Experimental craniosynostosis in growing rabbits

The role of the periosteum

Department of Orthodontics, School of Dentistry, University of Washington, Seattle, Washington

Reports on the role of the periosteum in premature sutural synostosis have been contradictory. The present study summarizes a series of six experiments designed to clarify these previously conflicting findings. Twenty-five male New Zealand White rabbits were divided into six experimental groups. In four of the groups, methyl-2-cyanoacrylate was used to glue the frontal and parietal bones together and temporarily immobilize the coronal suture. In the other two groups, the sutures were not immobilized. Polyethylene was used to separate the cyanoacrylate from the periosteum in two of the groups. The experiments were performed at 5 weeks of age, and the animals were killed at either 30, 45, or 180 days postoperatively. Metallic implants were placed in the frontal and parietal bones for monitoring growth and/or sutural immobilization. Sutural fusion was confirmed radiographically or histologically. Based upon the findings it seems that mechanical immobilization of a suture does not induce fusion of that suture in rabbits. Furthermore, it appears that the mere application of methyl-2-cyanoacrylate to the periosteum overlying a suture will consistently cause the formation of a bony bridge in growing rabbits but not in nongrowing animals. The adhesive does not consistently induce synostosis if the periosteum is excised.

KEY WORDS • suture • periosteum • cyanoacrylate • craniosynostosis • sutural immobilization • skull

RECENTLY, Graham and others2–4 proposed that fetal head constraint during the third trimester of pregnancy could produce isolated nonsyndromic craniosynostosis in human infants. These researchers believe that head constraint causes immobilization of the developing calvarial bones, resulting in premature fusion. The effect of mechanical immobilization on sutural development has been studied in rabbits1,6 and monkeys.5 In those studies, methyl-2-cyanoacrylate was used to glue the frontal and parietal bones together. The cyanoacrylate thus immobilized the coronal suture. This method consistently induced sutural synostosis in young animals but not in adults. Foley and Kokich1 suggested that the bone fusion was produced by the ectocranial periosteum, and somehow was related to the age of the animal or its stage of growth.

Other investigators have recognized an association between periosteal manipulation and premature sutural fusion, but the results have often been conflicting. Ritsilä, et al.,8 transplanted periosteum from the tibia to the zygomaticotemporal suture in rabbits and showed premature sutural synostosis. A similar study6 failed to duplicate these findings consistently in monkeys. Moss6 reported experimental craniosynostosis after excision of periosteum overlying sutures in rats. Williams9 was unable to induce fusion when he excised sutural periosteum in monkeys.

Why do previous attempts to experimentally induce sutural synostosis offer conflicting results? How important is periosteum in premature synostosis? Is mechanical immobilization alone sufficient to produce premature sutural fusion? What effect, if any, does the cyanoacrylate have on the periosteum? This paper summarizes a series of experiments which were designed to answer these questions.

Materials and Methods

A series of six experiments were formulated to evaluate various methods of intentionally inducing premature synostosis of the coronal suture. In order
General Protocol

The sample consisted of 25 male New Zealand White rabbits, which were divided into six experimental groups. At the time of surgery, the animals were 5 weeks of age, with a mean weight of 752 gm. During the experiment, the animals were housed in the University of Washington Vivarium where water and rabbit pellets were available ad libitum. The animals were caged singly in a 68° to 70°F environment with lighting from 8:00 a.m. to 5:00 p.m.

Initially, each animal's head was placed in a Kopf

**TABLE 1**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Experimental Protocol</th>
<th>Experimental Interval (days)</th>
<th>Experimental Sutures</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>immobilization,</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>adhesive touching</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>periosteum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>immobilization,</td>
<td>45</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>polyethylene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>between adhesive &amp;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>periosteum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>no immobilization,</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>adhesive touching</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>periosteum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>no immobilization,</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>peristeum removed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>immobilization,</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>peristeum removed,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>adhesive touching</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>suture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>immobilization,</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>peristeum removed,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>polyethylene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>between adhesive &amp;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>suture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. Illustrations of experimental procedures for Groups A and B. a: Dorsal view. In Groups A and B, implants (I) were placed anterior and posterior to the right (RC) and left (LC) coronal sutures. Strips of periosteum were removed (PR) anterior and posterior to each suture. b: Cross-sectional view of Group A animals. The periosteum (Pe) was left intact over the coronal suture. The cyanoacrylate (Cy) bonded the exposed surfaces of bone and immobilized the suture (S). c: Cross-sectional view of Group B animals. Polyethylene film (Po) was placed over the sutural periosteum (Pe) prior to application of the cyanoacrylate (Cy).

FIG. 2. Illustrations of experimental procedures for Group C. a: Dorsal view. Implants (I) were placed anterior and posterior to the right (RC) and left (LC) coronal sutures. b: Cross-sectional view. Cyanoacrylate (Cy) was applied to the periosteum (Pe) overlying the left and right coronal suture (S).
Periosteum and experimental craniosynostosis

rodent stereotaxic unit* for stabilization. The rabbits were anesthetized by inhaling methoxyflurane. The surgical sites were shaved and disinfected with a 10% providine-iodine solution. An incision was then made through the skin adjacent to the suture, and the superficial fascia was reflected laterally. After the suture was identified, metallic implants were placed into the bones on either side of the suture. The distance between implants was measured directly with a Helios caliper and recorded to the nearest tenth of a millimeter.

Experimental Groups

The protocols for the experimental groups are summarized in Table 1.

Groups A and B. In Groups A and B (Fig. 1a), small rectangular strips of periosteum (approximately 2 × 6 mm) were then excised anterior and posterior to the right and left coronal sutures. As described in previous studies, the right coronal suture of Group A was mechanically immobilized by applying a layer (10 to 15 drops) of methyl-2-cyanoacrylate adhesive† between these two areas of exposed bone (Fig. 1b). In Group B, a layer of polyethylene‡ 15 μ thick was placed over the sutural periosteum between the rectangles of exposed bone. The cyanoacrylate was then applied over the polyethylene and extended from one area of exposed bone to the other as in Group A (Fig. 1c). In both groups, no cyanoacrylate or polyethylene was placed over the left coronal suture, and it served as the sham-operated control side. After the bridge of cyanoacrylate had polymerized (5 to 10 minutes), the flaps were reapproximated and the incision was closed with 5-0 Ethicon sutures. The sutures were removed on the 7th postoperative day.

Group C. Protocol C was performed on the right and left coronal sutures of each animal in this group. After implant placement, a 6 × 10 mm rectangle of cyanoacrylate was applied directly onto the periosteum overlying the left and right coronal sutures (Fig. 2). The cyanoacrylate did not touch bone.

Group D. After the metallic implants were placed, a 6 × 10 mm area of periosteum overlying the right coronal suture (Fig. 3a and b) was removed and discarded. The unoperated left coronal suture served as the control. The skin flaps were sutured as described previously.

Groups E and F. Protocol E was performed on the right coronal suture, and Protocol F was performed on the left coronal suture of the same animals. After implant placement, a 6 × 10 mm rectangle of periosteum was removed and cyanoacrylate (Cy) bonded the exposed surfaces of bone and immobilized the sutures. Protocol F was performed on the left coronal suture of the same animals. After implant placement, a 6 × 10 mm rectangle of periosteum was removed and polyethylene film (Po) was placed over the sutural ligament. Cyanoacrylate (Cy) was then applied to the exposed bone surfaces to immobilize the sutures.

---

* Small animal stereotaxic unit, No. 900 (modified) manufactured by David Kopf Instruments, Tujunga, California.
† Eastman 910 adhesive manufactured by Eastman-Kodak, Kingsport, Tennessee.
‡ Polyethylene manufactured by Dow Chemical Co., Freeport, Texas.

---

Fig. 3. Illustrations of experimental procedures for Groups D, E, and F. a: Dorsal view. In Groups D, E, and F, implants (I) were placed anterior and posterior to the right and left coronal sutures. The sutural periosteum was removed (PR) from the right suture in Groups D and E. The sutural periosteum was removed (PR) from the left suture in Group F. S=suture. Pe=periosteum intact; S=suture. c: Cross-sectional view of Group E. Sutural periosteum was removed and polyethylene film (Po) was placed over the sutural ligament. Cyanoacrylate (Cy) was then applied to the exposed bone surfaces to immobilize the sutures.
Fig. 4. Graphs showing average interimplant distance changes across the experimental and control sutures at 30 days postoperatively (a), 45 days postoperatively (b), and 180 days postoperatively (c). The range of individual measurements for each group is seen to the left (experimental) and right (control) of the mean value.

teum was removed from both right and left coronal sutures and discarded. A 15-μ thick film of polyethylene was placed over the left coronal suture. Cyanoacrylate was then placed across both right and left sutures and covered the entire area where periosteum had been removed (Fig. 3a, c, and d). Thus, in Group E the cyanoacrylate was in direct contact with the right coronal suture and exposed bone (Fig. 3c), while in Group F polyethylene separated the bone and left coronal suture from the cyanoacrylate (Fig. 3d). The skin was reapproximated and sutured as described previously. The control suture of Group D served as the control for Groups C, D, E, and F.

Methods of Analysis

The animals were killed at 30, 45, or 180 days postoperatively (Table 1). The heads were fixed in 10% buffered formalin. In order to assess gross morphological changes as well as distances between the paired left and right implants, sequential radiographs were taken preoperatively, postoperatively, at sacrifice, and following impregnation with silver nitrate. Direct implant measurements were made at the time of surgery and at the time of sacrifice. These were measured with a Helios caliper to the nearest tenth of a millimeter.

A representative specimen from each experimental group was sectioned into tissue blocks. These were impregnated with a 0.5% aqueous silver nitrate solution and radiographed. These were then decalcified, cleaned, and double-embedded in paraffin for histological sectioning. The sutures were sectioned sagittally and stained alternately with Harris' hematoxylin and eosin, Mallory's aniline blue collagen stain, Alcian blue periodic acid-Schiff's reagent, and Verhoeff's elastic stain. Alizarin and dry skull preparations permitted an evaluation of gross morphological changes.

Results

The observations will be described separately for each group of animals and will be expressed as changes in distance between implants, radiographic appearance, and histological structure.

Group A

In Group A animals (suture immobilization, adhesive in contact with the periosteum), the mean change in interimplant distance was 0.4 mm across the experimental sutures and 2.1 mm for the control sutures (Fig. 4). At 30 and 45 postoperative days, three of the four experimental sutures showed radiographic evidence of localized bone union across the suture (Fig. 5a). At 180 postoperative days, the three experimental sutures were obliterated by bone on radiography, while the control sutures were evident as serpentine radiolucent articulations (Fig. 5b). Histologically, the fascia overlying the experimental sutural periosteum was chronically inflamed at 30 days postoperatively.
Periosteum and experimental craniosynostosis

Fig. 5. Radiographs of rabbit calvariae impregnated with 0.5% aqueous silver nitrate. Dorsal view, × 40.  
(a) Group A, 30 days postoperatively. The left coronal (LC) sutural morphology is irregular and very convoluted. The right coronal (RC) suture is fused and less irregular.  
(b) Group A, 180 days postoperatively. The left coronal (LC) suture (control) is still patent, while the right coronal (RC) suture (experimental) shows a broad area of sutural obliteration.  
(c) Group B, 30 days postoperatively. The immobilized right coronal suture (RC) is patent but shows some decrease in irregularity.  
(d) Group C, 45 days postoperatively. Both the left (LC) and right (RC) coronal sutures are experimental sutures in this group. Both exhibit decreased complexity and marked narrowing of the sutural ligament.  
(e) Group D, 45 days postoperatively. The left coronal (LC) suture is the control. The right coronal (RC) suture had periosteum removed.  
(f) Groups E and F, 45 days postoperatively. Group E is the right coronal (RC) suture and Group F is the left coronal (LC) suture. The application of cyanoacrylate directly to bone inhibits periosteal bone deposition.
FIG. 6. Photomicrographs of experimental coronal sutures. a: Group E, sagittal section of the experimental right coronal suture (RC). The cyanoacrylate (Cy) applied to the frontal (FB) and parietal (PB) bones following removal of the sutural periosteum caused fusion within the sutural ligament 45 days postoperatively. Verhoeff, × 30. b: Group F, sagittal section of the experimental left coronal suture (LC). After removal of sutural periosteum, a polyethylene film (Po) was placed prior to the application of the cyanoacrylate (Cy). The sutural ligament (S) under the polyethylene is normal and not fused. Necrosis (NT) over the cyanoacrylate is apparent. Verhoeff, × 25.

The mass of cyanoacrylate had been broken up, and was partially encapsulated by fibrous tissue. As early as 30 days postoperatively, the frontal and parietal bones were fused by a bridge of nonlamellar bone at the ectocranial surface of the experimental suture. At 180 postoperative days, the experimental suture was completely obliterated by bone, while the control suture was patent and showed an intact sutural ligament.

Group B

In Group B animals (suture immobilization, polyethylene between the adhesive and periosteum), the mean change in interimplant distance (Fig. 4a and b) was similar between the experimental (1.5 mm) and sham-operated control sutures (1.7 mm). Although sutural growth was limited slightly by the immobilizing effect of the adhesive, none of the experimental sutures in Group B showed radiographic evidence of bone union across the sutural space (Fig. 5c). Histologically, the width of the experimental suture was greater than the control; however, the structure of the sutural ligaments was similar. No synostosis was found. The periosteum overlying the experimental suture had a thicker fibrous layer than that seen in Group A.

Group C

In Group C animals (no suture immobilization, adhesive in contact with the periosteum), the interimplant distance showed a mean difference of 0.6 mm in the experimental group and 1.8 mm in the controls (Fig. 4a and b). Three of the four experimental sutures showed radiographic evidence of bone union between the frontal and parietal bones (Fig. 5d). Histologically, synostosis was confirmed at the ectocranial sutural surface in these three specimens.

Group D

In Group D animals (no suture immobilization, periosteum removed), a slight difference was recorded between the mean change in interimplant distance for the experimental (1.6 mm) and control (1.8 mm) sutures (Fig. 4a and b). Radiographically, the experimental and control sutures appeared nearly identical (Fig. 5e). At 30 days postoperatively, the excised periosteum had regenerated over the experimental suture; however, it appeared slightly thinner than on the control side. Microscopically, both right and left coronal sutural ligaments were similar and not synostosed.

Group E

In Group E animals (suture immobilization, periosteum removed, adhesive in contact with the suture), the mean change in interimplant distance was 0.9 mm for the experimental suture and 1.8 mm for the control (Fig. 4a and b). The difference was attributed to the temporary immobilizing effect of the glue. One of the six experimental sutures showed radiographic and histological evidence of localized bone fusion (Fig. 6a). The fusion appeared to be located at both the ectocranial and the internal areas of the sutural ligament. The remaining experimental sutures were not fused. Histologically, the fascia overlying the cyanoacrylate exhibited active inflammation. In addition, the application of glue directly to bone had limited the regeneration of the excised periosteum.
Periosteum and experimental craniosynostosis

Group F

In Group F animals (suture immobilization, periosteum removed, polyethylene between the adhesive and suture), the mean change in interimplant distance was 1.1 mm for the experimental sutures and 1.8 mm for the control sutures (Fig. 4a and b). Radiographically, the sutures appeared patent and, histologically, the sutural ligament was similar in both the experimental and control specimens (Fig. 6b). Chronic inflammatory infiltrate was found overlying the cyanoacrylate and polyethylene.

Discussion

Previous investigators have immobilized the coronal suture in growing rabbits with methyl-2-cyanoacrylate adhesive. In each of these studies, the adhesive overlaid the periosteum and extended between areas of exposed bone on either side of the suture. These studies showed consistent premature synostosis of the experimental suture in young animals. The authors attributed the synostosis to mechanical immobilization of the calvarial suture during a period of rapid cranial growth, resulting in the formation of a periosteal bone bridge. The protocol for Group A (suture immobilization, adhesive in contact with the periosteum) in the present investigation duplicated the methods of these previous studies, and the results are likewise identical, namely, premature sutural fusion. At that point it seemed plausible that restricting suture growth could result in its premature synostosis. However, when a sheet of polyethylene was placed between the periosteum and cyanoacrylate (Group B), sutural synostosis never occurred despite the sutural immobilization. This contradictory finding suggested that mechanical immobilization, as provided by cyanoacrylate, during calvarial growth might not be the primary initiator of premature sutural synostosis.

In order to further test this hypothesis, a third slightly different experiment was performed. Instead of the parietal and frontal bones being glued together, as in previous studies, the cyanoacrylate in Group C was merely applied to the periosteum over the coronal suture. The suture was not mechanically immobilized by the adhesive. However, the suture fused prematurely in each experimental animal. Based upon these findings, it seems that mechanical immobilization of a cranial suture with cyanoacrylate at 5 weeks of age in growing rabbits does not induce sutural synostosis. The fusion apparently results when cyanoacrylate interacts directly with the calvarial periosteum. The nature or type of interaction is still unknown but warrants further study.

Moss removed the periosteum from cranial sutures in both young and adult rats and reportedly induced ectocranial sutural fusion. In the present study, excision of sutural periosteum (Group D) resulted in a slight decrease in the amount of growth across the suture, but regeneration of the periosteum occurred rapidly and synostosis did not occur. When cyanoacrylate was applied to the exposed bone after periosteum removal (Group E), sutural synostosis occurred in one of six experimental animals. Similar findings were reported by Williams, who believed that the synostosis is due to osteogenic transformation of periosteal remnants by cyanoacrylate. To test this hypothesis, polyethylene film (which has good tissue compatibility) was used to separate the sutural ligament and cyanoacrylate after periosteal excision (Group F). Synostosis did not occur in these animals. This finding also suggests that sutural fusion is not the result of immobilization but may be due to an interaction between remnants of the periosteum and the adhesive.

Conclusions

We have reported and discussed the results of six different experiments. The studies were designed to shed further light on our understanding of sutural synostosis. Based upon the findings, it seems that mechanical immobilization of a suture does not induce fusion of that suture in rabbits. Furthermore, it appears that the mere application of methyl-2-cyanoacrylate to the periosteum overlying a suture will consistently cause the formation of a bone bridge in growing rabbits. The adhesive does not consistently induce sutural synostosis if the periosteum is excised. The specific mechanism of interaction between the periosteum and glue is not understood and should be studied further.

Acknowledgments

The authors wish to express their sincere appreciation to Drs. Stanton Hall, Benjamin Moffett, Leena Koskinen-Moffett, Eric Luschei, Peter Shapiro, and Gary Wolf for their valuable collaboration. Special gratitude goes to Mrs. Vonnie MacDannold and Mr. James Clark for their technical assistance.

References


Manuscript received June 1, 1982.
Accepted in final form August 31, 1982.
This research was supported by National Institutes of Health Biomedical Research Support Grant RR-05346-21, and by the University of Washington Orthodontic Memorial Fund.
This article is based on research submitted by the senior author in partial fulfillment of the requirements for the Master of Science in Dentistry degree, Department of Orthodontics, University of Washington, Seattle, Washington.
Present address for Dr. Nappen: United States Naval Regional Dental Center, Subic Bay, Republic of the Philippines.
Address reprint requests to: Vincent G. Kokich, D.D.S., M.S.D., School of Dentistry, Department of Orthodontics, University of Washington, Seattle, Washington 98195.