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Experimental alteration of the coronal sutural area: a histological and quantitative microscopic assessment

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INTRODUCTION

The growth of the calvarium is an integrated process of osteogenesis and of proliferation of intervening soft tissues within the originally membranous cranial capsule. A consideration of the functional role of the soft tissues or sutures separating the osseous elements of the calvarium presents several problems of major importance. One of these is the extent to which the shape of an individual calvarial bone is predetermined by the location of its sutures. As well, the mechanism of normal sutural growth and the determinants of normal sutural morphology and their behaviour under experimental alteration have not been adequately explained.

In the past the sutures have been thought to be sites of primary expansive growth of the flat bones of the vault (Weinmann & Sicher, 1955). This was denied by Scott (1967) and more recently Moss (1962) and Moss & colleagues (1960, 1969) have propounded the hypothesis that it is the growth of the 'functional matrix' (i.e. those tissues supported or protected by functionally related skeletal elements) which furnishes the primary growth force, the bones and the sutural tissues responding secondarily. Extirpation of various calvarial sutural areas by Moss (1954) and Selman & Sarnat (1957) without appreciable effects on skull morphology, stresses the secondary nature of sutural growth. A strong relationship between detailed sutural morphology (bevelling and interdigitation) and the different velocities and directions of bone growth in cranial sutural areas has been demonstrated by Isotupa, Koski & Makinen (1965) using alizarin red vital staining techniques: while an examination of gross sutural patterns by Moss (1961) after temporal myelectomy led him to conclude that 'extrinsic' factors such as muscle activity secondarily both produce and preserve the final moulding of bones in sutural areas. The mechanism of sutural changes occurring after implantation of growing tissues into the brain (Moss, 1957) or after skull damage (Girgis & Pritchard, 1957, 1958) has not been fully explained. For this reason, a study of the behaviour of sutural areas under normal and experimental conditions has been undertaken using histological, quantitative vital staining analysis and accurate photographic records of gross morphology.

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Table 1. *Animal groups for vital staining and histology*

Animal groups (vital staining)

Group	Plane of sectioning	Age postnatal (days)	Number of animals
Control	Sagittal	6	7
Control	Coronal	6	6
Repositioned	Sagittal	5	7
Extirpation	Sagittal and coronal	4	7
Incision	Sagittal and coronal	5	6

Animal groups (histology)

Group	Plane of sectioning	Age at sacrifice postnatal (days)	Number of animals
Control	Sagittal	4, 10, 23, 38	12
Experimental (repositioned)	Sagittal	4, 10, 23, 38	11

MATERIALS AND METHODS

Twenty five normal and 31 experimental Long–Evans strain male rats were divided into five groups: a control sagittal, control coronal, incision, extirpation, and a repositioned group. Subgroups of the control sagittal and repositioned groups were used for histological examination at 4, 10, 23 and 38 days. Using a multiple bone marking technique (Cleall, Perkins & Gilda, 1964), beginning at five days postnatally ± one day, all other groups received intraperitoneal injections of vital stains on the 1st, 8th, 18th and 28th experimental days and were killed on the 42nd experimental day. Table 1 lists these groups.

The surgical procedures at the left coronal sutural area used to create the experimental alterations are illustrated in Fig. 1. Group III had part of the sutural area extirpated involving both parietal and frontal bony margins. Group IV had a portion of the sutural area incised, detached from the underlying periosteum and repositioned. In Group V, a three-sided incision was made leaving the posterior parietal margin intact.

The method of analysis included examination of dried skulls using photographic records obtained with a standard 28 inch skull-camera separation. The records were printed on dimensionally accurate photographic paper (McKeown, 1972). Histology and quantitative vital staining analyses were carried out. In the latter technique, incremental measurements of bone growth at specific sites in both sagittal (Fig. 2) and coronal planes (Fig. 3) were subjected to statistical appraisal and the left and right side differences were compared between groups.

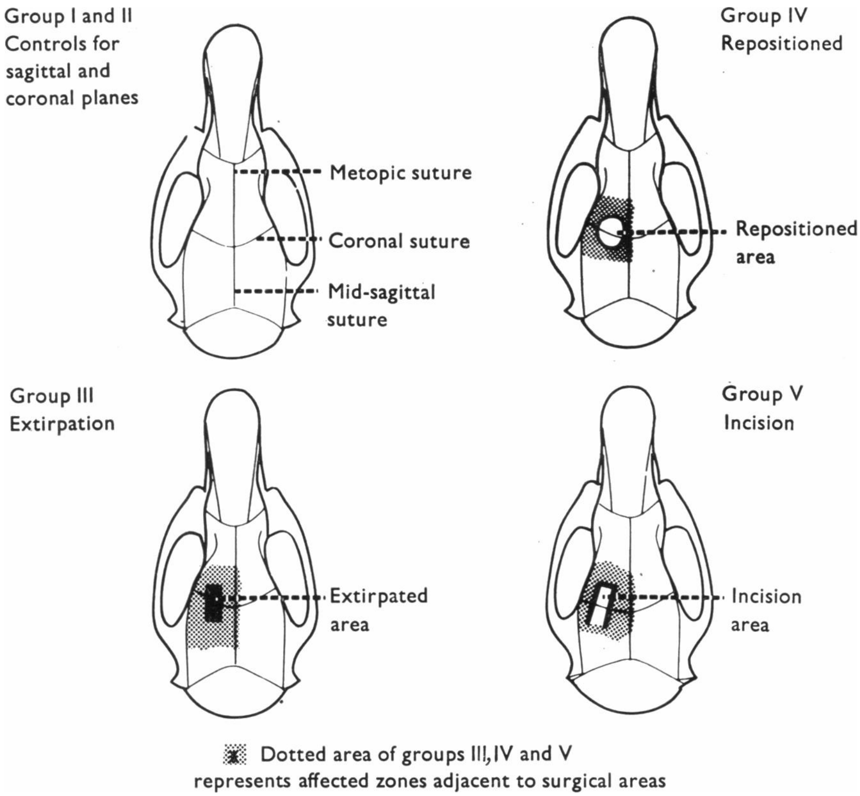


Fig. 1. Dorsal view of rat calvaria outlining animal groups.

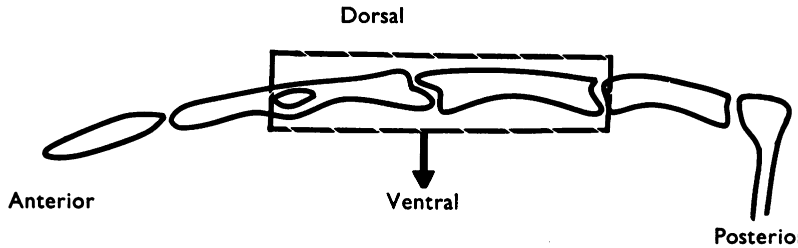
RESULTS

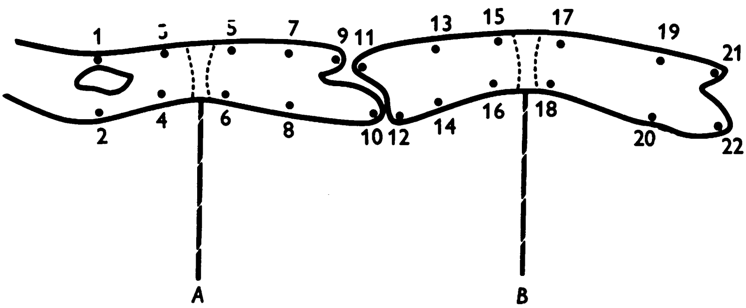
Examination of standardized photographs of crania control group

The normal coronal suture showed extensive interdigitations which appeared wider laterally and narrower medially, while both the metopic and mid-sagittal sutures had either smooth opposing margins or presented slight interdigitations.

Incision group

Complete bony union of the anterior incision in the frontal bone but only partial union of the lateral incision areas occurred (Fig. 1). The coronal sutural interdigitations were maintained. The bone margins in the incision area were elevated relative to adjacent bone surfaces: this effect will be referred to as 'marginal lipping'. Apparent sutural deviations to the non-operated side were observed at the metopic and mid-sagittal sutures close to bregma while the coronal suture medial to the incision area (Fig. 1) demonstrated a posterior relocation relative to the contralateral side.





A–B missing in group III

A cut bone edge in group V

A and *B* cut bone edges in group IV

Fig. 2. Linear sites for each of four sagittal planes studied (two on each side of the mid-sagittal plane).

Repositioned group

The repositioned segment was isolated from the adjacent calvarium by a fibrous tissue barrier and the coronal suture within this area lost its interdigitations resulting in a smooth-line articulation. All cut surfaces showed marginal lipping and the deviations in sutural locations were similar to the incision group.

Extirpation group

No repair of the extirpated area occurred and the marginal lipping and sutural deviations adjacent to the surgical area were similar to other groups.

Histology

Four day coronal sutural area

The control coronal sutural area at four days (Fig. 4) showed overlapping (more laterally than medially) of opposing bony margins. Active osteogenesis was occurring as evidenced by the clusters of osteogenic cells at the projecting bone margins (small

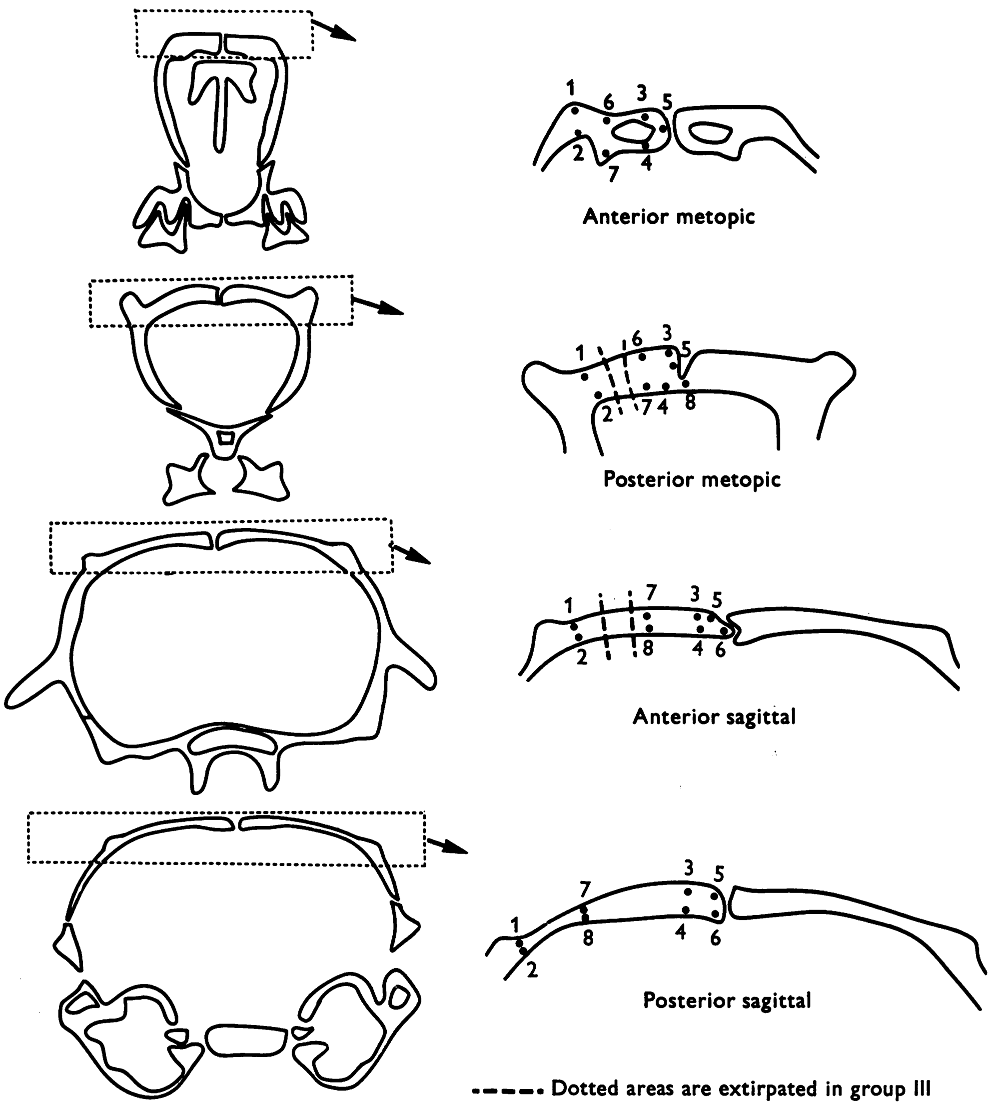


Fig. 3. Linear sites for each of four coronal planes studied.

arrows) and on the marginal surfaces (A). Open diploic spaces were noticed at the bone margins (D). The fibres of the intervening sutural layers between the opposing bony margins were predominantly parallel to the calvarial surfaces, and a close continuity existed between the intervening layers and the denser periosteal or uniting layers which extended over the superior and inferior aspects of the sutural area.

The isolated coronal sutural area immediately after surgery showed no apparent differences from the control.

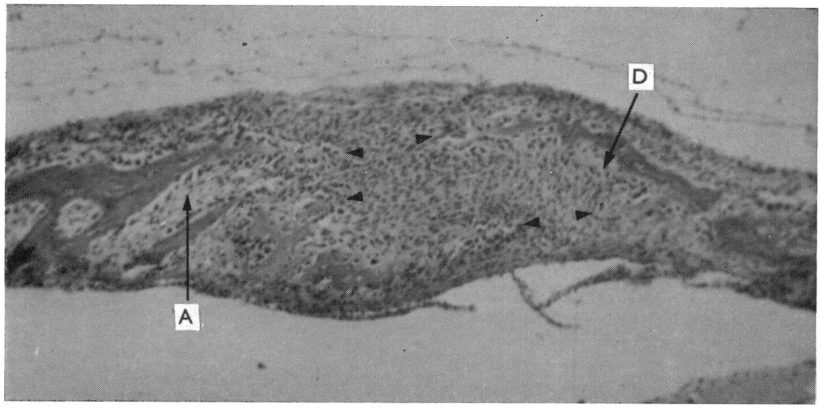


Fig. 4. Four day control coronal sutural area (lateral aspect). Left: frontal bony margin. Right: parietal bony margin.

Osteogenesis is occurring at sutural margins (small arrows) and adjacent surfaces (A). Note open primary marrow spaces (D). H and E. ×80.

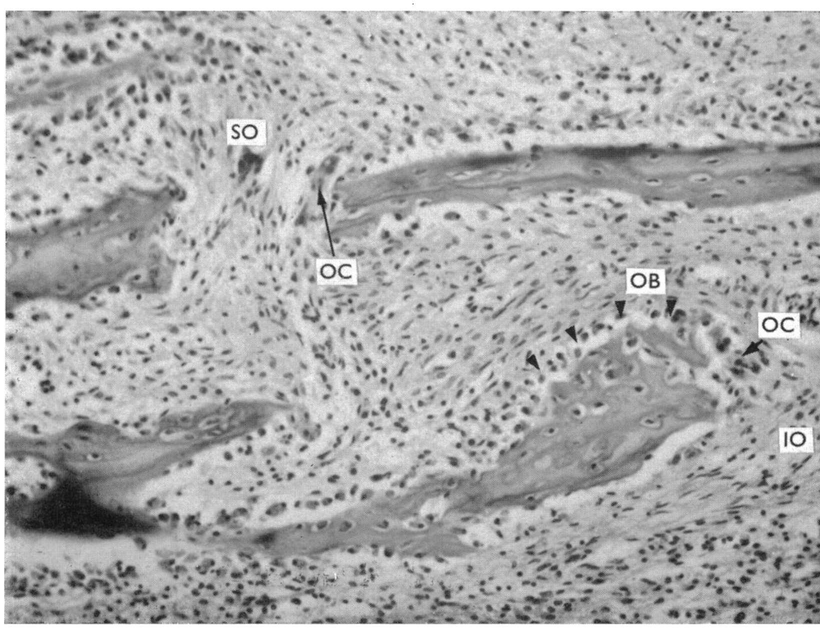


Fig. 5. Ten day repositioned coronal sutural area. Left: frontal bone. Right: parietal bone. Note perpendicular fibre orientation at superior and inferior sutural openings (SO, IO), osteogenic zones (OB), and osteoclasts at ends of bone margins (OC). H and E. ×200.

Ten day coronal sutural area

There was greater overlapping of the opposing margins in the control at ten days, and although the suture was more fibrous than at four days the direction of fibre orientation remained the same.

The repositioned segment was separated from the intact calvaria by inflammatory tissue which, at the sutural area, seemed to be limited to the superior calvarial

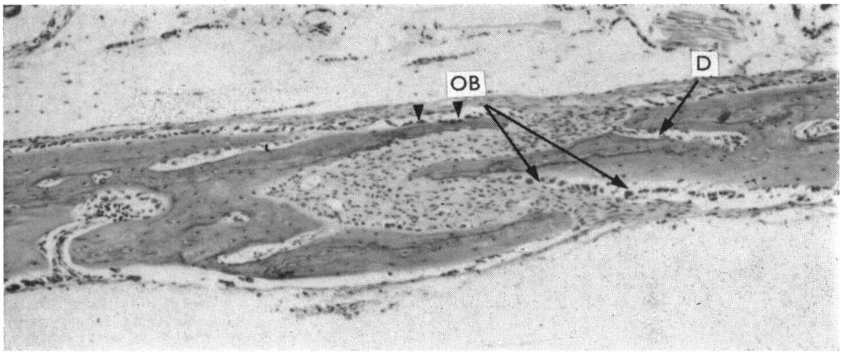


Fig. 6. Twenty three day control coronal sutural area (lateral aspect). Left: frontal bone. Right: parietal bone. Note shift in concentration of osteogenic cells to margin surfaces (OB) resulting in increased thickening of margins. Open marrow spaces (D) present but not conspicuous. H and E. × 80.

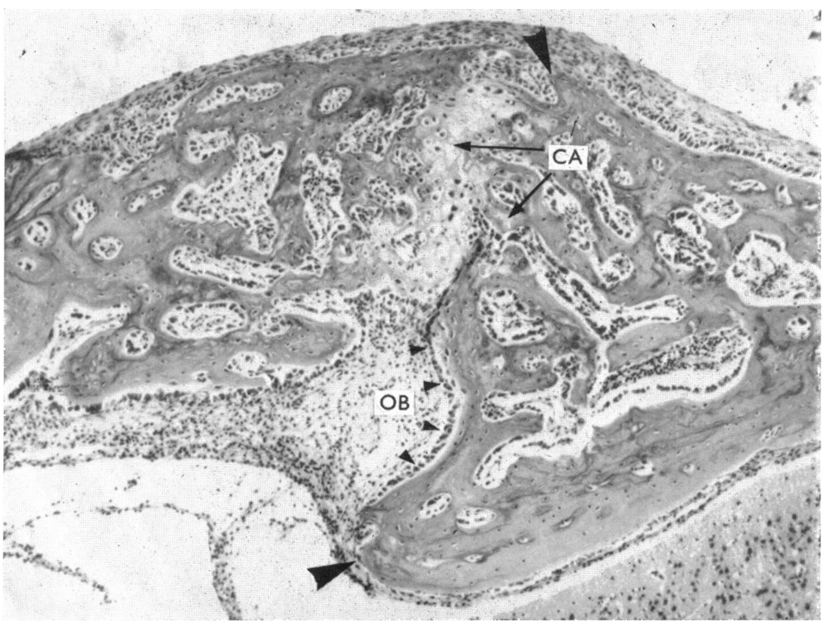


Fig. 7. Twenty three day repositioned coronal sutural area. Note cartilaginous fusion (CA), osteogenic areas within suture (OB) but little evidence of ossification at bone ends (large arrows). H and E. × 80.

surface. The overlapping of the opposing sutural margins (Fig. 5) was still present but they appeared more closely approximated. The direction of osteogenesis at the sutural area had shifted in favour of the margin surfaces (OB) rather than their projecting ends so that an increased thickness of these margins was occurring. This may account for their closer approximation. Resorptive remodelling, as shown by the presence of osteoclasts, was taking place at the ends of the opposing margins (OC). Fibre orientation at the superior (SO) and inferior (IO) sutural openings was

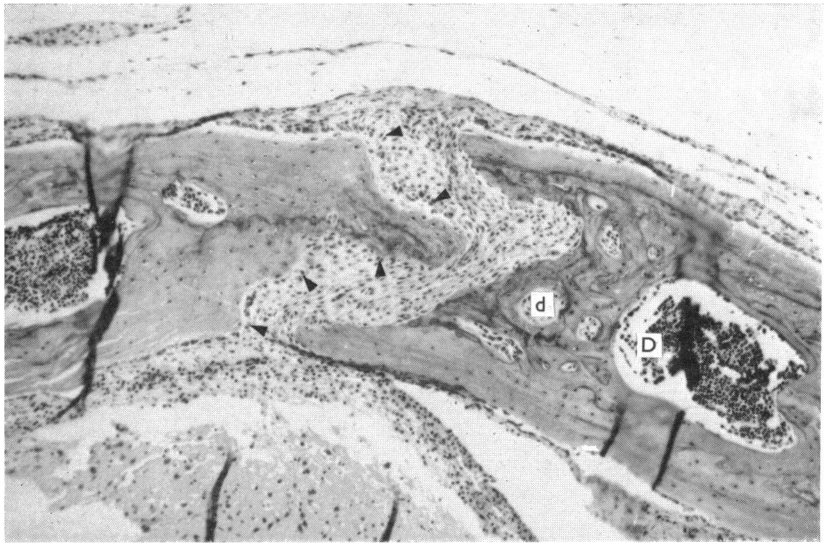


Fig. 8. Thirty eight day repositioned coronal sutural area. Minimal osteogenesis occurring within suture (small arrows) with increased number of diploic spaces (d) at margins and adjacent areas (D). H and E. ×80.

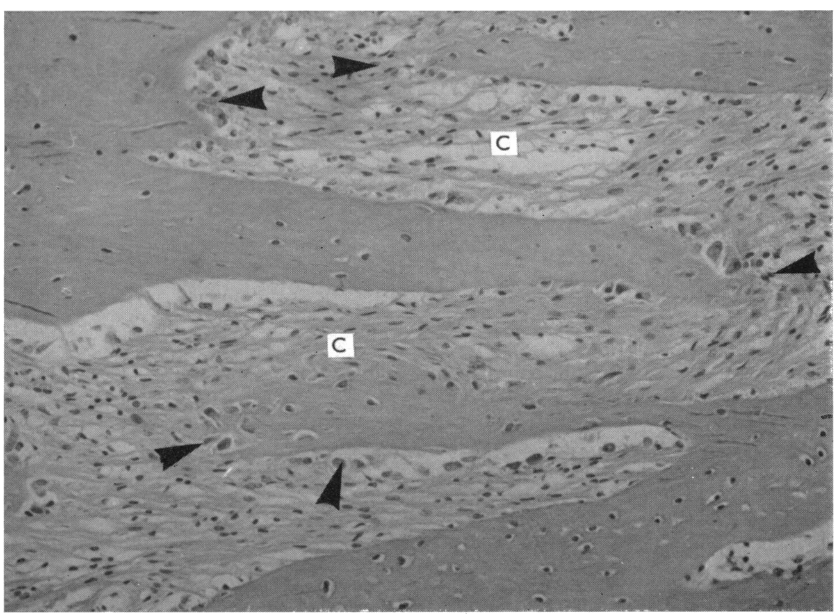


Fig. 9. Thirty eight day intact coronal sutural area medial to repositioned segment. Note the maintenance of bevelling and interdigitation, the increased osteogenic activity at sutural margins compared to repositioned area (arrows), and parallel orientation of central fibrous layers (C). Stained with trichrome. ×200.

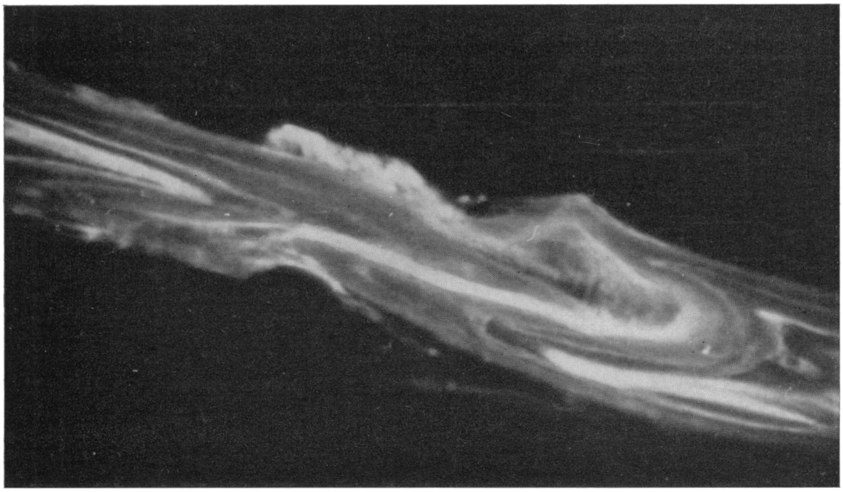


Fig. 10. Forty six day control coronal sutural area. Left: parietal bone. Right: frontal bone. Non-decalcified section photographed under ultraviolet light. ×80.

perpendicular to the calvarial surface and in the overlapped area was disorganized, but quite different from the control. In some areas complete bony fusion of the superior sutural margins was observed.

The intact coronal sutural area medial to the repositioned area demonstrated thickened sutural margins but differed from the repositioned suture in that the location of osteogenic cells and the fibre orientation were similar to the controls.

Twenty three day coronal sutural area

The control area showed more extensive overlapping and interdigitation than at ten days. The proportion of fibres to cells had increased, but fibre orientation remained the same (Fig. 6).

The repositioned sutural area demonstrated either minimal overlapping or a butt-end articulation (Fig. 7). It was thicker than in the control and in some areas cartilaginous sutural fusion occurred (CA). The concentration of osteogenic cells shifted away from the margin ends (large arrows) to sites within the suture (OB). Fibre orientation at the suture was perpendicular to the calvarial surface, whereas the intact coronal sutural area more medially was similar to controls in structure.

Thirty eight day coronal sutural area

Although the amount of bevelling and interdigitation was greater, the control was similar to the 23 day sutural area.

The isolated sutural area (Fig. 8) showed minimal overlapping and interdigitation with only a single layer of osteogenic cells at the sutural margin (small arrows). Fibre orientation was perpendicular to the calvarial surface and this contrasted with the parallel oriented fibres (C in Fig. 9) of the intact coronal sutural area more medially, which also showed similarities as regards the morphological pattern and the concentration of osteogenic cells at the bone margins (arrows) with the control.

Table 2. *Linear analysis showing normal growth increments in various sutural areas*

Injection sequence		Coronal: Frontal component S	Coronal: Frontal component I	Coronal: Parietal component S	Coronal: Parietal component I	Ant. metopic	Post. metopic S	Post. metopic I	Ant. sagittal S	Ant. sagittal I	Post. sagittal S	Post. sagittal I
A–B	Mean	—	266	224	—	—	—	—	—	—	—	—
	S.E.	—	—	—	—	—	—	—	—	—	—	—
B–C	Mean	143	205	182	155	30	35	Fused	47	41	40	44
	S.E.	9	21	26	2	3	11	—	25	13	13	20
C–D	Mean	243	252	241	173	29	15	—	108	87	58	65
	S.E.	27	32	33	22	7	8	—	18	22	9	10
D–E	Mean	226	206	268	165	23	7	—	97	88	98	92
	S.E.	28	19	30	18	7	4	—	14	15	14	13
A–E	Mean	—	807	1077	—	—	—	—	—	—	—	—
	S.E.	—	154	120	—	—	—	—	—	—	—	—

All figures in microns.
A–B (8 days), B–C (10 days), C–D (10 days), D–E (14 days), A–E (42 days).
S, Superior bone margin.
I, Inferior bone margin.
—, Missing value.

Fig. 11. Forty six day coronal sutural area within incision area. Left: parietal bone. Right: frontal bone. Note increased overlapping and interdigitation of opposing margins. Non-decalcified section photographed under ultraviolet light. ×80.

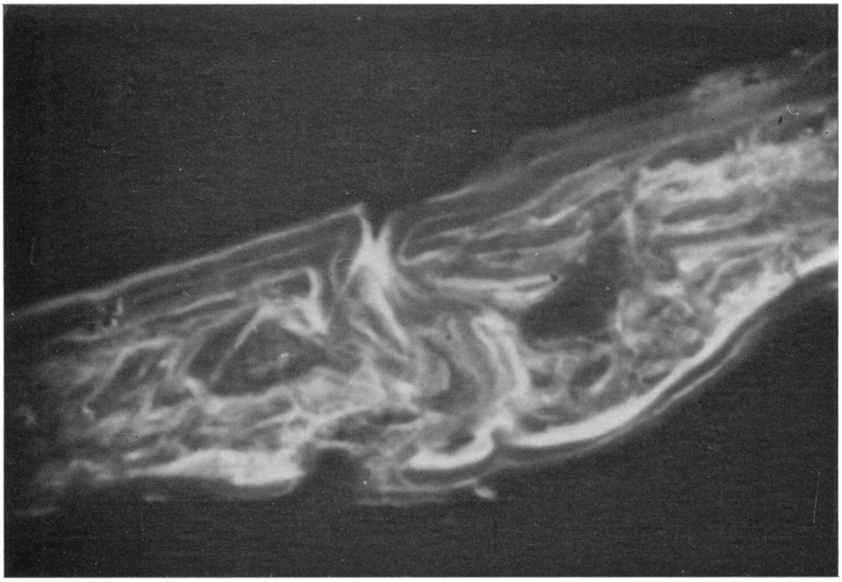


Fig. 12. Forty six day repositioned coronal sutural area. Note change toward butt-end type and thickened margins. Non-decalcified section photographed under ultraviolet light. ×80.

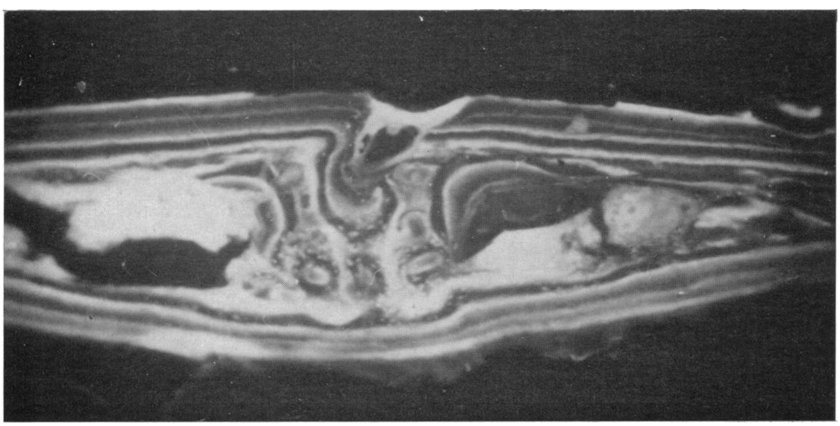


Fig. 13. Forty six day control posterior metopic sutural area. Note fused inferior border. Non-decalcified section photographed under ultraviolet light. ×80.

Linear analysis of vital staining

Control group

The normal increments of bone growth in the sutural areas studied are listed in Table 2. Differential growth was demonstrated between sutural areas and between opposing margins at a specific area. This is illustrated in the coronal sutural area (Fig. 10) where the superior (overlapping) parietal margin showed a greater total increment of growth than the inferior frontal margin during the period studied.

Growth on the superior margin of the posterior metopic sutural area progressively

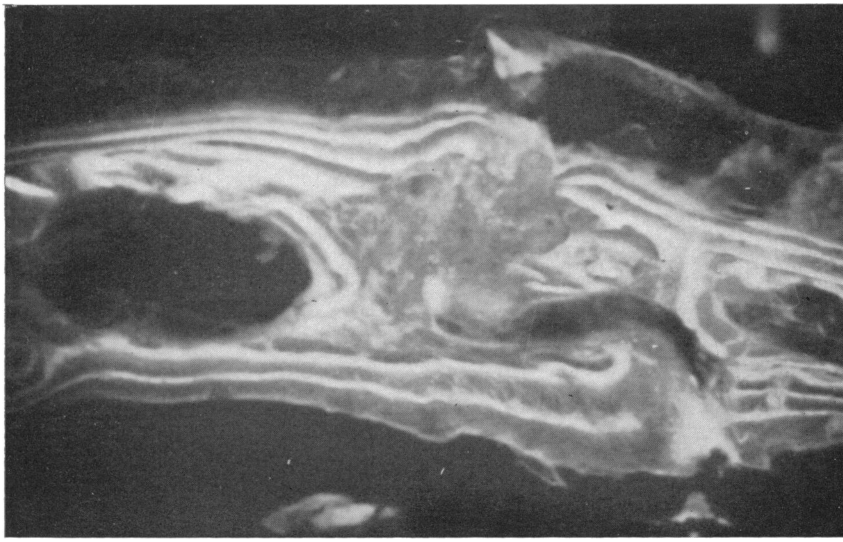


Fig. 14. Forty six day posterior metopic sutural area adjacent to extirpated area. Left: right side of skull. Right: left side of skull. Note lack of fusion of inferior margins and change to the bevelled type. Non-decalcified section photographed under ultraviolet light. × 80.

diminished from the 4th to the 46th day postnatally and fusion of the inferior margin was noted at this time. The pattern of vital staining revealed that fusion occurred between the 12th and 22nd days postnatally.

The mid-sagittal suture (Fig. 16) was of the butt-end type with equal growth occurring on superior and inferior bony margins.

Incision group

The coronal sutural area within the incision area (Fig. 11) was in continuity with the adjacent surfaces of the calvarium as the incision areas had healed except for isolated areas of the lateral incisions. The superior frontal sutural (Fig. 2, Site 9) margin showed an increased growth between the 12th and 32nd day postnatally, resulting in a posterior relocation of the superior opening of the suture. No statistically significant differences in growth increments on other sutural margins could be demonstrated.

Other sutural margins in the vicinity of the incision area showed marked differences in both morphology and growth rate. The posterior metopic sutural area (Fig. 14, representative of Groups III and V), close to bregma, was changed to the bevelled type, with no fusion on the inferior aspect. Linear analysis showed a greater increment of growth at the left superior sutural margin than the right (Fig. 3, Site 5) between the 12th and 22nd postnatal day, resulting in a relocation of the superior sutural opening to the right side.

The anterior sagittal sutural area (Fig. 17) also showed extensive overlapping of the left margin over the right. Linear analysis indicated increases in growth increments for the sutural margins between the 12th and 32nd days postnatally, resulting in a deviation of the superior sutural opening to the right side.

Table 3. *Summary of results from quantitative vital staining analysis*

Cranial sites adjacent to surgical areas	Control	Experimental	Morphological characteristics (experimental)
Coronal suture	++++	+++++	Increased overlapping and interdigitation
Metopic suture	+	++	No fusion of inferior border
Mid-sagittal suture	++	+++	Increased overlapping and interdigitation
Ectocranial surface	++	++++	Marked lipping
Endocranial surface	++	+++	Moderate lipping
Repositioned areas			
Coronal suture	++++	+	Butt-end type
Ectocranial surface	++	+++	Increased thickening
Endocranial surface	++	+++	Increased thickening

+ signs represent the amount of growth for a specific area.

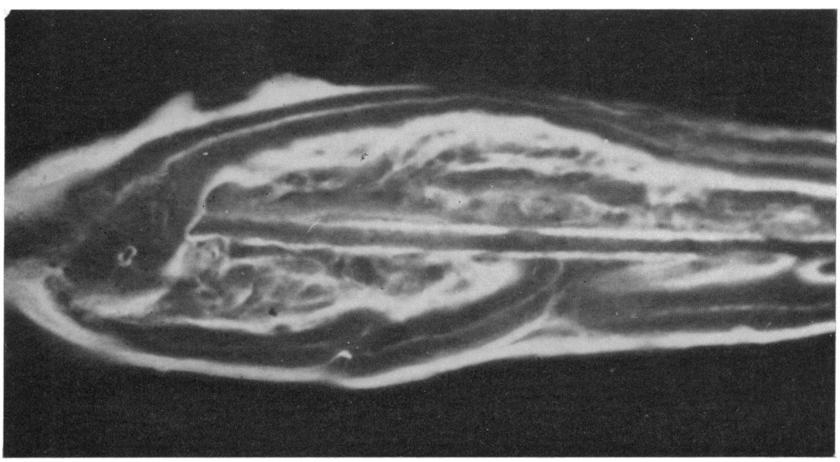


Fig. 15. Lipping of bone margin adjacent to extirpated area. Non-decalcified section photographed under ultraviolet light. × 80.

In the regions of the cut surfaces the linear analysis showed an increased growth from the 12th to the 22nd days postnatally. This thickening of the cut margins (Fig. 15) has been referred to as marginal lipping.

Extirpation group

The coronal sutural area that was extirpated did not re-establish itself and the defect was filled with fibrous tissue. The pattern of vital staining revealed that bone resorption took place in the first 22 days on the defect margins but that increased growth occurred on the bone surfaces (Fig. 2, Sites 3, 4, 17, 18) adjacent to the defect during this period, resulting in lipping. The sutural areas adjacent to the defect showed similar incremental growth and morphological changes to the incision group.

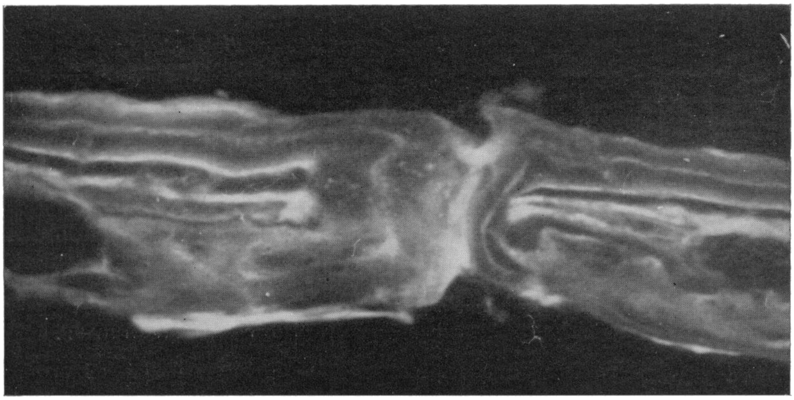


Fig. 16. Forty six day control anterior sagittal sutural area. Note butt-end type of sutural area. Non-decalcified section photographed under ultraviolet light. × 80.

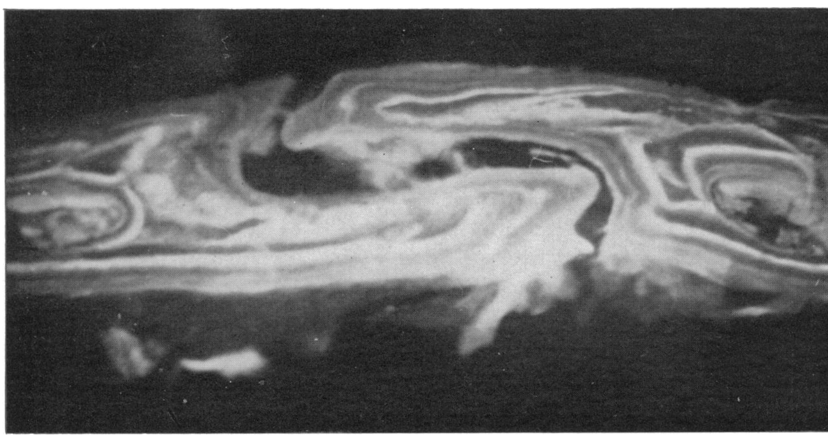


Fig. 17. Forty six day anterior sagittal sutural area adjacent to extirpated area. Left: right side of skull. Right: left side of skull. Note change to bevelled type of sutural area and deviation of superior sutural opening to right side of skull. Non-decalcified section photographed under ultraviolet light. × 80.

Repositioned group

The changes in growth rate and morphology in the sutural areas adjacent to the surgical site and the marginal lipping of the cut surfaces were similar to the changes seen in the other experimental groups. Significant changes occurred in the isolated coronal sutural area (Fig. 12). A marked reduction in growth rate on all bony margins (Fig. 2, Sites 9, 10, 11, 12) occurred throughout the experimental period. This contrasted with the adjustments in other intact sutural areas which occurred only during the first 22 days. The superior bone surfaces adjacent to the sutural area (Fig. 2, Sites 7, 13) demonstrated increased growth up to 22 days postnatally, resulting in an increase in thickness of these areas. For the other time periods these areas had similar growth rates to the control. Table 3 summarizes the results from the linear analysis.

DISCUSSION

The coronal sutural area during a period of rapid growth shows several approaching bony edges which are capped with clusters of osteogenic cells. Although the fibrous component is small at four days postnatally, by 46 days it comprises a significant part of the suture: at all times the fibres are parallel to the calvarial surface, indicating that the suture is under tension. The extent of bevelling and interdigitation increases with age and varies from one part of the coronal suture to the other. The finding of differential growth at different sutural margins and between opposing margins of the same suture agrees with Baer (1954) and Massler & Schour (1951), who maintain that it serves to change the relationship between adjoining bones during growth.

The mechanism of control of sutural development is debated. Since interdigitation continues long after the major expansion of the neurocranium is complete (20 days: Baer, 1954) this can scarcely be correlated with expansion of the brain.

Our experiments, in which the coronal suture area was damaged, indicated that injury can alter the rate of bone growth at a suture resulting in changes in sutural morphology. Presumably an inflammatory 'humoral' factor is involved (Cf. Pritchard, 1946).

Furthermore our results furnish evidence that cranial sutural morphology is not predetermined genetically, but can be changed when the mechanical milieu is altered. Thus the repositioned sutural area showed a decreased growth at its opposing bony margins at all time intervals throughout the experimental period and this is most easily explained by lack of normal tensile stresses on the suture tissues under the conditions of the experiment. This explanation is supported by the fact that the suture shows a fibre orientation which is opposite to that in the intact sutural area where normal tension is presumably acting. It should be pointed out that isolation of the suture must substantially reduce any influence due to (1) the forces of neurocranial growth, (2) muscle forces, (3) spatial relocations of adjacent bones during rotations of the cephalic components with growth. It may be concluded therefore that, when in functional continuity with the rest of the skull, the sutures respond to all of the factors mentioned, the degree of response naturally varying with the stages of growth. Another conclusion is that intrinsic factors evidently play a less important role than remote factors in determining sutural form.

Sutural fusion occurs in areas where the growth rate is markedly reduced and where absence of movement between adjacent bones exists. Moss (1958) attributes sutural fusion to extrinsic forces set up in the falx cerebri during rotations of the various cephalic components with growth. Such a mechanistic concept is partially supported by this study in view of the changes found in fibre orientation in the isolated suture. The presence of cartilage in the fused area may be due to mechanical forces associated with changes in fibre direction, or with conditions related to the inflammatory process which alters the response of the osteoprogenitor cells.

The results of the sutural extirpation experiments, together with sutural repositioning, indicate that the suture is not the primary, active site of growth of the calvaria. In the extirpation group, changes in adjacent skull areas were similar to those in the incision group in which the coronal sutural area was intact. In addition, only minimal changes in bone growth occurred at a distance from the coronal sutural area and these were associated with lipping of cut margins. If the sutural areas were primary sites of expansive growth, as suggested by Weinmann & Sicher (1955), then greater changes in skull form would have been expected in the extirpation group than in the incision group.

Further supporting the hypothesis that sutural areas have little autonomy of growth, is the decreased growth observed at all sutural margins of the coronal sutural area when it is isolated from its normal functional context. That these changes are associated with a lack of tension at the sutural area, and not with humoral changes initiated by the surgical intervention, is supported by 3 facts: (1) the inflammatory response which results in an increased thickness of the sutural margins is completed before the 22nd day postnatally, whereas the changes associated with a lack of functional continuity continue to the end of the experimental period (46 days); (2) the fibre orientation within the functionally intact coronal sutural area, where tension is acting, is opposite to the repositioned area adjacent to it; (3) the superior (ectocranial) and inferior (endocranial) bone surfaces of the repositioned segment showed increased osteogenic activity, whereas all sutural margins showed a decreased rate of growth throughout the experiment period.

This study lends support to Moss's theory of the 'functional matrix' (Moss, 1962) whereby the expanding neural mass is the *primary* force causing a separation of cranial bones during growth. Linear analysis, using vital dyes as markers, indicates that the sutural areas are the most active sites of bone growth in response to separation of the bones, and are chiefly responsible for the increase in size of the calvarium.

SUMMARY

The mechanism of normal sutural growth and its behaviour under experimental alteration was examined in the rat calvarium, using histological, quantitative vital staining analysis and accurate photographic records of gross morphology.

Twenty five normal and thirty one experimental Long–Evans strain male rats were divided into five groups: a control sagittal, control coronal, incision, extirpation, and a repositioned group. Subgroups of the control and repositioned groups were used for histological study. All other groups received intraperitoneal injection of vital stains.

The qualitative and quantitative evaluations of the results indicate that the sutural areas are sites of active bone growth, which occurs as a response to the separation of the bones resulting from expansion of the neural mass. The rates of growth vary between sutural areas and between opposing margins at a given area. Stimulation of sutural growth as a result of surgical intervention leads to morphological changes including delays in sutural fusion, increased bevelling and interdigitation, and sutural deviations. Isolation of the coronal sutural area from its normal functional connections results in a butt-end or minimal-overlapping type of suture due to both decreased growth at the sutural margins and resorptive remodelling within the suture. The sutural fusion observed was related to the growth rate at the opposing bony margins, the absence of movement between adjacent bones, and mechanical factors associated with changes in fibre orientation. Normal sutural growth and

morphology and sutural behaviour under experimental conditions evidently reflect the state of the surrounding soft tissues which in turn are influenced by remote mechanical determinants arising from growth of the skull as a whole.

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