, 1983a.

McCulloch, C.A.G. and Melcher, A.H.: Cell migration in the periodontal ligament of mice. J. Periodont. Res. 18:339-352, 1983b.

Melcher, A.H. and Bowen, W.H.: Biology of the Periodontium. Academic Press, London, 1969.

Merriam, R.W.: Standard chemical fixation as a basis for quantitative investigations of substances other than deoxyribonucleic acid. J. Histochem. Cytochem. 6:43-51, 1958.

Minkoff, R. and Engstrom, T.G.: A long-term comparison of protein turnover in subcrestal vs supracrestal fibre tracts in the mouse periodontium. Archs Oral Biol. 24:817-824, 1979.

Minkoff, R., Stevens, C.J. and Karon, J.M.: Autoradiography of protein turnover in subcrestal versus supracrestal fibre tracts of the developing mouse periodontium. Archs Oral Biol. 26:1069-1073, 1981.

Orlowski, W.D.: The incorporation of [H^3]-proline into the collagen of the periodontium of a rat. J. Periodont. Res. 11:96-100, 1976.

Picton, D.C.A.: On the part played by the socket in tooth support. Archs Oral Biol. 10:945-955, 1965.

Picton, D.C.A. and Davies, W.I.R.: Dimensional changes in the periodontal membrane of monkeys (macaca irus) due to horizontal thrust applied to the teeth. Archs Oral Biol. 12:1635-1643, 1967.

Quigley, M.B.: Perforating (Sharpey's) fibers of the periodontal ligament and bone. Ala. J. Med. Sci. 7:336-342, 1970.

Reitan, K.: Continuous bodily tooth movement and its histological significance. Acta Odont. Scand. 8:115-143, 1950.

Reitan, K.: Tissue rearrangement during retention of orthodontically rotated teeth. Angle Orthod. 29:105-113, 1959.

Reitan, K.: Tissue behaviour during orthodontic tooth movement. Am. J. Orthod. 46:881-899, 1960.

Reitan, K.: Effects of force magnitude and direction of tooth movement on different alveolar bone types. Angle Orthod. 34:244-255, 1964.

Rippin, J.W.: Collagen turnover in the periodontal ligament under normal and altered functional forces. J. Periodont. Res. 11:101-107, 1976.

Roberts, W.E. and Chamberlain, J.G.: Scanning electron microscopy of the cellular elements of rat periodontal ligament. Archs Oral Biol. 23:587-589, 1978.

Roberts, W.E. and Chase, D.C.: Kinetics of cell proliferation and migration associated with orthodontically-induced osteogenesis. J. Dent. Res. 60:174-181, 1981.

Rogers, A.W.: Techniques in radioautography. 2nd ed., Elsevier Scientific Publishing Co., Amsterdam, pp. 226-309, 1979.

Ross, R. and Benditt, E.P.: Wound healing and collagen formation. III. A quantitative radioautographic study of the utilization of proline-[H^3] in wounds from normal and scorbutic guinea pigs. J. Cell Biol. 15:99-108, 1962.

Rossman, L.E., Rosenbloom, J. and Robinson, P.: Biosynthesis of collagen in isolated periodontal ligament. J. Dent. Res. 54:1115-1119, 1975.

Rygh, P.: Ultrastructural vascular changes in pressure zones of rat molar periodontium incident to tooth movement. Scand. J. Dent. Res. 80:307-321, 1972.

Rygh, P.: Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement. Acta Odontol. Scand. 31:109-122, 1973.

Rygh, P., Moxham, B.J. and Berkovitz, B.K.B.: The effects of external forces on the periodontal ligament - the response to

horizontal loads. IN "The Periodontal Ligament in Health and Disease", B.K.B. Berkovitz, B.J. Moxham and H.N. Newman, Pergamon Press, Oxford, 1982.

Sauk, J.J. Jr., Brown, D.M., Corbin, K.W. and Witkop, C.J.: Glycoaminoglycans of predentin, peritubular dentin and dentin. A biochemical and electron microscopic study. Oral Surg. 41:623-630, 1976.

Schneider, V.G. and Schneider, G.: Qualitative und Quantitative Untersuchungen uber Stoffverluste bei Formol - und Carnoy-fixierung von Menschlichen Hirngewebe. Acta Histochem. 28:227-242, 1967.

Schubert, M. and Pras, M.: Ground substance protein polysaccharides and the precipitation of calcium phosphate. Clin. Orthop. 60:235-255, 1968.

Scott, J.E., Dorling, J. and Quintarreli, G.: Differential staining of acid glycosaminoglycans by Acian Blue in salt solutions. Biochem. J. 90:4-5, 1964.

Selvig, K.: The fine structure of human Sharpey's fibers cement. Acta Odontol. Scand. 23:423-441, 1965.

Shackleford, J.M.: The indifferent fiber plexus and its relationship to principal fibers of the periodontium. Am. J. Anat. 131:427-442, 1971a.

Shackleford, J.M.: Scanning electron microscopy of the dog perio-

dontium. J. Periodont. Res. 6:45-54, 1971b.

Shackleford, J.M.: Ultrastructural and microradiographic characteristics of Sharpey's fibers in dog alveolar bone. Ala. J. Med. Sci. 10:11-20,

Silbert, J.E.: Ground substance. IN "Textbook of Oral Biology", J.H. Shaw (Ed.), p. 453-469, W.B. Saunders, Philadelphia, 1978.

Skougaard, M.R., Frandsen, A. and Baker, D.G.: Collagen metabolism of skin and periodontal membrane in the squirrel monkey. Scand. J. Dent. Res. 78:374-377, 1970.

Smith, J.W.: The disposition of protein polysaccharide in the epiphyseal plate cartilage of the young rabbit. J. Cell. Sci. 6:843-864, 1970.

Sodek, J.: A new approach to assessing collagen turnover by using a micro-assay. Biochem. J. 160:243-246, 1976.

Sodek, J.: A comparison of rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a micro assay. Arch. Oral Biol. 22:655-665, 1977.

Stallard, R.E.: Utilization of [H^3]-proline by the connective tissue elements of the periodontium. Periodontics 1:185-188, 1963.

Stutzmann, J. and Petrovic, A.: Human alveolar bone turnover

rate: a quantitative study of spontaneous and therapeutically-induced variations. IN "Malocclusion and the Periodontium", J. McNamara and K. Ribbens (Eds.), Monograph No. 15, Craniofacial Growth Series Center for Human Growth and Development, University of Michigan, Ann Arbor, 1983.

Svejda, J. and Skach, M.: The periodontium of the human tooth in the scanning electron microscope (stereoscan). J. Periodontol. 44:478-484, 1973.

Thompson, H.E.: Preliminary macroscopic observations concerning the potentialities of supra-alveolar collagenous fibers in orthodontics. Am. J. Orthod. 40:485-497, 1958.

Thompson, H.E.: Orthodontic relapses analyzed in a study of connective tissue fibers. Am. J. Orthod. 45:93-109, 1959.

Tonna, E.A.: Application of the [H^3]-proline topographic method to dental tissues. J. Periodont. Res. 10:357-368, 1975.

Tonna, E.A. and Cronkite, E.P.: Histochemical and radioautographic studies on the effects of aging on the mucopolysaccharides of the periosteum. Biophys. Biochem. Cytol. 6:171-183, 1959.

Trott, J.R.: The development of the periodontal attachment in the rat. Acta Anat. 51:313-328, 1962.

Warshawsky, H. and Moore, G.: A technique for the fixation and decalcification of rat incisors for electron microscopy. J.

Histochem. Cytochem. 15:542-549, 1967.

Wink, C.S.: The effects of castration on size and appearance of Sharpey's fibers of rat femurs: A scanning electron microscopic and microradiographic study. Anat. Rec. 202:9-13, 1982.

Zengo, A.N., Bassett, C.A.L., Pawluk, R.J. and Gjelsvik, A.: Hemodynamic deformation of alveolar socket wall. J. Dent. Res. 53:28-32, 1974.