The morphologic and biochemical effects of tensile force application to the interparietal suture of the Sprague-Dawley rat


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The purpose of this study was to correlate the histologic and biochemical responses of the interparietal suture to a range of tensile forces. Stainless steel spring implants, calibrated to generate expansive forces from 50 to 250 g, were placed across the interparietal suture in 85 female Sprague-Dawley rats. After experimental periods from 2 hours to 14 days, the interparietal sutures were evaluated by radiography, histology, and biochemistry. An in vivo/in vitro system was used for the biochemical analysis; total protein, proline incorporated, percent collagen, and alkaline phosphatase activity were measured. The radiographs and histology showed that in vivo suture expansion was achievable with 50 to 70 g of force, but the heavier forces showed greater sutural opening, more cellular proliferation, and more bone formation. This increased biologic response by the heavier forces was substantiated by an increase in sutural protein and alkaline phosphatase activity but not in percent collagen. It was concluded that changes in the total protein content of the suture were not primarily caused by proliferation of osteogenic cells and fibroblasts but due to an influx of transudate. In contrast, the increase in incorporation of 3H-proline and alkaline phosphatase activity correlated with the observance of bone formation. This study indicated a positive correlation between the magnitude of tensile forces and osteogenic response. (Am J Orthod Dentofac Orthop 1987;92:123-33.)

Studies of orthopedic expansion of cranial sutures have carefully documented that the biologic response is a widening of the suture followed by the production of connective tissue components (Hinrichson and Storey, Storey, Cleall and associates, Murray and Cleall, Ten Cate, Freeman, and Dickinson). By this remodeling activity, the suture reestablishes a configuration similar to its original form. The remodeling appears to be chemically mediated, but this is not well understood. Because collagen is the major protein of connective tissue, the metabolism of this molecule is important in understanding how tensile forces function.

Many of the evaluations of collagen regulation in response to tensile forces were autoradiographic, using tritiated proline as a marker for collagen metabolism (Baumrind and Buck, Diaz, Crumley, Stallard, and Koumas and Matthews). These studies reported a transient decrease followed by a steady rise in the incorporation of label. Inferences were made regarding collagen metabolism, suggesting that tensile force specifically stimulated collagen production. An inherent problem in the methods used in these studies is the amino acid proline, which is incorporated into proteins other than collagen. With autoradiography, one cannot discern grains representing collagen from noncollagenous proteins. Consequently, the role of collagen metabolism is not well understood from these studies.

To clarify this question, in vitro models using sutures were developed. The sutures that have been studied most often are the interparietal and interpremaxillary sutures. The use of the in vitro aspect of the study permitted easy metabolic labeling and protein isolation.

With this approach, Meikle and associates reported nearly a threefold increase in 3H-hydroxyproline incorporation in the mechanically stressed coronal suture compared with control. However, the percentage of collagen versus noncollagenous proteins that were newly synthesized did not change. These results indicated that stress applied to the increase in collagen synthesis was part of a general increase in protein synthesis. Chang also corroborated these findings. In contrast, Yen and associates observed a specific increase in the percentage of collagen at day 3. They used an organ culture system involving higher oxygen tension than the other investigators, which may have affected collagen formation. Consequently, it is still unclear.
whether collagen synthesis is specifically stimulated as a result of tensile forces.

Other investigations of collagen synthesis following orthopedic expansion suggest that there is a phenotypic change rather than a change in the amount. Studies by Meikle and associates,13-15 and Yen and associates17,18 indicated an early appearance (days 3 and 4, respectively) of proportionately larger amounts of type III collagen with little increase in type I collagen in stressed sutures, whereas unstressed sutures contained little or none of the type III collagen. This change in collagen type may indicate an alteration in the metabolic activity of the suture.

The changes of alkaline phosphatase activity in the suture in response to tensile forces are also controversial. Takimoto, Deguchi, and Mori19 reported an increase in alkaline phosphatase activity on the tensile side 5 days after tooth movement, whereas Chang16 reported a rise only after 14 days of force. In contrast, Lilja, Lindskog, and Hammarstrom20 reported no change in activity on the tensile side. Because alkaline phosphatase activity is associated with bone formation,21,22 an increase in activity would be expected after tensile force application. Whether this increase is measurable is not clear. Measuring alkaline phosphatase activity may permit the determination of the initiation of bone formation and permit one to look at dose responses of different force levels.

The force levels that have been used in previous studies have varied considerably. Storey2,4 used 25, 50, and 100 g, Meikle and associates13-15 used 20 to 30 g, Chang16 used 75 to 100 g; Ten Cate, Freeman, and Dickinson7 used heavy forces. These heavy forces have been shown to cause in vivo opening of the interparietal suture,7 but it is not clear to what extent lighter forces can. Also, it is not well established whether the sutures are capable of being stimulated in a dose-response fashion. To achieve this, assays are required to quantitate the connective tissue response. This objective would best be achieved by correlating histologic and biochemical data.

MATERIALS AND METHODS

This study was divided into two sections: an anatomic portion to determine the magnitude of the in vivo suture opening and cellular response, and a biochemical portion to measure changes in the levels of protein, collagen synthesis, and alkaline phosphatase activity in the suture.
Anatomic evaluation

Forty female Sprague-Dawley rats weighing 200 to 225 g, approximately 53 to 58 days old, were divided into four groups. In three of the groups, the animals received helical spring implants (0.018-inch round stainless steel) designed to deliver an expansion force in one of three ranges: light (50 to 70 g), medium (100 to 150 g), and heavy (200 to 250 g). The animals of the fourth group served as controls and underwent a surgical procedure without spring implantation.

The surgical procedure involved ether anesthesia and a midsagittal anteroposterior incision through the scalp, exposing the interparietal suture. Two holes, 4 to 5 mm apart, were drilled equidistant from the suture. The spring implant was expanded 3 mm beyond the holes. The wound was then closed and a 0.1-ml subcutaneous injection of mycotorm (Duplocillin) was given.

After the designated experimental period of 1, 4, 7, or 14 days, the calvarium was dissected out leaving the spring intact. The explant was placed in Karnofsky’s fixative at 4°C and radiographed. After 1 hour, the explants were placed in EDTA (pH 6.9) for demineralization. The samples were then prepared for light microscopy by means of routine histologic procedures.

Biochemical evaluation

Forty-five female Sprague-Dawley rats weighing 200 to 225 g were divided into three groups. In two of the groups, the animals received helical spring appliances that generated expansion forces either in a low force range (50 to 70 g) or a heavy force range (200 to 250 g). The animals of the third group, the control group, received completely passive springs. The experiment involved in vivo/in vitro phases similar to the method of Chang.

Two hours before termination of the experiment, the rats were killed, and the calvaria dissected free and trimmed to 10 × 30 mm explants. The explants were cultured on grids with springs in place in Trowell-type
organ culture dishes (60 X 15 mm).* The pulse medium consisted of 1.5 ml of Dulbecco-Vogt medium† supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, † 50 μg/ml sodium ascorbate, and 10 μCi/ml 3H-proline (New England Nuclear,‡ 23.7 Ci/mM). The explants were cultured for 2 hours at 37° C with 95% O₂ and 5% CO₂ (Fig. 1). At the end of 2 hours, the explants were removed; the sutures were excised from the neighboring bones and transferred to 1.5 ml of a solution containing Dulbecco-Vogt medium only. The sutural sample was thoroughly homogenized,§ then centrifuged (780 X gravity) for 5 minutes. The resultant supernatant was collected and frozen at -20° C.

**Determination of total sutural protein**

Protein determinations were performed using the method of Lowry and associates.²⁴ The standards and samples were read in a spectrophotometer at 750 nm.

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**Fig. 5.** Radiograph of interparietal suture of light force group at 14 days. Note substantial in vivo opening had occurred at this time.

**Fig. 6.** Radiograph of interparietal suture of heavy force group at 14 days. Note substantial new bone formation (arrows).

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**Determination of proline incorporation**

Proteins were precipitated with 10% trichloroacetic acid (TCA) and 0.5% tannic acid at 4° C for 45 minutes. The precipitated samples were then collected on pre-soaked GF/A glass-fibered filters,* dried, placed in scintillation cocktail, † and analyzed in a scintillation spectrophotometer, ‡

**Determination of percent collagen**

A collagenase digest, according to the method of Peterkofsky and Dinglemann,²⁵ was used to determine the percentage of collagen that was synthesized during the 2-hour pulse. The samples were precipitated with 10% TCA and 0.5% tannic acid, and counted as described previously.

**Alkaline phosphatase activity**

The residual samples were exhaustively dialyzed against 0.1 M diethanolamine buffer (pH 10.3) at 4° C. Alkaline phosphatase activity was determined

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* Becton Dickinson Labware, Oxnard, Calif.
† Gibco, North Andover, Mass.
‡ New England Nuclear, North Billerica, Mass.
§ Tekmar Company, Cincinnati, Ohio.
RESULTS

Although variable, the radiographic evaluation indicated that the medium and heavy forces achieved similar levels of sutural opening at day 14—3.0 and 3.2 mm, respectively—whereas the lightest force achieved 2.1 mm of sutural opening (Figs. 2 through 6).

Anatomic analysis

The suture of the control animals showed minimal osteogenic activity as evidenced by the flattened profiles of the osteoblasts lining the suture and the sparsity of mitotic figures (Fig. 7).

At day 1, the suture of the light force group had localized areas of stretching of collagenous fibers, but very little widening of the suture had taken place. Few mitotic figures were observed. In the heavy force group, greater stretching and abundant mitotic figures, preferentially in fibroblasts over osteogenic cells, were noted (Figs. 8 and 9).

At day 4, the light force group displayed noticeable stretching of the collagenous fibers. Although the number of osteoblasts had increased, very little new bone formation was detectable along the borders of the suture. In contrast, the heavy force group displayed both numerous active osteoblasts and large amounts of new bone formation (Figs. 10 and 11).

At day 7, in the light force group, new bone formation was detected with mitotic figures still present throughout the suture. In the heavy force group, the configuration of the new bone changed from previous days. Rather than being deposited along the sutural margin in a block fashion, new bone was deposited as finger projections oriented along the collagenous fibers (Fig. 12).

At day 14, the light force group showed continued osteogenesis, similar bony projections were seen in the heavy force group on day 7. However, very few mitotic...
Fig. 9. Photomicrograph of the interparietal suture of the heavy force group at day 1. Note the widening of the suture, stretching of the connective tissue fibers, and the activity of the osteoblasts. Arrows indicate mitotic figures. (Stained with methylene blue and basic fuchsin. Original magnification ×210.)

Fig. 10. Photomicrograph of the interparietal suture of the light force group at day 4. Note the significant stretching of the connective tissue fibers and the inflammatory tissue that is limited to the periosteum. Arrows indicate new bone formation. (Stained with methylene blue and basic fuchsin. Original magnification ×210.)

figures were observed in the light force group; the heavy force group showed similar histology.

Biochemical analysis

The effect of tensile force on the total protein content of the suture is presented in Fig. 13 and Table 1. At day 1, both experimental groups demonstrated significant increases in total protein. By day 4, the light force group values diminished, whereas they remained high in the heavy force group.

The effect of tensile force on the amount of incorporated proline is presented in Table II. Both experimental groups demonstrated greater counts, with the heavy force group showing a higher and more sustained
increase. Two variables—the surgical procedure and implantation of the spring—both had a negative effect on proline incorporation. On day 1, in the control group, the number of counts dropped from 16.2 to 7.2 CPM/suture ($\times 10^{-3}$). By 2 weeks, the levels of the control group had returned to the original values. When the effects of the surgical procedure and metallic implant were analyzed separately (in a previously conducted experiment), both variables showed similar reduction in counts as was observed with the combined use of the surgical procedure and implant (data not shown).

The effect of tensile force on the percentage of collagen synthesized is presented in Table III. The only
Table I. Effect of tension on total protein within interparietal suture (mean μg protein/suture \( \times 10^{-3} \) ± standard deviation)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Light force</th>
<th>Heavy force</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2 ± 1.5</td>
<td>3.3 ± 1.4</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>1</td>
<td>2.7 ± 1.1</td>
<td>13.4 ± 8.6*</td>
<td>8.3 ± 0.1*</td>
</tr>
<tr>
<td>4</td>
<td>6.8 ± 2.8</td>
<td>4.8 ± 0.9</td>
<td>15.0 ± 3.2*</td>
</tr>
<tr>
<td>7</td>
<td>4.3 ± 0.9</td>
<td>4.5 ± 2.0</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>14</td>
<td>6.7 ± 4.9</td>
<td>7.7 ± 3.5</td>
<td>5.8 ± 2.8</td>
</tr>
</tbody>
</table>

Protein content of the suture was determined after 2-hour organ culture according to the method of Lowry and associates as described in MATERIALS AND METHODS. Data are presented as the mean for three independent experiments performed in duplicate. *Significantly different from control (P < 0.05).

Table II. Effect of tension on TCA precipitable counts of 3H-proline within the interparietal suture (mean CPM/suture \( \times 10^{-3} \) ± standard deviation)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Light force</th>
<th>Heavy force</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.2 ± 4.6</td>
<td>19.8 ± 1.8</td>
<td>17.1 ± 3.4</td>
</tr>
<tr>
<td>1</td>
<td>7.2 ± 0.0</td>
<td>13.9 ± 4.1</td>
<td>15.0 ± 2.6</td>
</tr>
<tr>
<td>4</td>
<td>10.3 ± 2.0</td>
<td>12.4 ± 1.4</td>
<td>25.8 ± 15.8</td>
</tr>
<tr>
<td>7</td>
<td>10.9 ± 6.7</td>
<td>10.6 ± 0.8</td>
<td>21.0 ± 11.8</td>
</tr>
<tr>
<td>14</td>
<td>10.7 ± 5.9</td>
<td>22.4 ± 2.2</td>
<td>19.2 ± 6.5</td>
</tr>
</tbody>
</table>

Each suture was pulsed with 10 μCi/ml of 3H-proline during the 2-hour organ culture. Counts per minute were determined by counting the precipitates from the reaction of the samples with TCA and tannic acid as described in MATERIALS AND METHODS. Data are presented as the mean for three independent experiments performed in duplicate. No significant differences were found among the three groups.

increase was at day 14, when both experimental groups showed approximately a 10% rise.

The effect of tension on the alkaline phosphatase activity is shown in Fig. 14 and Table IV. In both experimental groups, there were similar significant increases at days 7 and 14.

DISCUSSION

The connective tissue of the suture demonstrated a differential response to the tensile forces applied. Radiographically, heavy forces produced greater opening of the suture than light forces. Histologically, heavy forces produced more cell proliferation and differentiation, and greater bone formation. These processes also occurred earlier and persisted longer. Biochemically, heavy forces produced more protein content in the suture, greater proline incorporation, and an earlier rise in alkaline phosphatase activity. Collectively, these data suggest that the response of the connective tissue correlates positively with the amount of force applied.

However, this dose–response relationship between the connective tissue and the tensile force must be qualified. For example, Storey reported an increased rate of tooth movement with increasing force, but not the in amount of bone formation. With heavy forces Storey found bone formation to be less well organized and poorly calcified. In contrast, this study found more bone formation with heavy forces with no deficiency in the quality of the bone. Storey evaluated the intermaxillary suture rather than the interparietal suture, so com-
DIFFERENCES IN PROTEIN CONTENT BETWEEN EXPERIMENTAL AND CONTROL GROUPS

Fig. 13. The effect of tension on the total protein within the interparietal suture. Protein content of the sutures was determined after 2 hours of organ culture (as described in MATERIALS AND METHODS). Data are presented as the mean for three independent experiments performed in duplicate.

DIFFERENCE IN ALKALINE PHOSPHATASE ACTIVITY BETWEEN EXPERIMENTAL AND CONTROL GROUPS

Fig. 14. The effect of tension on alkaline phosphatase activity. Each sample was dialyzed against diethanolamine buffer with 2% mg Cl. Samples were tested for enzyme activity using a 10^{-3} M p-nitrophenyl phosphate substrate (as described in MATERIALS AND METHODS). Data are presented as the mean for three independent experiments performed in duplicate.

Comparisons are difficult. Still, the data from this study and Storey’s data suggest that there is a limited ability of the connective tissue to respond to tensile forces. This ability of the tissue to respond in a dose–response manner operates within a narrow range of force values and is dependent on the suture evaluated.

A surprising result of this study was the finding that the protein content of the suture and the amount of
incorporable proline did not change in concert. Instead, these two variables acted independently. Except for the transient rise in protein content at days 1 and 4, the protein content of the suture was stable. In contrast, the rate of proline incorporation in the heavy force group remained elevated throughout the 2 weeks. These data suggest that an alteration in the protein synthesis does not affect the total protein content of a suture of a rat whose growth has stabilized. Meikle and associates\textsuperscript{13-15} reported an increase in the protein content with time in the control suture, but they were using very young rabbits in their studies.

The fact that the protein content remained stable despite the increase in protein synthesis suggests there must be a concomitant rise in protein degradation to account for the unaltered amount of protein. Meikle and associates\textsuperscript{13-15} reported a rise in gelatinase, collagena se, and neutral metalloproteinase enzymes, but not in degradation products. Sodek\textsuperscript{27,28} reported an increase in collagen turnover in stressed periodontal tissues. With the elaboration of bony- and soft-tissue matrices, there must be a reorganization of these elements. As a consequence, degradation, as a part of the remodeling process, must occur parallel with, or directly following, synthesis.

Therefore, the observed early rise in protein content of the suture may not reflect an alteration in protein metabolism but some other cause: an influx of cells or transudate into the suture, or an increase in the cell number of the suture. The histology demonstrated a marked increase in cell synthesis as evidenced by numerous mitotic figures. This increase in cell proliferation lasted in the heavy force group 7 days. Roberts, Chase, and Jee\textsuperscript{29} reported an early peak (27 hours) in cell proliferation. This proliferative response lasted several days. Southard\textsuperscript{29} found the proliferative response was of greater magnitude but of shorter duration with heavy forces when compared with light forces. In the present study, the period of proliferation extended well beyond the observed peak in protein synthesis and was of greater magnitude in the heavy force group than in the light force group. Because the proliferative period extended beyond the rise in protein content, cell proliferation was most likely not responsible for the early rise in protein in the suture. Therefore, the early change in protein content was probably caused by an influx of transudate. The histology demonstrated no inflammatory cells in the sutural zone, but edema was present. The edema was particularly evident during the first few days.

As with the findings of Meikle and associates\textsuperscript{13-15} and Chang,\textsuperscript{16} this study found tensile forces did not elicit a specific increase in the percentage of collagen synthesized. Yen and associates\textsuperscript{17,18} reported otherwise. To clarify this discrepancy, the present study used culture conditions of 95% O\textsubscript{2}/5% CO\textsubscript{2}, similar to the methods of Yen and associates, and still found no specific increase in collagen synthesis. Possibly, the two variables—implantation and surgery—with their inhibitory effect on proline incorporation and percent collagen masked the detection of a shift in collagen synthesis. Both Yen and associates, and Meikle and associates did not report the effect of the surgical procedure nor the implant in their studies. Future studies on this subject should seek an alternative model that does not involve surgery or implantation to address this issue.

Previous biochemical studies that have examined expansion of the interparietal suture have not shown in vivo suture opening. Most studies have used extended organ culture periods (6 to 24 hours) before radioactively labeling the tissues. Dramatic in vitro suture opening has been observed during these extended organ culture periods. Therefore, the results of these previous studies have principally provided information of in vitro events. This study has provided radiographic evidence that in vivo opening of the interparietal suture was possible throughout a 14-day period with forces varying from 50 to 250 g. Because in vivo suture opening was confirmed, it was possible to reduce the total organ culture and labeling time to 2 hours. This reduced the time in which in vitro suture opening could occur and the time needed to maintain tissue viability.

The rat interparietal suture was used in an in vivo/in vitro model to examine the anatomic and biochemical changes that occurred after the application of tensional forces. With modifications, this model has been used by other researchers (Meikle and associates\textsuperscript{13-15} and Chang\textsuperscript{16}). Several advantages are offered with this model: it permits the analysis of tensional forces separate from compressive forces; it provides sufficient tissue for biochemical analysis; it permits in vivo analysis; and it is readily accessible. The principal disadvantage of this model is that both surgery and implantation are required, and both these manipulations affect the metabolism of the suture.

REFERENCES

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