Roentgen stereophotogrammetric analysis of neurocranial suturectomy in rabbits

PER ALBERIUS, D.D.S., M.D., GÖRAN SELVIK, M.D., AND LEIF EKELUND, M.D.
Departments of Anatomy and Diagnostic Radiology, University Hospital, Lund, Sweden

This investigation was conducted to further elucidate both the significance of a calvarial suture and the compensatory ability of the cranial vault. Four-week-old male New Zealand White rabbits were subjected to unilateral or bilateral extirpation of the coronal suture after insertion of metallic markers, and were then followed regularly by roentgen stereophotogrammetry until age 21 weeks. Bilateral extirpation of the normal coronal suture resulted in a dramatically increased initial rate of bone separation, which tended to remain supranormal for the rest of the investigation. Unilateral suturectomy showed differences in growth between the sides, the operated side initially separating significantly more than the other. Volumetric calvarial growth in rabbits with unilateral extirpation terminated similar to that in control animals, while volumes in rabbits with bilateral extirpations constantly exceeded control volumes, finally exceeding these by 65%. Responses at intact sutures confirmed the compensatory capacity of cranial vaults. The results indicate that the passive longitudinal and volumetric cranial vault bone growth responds quickly to growth disturbances, thereby demonstrating its plasticity, and that the neurocranial suture is a restraining and modulating component in cranial growth.

KEY WORDS: cranial suture, metallic implant, roentgen stereophotogrammetry, skull growth, suturectomy, craniosynostosis, brain growth

Advances in cranial surgery have stimulated interest in the nature of neurocranial sutures. These extremely adaptable fibrous articulations allow bone deposition in response to neural expansion during pre- and postnatal growth. The effect of prematurely terminated bone separation at the calvarial suture, whether primary or secondary, is clearly demonstrable in craniosynostosis, resulting in abnormal skull shapes. By early surgical re-creation of sutures, cranial form is often improved dramatically.

Studies to gain new knowledge of suture function have been performed by many means, one being extirpation of sutures. This operation has been utilized to confirm whether location of sutures is predetermined or not, and to study the growth potential of suture tissue, sutural morphology, dural bone regeneration, periosteal regenerative capacity, and embryonic sutural behavior. Much attention has also been focused on the influence of sutures on craniofacial development. Removal of various facial and neurocranial sutures has given divergent experimental results, with no or slight growth disturbances. These conflicting views call for new investigations performed with highly accurate techniques to elucidate the function of and interactions between sutures. This information is essential to optimize the extent of surgical intervention in cases of suture disturbance. The purpose of this investigation was therefore to clarify biometrically the effects of the absence of a neurocranial vault suture per se and on adjacent intact calvarial sutures, as well as on calvarial volumetric changes, in the hope of improving the design of neurocranial operations.

Materials and Methods

Experimental Animals

We used 20 male New Zealand White rabbits (10 in a control group and 10 in the experimental group), approximately 4 weeks old (32 ± 2 days). The animals were kept at 20°C (40% relative humidity) with light from 6 a.m. to 6 p.m. Standard food pellets and water were provided ad libitum. The rabbits were followed until 21 weeks (146 ± 7 days) of age since, according to Engdahl, a leveling off in their growth curve is shown at the age of 5 months.
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Operative Techniques

The animals were anesthetized by intramuscular neuroleptanalgesia, fluanisone 10 mg/ml and fentanyl 0.2 mg/ml, in a dose of 0.6 ml/kg body weight (Hypnorm vet.). The rabbit's head was shaved, after which an antiseptic solution (alcoholic chlorhexidine, 5 mg/ml) was applied over the operative area. The surgical site was then exposed by a paramidline incision from the parietal to the nasal region. The scalp and underlying tissues were reflected laterally and the calvarial sutures identified (Fig. 1). Spherical tantalum bone markers were implanted as illustrated in Fig. 2. Thereafter, 7 mm of periosteum on each side of the coronal suture was extirpated, and the suture, including 5 mm of adjacent parietal and frontal bone, was removed with a burr mounted on a dental drill.

Six bilateral coronal suturectomies extending to and including the adjacent part of the temporal bone, and four unilateral extirpations on the left side extending slightly across the midline, were made (Fig. 3). Extreme care was taken to avoid damaging the underlying dura. Bone wax was fixed to the bone edges and hemostasis secured. Zenker's modified solution (without glacial acetic acid) was applied to the dura with cotton swabs for 1 minute, avoiding the superior sagittal sinus, to retard bone regeneration. After copious irrigation of the surgical field with sterile saline, Nebocetin antibiotic powder (neomycin 0.33 gm, bacitracin 25,000 IU) was sprinkled over the wound. The incision was closed with 4-0 Ethicon sutures, and the surgical site finally covered with Nobecutan spray. The animals were left to recover on a warm electric blanket. The postoperative period was uneventful.

Roentgen Stereophotogrammetric Analysis

A method of roentgen stereophotogrammetric analysis has been developed to enable precise skeletal measurements in patients or experimental animals. This
method requires the implantation of metallic markers and certain roentgen calibration equipment in which the subject is examined stereometrically (Fig. 4). The equipment (a glass cage with markers of known internal positions, lying in two parallel planes), defines a three-dimensional (3-D) laboratory coordinate system, enabling determination of the subject’s markers as derived by computer calculations based on measurements from the exposed films. The two roentgen tubes with simultaneous exposure were positioned with an approximate focus-to-focus distance of 0.4 m and a focus-to-film distance of 1.0 m. Exposure data for a rabbit cranial vault examination were 48 to 51 kV and 4 to 16 mA depending on age.

Methodological accuracy has previously been determined. Complete reexaminations of differently positioned rabbit skulls were performed, exhibiting a technical error of 21.0 μm for growth measurements. Correspondingly, an error of 0.48% has been obtained for volumetric calculations.

Stereoroentgenograms were obtained on the day of implantation and at weekly intervals until age 10 weeks, thereafter at 12, 14, 16, and 21 weeks. Weight control was carried out at each examination.

**Analysis of Data**

The films were digitized in a precision instrument for aerial photogrammetry (Wild Autograph A8). The 3-D coordinates of the bone markers were obtained by computer processing and further analyzed by computer to ascertain volumetric as well as distance changes. Volume was calculated from a polyhedron with corners at all markers except the nasal bone markers, constructed as the closest to a convex polyhedron in a specific sense from the given marker configuration. Growth rates (means of left and right sides) were related to a mean day in the investigation interval and expressed in μm/day; volume changes were given as a percent of the initial volume.

To reduce the influence of technical errors (see below) on growth rate values, the coronal suture was observed for at least 2-week intervals. The sagittal suture, whose growth between succeeding measurements approached the technical error, was analyzed only for Weeks 4, 7, 12 and 21. The variations in numbers of animals in each analysis were due to non-optimal implantation procedures, which in some cases resulted in marker losses or oblique localization across a suture; thus, a few analyses were impossible to undertake. Also, some rate values were excluded to avoid influence from unrepresentative growth values, such as due to major illness (as determined by weight loss).

For reasons of simplicity the sutures studied are referred to as calvarial or cranial vault sutures, despite both the internasal and frontonasal sutures being connected to the rabbit’s facial skeleton. Statistical analysis was performed using the Student’s t-test, the results being presented in the diagrams. Since no specific hypothesis of differences between experimental groups was tested, only comparisons of the experimental groups versus controls were considered.

**Macroscopic Investigation**

At the termination of the experimental period, the degree of bone regeneration was controlled post mortem under a dissecting microscope. Dorsal radiographs were analyzed by protractor to determine whether snout deviation from the neurocranial midline was present. Any deviations were recorded to the nearest one-half of a degree in absolute figures. The mean total growth of sutures over the entire investigation period was used to compare growth at different sutures.

**Results**

**Macroscopic Findings**

The originally wide coronal suture extirpations remained mostly unossified. In all animals, these were covered by dense fibrous tissue without observable islands of bone. Generally, rabbits with unilateral resection exhibited bone growth from the extirpated margins to a greater degree than did rabbits with bilateral extirpation, especially adjacent to the temporal bone. The extirpation defects were bordered by protruding bone spicules, their long axes mainly oriented along the
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anteroposterior axis of the vault; the parietal and frontal bones appearing to react similarly. Overall morphology of unoperated parts of the relevant bones and sutures were comparable to that in the control group. The bone tissue seemed unaffected by the presence of tantalum markers, and the bone covering the markers appeared similar to adjacent areas. Marker loss was rare, and was almost completely restricted to the nasal bones.

Lateral deviation of the snouts varied in rabbits with unilateral extirpation from 1.0° to 2.0° (mean 1.6°) and in rabbits with bilateral extirpation from 1.0° to 1.5° (mean 1.1°). These ranges were similar to those in the control group (0° to 2.0°, mean 1.1°).

Frontonasal Suture

A pattern of rapid, almost linear, growth deceleration was observed in the frontonasal suture (Fig. 5). This was the site of fastest bone separation in the rabbit calvaria during the observation period. Initially, bone separation tended to slightly exceed control values in animals with bilateral extirpation, while those with unilateral extirpation showed somewhat subnormal rates of separation. Contrary to the control group, the fall in growth rate for rabbits with bilateral suturectomy tapered off during the last part of the investigation.

Coronal Suture

Generally, anteroposterior growth at the coronal suture was less pronounced during the experimental period as compared to the frontonasal suture (Fig. 6). Throughout the greater part of the investigation, animals with bilateral extirpation showed marker separation significantly exceeding that in the control and animals with unilateral extirpation, except in the final period when both unilateral and bilateral suturectomy resulted in marker separation grossly exceeding control values. Otherwise, growth rates in animals with unilateral extirpation nearly paralleled control values, the extirpated side initially exceeding the unoperated side.

Sagittal Suture

Despite fewer evaluations, a distinct pattern of growth deceleration at the sagittal suture (the internasal, interfrental and interparietal suture) could be detected (Fig. 7). The nasal bones were found to separate at a rate 1.9 to 5.9 times greater than the frontal or parietal bones during the whole observation period. Nevertheless, growth rates (and hence the shape of the curves of the three sections) were similar. Only a significantly reduced initial growth rate at the interfrental suture was observed following unilateral extirpation. Thus, coronal suturectomy only slightly changed sagittal suture growth.

![Fig. 5. Mean frontonasal suture growth rates. Bi = bilateral coronal suturectomy (10 sides, with non-optimal marker implantation excluded); Uni = unilateral (four extirpation sides); and c = controls (17 sides). Standard error of the mean is indicated by brackets. Significance levels (Student t-test): * = p ≤ 0.05; ** = p ≤ 0.01.](image_1)

![Fig. 6. Mean growth rates of coronal sutures. Bi = bilateral suturectomy (12 sides); Uni-e = unilateral suturectomy (four extirpated sides); Uni-no = unilateral suturectomy (three unoperated sides); c = controls (20 sides). significance levels (Student t-test): * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001. Standard error of the mean is indicated by brackets.](image_2)
Neurocranial Volume

The neurocranial (polyhedron) volume of control animals showed a tendency to increase slowly (Fig. 8). A slight fluctuation was observed for the different intervals during the whole observation period, ranging from 1.8% to 4.2% of the preceding volume. A minor relative increase was observed during the last part of the investigation. The expected leveling off in brain growth was apparently not followed by a reduction in the calvarial volumetric increase during the span of this investigation.

A similar initially elevated volumetric increase was observed for both experimental groups (Fig. 8). Therefore, bilateral extirpation produced a constantly elevated near-parallel increase compared to control values, finally rising beyond controls by approximately 65%. On the other hand, expansion following unilateral extirpation conformed to control values at the end of the investigation. The irregularity of the control curves was duplicated in the experimental groups. However, in animals with bilaterally extirpated sutures, neurocranial volume was observed to reach a plateau.

Mean Total Longitudinal Growth

Bone separation at the coronal suture was significantly increased by bilateral extirpation, as was the cranial vault width (Table 1). Unilateral suturectomy resulted in only minor deviations, and sagittal suture growth in both groups was not significantly affected.

Discussion

Bone Regrowth

To delay osseous regrowth in the extirpation defect and thereby minimize interference with bone separation, the calvarial peristome (dura mater and pericranium) was manipulated. The outer peristome was excised about 2 mm distant around the suturectomy, while modified Zenker’s solution (glacial acetic acid excluded) was applied to the dura. Anderson and Johnson first introduced this tissue fixative in treatment of craniosynostosis in order to minimize the risk of premature resynostosis.

Today there is little doubt that not merely cells of the outer calvarial peristome but also those of the dura exhibit osteogenic potential. The importance of the dura during normal development and bone redevelopment has been confirmed by many experimental investigations and various clinical reports on craniosynostosis. There has always been concern that the dural application of Zenker’s solution might damage the underlying cerebral cortex. This has now been experimentally verified in the cat and dog. In man, this manifests itself by seizures or neurological deficiencies. By using

Fig. 7. Mean growth rates of sagittal sutures. Left: Internasal sutures. Bilateral (bi) suturectomy (five sides); unilateral (uni) suturectomy (two sides); controls (c, six sides). Upper Right: Interfrontal sutures. Bilateral (six sides); unilateral (four sides); controls (c, 10 sides). Lower Right: Interparietal sutures. Bilateral (six sides); unilateral (three sides); controls (10 sides). Significance levels (Student t-test): * = p ≤ 0.05. Standard error of the mean is indicated by brackets.
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### TABLE 1

<table>
<thead>
<tr>
<th>FeatureMeasured</th>
<th>Unilateral</th>
<th>Bilateral</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td>Suture Extirpation</td>
<td>Growth Rate (µm/day)</td>
<td>No. of Animals</td>
<td>Growth Rate (µm/day)</td>
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<td>internasal suture</td>
<td>16.6 ± 1.5</td>
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<td>13.1 ± 1.8</td>
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<td>frontonasal suture</td>
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<td>4.9 ± 1.8</td>
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<td>41.6± ± 2.0</td>
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<td>interparietal suture</td>
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<td>5.3 ± 1.2</td>
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<tr>
<td>cranial vault width</td>
<td>17.1 ± 4.8</td>
<td>3</td>
<td>29.0± ± 3.0</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the mean. Significance levels (Student t-test): * = p ≤ 0.001; ** = p ≤ 0.05.

**Longitudinal Cranial Vault Growth**

When studying specific sutural reactions after suturectomy, adhesion to adjacent tissues and structures that may interfere with succeeding bone separation must be considered. As to the rabbits' frontonasal suture, Roskjaer, Freng, and Babler, et al., all commented on the results of Selman and Sarnat, who extirpated this suture without any observed changes in growth. Roskjaer explained this as due to maintained contact between the frontal and nasal bones through the ethmoturbinals, and Freng supposed the unaffected growth to depend on a strong connection between the suture and the maxilla. Babler, et al., on the other hand, considered that Selman and Sarnat had used animals that were too old. Additionally, they remarked that, although the coronal suture responds primarily to the increasing brain mass, the frontonasal suture is also influenced by midfacial growth. Thus, to minimize interference on the experimental area, the coronal suture was selected in the present study. This suture, when growing, provides the advantages of being influenced mainly by one functional matrix, and no restricting tissue unions seem to exist. Suture function could thereby be more accurately investigated.

After bilateral coronal suturectomy, the frontal and parietal bone separation showed a significantly accelerated initial pace, followed by an increased rate for most of this investigation, as compared to control values. This agrees with some previous observations; however, the latter generally exhibited higher growth values. These values can partly be explained by lacking compensation for geometric magnification; intrastrian and sex differences should also be considered.

Since complete removal of the coronal suture entailed bilateral removal of the adjacent parts of the temporal bone, lateral restrictions in respect to the frontal and parietal bones were eliminated. This elimination was manifested by increased cranial vault width. Changes of symmetry, on the other hand, were not observed. The average nasal deflection obtained was identical to our controls, and was similar to the findings of others, including Alhopuro (1.3°), and Bardach, et al. (0.98°).
The highly accurate method of roentgen stereophotogrammetric analysis also enabled the detection of increased bone separation on the extirpated side immediately after unilateral suturectomy, accompanied by a slightly increased snout deviation.

Presumably, these results express a liberation of biomechanical stresses due to neural mass growth. Thus, the suture tissue proper is a restraining component during normal growth, and has a modulating effect on skull growth. Furthermore, this supports the passive nature of cranial vault growth, pointing to the importance of full surgical release of fused sutures and an exact positioning of surgically created sutures in cases of synostosis so as to obtain optimal postoperative cosmetic results.

So far, only Levine, Persson, et al., and Babler, et al. have investigated removal of the normal rabbit coronal suture. Only Babler, et al., have reported on subsequent sutural interaction in parts of the rabbit cranial vault. Disturbance of normal skull growth is known to alter growth not only at the site of disturbance but also at adjacent “normal” sutures. We found that the remaining intact calvarial sutures showed somewhat different behavior, particularly when following bilateral suturectomy. The initial decrease of interfrontal suture growth in animals with unilaterally extirpated sutures demonstrated that even slow-growing vault sutures are sensitive sites of compensation. Macroscopically, though, skull morphology remained similar to that in control rabbits, indicating other sutural areas might have adjusted to the local overgrowth.

By its changes in growth rate, the frontonasal suture seems to be correlated with neural mass expansion. It can be speculated that the registered changes in calvarial growth pattern are due to spatial rearrangements of cranial bones which redirect brain growth, in combination with a larger capacity for calvarial adjustment due to its association with the later-maturing facial structures. Interestingly, Babler, et al., found that bilateral suturectomy caused reduced growth at the frontonasal suture; however, we noticed an initial stimulation of growth. These divergent results may be explained by variations in animal health. This is further discussed in a companion article.

It has recently been suggested that the responsive variations to diverse influences at various sutures may reflect suture-specific differences in reaction to disturbances of normal growth relationships. Considering the divergent responses observed between our experimental groups, this investigation tends to support the suggestion that vectorial changes in neural tissue expansion regulate the individual suture response.

**Volumetric Growth**

This is the first time the volumetric growth of a representation of the calvaria has been followed with great accuracy. To obtain a volume as representative as possible for cerebral volume, markers were introduced into the temporal bones. The nasal bone markers were subsequently excluded from the calculations. In control animals, the volumetric growth rate fluctuated continuously without any tendency to diminish during the observation period. The lack of initial volumetric increase may be attributed to the surgical trauma at bone marker implantation.

Both suturectomized groups showed a marked initial volumetric increase, presumably an effect of decreased sutural restraint. Following an irregular course, growth in unilaterally suturectomized animals culminated in a final volume similar to control values. On the other hand, a dramatic near-linear volumetric increase was observed following bilateral extirpation. This paralleled control values and finally reached a plateau, which was not observed in control animals during the same period. However, this increase in calculated volumes might be partly due to changed external morphology, giving a higher calculated polyhedral volume without a corresponding brain case increase. This is plausible, as neural tissue growth is considered to be almost completed in rabbits at 21 weeks, when this investigation ended.

Subsequently, as the intracranial volume is presumably increasing only minimally, a rearrangement of neurocranial bones may take place. This would imply a rotation of the cranial vault bones. By kinematic analysis, continuous rotations of rabbit calvarial bones were demonstrated during this interval, despite the successive diminution of longitudinal growth.

This may indicate that animals with bilaterally extirpated sutures reached the expected final spatial bone arrangement sooner after liberation of expansive forces. This suggests that spatial adjustments were more easily accomplished, and further supports Persson’s theory concerning the restricting capacity of structures associated with a suture. This possibility strongly emphasizes the necessity for careful planning of cranial surgery involving sutural manipulation during infancy, in order to avoid interference with normal neurocranial development due to excessive liberation of sutural restricting forces.

**References**

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Address reprint requests to: Per Alberius, D.D.S., M.D., Department of Anatomy, University of Lund, Biskopsgatan 7, S-223 62 Lund, Sweden.

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