Subtotal neonatal calvarieotomy

A radiographic and histological evaluation of calvarial and sutural redevelopment in rabbits


Department of Orthodontics, School of Dentistry, and Department of Neurological Surgery, School of Medicine, University of Washington, Seattle, Washington

A subtotal calvariectomy was performed on rabbits between 10 and 14 days of age. The animals were allowed to grow and were then sacrificed serially so that the sutural and skeletal redevelopment could be analyzed through a combination of gross, radiographic, and histological techniques. The results indicate that calvarial regeneration is a progressive process with a definite pattern and rate of development. During the regenerative process, bone was deposited both at the surgical margin and as islands within the surgical defect. The eventual approximation of these areas of ossification produced multiple fibrous articulations. The majority of these articulations were obliterated by bone union, except for the midsagittal, coronal, and metopic sutures, which were re-established in their appropriate anatomical positions. The maintenance of dural integrity during the surgical phase and the regeneration and establishment of pericranial continuity during the postoperative period were believed to be important in the re-estabishment of normal sutural and skeletal architecture.

Key Words • cranial suture • craniosynostosis • calvariectomy • periosteum • reskeletization

The cranial sutures are extremely adaptable fibrous articulations which allow the deposition of bone at the periphery of the calvarial bones in response to neural expansion during fetal and postnatal growth and development in humans. If one or more of these articulations become prematurely fused or synostosed before development of the brain is complete, the skull develops an abnormal shape. If all cranial sutures are prematurely synostosed, intracranial pressure (ICP) is greatly increased resulting in neurological deficits or in death. In order to alleviate the ICP, avoid the skeletal deformity, and re-establish functional sutures, clinicians have attempted various corrective surgical procedures. Methods include linear craniectomy bilateral to the affected suture, linear craniectomy and removal of the synostosed suture, removal of the suture and placement of a metal or cellulose covering over the adjacent bone margin, and a variety of partial craniectomy procedures followed by the reimplantation of autogenous morcellized fragments of calvarial bone. In the majority of these procedures, however, the cranium shows only a minimal improvement in shape and in many cases the affected suture will resynostose, requiring additional surgical intervention.

Recently, Hanson, et al.,4 reported their results following a subtotal calvariectomy procedure in which the synostosed calvaria of a newborn child was removed and a new calvaria was allowed to reskeletize without the implantation of morcellized fragments of bone. Since the surgical procedure eliminates the prematurely fused sutural articulations, Hanson, et al.,4 reported a subsequent remolding of the head to its normal shape. Furthermore, these researchers have provided clinical evidence that suggests that the calvaria and associated sutures redevelop in their proper anatomical relationship during the postoperative period.
Although previous researchers have attempted to document the radiographic and histological results of various types of calvariectomy procedures in animals, these investigators have not used the surgical procedure outlined by Hanson, et al.4 The purpose of the present investigation, therefore, is to duplicate the aforementioned procedure in the laboratory and to describe the long-term effects of subtotal calvariectomy on the redevelopment of the calvaria and associated sutures, using the rabbit as the experimental model.

Materials and Methods

Sixty male New Zealand white rabbits were used in the present study. Calvariectomy was performed on 30 rabbits between 10 and 14 days of age. Control animals of similar age comprised the remainder of the sample. Three control and three experimental animals were sacrificed at each of the following postoperative times: 1, 2, 3, 4, 6, 8, 12, 18, 26, and 39 weeks.

Experimental Protocol

The experimental animals were initially anesthetized with methoxyflurane. The operative area of the scalp was shaved and then disinfected with a benzylalkonium chloride antiseptic spray. A midsagittal incision was made and the scalp and underlying periosteum were reflected bilaterally. Initial penetration was through the left parietal bone with a sharp, discoid instrument. Extreme care was taken to avoid damaging the underlying dura. The surgical opening in the calvaria was then extended with small end-cutting ronguers to include a major portion of the parietal and frontal bones, midsagittal suture, metopic suture, and coronal suture. After a thorough irrigation of the surgical field with sterile saline, the periosteal flaps were reapproximated in the midline, and the scalp incision was closed with interrupted sutures. These were removed on the fifth postoperative day.

One control and one experimental animal from each group were given a 50 mg/kg injection of tetracycline hydrochloride 1 week prior to sacrifice and on the day before sacrifice. Animals younger than the 12-week postoperative group received intraperitoneal injections due to the small size of the blood vessels. The injections were administered intravenously to the animals examined 12, 18, 26, and 39 weeks postoperatively.

Postexperimental Examination

The animals were sacrificed according to the predetermained schedule. Following sacrifice, the specimen heads were removed and placed in 10% buffered formalin. One head from both the experimental and control groups was impregnated with a 0.5% aqueous silver nitrate solution to provide better radiographic visualization.6 The calvaria was then removed from each skull, radiographed, and divided into anterior, lateral, and posterior segments in a plane that allowed evaluation of skeletal and sutural healing. Histological sections were presented clear and stained alternately, using hematoxylin and eosin, Mallory's aniline blue collagen stain, alcian blue-periodic acid-Schiff's reagent, and Verhoeff's elastic stain.11-16

From those animals that received tetracycline injections, the calvariae were removed in a similar manner and radiographed from the same perspective. Each of these calvariae was divided anteroposteriorly parallel to the sagittal suture, and mediolaterally anterior to the coronal suture. Histological sectioning along these planes provided optimal evaluation of the rate of bone deposition at varying locations around the entire surgical margin. These calvarial segments were prepared as nondecalcified sections and were viewed under ultraviolet light to verify the presence and rate of bone deposition seen histologically.

The remaining specimens in each group were either stained in vitro with alizarin red S or prepared as dry skulls. These techniques provided additional information in three dimensions regarding reskeletization (bone formation and sutural redevelopment) of the calvaria that substantiated the histological and radiographic findings.

Results

Radiographic Findings

Minimal radiographic changes were observed at the surgical margins following 1 week of postoperative healing. However, a significant amount of bone deposition occurred during the second postoperative week. Bone was initially deposited at the surgical margin and also as islands of bone adjacent to this margin (Fig. 1 b). Little deposition occurred where the intact sagittal, coronal, and metopic sutures entered the surgical defect. This pattern of bone deposition continued during the third, fourth, sixth, and eighth postoperative weeks as the bone islands increased in size and began to coalesce with the ossifying surgical margin (Fig. 1 c).

From 8 to 18 weeks this pattern of reskeletization slowed down, and there was little decrease in the size of the surgical defect (Fig. 1 d). By 26 weeks postoperatively the calvaria was totally re-established. Sutures were apparent radiographically but difficult to trace throughout their entire length (Fig. 2 b); however, they were in their appropriate anatomical position compared to a similarly aged control animal (Fig. 2 a). At 39 weeks the bone of the calvaria showed no further changes. The sutures were not now visible radiographically in the experimental animals, while a definite sutural pattern existed in the control animals.

Histological Findings

A thin layer of unremodeled nonlamellar bone was observed at the surgical margin and also on the immediately adjacent ectocranial surface after 1 week of
postoperative healing. This newly deposited bone was arranged in isolated trabeculae directed toward the surgical defect (Fig. 3 a). The dura and pericranium which covered the exposed brain appeared thicker than the fibrous periostium covering the intact calvaria. At 2 weeks of healing the zone of newly deposited nonlamellar bone was significantly wider than that seen at 1 week. Trabeculae were observed on both ectocranial and endocranial surfaces, and were oriented toward the central surgical defect (Fig. 3 b). The deposition of bone at the endocranial margin appeared greater than at the ectocranial margin.

The isolated ectocranial bony trabeculae visible at 2 weeks were consolidated by intertrabecular bone deposition at 4 weeks of healing. The endocranial surface continued to demonstrate trabeculae similar to those seen after 2 weeks postoperatively. At this time interval bone formation appeared to be occurring more rapidly on the ectocranial margin than the endocranial margin. Although the ossifying surgical margin exhibited a significant amount of bone deposition at 4 weeks, the zone of unremodeled, nonlamellar bone was narrower than that observed after only 2 weeks of healing.

In isolated areas, the redeveloping bone margins closely approximated each other at 6 to 8 weeks of healing. Histologically, the soft tissue intervening between these ossifying plates of bone resembled a normal sutural ligament. A blunting and thickening of the bone margins occurred as the bones developed new sutures (Fig. 3 d). An isolated area of sutural fusion was seen in an unoperated portion of the left coronal suture in one animal 8 weeks postoperatively, whereas in the similarly aged control specimen none of the cranial sutures showed signs of fusion. The zone of unremodeled nonlamellar bone at the redeveloping sutural margins became narrower than that seen at 4 weeks of healing.

In isolated areas, the redeveloping bone margins closely approximated each other at 6 to 8 weeks of healing. Histologically, the soft tissue intervening between these ossifying plates of bone resembled a normal sutural ligament. A blunting and thickening of the bone margins occurred as the bones developed new sutures (Fig. 3 d). An isolated area of sutural fusion was seen in an unoperated portion of the left coronal
FIG. 3. Photomicrographs of rabbit calvariae, frontal sections. H & E. a: Experimental specimen at 1 postsurgical week. The newly deposited bone at the ectocranial surface was arranged in trabeculae (arrows) which were directed toward the surgical defect. B: brain; PB: parietal bone, × 62. b: Experimental specimen at 2 weeks. Medially directed bone trabeculae (arrow) are seen at both ectocranial and endocranial surfaces. Ossification is more advanced at the endocranial (EN) than at the ectocranial (EC) margin. PB: parietal bone. × 62. c: Experimental specimen at 2 weeks. A wide zone of newly deposited nonlamellar bone (NB) was evident at the redeveloping margin. A distinct boundary (arrow) was evident between the new bone and the previously existing remodeled calvaria (RB). B: brain. × 25. d: Experimental specimen at 12 weeks. The coalescence of developing bone islands resulted in the formation of several "functional sutures" (S), which histologically appeared similar. Resorption and perforations (arrows) of the ectocranial cortical plate were evident at 12 weeks. PB: parietal bone. × 25.

weeks. In the control specimen the medullary cavity appeared to be increasing in size through internal resorptive remodeling. However, the degree of internal remodeling in the experimental animal was significantly less than normal at 8 weeks of healing.

The zone of unremodeled nonlamellar bone at the redeveloping surgical margin was still narrower at 12 weeks of healing than in the 8-week group. The internal resorptive remodeling of the calvaria was close behind the advancing bone margin. A lack of continuity of the ectocranial cortical plate was evident in isolated areas at this age in both the experimental and control animals. However, the continuity of the endocranial cortical plate was maintained in both the control and experimental groups at 12 weeks postoperatively.

The calvaria was completely re-established by 26 weeks after the operation, including the reformation of sutures in their appropriate anatomical locations. Histologically they resembled the sutures of the control animal. An isolated area of sutural fusion was seen on the endocranial surface of the redeveloped right coronal suture of one animal, but a similar fusion was not observed in the control animal. At 26 weeks, the medullary cavity of both experimental and control animals had continued to expand relative to that seen at earlier postoperative intervals. The lack of continuity of the ectocranial cortical plate was also more pronounced (Fig. 3 d). In both control and experimental animals, fatty marrow completely filled the medullary cavity.

At 39 weeks of healing, the re-established sutures were fused along a majority of their length. No sutural fusion was observed in the control animals. The medullary cavities of both the experimental and control animals were similar to those seen at 26 weeks. The discontinuity of the ectocranial plate in both experimental and control groups was more extensive in the 39-week animals.

Discussion

The present investigation has clearly confirmed the osteogenic potential of the calvarial periosteum (dura mater and pericranium) following partial craniectomy in neonatal rabbits. Of perhaps greater significance, however, is the definite pattern of osseous regrowth
Subtotal neonatal calvariectomy

evident in this study. As the reskeletization process progressed, a "cross-shaped" soft-tissue area was consistently apparent within the central portion of the calvaria. The shape of this area corresponded with the normal anatomical positions of the midsagittal and coronal sutures. The defect progressively decreased in size but maintained a similar shape as the sutures were re-established in the experimental animals. This finding has received little attention in previous studies, but it is of importance in the understanding of normal calvarial development and subsequent sutural location. Embryologists have traditionally described the ossification of the rabbit cranial bones as a sequential deposition of bone originating from central areas and progressing toward one another. Pritchard, et al., claimed that the cranial sutures are established when the ossifying cranial bone margins come into close approximation during fetal development. During normal craniofacial development the anatomical position of these sutures is consistent. Previous experimental studies involving partial craniectomy in addition to intentional damage to the integrity of the dura caused the sutures to reform in abnormal locations. In the present investigation the dura was not damaged during the craniectomy procedure. Based on these collective findings, the maintenance of dural integrity during the surgical phase and the regeneration and establishment of pericranial continuity during the postoperative period are believed to be important in the re-establishment of normal sutural and skeletal architecture.

In the present investigation, the coalescence of bone islands evident after 18 weeks of healing resulted in the formation of multiple fibrous articulations. Histologically, all of these articulations resembled the normal sutures seen in the control animal. At 26 weeks, the bone margins that comprised the majority of these articulations had fused leaving only one patent frontoparietal, interparietal, and interfrontal suture. These sutures consistently appeared in their normal anatomical position relative to the underlying brain. This finding is in disagreement with those researchers who contend that the eventual location of a suture is not predetermined and raises several important questions. What factors influence the selective synostosis of several histologically similar "functional sutures"? Why is the surviving suture consistently located in the same anatomical position? Further research is needed to clarify these questions about sutural biology.

The rate of reskeletization of the calvaria in the present study tended to decrease as the ossifying bone margins came into closer approximation. The greatest amount of bone deposition occurred between the first and second weeks following surgery. This finding is substantiated by a significant increase in the width of the unremodeled nonlamellar bone deposited at the surgical margin during this time period. The zone of newly deposited nonlamellar bone progressively decreased in width between 2 and 26 weeks. A part of this decrease may be attributed to the normal age-related increase in the size of the medullary cavity. It is possible that another part of this decrease in depository activity is related to the neurocranial functional matrix. According to the functional matrix theory, the rate of calvarial growth is proportional to the volumetric increase in the neurocranium. In humans, approximately 70% of brain growth is completed by 2 years of age. Furthermore, Moss has reported that the growth of the brain of the rat is essentially complete at 34 days. Although no comparable information on the rabbit was available, it was estimated that approximately 85% of brain growth in the present study was completed by the fourth postoperative week. It is believed, therefore, that the expanding brain, through its influence on the overlying periosteum, plays a significant role in determining the rate of calvarial regeneration following partial craniectomy in the neonatal rabbit.

At 39 weeks, the midsagittal suture in the experimental animal exhibited histological evidence of sutural fusion along the majority of its length. In the control animal, sutural fusion did not occur in any of the cranial sutures during the experimental period. Several investigators have documented the age at which sutural fusion normally occurs in human cranial sutures, with postsurgical clinical observations in patients. However, previous researchers have not clearly determined the factors that promote normal age-related sutural synostosis, and therefore, the reason for earlier fusion of the redeveloped cranial sutures in the present study is unknown. Since the greatest increase in brain size occurred while the redeveloping sutural margins were widely separated, the influence of synostosis taking place earlier than normal on the size and final morphological configuration of the healed adult calvaria appeared to be significant.

In contrast with calvarietomized human infants, the present study involved animals without known calvarial or sutural abnormalities. However, the changes documented in these animals are consistent with postsurgical clinical observations in patients. Although histological evidence in humans is lacking, the present findings support the rationale for the subtotal calvarietomy procedure. The location of the cranial vault sutures is determined by factors that are present not only during embryogenesis and fetal life but also postnatally. Whether these factors are mechanical or due to local biochemical changes is unknown. Further studies are planned to identify the factors that lead to the formation of a suture and play a role in premature sutural fusion.

Summary

A craniectomy procedure was performed on 30 New Zealand white rabbits aged between 10 and 14 days. The animals were allowed to grow and were then...
sacrificed serially so that the sutural and skeletal redeveloped could be analyzed through a combination of gross, radiographic, and histological techniques. Based on a comparison with similarly aged control specimens, the following conclusions are proposed:

1. The expanding brain, through its influence on the overlying periosteum, may play a significant role in determining the rate of calvarial regeneration following partial craniectomy in the neonatal rabbit.

2. Although the redeveloped sutures in the present study exhibited earlier than normal synostosis, the resultant effect on the final morphology of the redeveloped calvaria was insignificant due to the normal age-related diminution in the rate of brain expansion.

3. The maintenance of dural integrity during the surgical phase and the regeneration and establishment of pericranial continuity during the postoperative period are believed to be important in the re-establishment of normal sutural and skeletal architecture.

Acknowledgments

The authors wish to express their gratitude to Dr. Lewis H. Tarrant for help during the experimentation; to Dr. A. C. Brown for use of the experimental facilities; to Dr. Leena Koskinen-Moffett for reviewing the manuscript; and to Mrs. Livia Molnar, Mrs. Vonnie MacDonald, and Mr. James Clark for their technical assistance.

References

15. Molnar LM: Modification of Verhoeff's elastic stain for sections from tissue double embedded with nitrocellulose and paraffin. Histo-Logic V:64, 1975c

This research was supported by the United States Public Health Service Research Grants DE-02918 and DE-02931, and by the University of Washington Orthodontic Memorial Fund.

This article is based on research submitted by Dr. Mabbutt in partial fulfillment of the requirements for the Master of Science in Dentistry degree, Department of Orthodontics, University of Washington.

Address reprint requests to: Vincent G. Kokich, D.D.S., M.S.D., Department of Orthodontics, University of Washington School of Dentistry, Seattle, Washington 98195.