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SYNTHESIS OF SHARPEY'S FIBER PROTEINS WITHIN RODENT ALVEOLAR BONE

R.B. Johnson[1]* and R.H. Martinez[2]

[1]Dept. of Diagnostic Sciences, Univ. of Mississippi School of Dentistry, 2500 North State Street, Jackson, Mississippi 39216-4505; [2]Departmento de Ortodoncia, Escuela Colombiana de Medicina, Bogotá, Colombia

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Abstract

Physiologic drift of teeth requires remodeling alveolar bone at the tooth socket wall. Remodeling must be regulated so that collagenous attachments are maintained as new tooth positions are established. Thus, alveolar remodeling must occur coincident to remodeling of the Sharpey's fibers which are embedded within that bone. Studies suggest that these fibers are severed at resorptive sites on the alveolar wall and continuity with periodontal ligament fibers is reestablished by splicing, *de novo* synthesis, or adhesion to the base of the Howship's lacuna at the alveolar wall; however, little information is available concerning the mechanisms of these events.

This article reviews types of periodontal Sharpey's fibers and quantifies and compares protein deposition into these fibers with that of adjacent bone. Effects of orthodontic tooth movement on the protein incorporation into the fibers were also studied. Coincident to orthodontic tooth movement, sites of bone matrix deposition (without previous resorption) and resorption/reversal were evident. At sites of deposition, periodontal ligament fibers were passively entrapped as Sharpey's fibers by the new bone matrix. Incorporation of ^{3}H-proline-labeled proteins into bone matrix adjacent to Sharpey's fibers was significantly increased; however, radiolabeled protein incorporation into the Sharpey's fiber was not affected. At resorption/reversal sites, Sharpey's fibers had been severed and periodontal ligament fiber attachments to the alveolar wall were becoming reestablished. Incorporation of radiolabeled proteins into both Sharpey's fibers and adjacent matrix was significantly increased at these sites. Thus, movement of adjacent teeth affect the relative metabolism of these fibers, assuring adequate support of the tooth during its relocation.

Key Words: Sharpey's fibers, periodontal ligament, proteins, alveolar bone.

*Address for correspondence:
Roger B. Johnson, address as above.
Telephone number: (601) 984-6010
FAX number: (601) 984-6014
E-mail: drrogerb@fiona.umsmed.edu

Introduction: Review of the Literature

Sharpey's fibers attach tendons and ligaments to bone. They consist of a bundle of collagen fibers and are usually partially mineralized (Selvig, 1965; Boyde and Jones, 1968; Shackleford, 1973; Jones and Boyde, 1974; Johnson, 1983). The fibers are surrounded by a mineralized, collagenous sheath (Johnson, 1983).

For many years, it was presumed that Sharpey's fibers were relatively inert. However, recent studies suggest that they readily adapt to stress/strain forces coincident to functional movements of adjacent teeth. The site of adaptation is at the periosteal surface of bone, where the collagenous fibers of the tendon or ligament enter the osteoid, becoming a Sharpey's fiber.

A convenient place to study the effects of functional forces on the metabolism of Sharpey's fibers is within the periodontal ligament of the rodent molar teeth. This ligament supports the tooth root and is composed of a large number of unmineralized collagen fiber bundles, which are embedded in root cementum and alveolar bone as Sharpey's fibers. In addition, the turnover rate of matrix components of alveolar bone is approximately 10 times more rapid in alveolar bone than at other sites (Vignery and Baron, 1980); thus, changes in the rate of metabolism of the Sharpey's fiber and its surrounding bone matrix are more readily evident in alveolar bone than at other skeletal sites.

Rodent molar teeth drift in a distal direction, requiring continuous remodeling of the periodontal ligament and alveolar bone to assure adequate tooth support. Bone deposition occurs on the distal surface of the interdental septum of alveolar bone, which maintains a positive bone balance (Stallard, 1963; Crumley, 1964; Baumhammers *et al*., 1965; Carneiro and Fava de Moraes, 1965; Anderson, 1967; Kraw and Enlow, 1967; Kenney and Ramfjord, 1969; Vignery and Baron, 1980; Dreyer and Sampson, 1984). The mesial, or remodeling, side of the interdental septum has focal regions of bone resorption and reversal, and has a negative bone balance (Baron, 1973; Vignery and Baron, 1980). However, there is always some synthetic activity evident on these surfaces (Johnson, 1987). For tooth movement to occur, remodeling of Sharpey's fibers at the bone interface is necessary (Garant, 1976; Beertsen *et al*., 1978; Deporter

and Ten Cate, 1980; Rygh, 1982). To effect remodeling, unmineralized periodontal ligament fibers are detached from the bone-embedded Sharpey's fiber (Deporter and Ten Cate, 1980) and reattached in several ways. They are classified with respect to their position relative to old bone (bone present before resorption), the reversal line, and new bone (bone deposited subsequent to reversal) (Johnson, 1987).

Resorptive surfaces

On resorptive surfaces, bone removal at the alveolar wall severs the attachment of periodontal ligament and Sharpey's fiber (Johnson, 1987). In many instances, reattachment never occurs and severed Sharpey's fibers remain evident within old bone, deep to the reversal lines.

There are three mechanisms for remodeling of periodontal ligament attachments to bone on the resorptive surface: (1) *de novo* synthesis of Sharpey's fibers and new bone, (2) formation of adhesive attachments, and (3) splicing of periodontal fibers to existing Sharpey's fibers.

Adhesive attachments Kurihara and Enlow (1980a,b) and Johnson (1986) proposed "adhesive" attachments, a fibrous meshwork composed of proteoglycans and randomly arranged unit collagen fibrils which reattach periodontal ligament fibers onto resorbed surfaces of alveolar bone. At these sites, the deep portions of the Sharpey's fibers appear to attach to the reversal line, and the fiber then extends into the adjacent periodontal ligament. This is the least common method for periodontal remodeling (Johnson, 1987). The bone matrix collagen subjacent to the adhesive attachment is often very dense.

***De novo* synthesis** Sharpey's fibers are often formed coincident to deposition of bone matrix onto a reversal surface. These fibers are embedded within new bone, superficial to the reversal line. There is no evidence that they are attached to the reversal line, as are adhesive attachments (Johnson, 1987).

Continuous attachments These are presumed to be splices between collagen of the periodontal ligament fibers and the Sharpey's fiber bundles and are the most common method for periodontal remodeling (Johnson, 1987). There is morphological evidence suggesting that when periodontal ligament and Sharpey fiber continuity had been severed during the resorption phase, the fibers were then rejoined by an unbanded protein (Johnson, 1987). This protein probably serves as a scaffold for collagen deposition and could be either masked or replaced by mature collagen. These fiber bundles cross the reversal line (Johnson, 1987).

Depository surfaces

There is morphological evidence suggesting that periodontal ligament fibers are passively entrapped by the advancing front of bone at depository surfaces (Kraw and Enlow, 1967; Kurihara and Enlow, 1980a,b; Johnson, 1984). Garant and Cho (1979) and Johnson (1986), in light microscopic radioautographic studies, suggest that Sharpey's fibers and alveolar bone may be simultaneously formed on these surfaces.

Effects of normal and altered functional forces on Sharpey's fibers of the periodontal ligament

Metabolism of Sharpey's fibers is affected by the function of adjacent teeth. Functional forces determine the mineralization patterns, diameters, and density (fibers/unit area) of Sharpey's fibers at the alveolar wall (Martinez and Johnson, 1987; Short and Johnson, 1990) and cementum (Akiyoshi and Inoue, 1963), which assures adequate support for teeth coincident to changes in their function. There is no evidence that functional forces determine the type of Sharpey's fiber present at the alveolar wall.

Physiologic forces There is no evidence to indicate that the compression and tensile forces coincident to physiologic tooth movements have significant effects on Sharpey fiber diameters or densities (Short and Johnson, 1990). However, unmineralized cores of Sharpey's fibers are larger at depository than at resorptive/reversal surfaces (Boyde and Jones, 1968; Shackleford, 1973; Johnson, 1983).

Hypofunction and partial function Short-term nonfunction of adjacent teeth results in significant increases in woven bone deposition at the alveolar crest of the rodent interdental septum (Glickman, 1945; Stallard, 1964; Levy and Mailland, 1980; Tran Van and Mailland, 1981; Johnson, 1990). In a recent study (Short and Johnson, 1990), non- and hypofunctional mandibular molar teeth were created by selective extraction of rat maxillary molar teeth (Cohn, 1966), which placed teeth in the contralateral quadrant in hyperfuntion. Sharpey's fibers supporting non- or hypofunctional teeth had larger unmineralized cores than controls. Mean Sharpey fiber diameters were significantly greater and their mean density significantly less in the periodontium supporting non- and in hypo- or hyperfunctional teeth. Establishment of partial function to nonfunctional teeth ameliorated the atrophic effects of nonfunction on mean diameter and density, but had little effect on the mineralization of Sharpey's fibers, suggesting that their mineralization may be controlled by factors other than occlusal forces on adjacent teeth (Short and Johnson, 1990).

Orthodontic forces A recent study has demonstrated changes on the diameter of Sharpey's fibers at the alveolar wall coincident to orthodontic forces. The study presumed that these fibers experienced tension when adjacent teeth were separated by an orthodontic appliance. These Sharpey's fibers had significantly greater diameters than untreated controls (Martinez and Johnson, 1987). However, their morphology was not significantly altered by the forces. It is possible that increased Sharpey's fiber diameters could be a biological attempt to prevent tearing of the fiber bundle and separation of the bundle from alveolar bone.

Although there is morphological evidence to suggest that synthesis of proteins of Sharpey's fibers and adjacent bone matrix is affected by movement of adjacent teeth, there is little functional data supporting these observations. In addition, there is little information concerning the effects of tooth movement on the relative synthesis of bone matrix and Sharpey's fiber proteins. To this end, we have conducted a radioautographic study to assess and compare the effects of normal physiologic and orthodontic tooth movement on the deposition of these matrix components.

Materials and Methods

Radioautography, high-voltage electron microscope

Male Swiss mice, aged 45 days, were weighed and given an intravenous injection of an appropriate dosage of ^{3}H-proline (2 μCi/g; L-[5-^{3}H] proline; specific activity, 40 Ci/mmol, Amersham, Arlington Heights, IL). Animals had been entrained to a cycle of 12 hours light/ 12 hours darkness, which was maintained throughout the experiment. Twenty-four hours after injections, animals were anesthetized with a 1:4 solution of ketamine/ xylazine (0.23 ml/100 g body weight) and perfused through the left ventricle with Ringer lactate solution containing 4.5 mg of "cold" proline/ml to compete with the free ^{3}H-proline remaining in the tissue (Marchi and Leblond, 1983). Perfusion continued with 5% formaldehyde in 0.1 M cacodylate buffer (pH 7.3) followed by Karnovsky's fixative (pH 7.3) (Karnovsky, 1965). The preceding fixation regimen has been suggested by Marchi and Leblond (1983) to reduce binding of free-labeled proline to cells and extracellular substances as a result of reactions between glutaraldehyde containing fixatives, free amino acids, and tissue proteins.

Mandibles were removed by blunt dissection, rinsed in 0.1 M cacodylate buffer (pH 7.3) and demineralized in 4.13% EDTA (ethylenediaminetetraacetic acid; pH 7.0) (Warshawsky and Moore, 1967). Interdental septae were removed and post-fixed in 2% cacodylate-buffered osmium tetroxide, rinsed in 0.1 M cacodylate buffer, dehydrated in ethanols, and embedded in Epon-Araldite.

Radioautography procedures were performed essentially by the techniques of Pylypas *et al*. (1990). Serial sections, 0.5 μm in thickness, were made in coronal and sagittal planes, collected on glass slides coated with 1% celloidin, carbon-coated, and prepared for radioautography. Slides were dipped into diluted Ilford L4 emulsion (1:3; Ilford, U.K.), drained, placed into light-tight boxes and exposed for 17 weeks at 4°C. Sections were developed (at 20°C) in either Microdol-X (to yield fine grains) or diluted D-19 (1:10) (to yield filamentous grains), fixed in 25% sodium thiosulfate (at 20°C), floated off slides, and collected on Formvar coated grids. Grids were stained with 5% uranyl acetate in methanol for two hours (37°C) and with aqueous lead citrate for one hour (20°C), carbon-coated, and viewed in a JEOL-1000B high-voltage electron microscope at an accelerating voltage of 1000 kV (JEOL, Tokyo, Japan).

Radioautography, light microscope

Physiologic tooth movement Male Sprague-Dawley rats, aged 12 weeks, were weighed and given an intraperitoneal injection of ^{3}H-proline (2 μCi/g; L-[5-^{3}H] proline; specific activity, 40 Ci/mmol; Amersham). Four animals were killed 48 hours after injections.

Orthodontic tooth movement Six-week-old female Sprague-Dawley rats were weighed and anesthetized; separating springs were placed between the right maxillary left first and second molar teeth (Hadj-Salem, 1971). Then the rats were given an intraperitoneal injection of ^{3}H-proline (5 μCi/g; L-[2,3-^{3}H] proline; specific activity, 33 Ci/mmol; Amersham). Four animals were killed 1-5 days later. The right maxillary quadrant was untreated and served as an internal control. In addition, eight rats received sham-operations and radioisotope injections, and their right maxillary arch served as an external control.

Radioautographic procedures Maxillae were removed by blunt dissection and immediately fixed in Bouin's fixative to minimize the loss of radioactive label (Beertsen and Tonino, 1975). Specimens were demineralized in 4.13% EDTA at pH 7.0 (Warshawsky and Moore, 1967), dehydrated in dioxane, and embedded in paraffin wax. Serial sections, 6 μm thick, were cut in a sagittal plane through the roots of the teeth, mounted on slides, hydrated, dipped in NTB-2 emulsion (Kodak), and exposed in a refrigerator for 14 days. Slides were developed in D-19 developer (Kodak) and stained by the Van Gieson method (Luna, 1968).

Analysis Grain counts were made using an ocular grid demarcating 100 μm^{2}. Counts were made on every sixth section (36 μm between each area of analysis) over either a Sharpey's fiber bundle or adjacent bone matrix. Counts were also made over the emulsion for detection of background and adjusted mean counts (total count minus background) calculated for each area of each section. Mean counts were calculated for each animal and were compared by factorial analysis of variance and Duncan's New Multiple Range test.

Results

Sites of Sharpey fiber protein synthesis during physiologic drift

The distribution of ^{3}H-proline-labeled protein was limited to periodontal ligament cells and fibers and new bone matrix (matrix synthesized subsequent to injection of the radioisotope). Cells and fibers of old bone (matrix present

before injection of radioisotope) were not labeled (Fig. 1).

Twenty-four hours after injection of ^{3}H-proline, numerous silver grains were evident at the distal surface of the interdental septum, suggesting bone matrix deposition there. Silver grains were located over the osteoid, but not over intrabony portions of principal fibers of the periodontal ligament (Sharpey's fibers). In this situation, periodontal ligament fibers were likely being passively entrapped by the advancing front of new bone (Fig. 2).

The mesial surface of the interdental septum had focal areas of bone resorption and deposition and numerous reversal lines. At sites of reversal, silver grains were over both principal fibers and new bone matrix (Figs. 3 and 4), suggesting that Sharpey's fibers and new bone matrix were being simultaneously formed. At some sites, Sharpey's fibers did not deeply penetrate alveolar bone and did not cross the reversal line. Their terminal ends blended with the bone matrix collagen (Fig. 3). These fibers were likely formed *de novo*.

At sites of matrix resorption prior to reversal and deposition of new bone, Howship's lacunae were being filled by an anastomosing network of unit collagen fibrils, forming a structure resembling an adhesive attachment (Fig. 5). Silver grains were over unit collagen fibrils in these areas. Fibrils appeared to attach directly onto the surface of the alveolar wall (Fig. 5). The collagenous bone matrix there appeared to be slightly denser than subsurface bone matrix (Fig. 5).

In many areas, Sharpey's fibers crossed reversal lines and became continuous with principal fibers of the periodontal ligament. At the alveolar wall, silver grains were over the junction of Sharpey's and principal fibers (Fig. 6). Unit collagen fibrils of the two fiber bundles were joined by fine, unstriated fibrils. Silver grains were over these fibrils (Fig. 7). Splicing of fiber bundles was evident in areas of new bone deposition (Fig. 6), but also occurred in areas where new bone was not being formed (Fig. 8).

Effects of orthodontic tooth movement

Placement of a separating spring between the first and second molar teeth tipped the first molar in a mesial direction, creating a region of tension at the alveolar crest and a region of compression near the root apices (Fig. 9). Thus, numerous areas of alveolar bone deposition and resorption/reversal were evident on both mesial and distal surfaces of the alveolus. Histomorphometric analysis of the area of the interdental septum demonstrated net interdental septal bone loss from 1-5 days following spring placement, probably resulting from tipping of the molar teeth and coincident compression of the interdental septum by the adjacent tooth roots (Fig. 10). In specimens prepared for radioautography, sites of protein deposition were marked by silver grains over both Sharpey's fibers and the adjacent bone matrix. In general, more silver grains were evident over bone at depository surfaces than at resorptive/reversal surfaces. Orthodontic tooth movement produced a significant increase in silver grains over the bone matrix at depository surfaces, but had no effect on numbers of silver grains over the adjacent Sharpey's fibers as compared to maxillary bone matrix of sham-operated external controls (Table 1). The number of grains over bone formed coincident to orthodontic forces was significantly greater than in sham-operated external controls. At resorptive/ reversal surfaces, the numbers of silver grains over both matrix and Sharpey's fibers was significantly increased by orthodontic forces compared to sham-operated external controls (Table 1).

(Figures1-4 on facing page)

Figure 1. Central region of alveolar bone 24 hours after injection of ^{3}H-proline, D-19 developer. There are no silver grains evident over either Sharpey's fibers (SF) or surrounding bone (B); Bar = 1 μm.

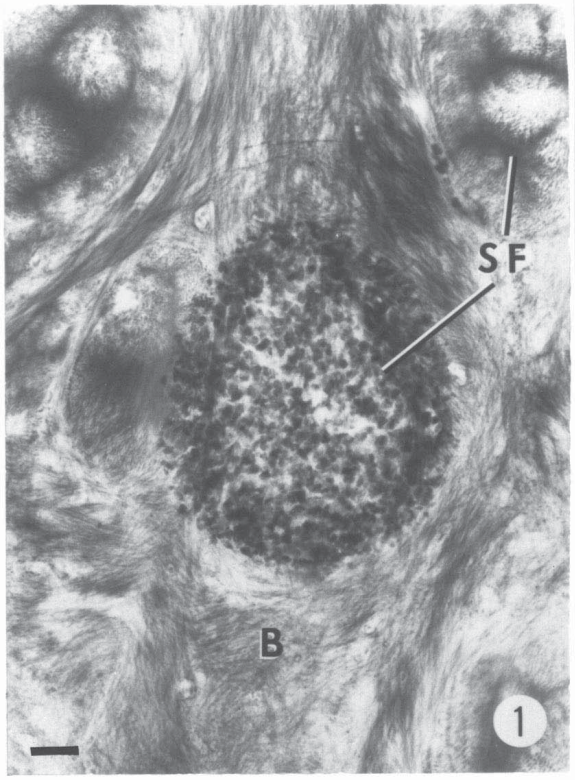
Figure 2. Depository surface of alveolar bone 24 hours after injection of ^{3}H-proline, D-19 developer. In some areas, silver grains are located over both principal fibers (PF) of the periodontal ligament and newly deposited osteoid (NB). There are no silver grains over either bone matrix present prior to injection of the radioisotope (OB) or Sharpey's fibers; Bar = 1 μm.

Figure 3. Reversal surface of alveolar bone 24 hours after injection of ^{3}H-proline, D-19 developer. Silver grains are over recently deposited bone matrix (NB) and principal fibers (PF) of the periodontal ligament. These fibers are an example of Sharpey's fibers formed *de novo*, as they are being synthesized coincident to osteoid and are not becoming linked to a severed Sharpey's fiber within old bone (OB). There are no silver grains over bone matrix prior to the injection of radioisotope (OB); Bar = 1 μm.

Figure 4. Alveolar bone near a reversal surface 24 hours following injection of ^{3}H-proline, Microdol-X developer. Silver grains are over both new bone matrix (NB) and Sharpey's fibers; Bar = 1 μm.

Discussion

The present study describes the pattern of ^{3}H-proline incorporation into bone and Sharpey's fibers at depository and resorptive/reversal surfaces of the interdental septum of the rodent during both physiologic and orthodontic tooth movement. Since the time after isotope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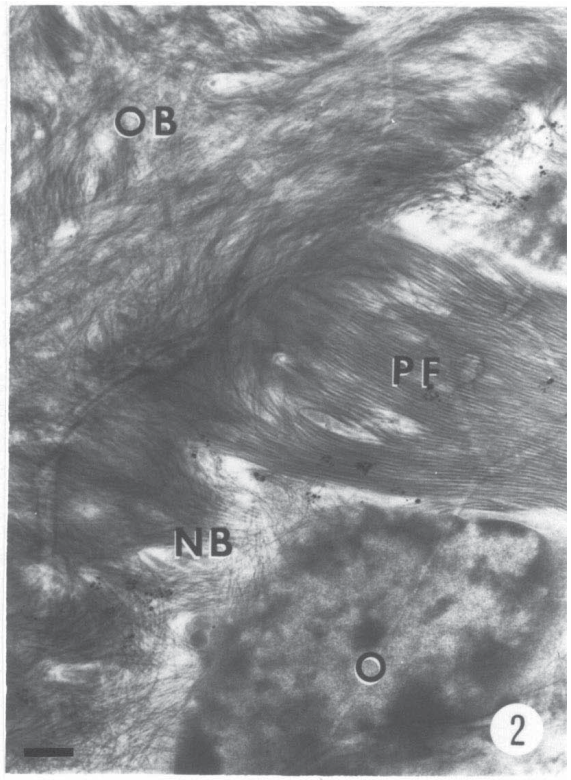
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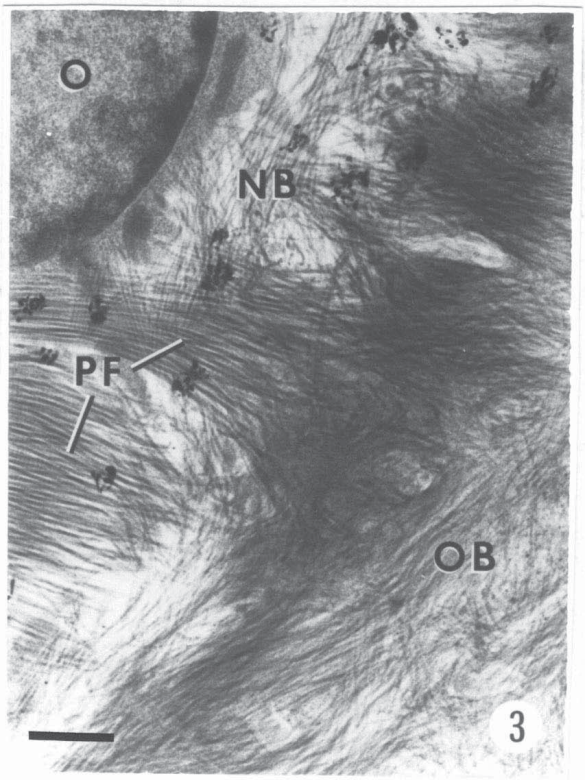
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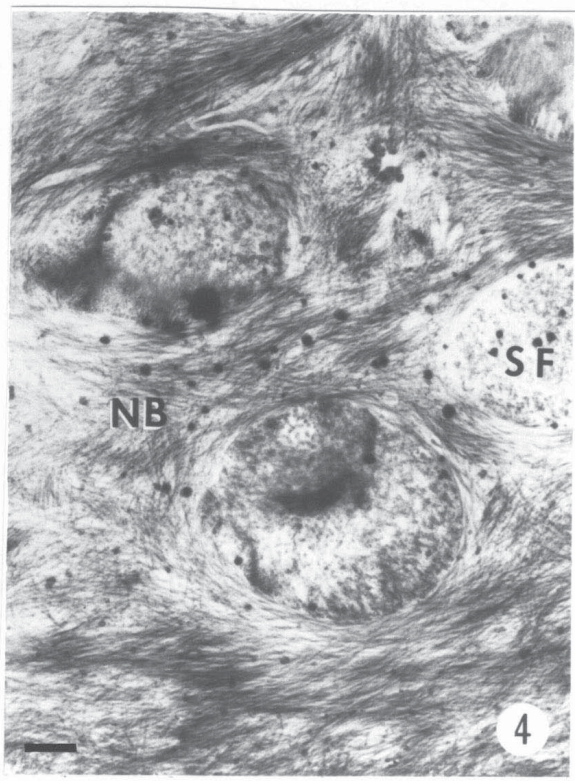
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Table 1. Mean silver grain counts (± standard error of mean) over 100 μm^2 areas of Sharpey's fibers and adjacent alveolar bone matrix from animals experiencing orthodontic tooth movement for 2 days and sham-operated external controls. N = 4 for each group. All counts from reversal/resorption sites were significantly different from the corresponding deposition site, $p < 0.001$.

Site	Tissue	Treatment	Count
Deposition(D)	Bone Matrix(M)	Experi-mental (E)	36.69 ± 3.58*,tt
D	M	Control©	19.00 ± 0.88tt
D	Sharpey's fibers(F)	E	24.78 ± 0.98
D	F	C	24.04 ± 0.93
Resorption /Reversal (R)	M	E	11.23 ± 1.14**
R	M	C	6.52 ± 0.74
R	F	E	12.22 ± 1.79**
R	F	C	7.35 ± 0.92

Significantly different from controls (C):
*$p < 0.001$; **$p < 0.01$
Significantly different from Sharpey's fibers (F):
t$p < 0.001$; tt$p < 0.01$

injection into rodents in our experiments was standard, we can assume that differences in quantity of silver grains within a tissue represented the net rate of incorporation of proline into those proteins. To maintain attachment of teeth to bone on remodeling surfaces of the alveolar wall, periodontal ligament fibers must be severed, and the fibers then reattached to the alveolar wall bone matrix or to severed Sharpey's fibers. To assess this phenomenon, semi-thin tissue sections were studied using high-voltage electron microscopic techniques. These techniques allowed study of relatively thick sections and provided more confident assessment of periodontal/Sharpey fiber continuity. In addition, we could more readily determine whether fibers had been severed or had passed from the plane of section, producing severance artifacts.

Patterns of deposition of ^{3}H-proline-labeled proteins herein suggest three types of periodontal ligament attachments to resorptive bone surfaces: (1) *de novo* synthesis of Sharpey's fibers and new bone, (2) formation of adhesive attachments, and (3) splicing of periodontal fibers to existing Sharpey's fibers, and confirmed and extended observations of Johnson (1987), who proposed these patterns based only

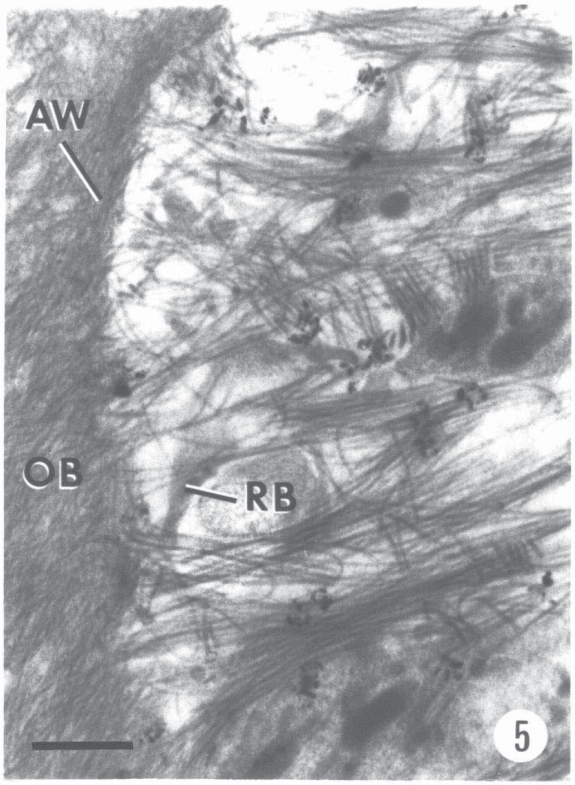
Figure 5. Alveolar bone near a reversal surface 24 hours following injection of ^{3}H-proline, D-19 developer. Within Howship's lacunae, an anastomosing network of unit collagen fibrils adheres to the alveolar wall (AW). Cellular membranes (RB) are evident within the meshwork. The alveolar wall (AW) has denser packing of matrix collagen than the underlying bone (OB). Silver grains are over the anastomosing network of unit collagen fibrils. The alveolar wall and subjacent bone are unlabeled; Bar = 1 μm.

Figure 6. Alveolar bone near a reversal surface 24 hours following injection of ^{3}H-proline, D-19 developer. In some areas, Sharpey's fibers (SF) maintain continuity with principal fibers (PF) of the periodontal ligament by fibril splicing at the bone surface. Silver grains are over recently deposited osteoid (NB) and the junction of principal and Sharpey's fibers (arrow). The remainder of the bone (OB) is unlabeled. O indicates osteoblast; Bar = 1 μm.

Figure 7. Reversal of a resorptive surface of the alveolar wall 24 hours following injection of ^{3}H-proline, D-19 developer. Higher magnification of junction between principal fiber (PF) and Sharpey's fiber (SF) at the bone surface. Silver grains are over non-crossbanded fibrils connecting unit collagen fibrils of the two fiber bundles; at 12,000x. Bar = 1 μm.

Figure 8. Alveolar bone near a depository surface 24 hours after injection of ^{3}H-proline, Microdol-X developer. Silver grains are over Sharpey's fibers (SF). There are no silver grains over the remaining bone (OB); at 8,000x. Bar = 1 μm.

on morphological evidence. The present work represents the only electron microscopic radioautographic study of these mechanisms and the only light microscopic radioautographic study of the effect of orthodontic tooth movement on Sharpey fiber protein metabolism. This data demonstrates (1) the development and maintenance of the various types of Sharpey's fibers, and (2) the effects of the tissue stress/strain microenvironment on patterns and quantities of Sharpey fiber and bone matrix proteins. This study extends other experiments by reporting changes in patterns of protein synthesis in both Sharpey's fibers and their adjacent alveolar bone matrix coincident to movement of adjacent teeth and suggests that attachment of periodontal ligament fibers to bone requires continuous and coordinated synthesis of collagenous fibers of both alveolar bone matrix and Sharpey's fibers. Separation of teeth by orthodontic force altered the stress/strain microenvironment of the supporting periodontium, which adapted to these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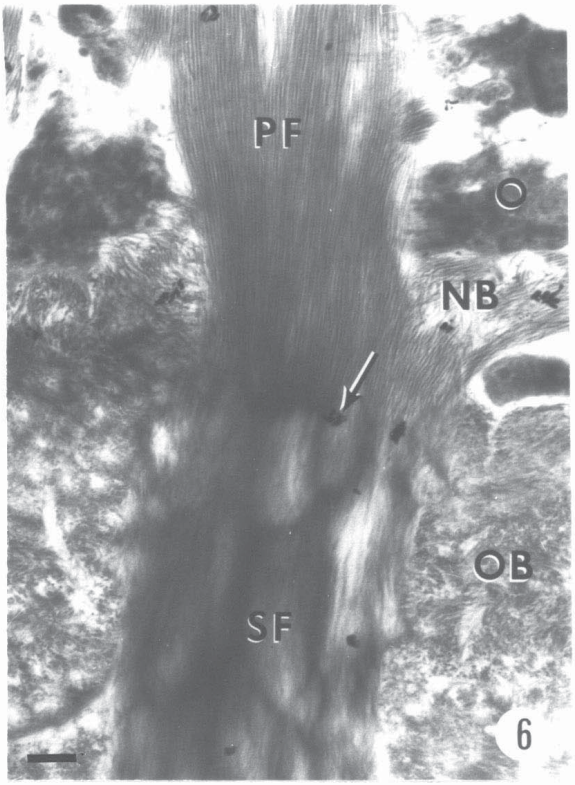
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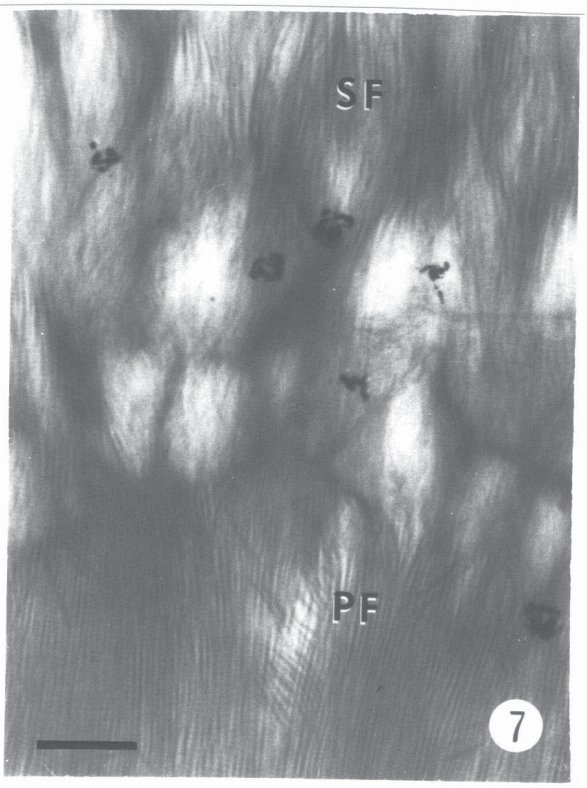
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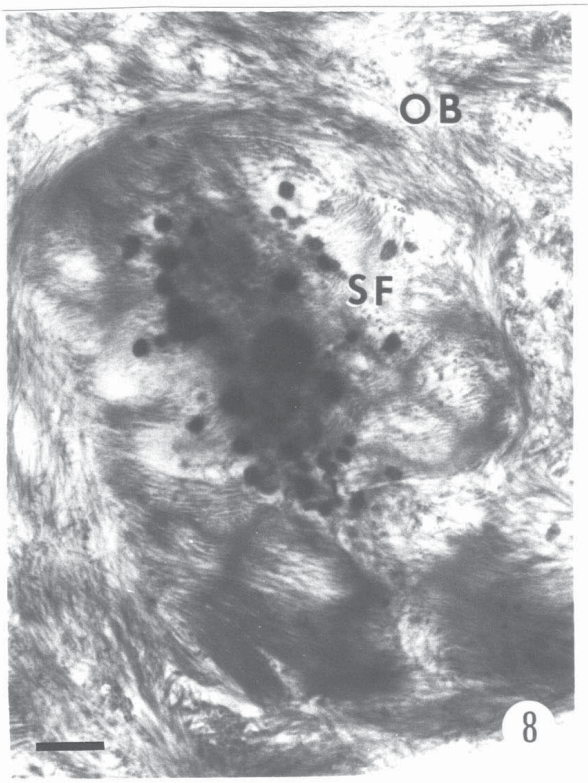
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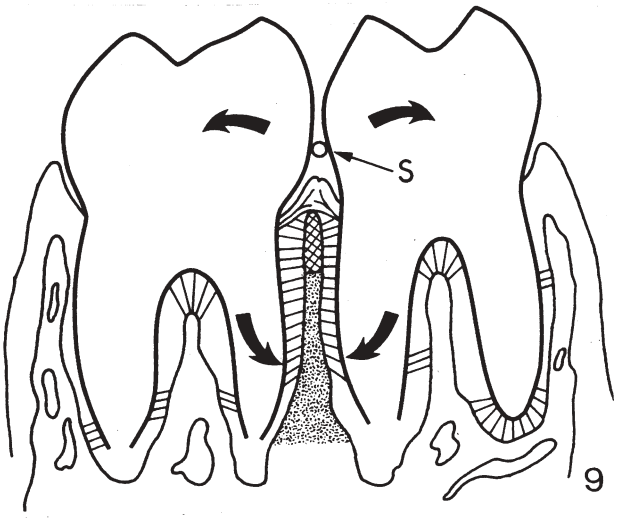

Figure 9. Diagram of spring (S) placement between the first and second molar teeth of a rat. Arrows suggest the movement of the teeth; separation of the crowns creating a tensile force in the cervical third of the periodontal ligament and approximation of the roots creating a compressive force in the apical third of the periodontal ligament (Martinez and Johnson, 1987).

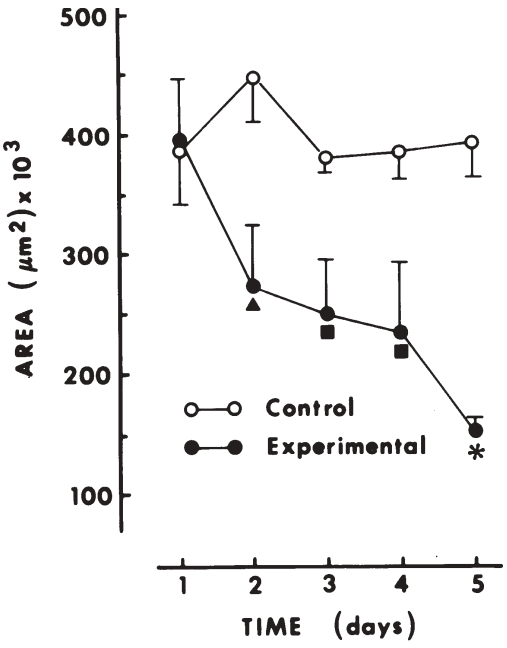

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Figure 10. Effects of tooth separation on the area of the interdental septum of the rat {$\mu m^2 \pm$ standard error of mean} one to five days after placement of a separating spring (Martinez and Johnson, 1987).

changes by alteration of the pattern and rate of synthesis of matrix proteins.

Our study also suggests how separation of adjacent teeth might alter Sharpey fiber metabolism. Placement of an orthodontic spring created areas of tension (cervical third) and compression (middle and apical thirds) of the periodontium. In areas of tension, bone deposition was evident, and there was little evidence of resorption/ reversal. There was no effect on resultant protein incorporation into the Sharpey fiber bundle at those sites, but a nearly doubled protein incorporation into the adjacent matrix was evident. Thus, both the fiber bundle and adjacent matrix likely became more resistant to damage from the tensile force. These results also suggest passive entrapment of pre-existing periodontal ligament principal fibers by the new bone matrix to become a Sharpey fiber, as protein incorporation into the periodontal and Sharpey fiber bundles were similar. At sites experiencing resorption/reversal, incorporation of proteins into bone matrix and Sharpey's fibers subsequent to reversal was nearly doubled as compared to sham-operated external controls; however, the quantity of proteins incorporated into the matrix and Sharpey's fibers was equivalent, suggesting simultaneous deposition of protein into the two tissues (and not periodontal fiber entrapment). Thus, the attachment of periodontal ligament to new bone was likely strengthened to resist damage from adjacent tooth movements.

Several studies have suggested that Sharpey fiber diameters were correlated to functional forces on adjacent teeth and that fibers became larger when these forces were increased and smaller when the forces were decreased (Martinez and Johnson, 1987; Short and Johnson, 1990). Neither study suggested a mechanism for this change in diameter. Our study demonstrated an increased quantity of protein incorporated into alveolar bone matrix and adjacent Sharpey's fibers coincident to orthodontic tooth movement only at areas of resorption/ reversal, suggesting that Sharpey fiber diameter may be more dependent on the extent of adjacent tooth movement than on the type of functional force on the adjacent teeth.

Thus, the movements of adjacent teeth affect both the quantity and ratios of protein incorporation into Sharpey's fibers and adjacent alveolar bone, which is dependent on the characteristics of the stress/strain microenvironment produced by these movements.

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Discussion with Reviewers

A.R. Ten Cate: It would be helpful if the authors could explain why they chose not to examine bone surfaces with high voltage electron microscopy (HVEM) following "orthodontic" tooth movement in addition to preparing conventional paraffin embedded sectioned for counting purposes. While it is likely that changes on these surfaces following orthodontic movement reflect enhancement of physiological adaptations, this assumption cannot be fairly made.

Authors: It was very difficult to quantify the radiography in HVEM due to low density of the marker. Since we could distinguish the type of Sharpey's fibers and the surrounding tissues using a light microscope, we chose this method. Light microscopy also allowed more extensive evaluation of the pattern and quantity of protein deposition into those tissues.

C.A. McCulloch: How can one explain increase of Sharpey's fiber diameter on both compression and tension zones with the same mechanism?

Authors: We do not believe that tensile or compressive forces alone determine the diameter of the Sharpey's fiber. The magnitude of the force seems to be more important. Our present data, in addition to that in a study of the hypofunctional periodontium by Short and Johnson (1990), suggests that the magnitude of the load exerted by teeth on the periodontium is the most important determinant of the diameter of the periodontal ligament and Sharpey's fibers. Whether the tooth movement enhances tensile forces (as in reactivated eruption) or compressive forces (as in orthodontic tooth movement) seems to have little effect on the resultant fiber diameters. Thus, in our model of orthodontic tooth movement, the magnitude of the forces to the periodontium produced by tipping the adjacent teeth would be equivalent in areas of tension and compression, producing increases in Sharpey's fiber diameter at both tension and compression zones.

S.B. Jones: Which cells do you think are contributing (precursors of) collagen to the intrinsic fibers and the Sharpey's (extrinsic) fibers? Are they always the same, or are they variable?

Authors: Since ^{3}H-proline is incorporated into collagen produced by both fibroblasts and osteoblasts, the source of the collagen to the intrinsic and Sharpey's fibers could not be determined. Another biomarker would be required to study this process.

S.B. Jones: At a reversal line, have you seen any evidence in your electron microscopy studies that demineralized collagen of Sharpey's fibers, as well as collagen that has never mineralized, is available for splicing? Or does any demineralized collagen self-destruct or be removed before new collagen assembles?

Authors: We reported indirect evidence of unmineralized cores in Sharpey's fibers at reversal lines in HVEM and a suggested incomplete severance of Sharpey's at the alveolar wall (Johnson, 1987). Although incomplete severance is difficult to prove, it would provide a template for slicing of new collagen to existing Sharpey's fibers to form continuous fibers.

S.B. Jones: Where a Sharpey's fiber crosses a reversal line, does part of the fiber in the old bone have an unmineralized core always, sometimes or never?

Authors: We believe that the fiber within old bone always has an unmineralized core (Martinez and Johnson, 1987; Johnson, 1987). However, the cores become reduced in size as the fiber becomes more deeply embedded in alveolar bone (Martinez and Johnson, 1987).

H. Warshawsky: The radiographic data show sites of protein synthesis and matrix deposition. How does this data confirm the morphological evidence that suggests three types of periodontal attachment to resorptive bone surfaces?

Authors: Our morphological data did not confirm formation of three types of periodontal attachments, but suggested that there may be three types of patterns of detachment of periodontal ligament fibers from alveolar bone. Radiographic data proved that these attachments are under constant renewal.

H. Warshawsky: How do you explain splicing of collagen filaments to existing cut ends of Sharpey's fibers in terms of

the quarter-stagger mechanism of collagen fibrillogenesis?
Authors: We cannot explain this from our data. There must be some coordination between collagen removal and deposition within these fiber bundles. We could only speculate on that mechanism.