The effects of mechanical immobilization on sutural development in the growing rabbit


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Methyl-2-cyanoacrylate was used to mechanically immobilize the coronal suture unilaterally in a series of New Zealand white rabbits at varying ages. The animals were separated into groups; some were sacrificed at 30 days and some at 60 days postoperatively. Amalgam markers were placed in the parietal and frontal bones across the coronal suture, and were measured immediately after surgery and at the time of sacrifice to confirm mechanical immobility. The animals were studied radiographically and histologically in order to document the presence or absence of sutural bone union. Based on the results of this study, it appears that immobilization of the coronal suture results in the formation of an ectocranial periosteal bone bridge in rabbits less than 8 weeks of age. Bone union was not found in animals older than 8 weeks of age. This age-related difference in response is believed to be due to decreased periosteal depository activity on the ectocranial surface of the calvaria once the brain ceases to expand actively. Furthermore, bone union or synostosis was never seen within or across the internal portion of the sutural ligament. It is suggested, therefore, that sutural immobilization at young ages in the rabbit does not result in sutural synostosis and that the term "periosteal bone bridge" should be used when referring to this biological response.

KEY WORDS • suture • craniosynostosis • periosteum • cyanoacrylate

The etiology of premature craniosynostosis has been studied by many investigators, and a variety of possible factors have been implicated as causative mechanisms for the introduction of sutural bone union. Recently, Graham and associates have reported several cases of isolated, nonsyndromic craniosynostosis in human infants. Based on exhaustive prenatal clinical histories, they have suggested that premature cranial sutural fusion may be a result of head constraint during late fetal life, resulting in immobility and eventual bone union across various isolated sutures. In support of this hypothesis, Persson, et al., have successfully immobilized the coronal suture with methyl-2-cyanoacrylate in 9-day-old rabbits, and have consistently demonstrated premature synostosis of the affected suture. Williams, however, used a similar methodology to immobilize various sutures in 4-year-old monkeys and was unable to produce sutural fusion. Furthermore, Elder and Tuenge and Brandt, et al., have experimentally generated substantial compressive forces across various facial and cranial sutures in 3-year-old and 8-year-old monkeys, respectively, and were unable to initiate fusion of the compressed sutures.

What is the reason of these differing responses seen in previous investigations? Is the production of sutural bone union following immobilization possibly related to age? Are sutures more susceptible to premature bone union following mechanical immobilization at younger ages rather than older ages? The answers to these questions are of critical importance to our understanding of the cause and effect relationship between sutural immobilization and premature sutural synostosis. The purpose of the present investigation, therefore, is to evaluate the response of the coronal suture to mechanical immobilization at varying ages in rabbits.

Experimental Animals

Thirty-two male New Zealand white rabbits were used in this study. The animals were separated into eight groups based on their age at the time of sutural immobilization: 2, 3, 4, 5, 6, 8, 12, and 16 weeks. Each group consisted of four similarly aged animals (Table 1). Following the immobilization procedure, two animals were allowed to grow for 30 days and two for 60 days. During the entire experimental period, the
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TABLE 1
Summary of experimental groups

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Groups</th>
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<tr>
<td>A B C D E F G H</td>
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<tr>
<td>age at surgery (wks)</td>
<td>2 3 4 5 6 8 12 16</td>
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<tr>
<td>no. sacrificed:</td>
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<tr>
<td>30 days postop</td>
<td>2 2 2 2 2 2 2 2</td>
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<tr>
<td>60 days postop</td>
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animals were housed in the University of Washington Vivarium in a 68° to 70°F environment with lighting from 8:00 a.m. to 5:00 p.m. The animals were fed stock rabbit pellets and were provided with water ad libitum.

Experimental Protocol

The animals were anesthetized with methoxyflurane during the surgical procedures. The cranium was initially prepared by shaving the scalp, and a benzalkonium chloride antiseptic spray was then applied to the surgical site. An incision was made through the scalp parallel to and over the midline region on the dorsum of the head. The skin and underlying fascia were reflected laterally, and the right and left coronal sutures were identified. A small region of periosteum was then abraded in the parietal and frontal bones anterior and posterior to the coronal sutures, and a cylindrical burr in a rotary handpiece was used to create small holes in these areas of exposed bone. Dental amalgam was condensed into each of the holes to serve as radiopaque markers (Fig. 1a).

Small rectangular strips of periosteum (approximately 2 × 6 mm) were then excised anterior and posterior to the right and left coronal sutures. A thin layer of methyl-2-cyanoacrylate was applied over the experimental right coronal suture, extending from one area of exposed bone to the other in an anteroposterior direction (Fig. 1b). No cyanoacrylate was placed over the left coronal suture, which thus served as the sham-operated control side in each animal (Fig. 1a). The cyanoacrylate was allowed to polymerize, the flaps were reapproximated, and the incision was closed with 5-0 Ethicon sutures that were removed on the 7th postoperative day.

Methods of Analysis

The animals were sacrificed according to the schedule shown in Table 1. After sacrifice, each specimen was fixed in 10% buffered formalin. The calvaria containing the cranial sutures was then removed from each specimen. Two calvariae from each group (30 and 60 postoperative days, respectively) were stained in vitro with alizarin red S or prepared as a dry skull. These techniques permitted a three-dimensional evaluation of sutural morphology. The other two calvariae in each group were impregnated with 0.5% aqueous silver nitrate solution to enhance radiographic visualization of the coronal suture.

In order to evaluate changes in the distances between the paired implants across the right and left

![Fig. 1. Illustrations of the experimental procedures. a: Dorsal view. Implants (I) were placed anterior and posterior to the right (RC) and left (LC) coronal sutures. Strips of periosteum (P) were removed anterior and posterior to each coronal suture. Methyl-2-cyanoacrylate was applied across the right coronal suture, extending from one area of exposed bone to the other in an anteroposterior direction (Fig. 1b). No cyanoacrylate was placed over the left coronal suture, which thus served as the sham-operated control side in each animal (Fig. 1a). The cyanoacrylate was allowed to polymerize, the flaps were reapproximated, and the incision was closed with 5-0 Ethicon sutures that were removed on the 7th postoperative day.](image)
coronal sutures, radiographs were taken at the following intervals: immediately after immobilization, at the time of sacrifice, and after impregnation with silver nitrate. Direct interimplant measurements of the specimens and indirect measurements from radiographs were made and recorded to the nearest tenth of a millimeter at the time of surgery and also at the time of sacrifice for comparison (Fig. 2).

Tissue blocks containing the right and left coronal sutures were removed from the specimens impregnated with silver nitrate, decalcified, and embedded in paraffin for histological processing. Sagittal histological sections, 7 μ thick, were prepared and stained with Harris’ hematoxylin and eosin, Mallory’s aniline blue collagen stain, Verhoeff’s elastic stain, and alcian blue-periodic acid-Schiff’s reagent. The histological sections provided microscopic documentation confirming the presence or absence of bone union across the sutural ligament.

**Results**

The results of the present investigation clearly demonstrated a correlation between a suture’s response to mechanical immobilization and the respective animal’s age. Consistently similar radiographic

![Fig. 2. Graphic representation of mean changes in distances between paired implants across the immobilized right and the sham-operated left coronal sutures, at 30 postoperative days (left) and 60 postoperative days (right).](image1)

![Fig. 3. Radiographs of rabbit calvariae impregnated with 0.5% aqueous silver nitrate. Dorsal view. A: Group E, 6 weeks of age. The morphology of the left coronal suture (LC) is very irregular and complex. The immobilized right coronal suture (RC) exhibits decreased complexity. B: Group F, 8 weeks of age. The experimental right coronal suture (RC) exhibits a slight decrease in morphological irregularity when compared to the sham-operated left coronal suture (LC). C: Group G, 16 weeks of age. The complexity and irregularity of the right (RC) and left (LC) coronal sutures is nearly the same.](image2)
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and histological observations were noted in those animals that were less than 8 weeks of age at the time of sutural immobilization (Groups A, B, C, D, and E). A different response was observed in animals that were older than 8 weeks of age (Groups G and H). Therefore, in order to avoid unnecessary repetition, the results will be collectively grouped and subdivided according to observable radiographic and microscopic similarities between animals.

**Groups A, B, C, D, and E**

Radiographically, the sutural morphology of the nonimmobilized left coronal suture was extremely serpentine and irregular. The experimentally immobilized right coronal suture, however, exhibited decreased irregularity. The number of sutural bone projections had diminished after 30 and 60 postoperative days, and the sutural margins appeared straight and not convoluted (Fig. 3). In addition, the sutural space on the right side appeared more radiopaque, while a continuous radiolucent sutural space was easily recognizable radiographically between the frontal and parietal bones on the left or control side. The interimplant measurements across the right coronal suture were unchanged during the experimental period, confirming the immobilizing effect of the cyanoacrylate. Measurements of implants on the left side continued to increase during the same experimental interval (Fig. 2).

Histologically, the immobilized right coronal suture exhibited nonlamellar bone union across the ectocranial surface between the parietal and frontal bones after 30 and 60 days of postoperative healing (Fig. 4d). Sutural synostosis was not found, however, across the sutural bone margins within the internal portion of the sutural ligament. The nonimmobilized left coronal suture appeared patent and unfused after 30 and 60 postoperative days (Fig. 4b). Microscopically, the cyanoacrylic bridge was interrupted and had been broken down and encapsulated by fibrous tissue 30 days after its initial placement (Fig. 6).

**Group F**

The animals in Group F (8 weeks of age) exhibited variable responses to the experimental procedure. The radiographic and histological observations seen in the immobilized right coronal sutures from two of the specimens were identical to those described for the

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**Fig. 4. Group E, 6 weeks of age.**

*a:* Microradiograph of the nonimmobilized left coronal suture (LC), dorsal view. The radiolucent sutural space is continuous across the suture, indicating sutural patency. FB: frontal bone; PB: parietal bone. × 8.

*b:* Photomicrograph of the nonimmobilized left coronal suture (LC), sagittal section. The sutural ligament appears normal. No synostosis is evident. FB: frontal bone; PB: parietal bone. H & E, × 45.

*c:* Microradiograph of the immobilized right coronal suture (RC), dorsal view. A distinct radiolucent sutural space is not evident radiographically, and the suture appears fused. FB: frontal bone; PB: parietal bone. × 8.

*d:* Photomicrograph of the immobilized right coronal suture (RC), sagittal section. A bone bridge developed between the parietal (PB) and frontal (FB) bones at the ectocranial surface over the coronal suture. H & E, × 45.
FIG. 5. Group H, 16 weeks of age.  

**a**: Microradiograph of the nonimmobilized left coronal suture (LC), dorsal view. The continuous radiolucent sutural space suggests that the suture is patent. FB: frontal bone; PB: parietal bone. × 8.  

**b**: Photomicrograph of the nonimmobilized left coronal suture (LC), sagittal section. The suture is not synostosed, and the collagenous ligament appears normal. FB: frontal bone; PB: parietal bone. H & E, × 8.  

**c**: Microradiograph of the immobilized right coronal suture (RC), dorsal view. The suture exhibits slightly less morphological irregularity, but appears patent based on the radiolucent space. FB: frontal bone; PB: parietal bone. × 8.  

**d**: Photomicrograph of the immobilized right coronal suture (RC), sagittal section. The sutural ligament appears normal and resembles that seen in the nonimmobilized suture. Bone fusion is not evident between the parietal (PB) and frontal (FB) bones. H & E, × 45.

younger animals in groups A, B, C, D, and E, and included the development of an ectocranial bone bridge between the parietal and frontal bones. However, the other two animals in Group F exhibited a response to sutural immobilization which was identical to that observed in the older specimens (Groups G and H).

**Groups G and H**

Radiographically, the sutural morphology of the immobilized right coronal suture did not exhibit decreased complexity. The degree of irregularity and number of sutural bone projections visible on the right side were similar to those seen radiographically in the nonimmobilized left coronal suture (Fig. 5a, c). In addition, a continuous radiolucent sutural space was evident between the parietal and frontal bones on both the experimental and sham-operated sutural radiographs (Fig. 5a, c). The interimplant distances increased across the right and left coronal sutures in all specimens in Groups G and H. However, the absolute amount of interimplant change was considerably less across the immobilized right coronal suture (Fig. 2).

Histologically, no ectocranial bone bridge was found across the immobilized right coronal sutures in any of the specimens. The sutural ligament of both the nonimmobilized control and the immobilized experimental sutures appeared similar microscopically (Fig. 5b, d). As noted previously in rabbits of younger ages, the cyanoacrylic bridge was similarly interrupted and encapsulated by fibrous tissue after 30 postoperative days in Groups G and H (Fig. 6).

**Discussion**

Previous investigators have successfully immobilized the coronal suture with methyl-2-cyanoacrylate in 9-day-old rabbits, and have consistently reported premature synostosis of the affected suture. Recently, Williams used a similar methodology, and was unable to produce sutural fusion in monkeys at 4 years of age. In the present investigation, methyl-2-cyanoacrylate was used to mechanically immobilize the coronal suture unilaterally in a series of rabbits aged 2 to 16 weeks. Interbone fusion consistently occurred in animals less than 8 weeks of age, but was not
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In the present investigation, sutural immobilization in the younger animals resulted microscopically in the production of a skeletal bridge between the parietal and frontal bones. This bone union consistently occurred at the ectocranial margin immediately over the suture. Premature synostosis was never seen within the sutural ligament. This finding may suggest a possible explanation for the age-related difference in response seen in this study. Previous investigators have studied the growth of the cranium in various mammalian species, and have clearly shown that lamellar bone deposition continues to occur on the entire ectocranial surface while compensatory resorption takes place on the endocranial surface in response to expansion of the developing brain. As brain growth ceases, the need for a further increase in the size of the calvaria at older ages is reduced, and the amount of ectocranial bone deposition decreases proportionally. In the present study, the coronal suture was effectively immobilized in young animals during the period of active neural expansion. The periosteal envelope, therefore, continued to deposit bone on the ectocranial surface, and eventually bridged the gap between the frontal and parietal bones immediately over the suture. In the older animals, by at least 8 weeks of age, the rate of brain growth had decreased markedly and the calvaria had nearly reached its adult size, resulting in a decreased amount of ectocranial bone deposition. At this stage, immobilization of the suture was relatively ineffective in producing an ectocranial bone bridge, and the suture remained unfused. Therefore, based on the results of the present study, it is believed that mechanical immobilization of the cranial sutures at younger ages does not result in premature sutural fusion within the sutural ligament, but it does produce a periosteal bone bridge at the ectocranial surface. The bridging does not occur at older ages due to a diminished amount of ectocranial bone deposition once active neurocranial expansion has ceased.

Recently, Graham and co-workers studied a sample of neonatal human infants with isolated nonsyndromic premature craniosynostosis. Based on careful prenatal clinical histories, these researchers showed that alterations in intrauterine fetal position during the third trimester of pregnancy may result in a restriction of the normal calvarial response to neural expansion at certain sutural articulations, thereby producing premature synostosis of affected sutures. Subsequent linear craniectomies to remove the synostosed sutures in these patients apparently result in normal development of the calvaria with concomitant redevelopment of the sutural ligament. The observations made in the present study would suggest that the mechanical restriction during late fetal life in these infants may have resulted in the production of a periosteal bridge of bone at the ectocranial surface, which eventually caused the premature bone union of an apparently normal suture. In this situation, it would seem logical that removal of the fused suture could eventually result in redevelopment of a normal sutural ligament.

In the present study, the sutural morphology became less complex with decreased irregularity during the period of mechanical immobilization. Kokich and co-workers, and Miroue and Rosenberg, have documented the morphological aging changes in human and nonhuman primate sutures. These researchers reported an increase in the morphological irregularity of sutures with advancing age. Other investigators have proposed and documented experimentally a correlation between the development of

Fig. 6. Photomicrographs of the pericranium over the immobilized right coronal suture, sagittal sections. a: Group E, 6 weeks of age. The cyanoacrylate bond has been interrupted, and portions of the adhesive material (CY) are seen within the fibrous pericranium (P). H & E, × 45. b: Group F, 8 weeks of age. At a higher magnification, the fibrous tissue encapsulation and some inflammatory cellular infiltration are evident. H & E, × 180.
interdigitations in various sutures and the extrinsic forces existing about these sutures.11,16,20 Furthermore, according to Massler and Schour,18 the degree of development of the serrations at the suture is related to the length of time the suture is active. Hinrichsen and Storey* found a reduction in sutural serrations following immobilization of the midsagittal suture in guinea pigs. These findings are in agreement with those of the present study, and it is clearly apparent that the fine details of sutural morphology, such as interdigitations, are secondary responses to the normal, functional, and extrinsic forces imposed on the bones.

In this study, amalgam markers were placed in the parietal and frontal bones across the right and left coronal sutures to document sutural immobility radiographically. In those specimens that exhibited an ectocranial periosteal bone bridge across the suture, the interimplant distances on the experimental side were constant, while the implants on the control side continued to separate in response to neural and calvarial growth. In older specimens that did not develop a bone bridge, slight increases in the interimplant distances were recorded on the apparently immobilized side, while greater increases occurred between the control implants. This perplexing finding prompted a careful microscopic examination of the methyl-2-cyanoacrylic bond over the sutural area. It was interesting to note that by 30 days after operation, the cyanoacrylic bridge in both young and old specimens had been interrupted, and the foreign material had been broken down and was encapsulated by fibrous tissue. The effectiveness of the cyanoacrylate for mechanical immobilization is, therefore, merely temporary. This observation helps to explain the differences seen in the interimplant measurements between younger and older animals. In specimens younger than 8 weeks, the period of mechanical immobilization afforded by the cyanoacrylate was long enough to allow the formation of an ectocranial bone bridge. The bridging response at younger ages may be attributed to the greater inherent periosteal activity in response to the expanding brain at that age. Once the parietal and frontal bones were skeletally united, breakdown of the cyanoacryllic bond had virtually no effect on the interimplant distance. In older specimens, however, ectocranial bone deposition had diminished, the periosteal bone bridge had not formed, and the parietal and frontal bones were able to separate slightly following breakdown of the cyanoacrylate. The separation of the bones at the suture led to slight increases in the interimplant distance on the experimental side. However, the increases were consistently less than those recorded on the control side, indicating that the immobilization was definitely effective for a certain period of time.

The results of this study have expanded our knowledge regarding sutural biology, and tend to dispel some misconceptions regarding the cause and effect relationship between sutural immobilization and premature sutural fusion. Bone deposition at the sutural bone margin is merely adaptive and is influenced by the environmental forces that affect the sutural ligament during growth and development. Jackson, et al.,18 have demonstrated increased sutural bone deposition when tension was applied across the sutural ligament. Other investigators have clearly shown resorption of bone at the sutural margin when a suture is compressed.1,8 Based on the present study, it appears that sutural immobilization results in minimal resorptive or depositional activity at the quiescent sutural bone margin. The bone union that does occur subsequent to immobilization is found external to the suture, and is due to ectocranial periosteal deposition rather than synostosis of the internal sutural bone margins. Therefore, the term "periosteal bone bridge" is probably more appropriate than "sutural synostosis" or "craniosynostosis" when referring to this biological response.

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