

The effects of force magnitude on a sutural model: A quantitative approach

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In an effort to quantify the biologic effects of an orthodontic tensile force, the rat interpremaxillary suture was investigated as a model for the periodontal ligament and expanded *in vivo* with a helical spring across the maxillary incisors. Three levels of force were used: light (50 to 75 g), medium (150 to 175 g), and heavy (250 to 300 g). Thymidine labeling and histologic studies after 12 hours and 1, 2, and 4 days of force delivery are described ($n = 48$ rats), as are biochemical studies after 2 and 4 days including a 6-hour organ culture ($n = 32$). The percentage of labeled cells increased significantly in all force groups at 1 day, followed by a rapid decline at 2 days, to a value at 4 days not significantly different from the controls. Biochemical studies showed significant increases in proline incorporation and alkaline phosphatase activity after 2 days of heavy force application. Histologic examinations showed obvious tissue changes beginning by day 1 and involving increases in suture width, vascularity, size and number of cells, amount of osteoid production, and changes in suture morphology. The experimental system was convenient, inflammation-free, and appeared to be reliable as evidenced by characteristic, synchronous tissue and autoradiographic changes in all experimental sutures through 4 days. (AM J ORTHOD DENTOFAC ORTHOP 1988;93:460-6.)

Orthodontic tooth movement is dependent on the induction of a bony remodeling response by a mechanical force. Although sophisticated advances have been made in force delivery systems, relatively little is known to explain how changes in the applied forces affect the biologic system.

For many years knowledge of the biologic response to an orthodontic force had been limited to information gained only from histologic studies. Beginning with Sandstedt¹ in 1904, several investigators studied the histology of orthodontic tooth movement,²⁻⁵ which was described in terms of compressive (resorptive) and tensile (formative) responses. Unfortunately, the cellular mechanisms involved in the activation and control of bone-forming and resorbing processes were not well understood.

Recently, biochemical and cellular biology studies have helped to characterize the bony remodeling response. The means by which a mechanical force signals the induction of a biologic response is under scrutiny.^{6,7} The cellular kinetics of a proliferative response⁸⁻¹⁰ and the synthesis of collagen associated with tooth movement have been examined.¹¹⁻¹⁶

Little has been done to try to quantify the degree of biologic response to given force magnitudes, and clinical and histologic studies offer conflicting data.¹⁷ Storey showed that there is an optimal range of force required to produce a maximal rate of tooth movement and that forces above or below this optimum result in a decreased rate of tooth movement.¹⁸⁻²¹ However, others have not been able to support this concept. Hixon,²² using a canine retraction system, could not substantiate the theory of optimal force and concluded that the present state of investigation "does not permit formulation of strong theories regarding force and rate of tooth movement." Burstone²³ similarly has pointed out the complexity of the relationship between force magnitude and rate of tooth movement, and emphasizes the need to better understand the biochemical mechanisms in resorption and apposition.

The use of animal periodontal models to study the cellular and subcellular effects of orthodontic tooth movement has several limitations, including lack of substantial amounts of tissue for analysis and the inability to isolate areas under tension and those under pressure. Baumrind and Buck²⁴ have noted additional limitations with rat periodontium used as an orthodontic model—notably, substantial individual variations among standardized animals, statistically significant changes in untreated areas of experimental animals, and

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the lack of difference in character in cellular metabolic activity between areas considered to be those of pressure and tension.

The purpose of this work was to establish a model to try to quantify the appositional response to an orthodontic force. The specific aims were to evaluate the biomechanics, cell biology, and cell biochemistry in response to different magnitudes of a tensile force with a fibrous sutural model for the periodontal ligament.

MATERIALS AND METHODS

Eighty female Sprague-Dawley rats, 200 to 225 g in weight and 53 to 58 days of age, were obtained according to the laboratory's standard used in previous studies. All animals were treated identically except that experimental animals received a helical spring that delivered one of three forces: light (50 to 75 g), medium (150 to 175 g), or heavy (250 to 300 g). Animals were supplied with soft diet and water ad libitum.

Helical springs were fabricated from 0.010-inch, 0.014-inch, or 0.016-inch stainless steel and calibrated using a tension gauge to the appropriate force levels. Appliances were attached to maxillary incisors of experimental animals under general anesthesia via one-quarter round burr holes and 0.009-inch ligature wire. The mandibular incisors were cut on all animals to prevent abrasion of the maxillary incisors and subsequent loss of the appliance (Fig. 1).

Forty-eight of the animals were equally divided into a control and three experimental groups for *in vivo* labeling of DNA. Each group was subdivided further to conduct experiments of 0.5, 1, 2, and 4 days' duration; the experimental groups were matched with a control group for each time period and all animals for a particular interval were treated at the same time. One hour before killing the animals, they were anesthetized and injected with 1 μ Ci/g body weight tritiated thymidine ([methyl-³H]-thymidine, specific activity 81.9 Ci/mmol) via intraperitoneal injection.

Autoradiography and histology

At the termination of the experiment, the rats were killed, the spring was removed, and the anterior portion of the maxilla was dissected out and fixed overnight in Karnovsky's fixative.²⁵ The tissues were then decalcified in a large volume of 15% ethylenediaminetetraacetate solution at pH 6.9 and embedded in plastic. Slides of sections at 4 μ m were prepared, dipped in emulsion,[†] and exposed in a light-tight container for 16 days, then developed, fixed, and stained with meth-



Fig. 1. The appliance in place on the maxillary incisors of the rat.

ylene blue and basic fuchsin. Additional sections were prepared for standard histologic study.

The autoradiographic slides were examined with an ocular grid under magnification $\times 100$. Confirmation of thymidine labeling was made by checking for a consistent level of labeled cells in bone marrow. All fibroblast-like and osteoblast-like cells in 16 representative areas of each suture were counted and the number of cells with at least five grains per nucleus was determined to calculate a thymidine-labeling index.²⁶⁻²⁹

Biochemical assays

The remaining 32 animals, equally divided into a control and three experimental groups, were used to measure *in vitro* tritiated proline incorporation and alkaline phosphatase activity. A force was applied to each of the experimental groups for 2 and 4 days, and experimental groups were matched with a control group for each time period. At the time the animals were killed, the anterior portion of the maxilla (including appliance) was grossly dissected. The explant was cultured in a Trowell-type organ culture dish* resting on a stainless steel grid. The medium used was Dulbecco-Vogt[†] supplemented with an antibiotic mixture consisting of 50 U/mL penicillin and 50 μ g/mL streptomycin. The ex-

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†Gibco, Grand Island, N.Y.

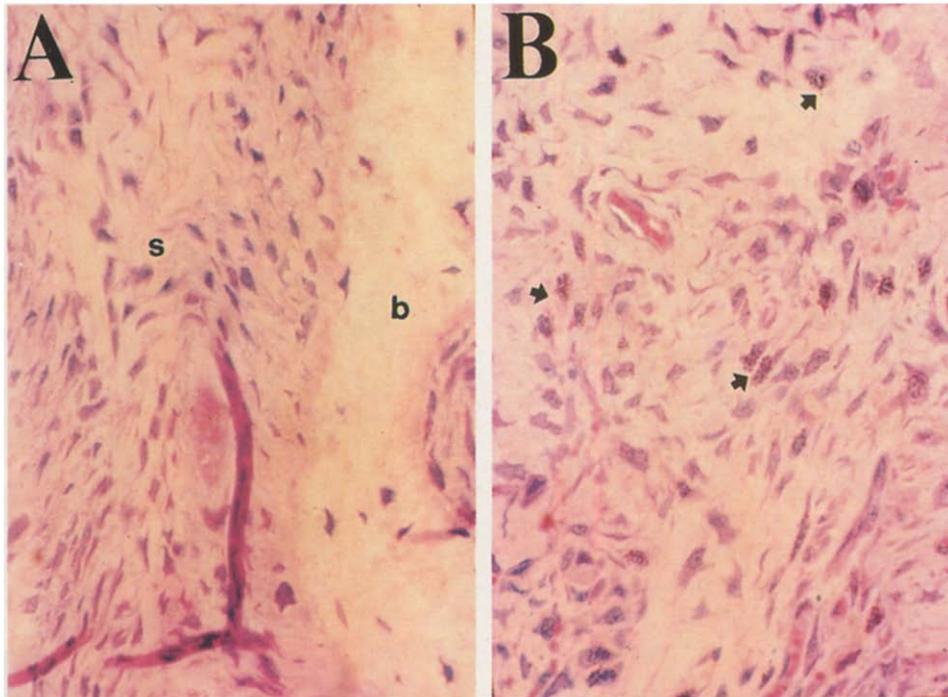


Fig. 2. Photomicrographs of autoradiography of the interpremaxillary suture demonstrating increases in labeled cells with tensile force application. **A**, One-day control. Note the lack of labeled cells in the suture (*s*), the straight course of the suture, and margin of bone (*b*). **B**, One-day light force. Note the increased number of labeled cells (*arrows*). (Stained with methylene blue and basic fuchsin. Original magnification $\times 400$.)

plants were incubated for 6 hours at 37° C in 95% O₂ and 5% CO₂.

Incorporated into the medium for the entire length of the organ culture was 20 μ Ci/mL of tritiated proline (L [2,3,4,5-³H]-proline, specific activity 142.1 Ci/mmol*). The pulse mixture consisted of 1.75 mL Dulbecco-Vogt with 20 μ Ci/mL [³H]-proline and 50 μ g/mL ascorbate. At the end of 6 hours, the sutures were rinsed with 0.5 mL cycloheximide 5 mg/mL, dissected out, and placed into 1.5 mL of fresh Dulbecco-Vogt medium. This 1.5-mL sample was homogenized, centrifuged (10,000 g for 30 minutes), and supernatant collected and frozen at -20° C for analysis.

Tritiated proline incorporation

A 100- μ l aliquot, in duplicate, for each of the sutural samples was added to an equal volume of 20% trichloroacetic acid in 1% tannic acid at 4° C for 45 minutes. Samples were then filtered, dried, and a scintillation cocktail was added to each sample. The samples were analyzed on a Beckman scintillation counter.

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Alkaline phosphatase assay

Following the method of Lowry,³⁰ 10 μ l of sutural sample, in duplicate, was added to 1 mL of 0.1 M diethanolamine buffer pH 10.3 with 10 mM p-nitrophenyl phosphate substrate. These samples were incubated at 37° C for 30 minutes. After the incubation period, 1 mL of 0.1 N NaOH was added for quenching the reaction. Standards were calculated using p-nitrophenol. The standards and samples were read at 405 nm in a Hitachi spectrophotometer.

Means and standard deviations were determined for all labeling indices and assays. The significant differences for force versus time ($\alpha = 0.05$) were tested using a two-way analysis of variance along with the Scheffé discrimination test.

RESULTS

Approximately 75% of all animals lost some weight. The weight loss was highly variable and did not correspond with a particular treatment or control group.

All control sutural tissues had very few labeled cells. Within 12 hours of tensile force application, labeling

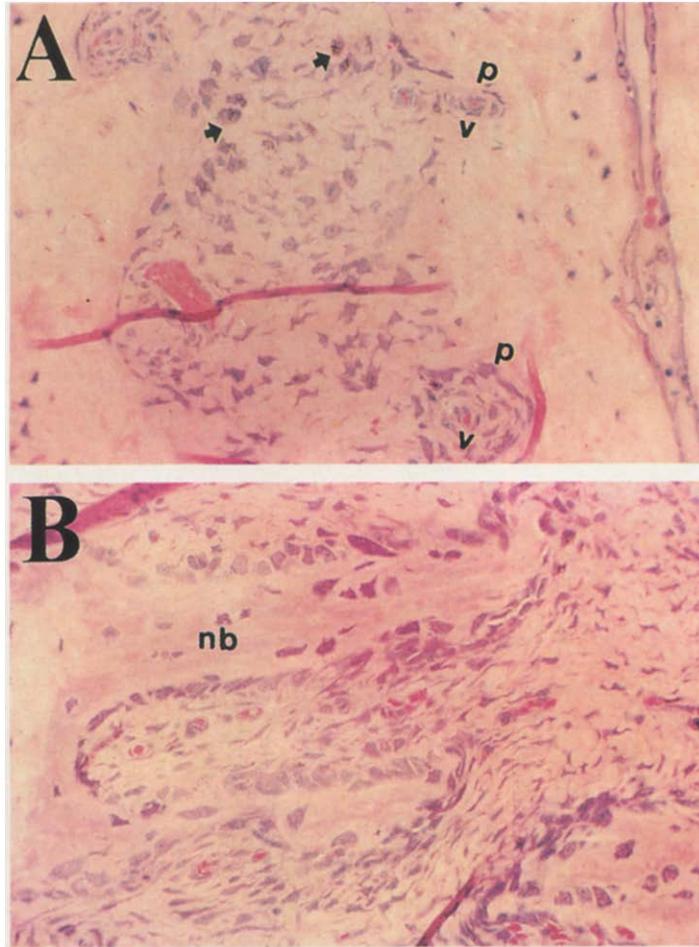


Fig. 3. Photomicrographs of autoradiography of the interpremaxillary suture demonstrating typical morphologic tissue changes seen with the application of a tensile force. **A**, One-day light force. Note the areas of vascularity (*v*) within sutural projections (*p*). Also note the number of labeled cells (*arrows*). **B**, Four-day heavy force. Note the projections of the suture with trabeculae of new bone (*nb*). (Stained with methylene blue and basic fuchsin. Original magnification $\times 240$.)

indices of the experimental groups increased. By 24 hours there was approximately a 30-fold increase in the percentage of labeled cells compared with controls (Fig. 2). This was followed at 2 days by a quick recovery and significant decline in the labeling index in all force groups; by 4 days there was a further decline in indices in all force levels to approximately 20% of the 24-hour level. There was a tendency toward increased labeling with heavier forces at 24 hours followed by a sharper decline toward the control value, but this did not achieve significance statistically (Table I).

Microscopic examination showed that starting at day 1, all experimental sutures underwent definite, synchronized tissue changes that involved increases in suture width, vascularity, size and number of cells, amount of osteoid production, and changes in suture

morphology (Fig. 3). Throughout all the time periods examined, neither experimental nor control specimens showed any evidence of significant inflammation.

Proline incorporation

The results of the assays for proline incorporation showed a statistically significant increase in the heavy force group at 2 days (Table I).

Alkaline phosphatase activity

The results of assays for alkaline phosphatase activity at 2 and 4 days showed a statistically significant increase in the enzyme activity in the heavy force group at 2 days. At 4 days there was a decline in activity but to a value that was still greater than that of controls (Table I).

Table 1. The effects of light, medium, and heavy tensile forces across the interpremaxillary suture expressed as mean (standard deviation) values

Force	Treatments			
	12 hours	1 day	2 days	4 days
<i>Control</i>				
Percentage of labeled cells ¹	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)
Proline incorporation ²	—	—	9.2 (2.2)	8.9 (2.5)
Alkaline phosphatase activity ³	—	—	18.8 (3.7)	18.8 (3.7)
<i>Light force</i>				
Percentage of labeled cells ¹	1.2 (0.6)	14.6 (3.0)*	7.3 (0.6)*	4.2 (1.2)
Proline incorporation ²	—	—	9.1 (2.1)	6.3 (0.7)
Alkaline phosphatase activity ³	—	—	25.4 (4.4)	21.3 (4.7)
<i>Medium force</i>				
Percentage of labeled cells ¹	2.7 (0.7)	13.1 (1.9)*	5.5 (0.9)*	3.0 (2.6)
Proline incorporation ²	—	—	10.2 (0.4)	5.6 (1.2)
Alkaline phosphatase activity ³	—	—	23.1 (1.3)	18.6 (3.0)
<i>Heavy force</i>				
Percentage of labeled cells ¹	1.3 (0.5)	17.5 (1.8)*	4.5 (0.5)	2.8 (1.2)
Proline incorporation ²	—	—	12.4 (1.4)*	6.7 (0.6)
Alkaline phosphatase activity ³	—	—	29.1 (6.1)*	26.8 (1.4)

¹Mean percentage labeled cells per high-power ocular grid in 16 representative areas of each suture (n = 3).

²Mean counts per minute/suture ($\times 10^{-4}$).

³Mean alkaline phosphatase activity in micromoles p-nitrophenol/suture (n = 4).

*Significantly different from control ($\alpha = 0.05$).

DISCUSSION

Orthodontic tooth movement is mediated via the remodeling response of the periodontal ligament after equilibrium disruption. With force application, areas of tension and compression result in distortion of the periodontal ligament inducing a biologic chain of events leading to bony remodeling that allows the PDL to restore itself to its original architecture. The clinical result is a new tooth position.

The interpremaxillary suture appears to offer several advantages as a model to provide a suitable facsimile of the periodontal ligament. Although there has been debate as to the exact morphologic nature of the fibrous suture, there are great structural and functional similarities between it and the periodontal ligament.³¹⁻³⁴ Application of a purely tensile force was accomplished via the maxillary incisors and appliances were virtually 100% retentive. Use of the interpremaxillary suture, in particular, allowed for noninvasive force application and all histologic samples were without signs of significant inflammation. In contrast, the use of other cranial sutures requires a surgical procedure with metallic implant.^{13,16,35} Histologic and thymidine labeling studies showed that the experimental tissues went through discrete, synchronous changes in morphology and in the number of cells undergoing DNA synthesis with little interanimal variation. The peak rise in the labeling in-

dex at 24 hours is in close agreement to that reported by Roberts and associates,⁸⁻¹⁰ who found increased labeling indices from 1.4% in a control group to 25.2% in experimental animals at 27 hours with an orthodontic force applied to rat periodontal ligament.

Substantive clinical implications would stem from finding a difference in response among light, medium, and heavy forces. Miyawaki³⁵ found a difference in response to force magnitude with the interparietal suture. Heavier forces produced earlier opening of the suture, earlier and increased cell proliferation, and earlier and increased bone deposition without adverse cellular response. The present study showed an increased response in proline incorporation, alkaline phosphatase activity, and thymidine labeling with heavy forces. However, no difference was clearly evident. One plausible explanation for the absence of greater differences is that all the forces used may have approached the maximal tissue response. This sutural system may respond to much lighter forces because the incisors, which extend far into the maxilla, act as long lever arms. Supporting this is the fact that maximal tooth separation appeared to be very rapid, occurring within several hours.

Storey,⁴ using a similar system with forces of 25, 50, and 150 g in rats, and 25, 50, and 250 g in rabbits, reported different qualities of bone deposited in re-

sponse to different forces. With light forces short, thick trabeculae developed with an intercellular distance equal to the original bone. With increased force there was an increased rate of cellular activity with long, thin trabeculae. With heavy forces the intercellular distance between osteocytes became smaller and the resultant bone was poorly mineralized. In contrast, we could find no difference among the three force levels in the quality of the bone deposited. However, Storey's observations were made over a longer period of time and his helical springs were active over a greater distance, producing a larger separation of the suture.

The purpose of this study was to establish a model for quantifying the effects of an orthodontic force. A fibrous suture under tension was used and some known biologic parameters were measured. The model proved to be convenient, and the biologic response reproducible. Although thymidine labeling studies did not demonstrate significant interforce differences, the system warrants further examination. Threshold forces, if they exist, need to be determined and further study with lighter forces is underway.

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