EXPERIMENTAL ALTERATION OF THE CORONAL SUTURAL AREA: A HISTOLOGICAL AND QUANTITATIVE MICROSCOPIC ASSESSMENT

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by

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EXPERIMENTAL ALTERATION OF THE CORONAL SUTURAL AREA: A HISTOLOGICAL AND QUANTITATIVE MICROSCOPIC ASSESSMENT

ABSTRACT

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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 31 experimental Long-Evans strain male rats were divided into five groups: a control sagittal, control coronal, incision, extirpation, and a repositioned group. Subgroupings of the control and repositioned groups for histological study were made. All other groups received intraperitoneal injections of vital stains.

The qualitative and quantitative evaluation of the results from this study suggest the following new findings:

(1) Stimulation of the growth rate at sutural margins experimentally, results in morphological changes, including an increased amount of bevelling and interdigitation, delays in sutural fusion, and sutural deviations, as observed from the dorsal aspect of the calvarium.

(2) Isolation of the coronal sutural area from its normal

functional demands results in a change in shape to minimal overlapping and interdigitation or to the butt-end type. Resorptive remodelling associated with changes in fibre orientation, and alteration in both the rate and direction of growth at the sutural margins are some responsible factors.

(3) Sutural fusion appears to be dependent on the growth rate at the opposing bony margins, the direction of fibre orientation within the suture, and the absence of movement between adjacent bones. The presence of cartilage in the fused area may be an intermediate step in complete osseous union.

(4) Experimental alteration of sutural morphology suggests that external rather than local intrinsic factors are the more important determinants of sutural form.

The following results lend support to findings from other investigations:

(1) The coronal suture consists of five intervening layers between opposing bony margins and two periosteal or uniting layers when studied from the 4th to the 38th day postnatally.

(2) Differential growth was demonstrated at various calvarial sutural areas and between different margins at a specific sutural area.

(3) The sutural areas are the sites of most active bone growth in the calvarium and the direction of fibre orientation suggests that tension is acting at the sutural area. Results from extirpative and repositioning procedures

demonstrated the sutures to have little autonomy of growth and were not primarily involved in the expansion of the neurocranium.

(4) The location of calvarial sutures is determined by the relative growth rate of the adjacent bones and can be altered experimentally.

(5) Under normal conditions, sutural area morphology is influenced by a variety of factors, including the velocities and directions of growth in different cranial regions, and the relative displacement or the absence of movement between adjacent bones.

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INTRODUCTION

It is commonly agreed that a comprehensive knowledge of cranial growth is necessary for any rational approach to orthodontic therapy. Many aspects of orthodontic diagnosis, therapy and prognosis are based firmly on this aspect of cranial biology. Let us consider the skull to be a series of articulations between osseous structures which increase in area, change their shape and alter their position relative to each other during growth. The soft tissues or sutures, separating these osseous elements must therefore have some functional significance in the growth of the skull. In the past, the sutures were thought to be sites of primary expansive growth of the flat bones of the vault, similar to the nasal, mandibular, condylar and spheno-occipital cartilages, while the processes of osseous deposition and resorption completed the roster of factors involved with cranial growth. Controversy still exists regarding the functional role of the suture, mainly because of the limited use of thorough investigative techniques and the utilization of experimental animals of one species and one age group and extrapolating the results to apply to other species and other age groups. A principle fault of many previous investigations, according to Moss _et al._ (1972) is that they have described the results of cranial growth but have mistakenly confused these results with the causes of such growth.

The focal point of the present study will be an examination of the coronal sutural area under normal and experimental conditions, in the hope of understanding better the functional role of sutural areas in general in the growth of the rat calvaria. Certain problems, such as the determination of sutural locations, the controlling factors involved in growth and morphological patterns at sutural areas, and the age changes, including sutural fusion, are not adequately answered.

Referral to the term "sutural area" is to be regarded as the entire complex of cellular and fibrous tissues intervening between and surrounding the definitive bone edges, as well as the opposing bony margins. The intervening soft tissues between the bony margins are termed "the suture".

Specifically, this study will examine:

(1) Normal morphological and histological structure, the amount of bone growth of different regions of the coronal sutural area, and the relationship between morphology and growth.

(2) Changes in structure and growth pattern of this area as a result of experimental alteration.

(3) Changes in form, and growth pattern in other vault areas, including sutural areas indirectly affected by the surgical intervention of the coronal sutural area.

By means of qualitative, quantitative, macroscopic and microscopic techniques, this study will re-examine the functional role of the suture in the morphogenesis of the rat neurocranium.

CHAPTER II

REVIEW OF LITERATURE

Origin of Bone in the Membranous Brain Capsule

Various theories have been advanced to explain the origin of bone in the membranous brain capsule. Thoma (1911, 1913) believed that the membranous brain capsule grew as a reaction to internal pressure exerted by growth of the brain. In his opinion, the first sign of bone should be regarded as a reaction to this pressure or a reaction to an intensifying of this pressure. Furthermore, the bending of the cranial base in a certain stage of development may cause an increase of the stress in certain parts of the brain capsule, resulting in an induction of bone formation. Kokott (1933) worked out a theory that comes close to the one developed by Thoma. By macroscopic investigation of the fibre orientation in the brain capsule of human embryos and by experimenting with a model (a balloon) of the brain capsule, Kokott arrived at a specification of the starting point of bone formation. According to him, the fibrous tracts connecting the brain capsule with the cranial base act as a reinforcement of the capsule and distribute the tension in the capsule in correlation with the later formation of the first bone trabeculae. Moss and Young (1960) proposed a similar idea. They demonstrated that differentiation of the dural fibre systems divides the calvarial surface of the cerebral capsule into a number of "fields". Approximately at the centre of these fields ossification begins in regions which have been termed "pressure poles". Bone trabeculae spread out immediately above the dural layer from the ossification centre. This osseous expansion occurs by transformation of previously undifferentiated connective tissue cells into osteoblasts. Even when the cerebrum is virtually absent, the vault may appear to have a normal size and shape, if the cerebral capsule has become filled with an equivalent amount of fluid (i.e., hydranencephaly). Maximow and Bloom (1957) indicate that the starting points for bone formation in the brain capsule are determined to a large extent by the presence of blood vessels. Weinmann and Sicher (1955) are of the opinion that intramembranous bone formation starts, and develops, in a condensation of mesenchyme where immature coarse fibrillar bone trabeculae are formed radiating from a centre. They consider the sutures as remnants of the membranous capsule of the brain.

McLean and Urist (1968) do not clarify the initiating factor responsible for intramembranous bone formation, but state that under appropriate conditions, in areas where bone is about to appear, the intercellular substance between the cells of the connective tissue, previously indistinguishable from other connective tissue, increases in amount and density. The connective tissue cells increase in size and assume the form of osteoblasts. Once osteoblasts have formed they produce collagenous fibres and deposit the ground substance of bone.

Unfortunately, all these theories lack adequate proof and more histochemical and tissue culture investigations, including a better understanding of the piezo-electric properties and blood vascular changes within the capsular tissues are needed before an acceptable explanation can be given for the origin of bone in the membranous brain capsule.

Mechanism of Calcification

Local osteoprogenitor cells differentiate into osteoblasts which make and secrete collagen and mucopolysaccharide molecules. The collagen molecules then line up and "crystallize" as fibres and fibre bundles, the mucopolysaccharide forming a cement in between the fibres. This combination of fibres and cement is known as "osteoid". Under normal circumstances calcium and phosphate ions from the body fluids almost immediately combine together and precipitate in the osteoid as amorphous calcium phosphate of which only a small portion is converted to hydroxyapatite crystals. The result is bone. The reason for this dense precipitation of hydroxyapatite is denoted.

Currently it is believed that the body fluids generally are supersaturated with calcium and phosphate ions and are only kept in solution because of the presence of an inhibitor thought to be a pyrophosphate. When bone is being formed, it is thought that osteoblasts secrete an enzyme (pyrophosphatase) which destroys the pyrophosphate and allows the calcium and phosphate ions to come together and crystallize out. In addition, it is believed that parts of the collagen molecule act as "nucleation" sites where the molecule has the right shape and electrical charges for attracting and holding on to the calcium and phosphate ions long enough for them to begin building up a crystal. Crystal growth then occurs whereby the crystals attain their full size and orientate their long dimensions in the axis of the collagen fibres (McLean and Urist, 1961; Pritchard, 1971).

Fundamental Structure of Cranial Sutures

Pritchard, Scott and Girgis (1956) have studied the structure and development of craniofacial sutures in a variety of experimental animals including the rat and in human specimens. They discussed the development of sutures by stages rather than by chronological age of each suture, since all the sutures examined went through essentially similar stages. At the time when the bony margins of the vault bones approach each other, the definitive structure of a suture appears. Pritchard describes the suture as consisting of five intervening layers and two uniting layers. The intervening layers consist of two cambial or cellular layers, two capsular or fibrous layers whose fibres are at right angles to the vault surface, and a middle zone consisting predominantly of blood vessels or fibres depending on the stage of development. The two uniting layers join the opposing bony margins on their external and internal surface. All these layers have, at one time or another, been described by previous workers but no single worker seems to have recognized them all. Troitsky (1932) describes the uniting as well as the cambial layers. He also mentions a "non-osteogenic" middle layer, but he does not differentiate this into capsular and middle zones. Weinmann and Sicher (1955) describe a three layered suture with two peripheral layers adjacent to the bones consisting of dense connective tissue and a central layer which is a site of cell proliferation and new formation and rearrangement of fibres. Scott (1954) illustrates a suture with four zones but soon developing into three as the two middle fibrous zones unite. He does not mention a middle zone. Moss (1954) describes a suture as possessing three layers at an early stage of development, corresponding to Pritchard's capsular and middle layers, but in the adult he recognizes only a single fibrous layer. It is probable that the authors mentioned missed some of the layers because selective fibre stains were not employed or because they described a suture at one developmental level in one animal and made a general statement regarding the definitive structure of sutures in general. Troitsky, Scott and

Pritchard have all stressed the continuity of the sutures' cambial and capsular layers with the corresponding cambial and fibrous layers of the periosteum. This important fact for the understanding of bone growth at sutures seems to have been overlooked by other investigators.

Development of Cranial Sutures

Pritchard *et al.* (1956) divides the development of sutures into five stages rather than to attempt to give the chronological history of each suture separately.

1) Stage of approaching bone territories.
2) Stage of meeting of the bone territories.
3) Early growing stage.
4) Late growing stage.
5) Adult stage.

In Stage 1 the vault bones approach each other within a preformed fibrous membrane. The cambial layers are sites of very active osteogenesis with the new bone being of the woven variety. The parallel fibres of this presumptive suture are continuous with the pericranium and dura mater of the approaching bone territories. The meeting of the vault bones is different from the facial bones in that the approaching bone territories are not separated by loose mesenchyme. The width of the fibrous layers which unite the vault bones decreases until the cambial layers almost fuse but the rapid appearance of a pair of encapsulating fibrous layers prevents this. Between these tangentially oriented capsular layers is a loose middle zone. At this stage the suture is represented by the five layers described earlier. A possible reason for the confusion regarding sutural morphology is the wide variety of stages that the suture goes through to reach its definitive form, any one of which may have been taken as representing the definitive form of the suture. Stages 3, 4 and 5 vary only in the amount of mitotic cells in the cambial layer which decreases and the structure of the loose middle zone which becomes more fibrous. Diploic spaces are formed, and instead of trabeculae of woven bone projecting radially toward the suture as in the early growth period, compact lamellar bone is added tangentially containing Haversian systems. Moss (1954) investigated the developmental stages of calvarial sutures in rats and suggested three stages:

1) Histodifferentiation.
2) Growth (early and late).
3) Definitive form.

Stages 1 and 2 (presumptive suture) involve the greatest amount of growth which extends the area of the vault bones to its normal shape. The area of the presumptive suture becomes limited in the late growth phase but osteogenesis is still active on all bone surfaces. The presumptive suture allows for continued soft tissue growth (neural mass, etc.) and for compensatory adjustments between bones during the growth period. The period of transition to the definitive form takes about three weeks in the rat at which time the three layered suture (two capsular layers and one middle zone) described by Moss (1954) and Weinmann and Sicher (1955) exists. The definitive suture now serves as the chief site of further minor adjustment in the growth processes. Moss feels the adult stage of the suture consists of a continuous fibrous layer and he fails to recognize a loose middle zone. As will be seen later, the function of this middle zone as either a primary growth site (Weinmann and Sicher, 1947) or an adjustment site (Moss, 1954 and Pritchard et al., 1956) is a controversial point.

Sutures have also been described from a gross morphological aspect in contrast to the histological. Moss (1957) describes two basic types of sutural morphology; an end-to-end or butt joint, and an overlapping or bevelled type. Both of these may be modified during growth by secondarily developed interdigitations.

Herring (1972) studied sutural morphology in pig skulls. Various families and a number of genera within each family were assessed for the degree of interdigitation and the sequence of closure for different cranial sutures. The degree of interdigitation was related to the ability to resist compression, tension, and shearing forces of adjacent bones. The functional significance of the sutural area morphology will be discussed later.

Location of Sutures

It was the classical opinion, supported by Troitsky (1932), who removed whole calvarial bones or portions of bones, in rabbits, guinea pigs, and dogs that in no case did the new bone transgress the suture lines which it previously occupied. He concluded that not only the existence of the vault sutures but also their position and shape was regulated by intrinsic or genetic factors which lay outside of the growth and structure of the bones themselves. In other words, the sutures were topographically distinct elements which primarily predetermined the shape of the individual bones of the skull vault. The work of Simpson *et al.* (1953), who demonstrated the re-establishment of normal osseous and sutural morphology following partial and total calvariectomy in juvenile and adult animals, appears to support this concept. However, neither of these investigators used young growing animals. The work of Moss (1954) and Girgis and Pritchard (1956, 1957, 1958) showed that Troitsky's views of a fixed site of vault sutures required modification. Moss (1954) carried out experiments involving osseous extirpation and ectocranial periosteal burnishing of bones adjacent to various sutures in the rat. Osseous overgrowth following extirpation of an adjacent bone in the first postnatal week demonstrated that sutural displacement was possible but depended to a certain extent on the developmental stage of the suture and adjacent bone margins. This fact was made clear in that no osseous overgrowth occurred after the first week when the suture had passed into the late growth period stage. Damage to the periosteum by burnishing produced similar results. This timely extirpation and periosteal interference with sutural margins demonstrated to Moss that sutural location was not predetermined but could be altered experimentally. The results did not, however, disprove Troitsky's findings because his experiments were carried out in a later developmental period. Girgis and Pritchard (1956, 1957, 1958) caused sutural deviations by cautery of bone adjacent to sutures in the rat. They felt that this destroyed the osteogenic cells in the periosteal tissues and altered the growth rate on that side. Osteogenesis occurred on the adjacent bone which resulted in overgrowth and sutural deviation. Sutural position then reflected the growth rate of the bones adjacent to the suture. But, it has been shown that growth rate of these bones adjacent to a suture may be quite different, dependent perhaps on intrinsic or genetic factors (Giblin and Alley, 1942; Moss, 1954; Baer, 1954: Cleall _et al._, 1968). Therefore, in addition to environmental factors, there must be strong genetic determinants concerned with the shaping of the skull and with the suture's location.

Incremental Growth at Sutures and on the Surfaces of Flat Bones

The problems relating to the study of the ontogenetic development of the skull have arisen due to inadequate investigative techniques. For instance, while the use of anthropometric techniques have delineated the major proportional changes in the skull, interpretation of the significance of these changes is contingent upon an understanding of the processes of bone growth, the units of growth, and the direction of growth. Even investigators using mathematical analysis to describe morphological changes in the skull cannot be sure that the measurements submitted to test the allometric relationship (Huxley, 1932) between different parts represents measures of discrete morphological elements or units of growth. The vital-staining technique has the advantage of specifying sites and direction of growth. John Hunter (1771) was one of the first researchers who described the appositional nature of bone growth in the mandible of madder-fed pigs. Brash's (1934) analysis of skull growth, also based on madder-fed pigs, led him to the conclusion that growth in size of the cranium can be explained by external apposition and internal resorption without any significant growth at sutural lines. He considered that the apparent growth taking place at the sutures was designed to maintain the relative position of the suture during increase in cranial size. Brash's findings were significant in older animals only and could not be applied to young growing ones. Massler and Schour's (1951) study of cranial vault growth in the rat using vital-staining techniques, does not support Brash's contention but demonstrates that vault growth takes place by a process of incremental growth at the sutures. They injected animals at different ages with alizarin dye and measured the new bone deposited during intervening periods. This revealed a greater amount and duration of growth at sutures contributing to growth in length than those contributing in breadth of the vault. Moore (1943) studied sites of cranial growth by alizarin staining in the monkey and also stressed the suture to be the site of bone deposition responsible for the increase in size of the vault. Moore noted the presence of dye on the internal surfaces of the vault bones but attributed much of this to remodelling changes. Baer (1954) carried out an extensive study revealing the patterns of skull growth in the rat by alizarin vital-staining. He attributed the proportional changes in the brain case to differential growth at the sutures which caused a spatial reorientation of the component bones. Differential apposition on the surface of the bones was not large enough to account for this radical change in relationship of the vault bones. He demonstrated two basic systems of growth in the rat skull:

a) early rapid expansion of the brain case in conjunction with growth of the brain, and

b) slow growth of longer duration, resulting in elongation of the cranial base and face.

Mednick and Washburn (1956) filled in what was considered to be the gap in Brash's findings by using alizarin red injections in 2 to 3 day old pigs compared to Brash's who were 20 weeks old. They supported the idea of incremental growth at sutures.

Vilmann (1968), who studied growth of the parietal bone in 14 to 60 day old rats, supported Baer's and Massler and Schour's findings that the principle mechanism in the change of calvarial form is differential sutural growth. By supplementing their vital-staining technique with a cephalometric method of superimposing radiographs on the cribriform plate they found a discrepancy between the change in form within the parietal bone and its spatial reorientation. The posterior upwards rotation of the parietal bone contrasted with the posterior downwards rotation within the bone itself. The point here is that some mechanism of altering the position of the cranial bones in space other than incremental growth at the sutures, as suggested by Baer (1954), is implied.

The one main fault in all these studies which utilized a single vital-staining technique was that no attempt was made to verify histologically that the red edges of bone used in measuring growth at a sutural area were not sites of resorption or were sufficiently unchanged over a period of time to permit their use as fixed lines (Hoyte, 1957, 1958, 1960, 1964). However, these techniques did demonstrate general tendencies for the sites of the most active growth even with the inaccuracies of the staining technique and the cross sectional nature of the studies.

Cleall, Perkins and Gilda (1964) outlined a longitudinal vital-staining technique utilizing a combination of agents, including terramycin, alizarin red S, trypan blue and others. As a consequence, a more accurate method of studying incremental growth at specific sites which could better detect areas of resoption and remodelling was obtained. Utilizing such a technique, Cleall et al. (1968, 1971) investigated the normal craniofacial growth of the rat in both sagittal and coronal planes. These studies were not concerned with factors responsible for growth but with the amount and direction of bone formation in the face and cranium. Generally, appositional growth of varying degrees occurred in both inner and outer tables and localized areas of resorption along with extensive remodelling changes in the intervening cancellous spaces were noted. Increase in intracranial diameter was largely due to differential growth at the sutures and by growth at the synchondroses. It must be remembered that even though the multiple bone marking technique is superior to the one stain method, it is still subject to criticism as is any sectioning technique that measures a three dimensional object in only one or two planes of space.

Mechanism and Control of Growth in Cranial Sutural Areas

It is clear that cranial dimension's increase with age but many theories have evolved regarding the controlling mechanisms. Two basic philosophies will be mentioned here. The first is that the sutural tissues themselves initiate a separating force which causes expansion of the brain capsule. If this is true, the sutures would have primary morphological significance, while the shape and size of the bones would be secondary and derivative (Toitsky, 1932; Baer, 1954; Weinmann and Sicher, 1955; Prahl, 1968). The second theory is opposite to the first and considers the sutures to be sites of secondary, compensatory bone growth. According to Moss (1956, 1960, 1962) the growth of bone and the maintenance of skeletal size and shape is dependent primarily on the functional state of related soft tissues for which was introduced the concept of "the functional matrix". This theory was inspired by the hypothesis of functional cranial components developed by Van Der Klaauw (1952) on the basis of comparative anatomy studies.

In support of the first theory, Troitsky (1932), who believed that the sutural tissues predetermined the size and shape of the bones, went further than this and postulated that the fibrous sutural barriers were present in the primitive brain capsule before the bones had met and therefore the eventual meeting places of the bones were predetermined. Baer (1954) felt from his studies on growth sites in rat calvaria with alizarin dye, that the enlargement of the brain case was due to separating growth at the sutures. This conclusion illustrates the point Moss (1969) makes when he says, "However reluctantly, we must turn our attention away from the all-too-obvious results of cranial growth and towards its causes". Baer (1954) was right in establishing the sutures as significant sites of growth but not in the assumption that they were the primary separating force in the cranial capsule. Weinmann and Sicher (1955) feel that sutural growth is a primary spreading of the suture, since apposition of bone can be observed over the entire area of a sutural surface. They feel that the central layer of a suture is the site of cell proliferation where this separating force is generated. An important consideration which refutes this theory is that the sutures are still in the stage of the presumptive suture throughout the growth period when the greatest amount of calvarial expansion is occurring (Moss, 1954). The sutural areas do not possess the histological structure, as outlined by Weinmann and Sicher (1955), necessary for the existence of an expansive force during the most active growth phase. Prahl (1968), who carried out a detailed study of the coronal suture in rats, supports the theory that the sutural area has great autonomy of growth. Her conclusions are based on the fact that the fibre orientation of the overlapping edges of the suture is opposite to the direction that should be observed if tension were operating at the sutural margins. The problem here is that Prahl is examining an 11 day old coronal suture. As Moss (1954) points out, the major growth of the rat calvaria has already occurred in the first week and the suture may be serving more as an adjustment site by 11 days, whereby other forces may be instrumental in altering the sutural morphology and fibre orientation. Also, it is difficult to base a theory regarding cranial growth on fibre orientation in a suture since sectioning and histological procedures may have altered the pattern. As Prahl points out herself, variation can occur in sutural patterns along different planes of the suture. Prahl (1968) further supports her theory of autonomy of sutural tissues by stating that the suture continues to grow after implantation, although not as much. She fails to give any functional significance to the reduction in the amount of overlapping at the opposing bony margins. Prahl did not find any influence on the enlargement of the cranial vault following induction of microcephaly and concluded that the brain has supporting function only and has only a very limited influence on the growth potency of the sutural area. However, as Moss (1960) points out, "even when the cerebrum is virtually absent, the vault may appear to have a normal size and shape, if the cerebral capsule has become filled with an equivalent amount of fluid (i.e., hydranencephaly)."

In the second hypothesis as supported by Moss (1960), 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 respond secondarily. In the neurocranial capsule the functional matrix is formed by the brain, leptomeninges, cerebrospinal fluid, blood vessels, scalp and muscles. The volumetric growth of this neural mass is genetically determined and it causes a secondary expansion of the surrounding neurocranial capsule as a whole by mitotic activity of the capsular connective tissues. In other words, the cranial capsules, within which the bones are formed and maintained, are spatially reorientated into different positions (translative growth) without involving any osseous apposition and resorption (active transformation) of the osseous elements which would tend to alter their form. That translative growth processes are primarily involved in neurocranial growth is suggested by Moss (1972) when he points out some discrepancies between the two processes (active transformation verses translation). The supraoccipital bone of the rat is translated upwards and posteriorly as shown by Vilmann (1968) but the processes of active transformation are opposite. Cleall et al. (1968) demonstrated this anterior "drift" of the supraoccipital bone with their multiple vital-staining technique whereby apposition was observed on the endocranial surface and resorption on the ectocranial. Not only does passive translation of the bones occur in response to brain growth, but it must also overcome the opposite effects of the transformative growth processes in this instance. These localized responses of the skeletal units are due to the constantly changing demands of their respective periosteal matrices which are themselves also undergoing passive spatial translation. Experiments by Moss (1954) on extirpation of various cranial sutures in the rat, and by Selman and Sarnat (1957) of the frontonasal suture in the rabbit further support the concept of the functional matrix. In no case did any alteration of the skull morphology occur, illustrating the secondary nature of sutural growth.

To what extent do the soft tissues control the morphological structure of a suture? According to Moss (1961), bone morphology is primarily determined by "intrinsic" (genetic) factors, while "extrinsic" factors, such as muscle activity, secondarily both produce and preserve the final molding of those bones in the form of crests, ridges, processes, tuberosities, and interdigitation and bevelling of sutures. He demonstrated that sagittal suture isolation resulted in complete loss of interdigitation.

Moss feels that muscle forces and not forces imposed by growth of the brain are responsible for these interdigitations. This is shown by the lack of sutural interdigitation and the smooth edges which develop in the sagittal sutural area upon cessation of neural growth. Muscles cannot bring about any morphogenic expression of their activity until the predominantly expansive force of the brain has expended itself. Proof of this is shown in the fact that only after the growth phase do interdigitations develop. Young (1959) supported this theory by demonstrating an inhibition of interdigitation in hydrocephalic rats. If growth of the brain were a factor one would expect greater interdigitation. Results of coronal suture isolation were more variable, and Moss feels that other factors may be involved. The coronal suture interdigitation and bevelling may be an adaptation of the suture to the relative displacement of adjacent bones brought about by spatial relocations of other parts of the craniofacial complex. These latter extrinsic factors become very important in regulating fusion of the metopic suture in the rat (Moss, 1958). Moss feels that changes in spatial orientation of the various cephalic components such as the splanchnocranium, cribriform plate, cranial base and cranial vault brings about corresponding angular changes in the attachments of the dura. Stresses are set up and transmitted through the fibrous dura tracts to their attachments. All of the dural layers find their firmest calvarial attachments at the sutural areas. Moss relates the endocranial ridging and the ultimate fusion of the posterior metopic suture in the rat to these extrinsic forces.

Giblin and Alley (1944) inserted a solid disc of bone taken from the parietal bone into a portion of the coronal sutural area of young puppies. They induced premature fusion of adjacent parts of the coronal suture and concluded that a primary condition for sutural fusion was the absence of movement. Herring (1972) examined the sutural patterns and gross morphological structure in different species of pigs. She concluded that suture morphology indicated the existence of stress from muscles, and use of the upper canines. Variations in different species were correlated with the relative contribution of these external forces. She demonstrated that many interdigitated sutures exhibited complex spicule patterns at the bone edge which may have resisted forces from all directions. Isotupa _et_ _al._ (1965) investigated the bone trabeculae patterns adjacent to various craniofacial sutures in rabbits by injection of alizarin red S and examination under a dissection stereomicroscope. In the coronal sutural area, bone trabeculae radiated out from the ossification centres of the frontal and parietal bones toward the suture but became more perpendicular to the sutural line with increasing age. The bone trabeculae at the interparietal suture varied depending on whether the middle of the suture (trabeculae perpendicular) or the anteroposterior limits of the suture (trabeculae parallel to the sutural line) were examined. The anterior trabeculae became more parallel with age. Although only the surface areas of the bone trabeculae could be examined due to limitations imposed by the bone thickness, it appears that the bone trabecular pattern adjacent to sutures changes with age and these changes are associated with the different directions and velocities of bone growth at the cranial sutural areas. This is in contradiction to Moss who feels that sutural area morphology (interdigitations) is associated more with muscle forces than with growth at the sutural area in response to an expanding brain.

It is apparent that suture morphology is under control from a variety of external forces. When sutures are removed from their normal environment by autotransplantation experiments, the significance of these external forces can be indirectly demonstrated. Watanabe, Laskin and Brodie (1957) carried out subcutaneous transplants of the zygomatico-maxillary suture of guinea pigs and found no evidence of growth as measured by metallic implants. Moss (1957) implanted various cranial sutures of the rat into the cerebral substance under a parietal flap. The dura mater and periosteum were strongly adherent to the implants which were positioned at right angles to the host calvaria. These implants were viable and they grew but they all consistently showed a flat, butt joint type of morphology, without interdigitations regardless of the duration of implantation. In all other respects, the sutural areas were normal. Moss concludes that the flat sutural area type is intrinsic to all these areas and is secondarily modified by normal extrinsic forces which are imposed on the neurocranial capsule from the moment of initiation. Prahl (1968) felt that the change in morphology in Moss's implantation experiments should be taken with reserve. Her results of intracerebral implantation of the coronal suture from the 4th to the 11th day demonstrated a lower degree of overlapping but only rarely did the butt end type occur. She attributes this type with individual variation or with a more medial plane of section as the coronal suture shows less overlapping in the more medial areas. However, one might suspect that the discrepancy between these results is due to the differences in time of implantation. Had Prahl allowed a longer period of implantation she might have obtained different results, and might not have attributed so much autonomy of growth to the sutural area. As Moss (1957) points out, "the adjacent bone edges never simply spring apart, they must grow apart", and this requires time.

Cartilage in Sutures

Cartilage has been found at the margins of the bones or in the sutural tissues proper. In investigations by Pritchard et al. (1956) it was observed in the sagittal and mid-palatal sutures at the end of the period of rapid growth. It has been found as irregular islands of large-celled cartilage interspersed with trabeculae of woven bone, or as more organized epiphysis-like masses covering the margins of the bones. Its significance is debated. Sitsen (1933) found it in infants under six months of age in the lambdoid suture, and regards it as the result of particularly strong pressure and shearing stresses between the bones. Pritchard et al. (1956) suggest a protective function for such cartilage, as it is well known that growing bone is intolerant of pressure and shearing stresses. That cartilage is not present at all stages in suture development, especially the early stages, is compatible with the theory that tension is normally believed to be present in growing sutural tissues and shearing and compressive forces are minimal or absent. Moss (1954) also demonstrated two types of secondary cartilage during frontal suture fusion in the rat; definitive secondary cartilage and an intermediate form. The term "secondary" is used to describe a later form of cartilage distinguished from the embryonic cartilage which acts as a cartilagenous anlage, later to be replaced by endochondral bone formation. The first type is transitory in nature and is replaced by the processes of endochondral bone formation. The intermediate form of secondary cartilage is a lower order of differentiation similar to that noted in the sagittal sutural area of the rat by Pritchard *et al.* (1956). The cells are larger and more irregular and are transformed directly into bone without endochondral replacement. The process of direct osseous transformation has been shown in fracture healing in frogs, lizard and rat by Pritchard and Ruzicka (1950). The fact that the fusion of the frontal suture may also be influenced by stresses set up in the dural attachments (Moss, 1958) lends support to the previously conceived relationship between cartilage formation and stress. Girgis and Pritchard (1956) believed that ischaemic conditions promote cartilage formation during repair. They made multiple cuts in the skull of neo-natal rats and scraped the pericranium to induce ischaemic conditions. Cartilage was formed between the 6th and 17th day after fracture in seven of the thirty-five skulls. Pritchard (1946) attributed the lack of cartilagenous "callus" in repair of fractures in the parietal bone to the developmental background of the cranial vault and to the immobility of the rigid skull bones which prevents pressure and movement between the fragments.

CHAPTER III

MATERIALS AND METHODS

I. SAMPLE

The sample consisted of 25 control and 31 experimental Long-Evans strain male rats. The control groups were divided according to the method of analysis (vital-staining or histological investigation) and the plane of sectioning (sagittal or coronal). The experimental animals (Fig. 1) were divided into three groups according to the method of surgical intervention; a repositioned, an extirpation, and an incision group. Subgroupings were made of the repositioned group for histological investigations.

The period of study for all groups with the exception of those used in histological investigation was 42 days beginning five days ± one day postnatally. Those groups undergoing histological study were sacrificed at 4, 10, 23, and 38 days postnatally.

During the study period, animal weights were recorded. If any particular animal of a litter varied markedly in weight from the mean litter weight, either immediately after intraperitoneal injections or at intervals between injections, it was discarded from further study. Careful control over housing environmental conditions existed regarding temperature, humidity, lighting and feeding procedures. The rats were maintained on a diet of Purina Laboratory Rat Chow and water ad libitum. Table I summarizes the animal groupings.

TABLE I

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

II. VITAL STAINING

A series of four vital stain lines was achieved by intraperitoneal injection of oxytetracycline, alizarin red S, or combinations of these at the intervals shown in Table II. The determination of appropriate stain dosages and the effect of these agents on bone growth has been described elsewhere (Cleall et al., 1964). The dosages of vital stains used in this study were within the range considered to be non-toxic (as judged by total body weight gain) by Cleall, and since both the control and experimental groups received the same dosages, the effects of these stains on the results were considered insignificant.

These stains are incorporated into newly deposited bone by means of a chelation mechanism whereby the carboxyl and hydroxyl groups of the alizarin and tetracycline molecules (Myers, 1968) form a chemical bond with the apatite crystals of bone. Thus new bone formation on a growing bone surface is represented by a thin stain line, the colour of which is specific for each agent. The thickness of the stain line increases as the plane of sectioning varies from 90 degrees to the bone surface, thereby complicating the interpretation and measurement of stain markings. Certain sectioning procedures will be described later to compensate for the curvature of the cranium so that stain lines will be as thin as possible.

TABLE II

VITAL STAINING SEQUENCE

Stains used	Code	Injection Day	Dosage
Terramycin (Oxytetracycline)	A	Day 1	70 mg./Kg.
Alizarin Red S (2% solution)	B	Day 8	200 mg./Kg.
Terramycin + Alizarin	C	Day 18	70 mg./Kg. 200 mg./Kg.
Terramycin	D	Day 28	70 mg./Kg.
Bone Edge (No stain)	E	Day 42	Sacrifice

Injection sequence began at 5 days ± 1 day postnatally.

III. SURGICAL PROCEDURES

General Remarks

In all animals subjected to surgical intervention, refrigeration for 15 minutes at 32°F was the method of anaesthesia. This method was used by Moss (1954) in rats up to four days of age, whereas older animals received ether anaesthesia since the depth of anaesthesia could be better controlled. A skin incision, 1 cm long, was made in the centre of the skull parallel to the mid-sagittal suture. The left coronal sutural area was exposed. One of three surgical procedures was carried out. Although care was taken not to damage the remainder of the calvarium during surgery, herniation of the underlying neural mass was sometimes encountered due to excessive holding pressures during the operation. Animals in which considerable herniation or bleeding, due to dural sinus puncture, occurred were discarded from the experiment. Upon completion of the operation, the skin was carefully replaced over the calvarium and a thin coat of Permabond contact cement[1] was used to seal the incision. Remnants of the cement disappeared after 3-4 days, thus no interference with cranial growth was anticipated.

Previous studies (Moss, 1954) showed that when part of a calvarial bone of young rats was removed, together with the periosteal layer of the underlying dura, regeneration of this bone did not occur. In this experiment, extirpation of the coronal sutural area was done to study the effect of this

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[1] Permabond (Pearl Chemical Company, Tokyo, Japan).

removal on the morphology of the adjacent growing bones and sutural areas, and the effect of removal and non-regeneration of part of the coronal sutural area.

It was felt that repositioning procedures, whereby part of the coronal sutural area was isolated from the rest of the calvarium, would reduce the effects of mechanical or other external influences and allow the local intrinsic (genetic) factors controlling sutural morphology to be expressed.

Incision of the coronal sutural area, leaving the posterior parietal surface intact, was carried out to act as a sham operation, involving minimal trauma to the sutural area, from which comparisons of the effects of other surgical procedures on both local and more remote cranial vault areas, could be made.

Extirpation

(1) A four sided incision through the calvaria in the coronal sutural area was made.

(2) The incision extended equally into the frontal and parietal bones.

(3) The area corresponding to the black square in Group III (Fig. 1) was carefully removed from the calvarium along with the underlying attached dura mater.

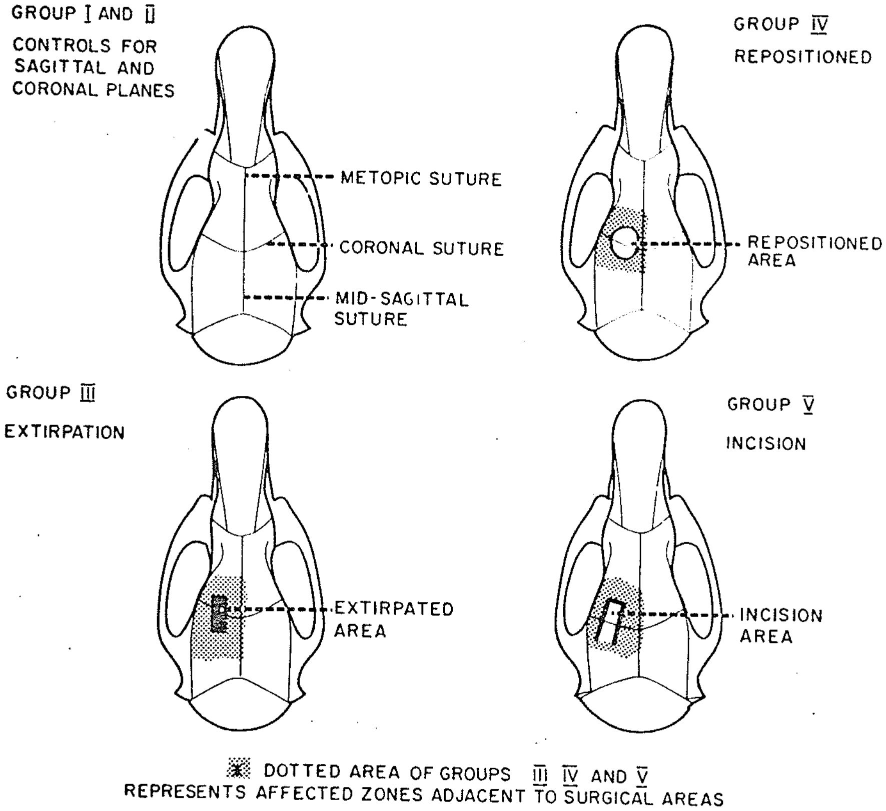

Figure 1. Dorsal view of rat calvaria outlining animal groups.

Repositioning

(1) A circular incision corresponding to the white circular area in Group IV (Fig. 1) was made in the coronal sutural area.

(2) The area outlined extended equally into the frontal and parietal bones but did not involve the mid-sagittal suture.

(3) The area was carefully detached from the underlying dura mater and removed completely from the calvarium.

(4) It was immediately inserted back into the calvarium in the same place so that all the cut margins of the repositioned section and the remaining calvarium coincided.

(5) Care was taken during skin closure not to disturb the position of the repositioned segment.

Incision

(1) A three sided incision, as outlined in black in Group V (Fig. 1), was made in the coronal sutural area.

(2) The incision involved both the frontal and parietal bones laterally and medially, but only the frontal bone anteriorly. The posterior parietal surface was left intact.

(3) Care was taken not to jiggle the surgical site so that no alteration of the incision area with the underlying dura mater was suspected.

(4) This procedure served as a sham procedure for the repositioning procedure where the attachment of the coronal sutural area with the underlying dura mater was temporarily disturbed.

IV. HISTOLOGY

Twenty-one animals were subjected to histological investigation which centred around the coronal suture only. Twelve control animals and 11 experimental animals were used. Animals in the control group were selected cross-sectionally from different litters so that normal coronal sutural histology at 4, 10, 23 and 38 days could be established. The 11 experimental animals selected from two litters at 4 days of age had a surgical repositioning procedure done at the coronal sutural area as described previously. These animals were sacrificed cross-sectionally to correspond chronologically with the normal group.

V. SPECIMEN PREPARATION

Vital Staining

The animals were sacrificed by decapitation under ether anaesthesia. The heads were placed in a pressure cooker at 30 lb./sq.in. for five minutes. After the soft tissues were removed from the bones, the cleaned crania were stored in 70% ethyl alcohol.

The embedding procedure using Ward's Bioplastic[2] was similar to that used by Ryll (1972).

Sectioning was done with a Gillings-Hamco Thin Sectioning machine with a diamond wheel[3]. Plastic blocks were prepared for this step by trimming, polishing, scoring guidelines, and mounting on a plastic holder in a precise orientation. Sections of 120-180 microns were cut and placed on microscopic slides under glass covers.

In both the sagittal and coronal planes, four regions were chosen for study. Three consecutive sections were taken in each region. Figure 2 illustrates the regions of sectioning.

Histology

(1) Upon sacrifice the skin was removed from all cranii by separation from underlying periosteum by blunt dissection.

(2) The cranii were fixed in 10% neutral buffered formalin for 48 hours.

(3) The left coronal sutural area was detached from the remaining calvarium by means of a high speed dental handpiece.

(4) The specimens were decalcified for three weeks in E.D.T.A. decalcifying solution.

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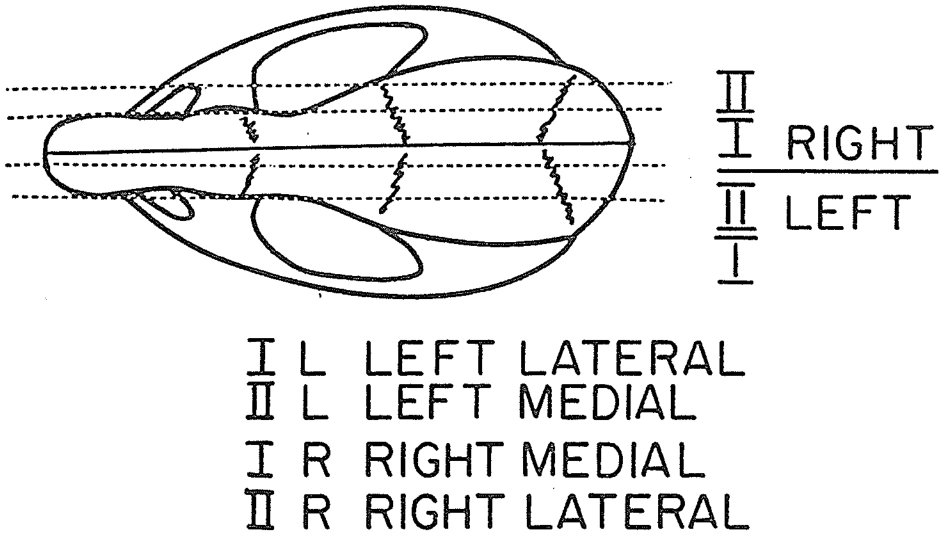
[2] Bioplastic (Ward's Plastic Centre Incorporated, Rochester, New York, 14606).

[3] Gillings-Hamco, Rochester 20, New York.

CRANIAL SAGITTAL REGIONS



CRANIAL CORONAL REGIONS

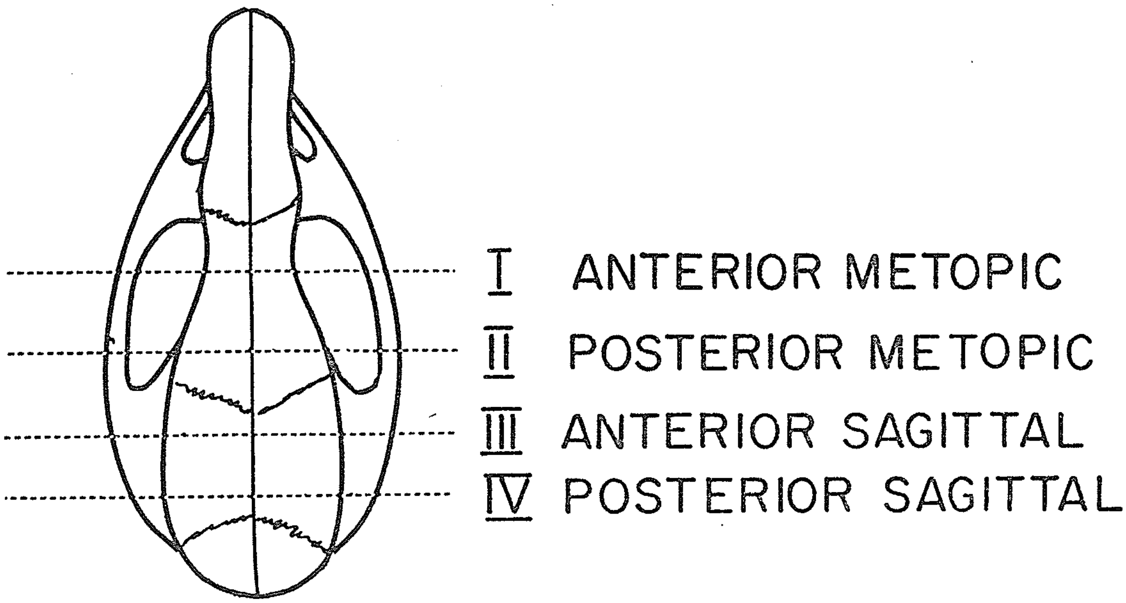


Figure 2. Regions (Blocks) of sectioning for linear analysis.

(5) Specimens were embedded in paraffin wax and oriented for sectioning.

(6) Sectioning was done on a rotary microtome[4] (Sections were cut at 6 μ thickness.).

(7) Sections were mounted on glass slabs and then deparaffinized for three hours.

(8) Staining techniques comprised of:

(a) Hematoxylin and Eosin (Y) for standard histological examination.

(b) Masson's Trichrome and Van Gieson's stains specific for collagen fibres.

VI. METHOD OF ANALYSIS

Skull Photographs

Standardized photographs were taken of the dorsal aspect of all cranii before embedding in Bioplastic. The cranii were positioned on a specially designed plastic mount so that the dorsal surfaces of all cranii were in the same horizontal plane and the same distance from the camera. A millimetre scale was positioned on the plastic mount so that enlargement of the prints during film processing and developing would also be standardized. Prints were made on dimensionally accurate photographic paper. The photographic setup is illustrated in Figure 3.

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[4] American Optical Sliding Microtome with a 120 mm Thomas-Schmid knife.

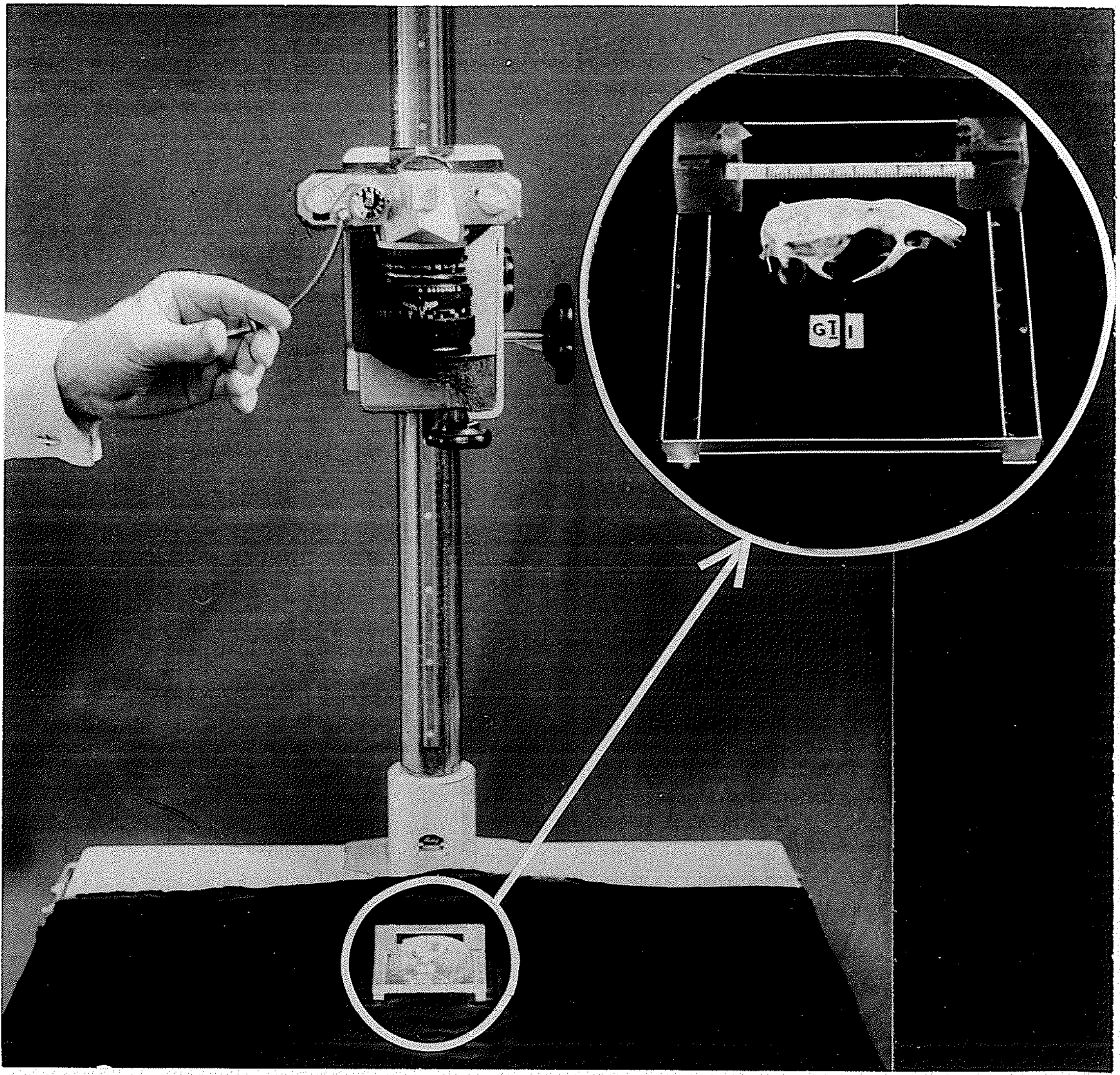

Figure 3. Photographic setup using a constant film distance, plastic mount for positioning skulls, and millimetre rule for standardized print enlargement.

Photomicrographs

Photomicrographs of slides representing the vital staining and histological characteristics of the various sutural areas were made. Photomicrographs of the vital stained slides were taken under ultraviolet light using high speed Ectochrome[5] film from which black and white prints were made. Photomicrographs of the histology slides were taken in black and white using Panatomic-X[6] film and black and white prints were made. Various filters were used so that good contrast on the micrographs could be obtained for the different staining techniques.

Linear Analysis

Two planes were used in the linear analysis, and four regions were studied in each of these planes (Fig. 2). In the sagittal plane, 22 sites were subjected to linear analysis in each of the four regions compared to a maximum of 8 sites for the coronal regions. The site locations in both these planes are illustrated in Figures 4 and 5, respectively. The site locations are clearly defined in the Glossary.

The specimens subjected to linear analysis were examined under ultraviolet light. The eyepiece scale, which was graduated in units of 5 microns at the magnifi-

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[5] Kodak High Speed Ectochrome ASA 160.

[6] Kodak Panatomic-X ASA 32.

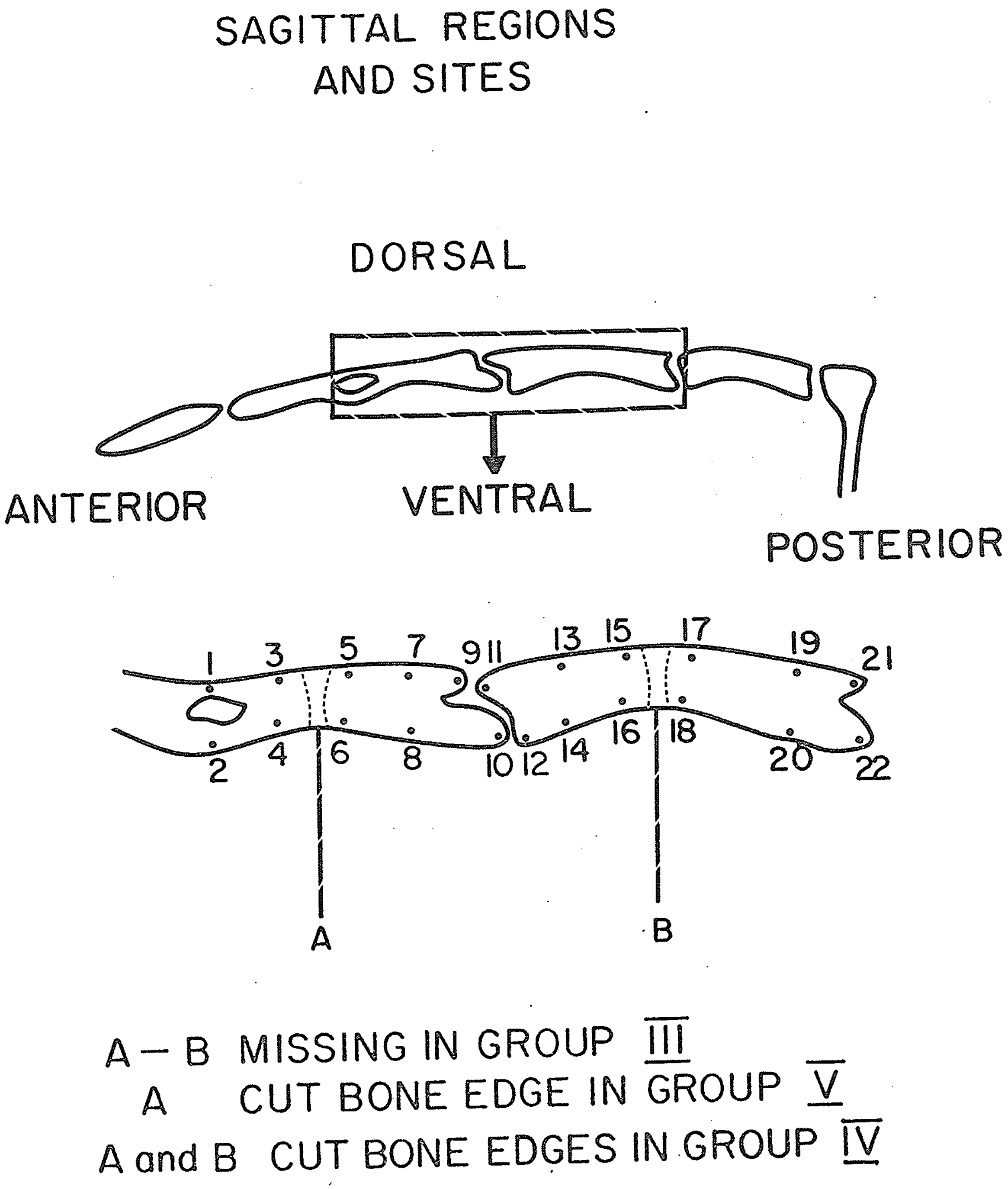


Figure 4. Linear sites for each of four sagittal blocks.

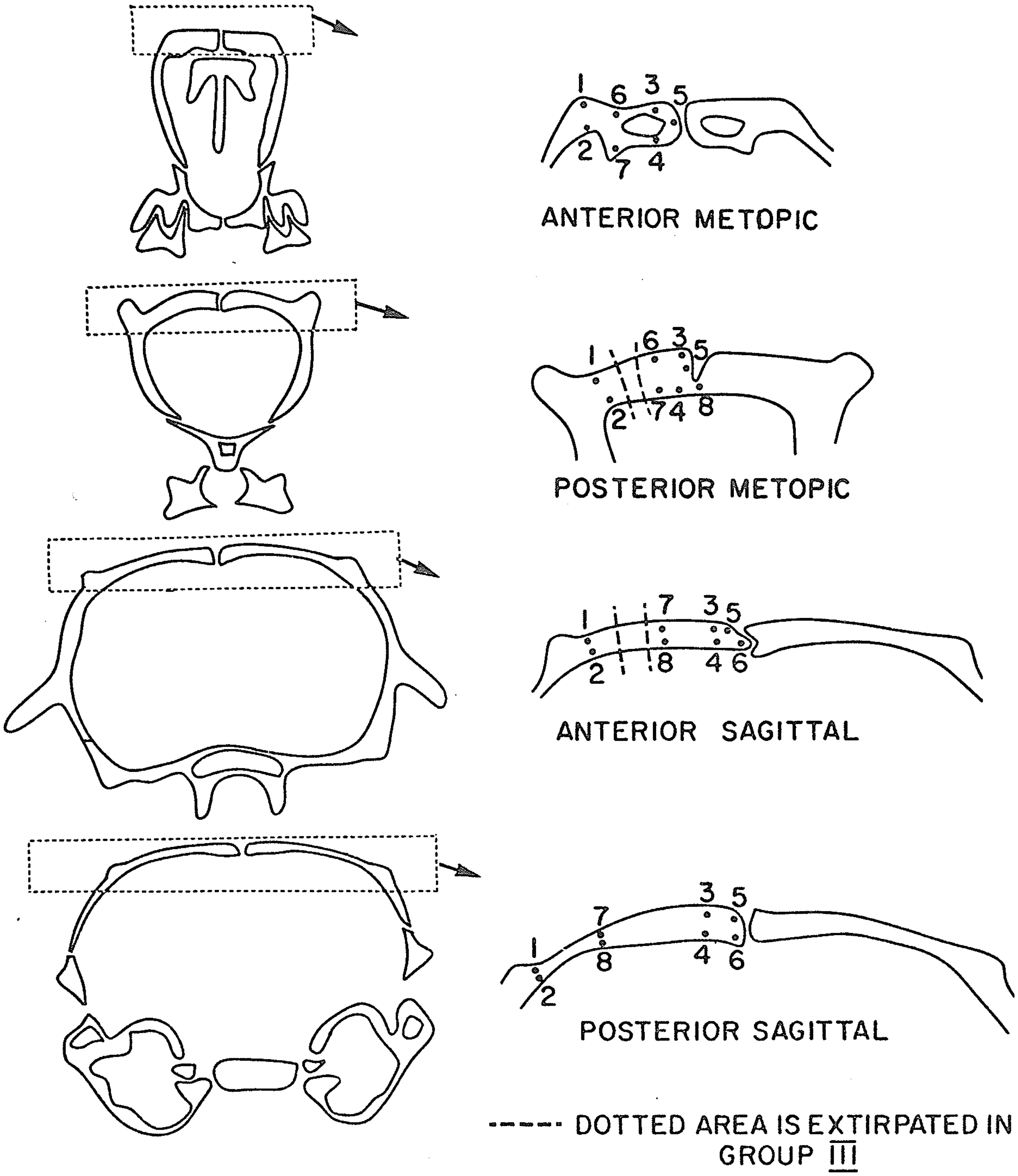


Figure 5. Linear sites for the four coronal blocks studied.

cation used, ranged from 0 to 500 units. The stain line intervals at each site were measured by first placing the 0 mark in the centre of the first stain, then recording the scale values at the centres of the subsequent stain lines, and finally the bone edge in accumulative fashion. The measurement error of the linear analysis varies with the width of the stain lines and the ability to relocate a measurement site. Errors ranged from ± 2.5 microns to ± 25 microns, depending on the site. On the average, a measurement error of ± 10 microns was expected which was similar to that outlined by Ryll (1972).

The computer program for the linear analysis (Cleall *et al.*, 1971) yielded the means and standard errors for the various stain intervals at each site for each region studied. The mean differences between left and right sides and between control and experimental groups for each site were compared by means of a paired t test for significance.

Histology

The normal histological characteristics and the fibre orientation at the coronal sutural area at four different time intervals (refer to Table I) was studied. These characteristics will be compared to those of the repositioned coronal suture for similar time intervals.

CHAPTER IV

RESULTS

QUALITATIVE

I. EXAMINATION OF STANDARDIZED PHOTOGRAPHS OF CRANIA

II. HISTOLOGY

QUANTITATIVE

I. LINEAR ANALYSIS OF VITAL STAINING

QUALITATIVE I

EXAMINATION OF STANDARDIZED PHOTOGRAPHS

General Description

Standardization of the photographs allows for a more positive appraisal of gross sutural patterns. Three sutures will be described from the photographs including the metopic, the coronal and the mid-sagittal sutures, as well as any gross alteration of the coronal sutural area and adjacent sutures as a result of surgical intervention.

Control Group

The metopic suture (Fig. 6, 1) shows minimal deviation from the midline and has relatively smooth edges on opposing bony margins. In the posterior metopic suture close to bregma some slight interdigitation is noticed.

The coronal suture (Fig. 6, 2) shows extensive interdigitations (although not as marked as the nasal frontal suture) which appears to be wider on the lateral and narrower on the medial aspects of the suture. The suture is angulated such that the lateral aspects are anteriorly positioned relative to the medial.

The mid-sagittal suture (Fig. 6, 3) shows varying degrees of interdigitation but to a lesser degree than the coronal suture. It seems that moderate interdigitation occurs in the central aspect of the suture while smoother opposing bony margins appear at the anterior and posterior

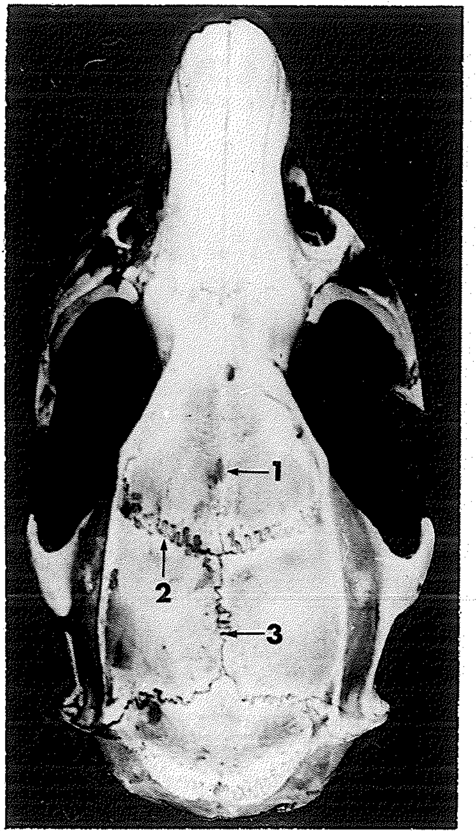


Figure 6. Photograph of 46 day rat skull (dorsal view) from control group. Arrows represent the sutural areas studied: (1) metopic, (2) coronal, (3) midsagittal.

aspects. However, due to the amount of individual variation in sutural pattern no specific description can be applied to any one part of the mid-sagittal suture.

Incision Group

In most animals the incision area shows some evidence of repair. There is complete bony union of the anterior incision in the frontal bone (Fig. 7, 4) but incomplete union of the medial and lateral anteroposterior incisions (Fig. 7, 3). In the cases where union occurs, a slight indentation in the skull is present, while non-union results in a fibrous tissue junction between bony segments. It could not be determined from the photographs whether bony union of both ectocranial and endocranial vault surfaces exists. The lighter appearance of the bone surrounding the lateral incisions indicates relatively extensive bone growth. The bone margins are elevated relative to the surrounding bone (observed on dried skulls) and these elevations will be referred to as marginal lipping.

The metopic suture appears the same morphologically as the control but there is an apparent deviation of the suture in the region of bregma toward the unoperated side of the animal (Fig. 7, 1).

The coronal suture on the operated side appears to have a similar interdigitating pattern as the control side but the medial part of the suture in the area of bregma

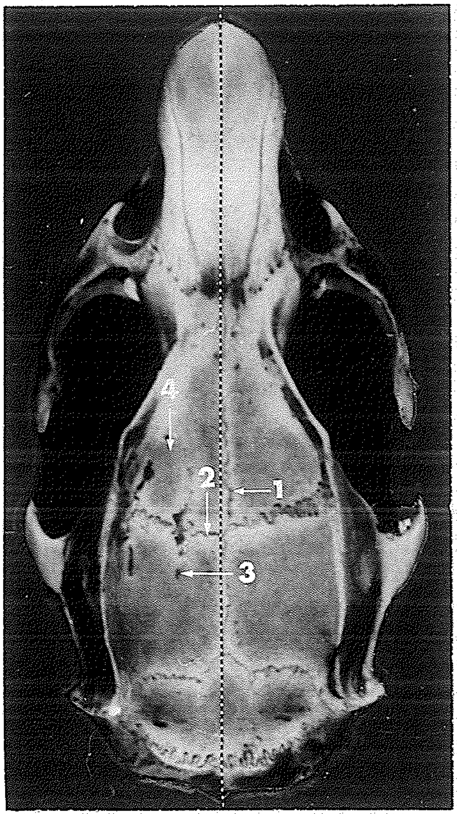


Figure 7. Photograph of 46 day rat skull (dorsal view) from the incision group. Dotted line represents midline of skull. The mid-sagittal (1) and coronal sutures (2) show an apparent deviation. The lateral incisions are not completely repaired (3) while the anterior incision in frontal bone shows bony reunion (4).

(Fig. 7, 2) is located more posteriorly relative to the contralateral coronal suture. The width of the unstained bone in the coronal sutural area seems to be greater on the operated side compared to the control side. This might indicate an increased rate of growth at the suture compared to the unoperated side. No differences can be seen between the coronal suture within and the suture medial to the incision area regarding their position and pattern of interdigitation.

No significant changes can be detected in the position or interdigitating pattern of the mid-sagittal suture.

Repositioned Group

The repositioned segment is separated from the adjacent calvarium by a fibrous tissue barrier (Fig. 8, 1). The width of the barrier varies and in some animals, partial bony union between the repositioned segment and the calvarium exists. It can not be determined whether this union extended through the full thickness of the calvarium from photographic examination. Marginal lipping around all cut surfaces is evident from the dried skulls.

The metopic suture in the anterior aspect appears normal but the posterior part shows an apparent deviation to the non-operated side (Fig. 8, 2). The pattern of interdigitation is variable.

The coronal sutural area within the repositioned

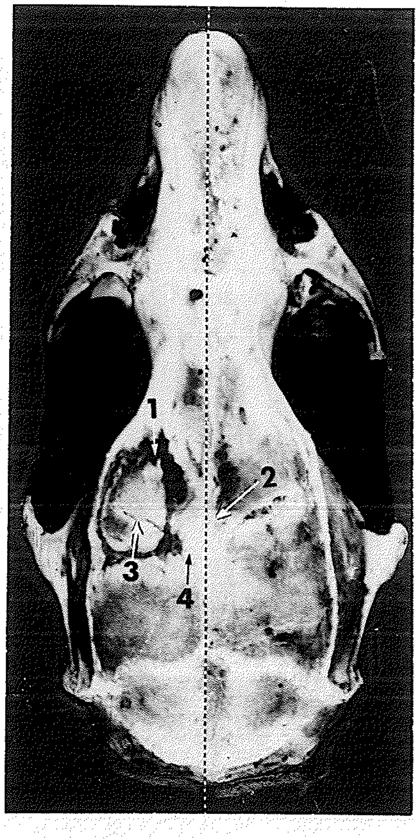


Figure 8. Photograph of 46 day rat skull (dorsal view) from the repositioned group. Dotted line represents midline of skull. The mid-sagittal suture (2), and the coronal suture medial to repositioned area (4) show apparent deviations. The repositioned segment shows fibrous union with rest of skull (1) and coronal suture within segment (3) shows loss of interdigitations.

segment (Fig. 8, 3) has a smooth appearance to the opposing bony margins compared to the contralateral coronal suture. In one-half of the animals, the intact coronal suture medial to the surgical area (Fig. 8, 4) demonstrates an apparent posterior deviation relative to the coronal suture on the unoperated side. The extent of this relocation is variable. In one animal, where almost half of the repositioned segment shows bony union, the coronal suture within this segment demonstrates a more extensive pattern of interdigitation.

The anterior mid-sagittal suture also shows an apparent deviation to the non-operated side in some animals.

Extirpation Group

The extirpated area shows no signs of repair (Fig. 9, 1). All defect margins demonstrate marginal lipping. The apparent sutural deviations of the posterior metopic and anterior mid-sagittal sutures to the non-operated side are clearly evident but to a greater degree than in other groups. The position of the intact coronal suture medial to the extirpated area is variable as some animals show an apparent posterior relocation and others do not.

In all surgical groups, variation in the position of the sutures adjacent to the surgical site is present. There seems to be a gradient concerning the amount of sutural relocation, from minor changes in the incision group, to more extensive changes through the repositioned and extirpation groups, respectively.

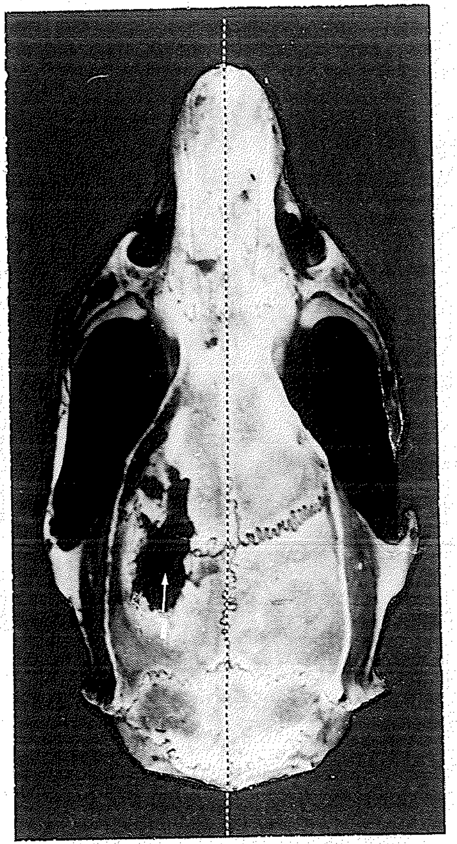


Figure 9. Photograph of 46 day rat skull (dorsal view) from the extirpation group. The extirpated area (1) is not repaired.

QUALITATIVE II

HISTOLOGY

The general histological characteristics of the coronal sutural area will be described at the various time intervals for both the control and the repositioned groups with special reference to: (1) morphology of the opposing bony margins, (3) distribution of the cellular component, and (3) fibrous tissue components and the direction of fibre orientation.

Four Day Control

The general morphological structure of the sutural area at four days is the overlapping type. The lateral aspects of the sutural area are overlapped to a greater extent than the medial aspects. In both lateral and medial parts of the sutural area, the parietal bone overlaps the frontal bone. On the lateral aspects of the suture (Fig. 10), there are several projecting trabeculae on each opposing bony margin. Although these projections demonstrate the regions of the most active osteogenesis, all bony surfaces of the opposing margins show active growth (Fig. 10, A). The total thickness of bone at the sutural area is greater on the lateral aspects relative to the medial (Fig. 11). Associated with this increased thickness laterally are the presence of open diploic spaces at the bony margins (Fig. 10, D) which are not seen medially. Sections taken more

Figure 10. 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 diploic spaces (D).

Stained with H and E (X 80).

Figure 11. Four day control coronal sutural area (medial aspect).

Left: Frontal bony margin.

Right: Parietal bone margin.

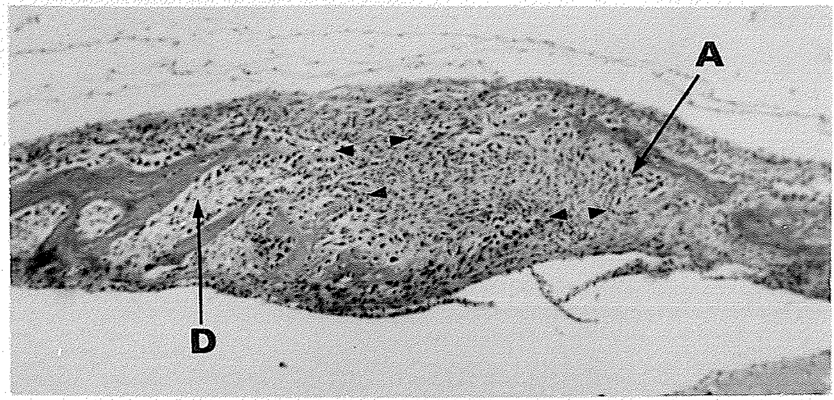
Note projecting bony margins (arrows).

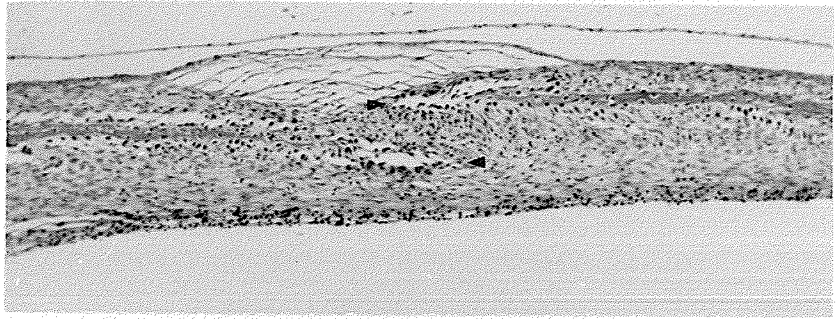
Stained with H and E (X 80).

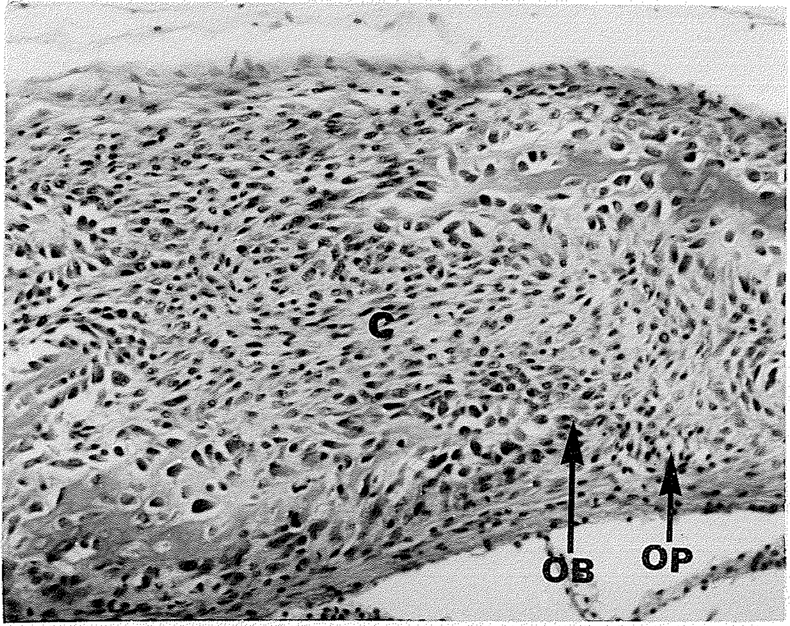
Figure 12. Magnification of Figure 10.

Note clusters of osteoblasts (OB) and osteoprogenitor cells (OP) and central fibrous layer (C).

Stained with H and E (X 200).


A
D




C
OB
OP

medially have only one projecting bony margin demonstrating active osteogenesis (Fig. 11) and these are usually slightly overlapping. Growth is occurring, however, on all surfaces of the bones at the sutural area. The variation in bone thickness may be associated with the greater curvature of the skull in these areas. The fact that more projecting bony surfaces are present on the lateral compared to the medial aspects of the suture is consistent with the observation on the 38 day skulls whereby more interdigitation occurs at the lateral portion of the coronal sutural area than the medial.

The cellular detail of the lateral aspect of the suture is represented in Fig. 12. Large clusters of cells are noted at the proximal portions of the projecting bony margins. Immediately adjacent to the bone edge these cells are the very large and plump osteoblasts with their eccentrically disposed nucleus and basophilic cytoplasm (Fig. 12, OB). Between the osteoblastic layer and the central fibrous layers of the suture are several layers of cells which are flattened or fusiform in shape. The cytoplasm has less basophilia than the osteoblast. These are the osteoprogenitor cells, the precursor to the osteoblast (Fig. 12, OP). Pritchard (1971) refers to these two types of cells close to the bone surfaces as osteogenic cells so for convenience sake this layer of cells will be referred to as the osteogenic layer. The extent of this layer in various regions gives some idea of the amount of osteogenesis occurring in these regions at a particular time. At the medial part of the coronal sutural area, osteoblastic activity is extensive and clusters of osteogenic cells are present at the proximal portions of the projecting bone margins. Overall osteogenic activity seems to be greater the more laterally one examines at the sutural area. Within the central areas of the suture numerous spindle shaped fibroblasts are seen within the fibrous connective tissue (Fig. 12, C).

There are three main fibre systems according to density and fibre orientation at the sutural area. The dense uniting layers of the fibrous periosteum covering the superior and inferior bone surfaces of the calvaria run from one bony sutural margin to the other. The fibres are orientated tangentially to the bone surfaces. These fibres stain heavily with Masson's Trichrome stain and are infiltrated with numerous fibroblasts (Fig. 13, P).

A central fibrous layer within the suture makes up the greatest proportion of the fibrous tissue between the opposing bony margins (Fig. 13, C). It is present at three main areas within the suture; the superior sutural opening, the overlapped area, and the inferior sutural opening. The fibres are moderately dense compared to the uniting layers and are orientated predominantly parallel to the calvarial surface. The central layer joins all opposing sutural bony margins and is continuous with the uniting periosteal layers.

Figure 13. Four day control coronal sutural area (lateral aspect).

Note diffuse fibrous layers (B), periosteal layers (P), and dense central fibrous layers (C).

Stained with Trichrome (X 200).

igure 14. Four day repositioned coronal sutural area.

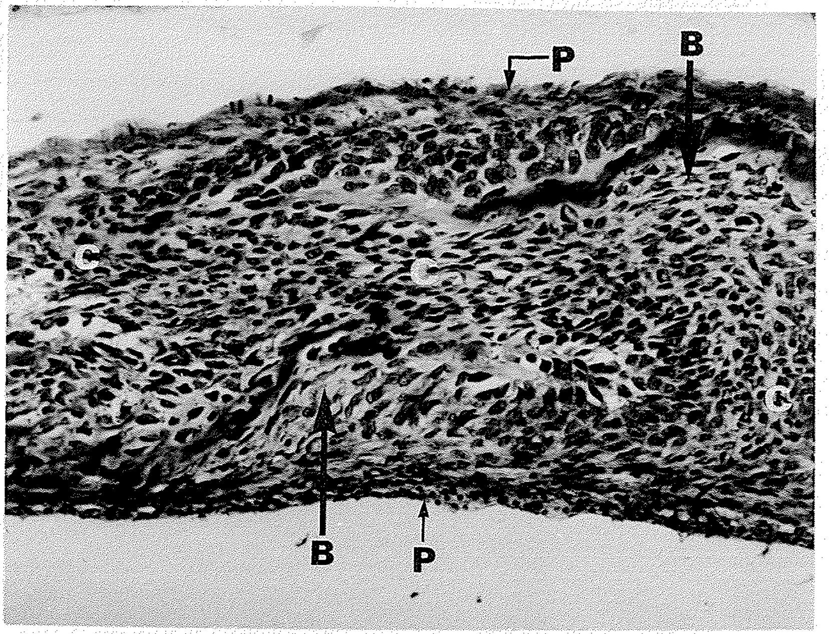
Stained with Trichrome (X 200).

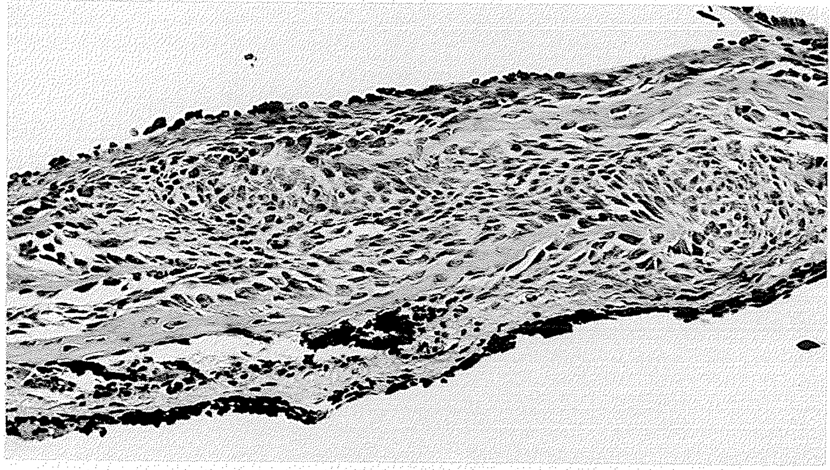
igure 15. Section of cut bone edge of frontal bone (A).

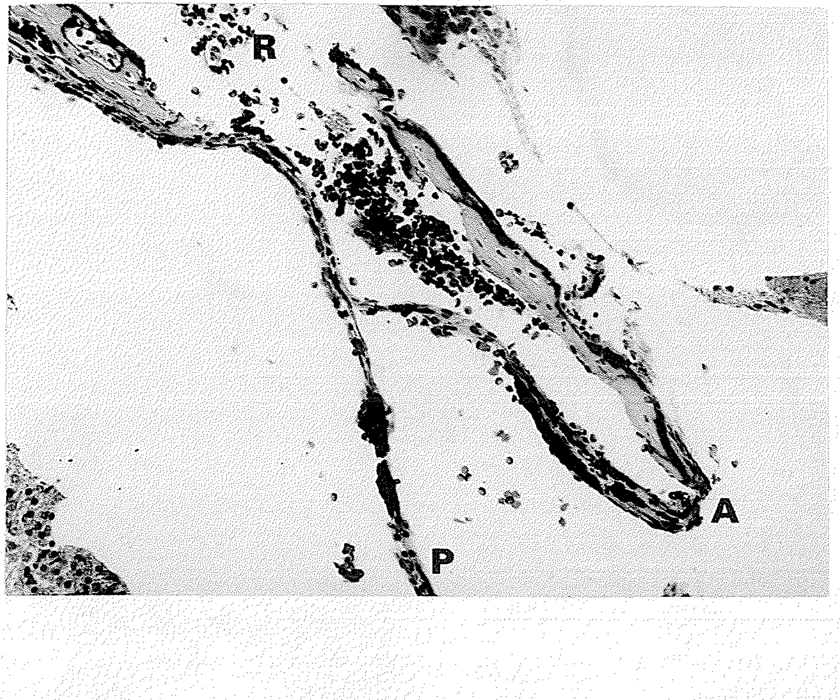
Top - Superior surface of frontal bone.

Note red blood cells (R) released from fracturedbone and detached periosteum (P)

Stained with Trichrome (X 200).


P
B
C
C
C
B
P




R
A
P

This description is relevant whether the lateral or the medial aspects of the sutural area are examined. Unfortunately, it is not always possible to see this layer clearly or to establish fibre orientation due to the great numbers of cells at this stage.

A diffuse, more delicate network of fibres which are somewhat disorganized in their orientation connects the bone surfaces at the sutural area to the tangentially orientated central fibrous layer (Fig. 13, A), and to the fibrous periosteum on the superior and inferior calvarial surfaces (Fig. 13, B). The fibrous layer is closely associated with the osteogenic layers next to the bone surfaces and tends to infiltrate between the cellular component.

In general, it appears that at four days of age the coronal sutural area of the rat demonstrates rapid bone growth. The evidence for this is the presence of projecting opposing bone margins which are capped by clusters of osteogenic cells. There are five intervening layers between the opposing bone margins; two osteogenic cellular layers, two delicate fibrillar layers, and a more dense central layer which makes up most of the intervening fibrous component. Running from one bony margin to the other on the superior and inferior calvarial surfaces are the uniting fibrous periosteal layers. The osseous, fibrous and cellular components at the sutural area demonstrate a morphological pattern which is well organized at the histological level.

The tangentially orientated central fibres of the suture would seem to indicate that tension is acting at the sutural area.

Four Day Experimental (Immediate Post-Surgery)

The repositioned segment is not always closely approximated with the adjacent bony calvarial surfaces. In some cases close approximation does occur. It is felt that the relationship of the repositioned segment may have been altered during sectioning procedures due to the fact that no fibrous attachment with adjacent bone surfaces occurs at this time.

The sutural area appears normal as regards cellular detail and fibre orientation (Fig. 14). The cut ends of the bones are damaged (Fig. 15, A) and severing of the blood vessels results in a release of red blood cells from the marrow spaces in the area of the cut bone edge (Fig. 15, R). The periosteum is separated from the bone surfaces close to the cut edge (Fig. 15, P). The periosteum is also separated minimally farther away from the cut edges. This could result either from skin retraction during the operation or from bending of the bone away from the periosteum at a distance from the incision area during surgery. It must be noted that the rat calvarium is extremely fragile at four days of age, being only a few microns in thickness and bone bending is very likely to occur.

Ten Day Control

The coronal sutural area at ten days (Fig. 16) presents the same general morphological pattern as the four day sutural area. Certain differences are apparent. There is a greater amount of overlapping of opposing bony margins although no definite differences can be seen between the lateral and medial portions of the sutural area. However, the more lateral portions demonstrate interdigitation of opposing bony margins (Fig. 17) while the medial portions do not. Large diploic spaces open directly in the suture at the bony sutural margins (Fig. 16, D). The portions of the frontal and parietal away from the sutural area are devoid of diploie at this stage.

The cellular component of the suture differs in that the clusters of osteogenic cells at the ends of the projecting bony margins (Fig. 17, OB) were not as large as the four day suture. The presence of these cells adjacent to all opposing bony margins is an indication that the suture is still in a stage of very active osteogenesis.

The fibre direction of the central fibrous layer is predominantly parallel to the calvarial surface. The five intervening sutural layers can be identified.

Ten Day Experimental

The repositioned segment is separated from the intact calvaria by an inflammatory response at the cut

Figure 16. Ten day control coronal sutural area.

Left: Frontal bone.

Right: Parietal bone.

Note open diploic spaces (D).

Stained with H and E (X 80).

Figure 17. Ten day control coronal sutural area (lateral aspect).

Left: Parietal bone.

Right: Frontal bone.

Note osteogenic cells (OB) and parallel oriented central layers (C).

Stained with Trichrome (X 200).

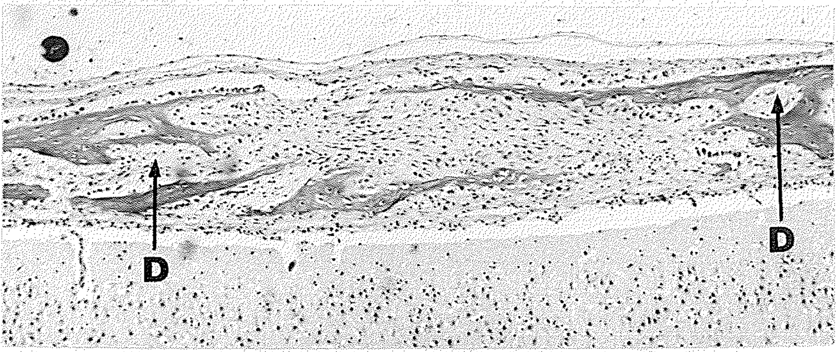
Figure 18. Ten day repositioned coronal sutural area.

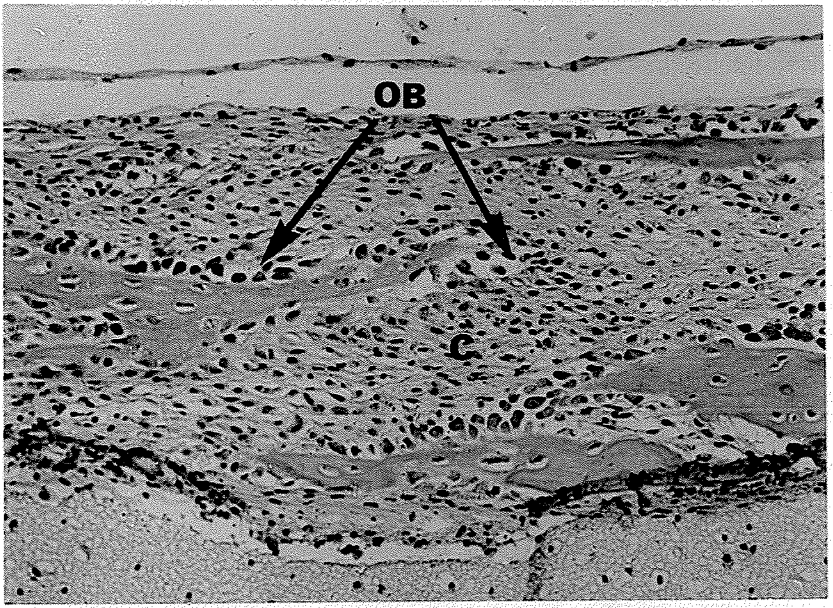
Left: Frontal bone.

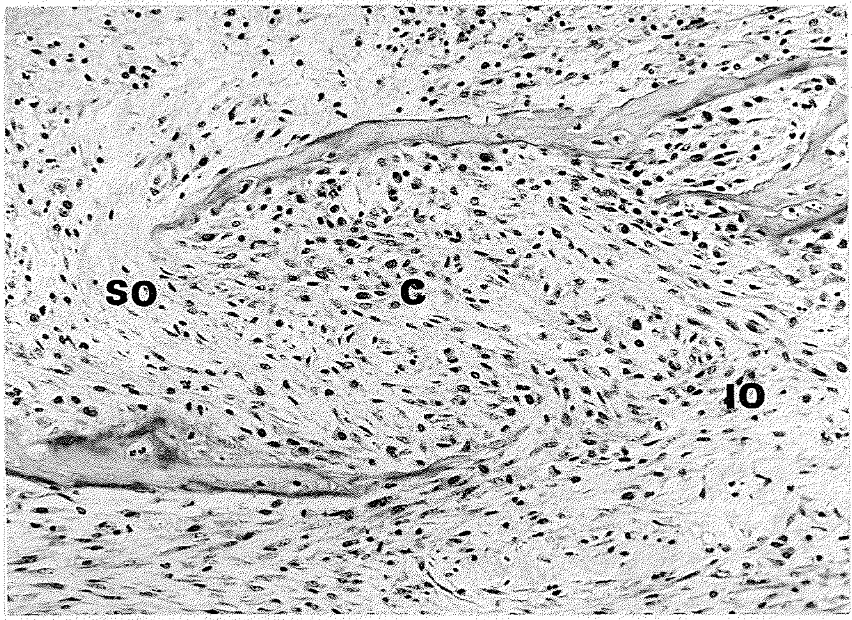
Right: Parietal bone.

Note perpendicular fibre orientation at superior and inferior sutural openings (SO, IO) and oblique orientation in central overlapped area (C).

Stained with Trichrome (X 200).


D
D


OB
C


SO
C
IO

surfaces. In most cases the repositioned segment is at a slightly superior level relative to the adjacent calvaria. A mild inflammatory response is limited mainly to the superior calvarial surfaces of the repositioned area. The severity of the response decreases the farther one examines from the sutural area.

The sutural area poses several histological differences from the control. There is still an overlapping of the opposing bony margins but it appears as if they are more closely approximated (Fig. 18). Open diploic spaces are present at the sutural area, and these appear to be more extensive and more numerous than in the control.

The fibre orientation at the sutural area is perpendicular to the calvarial surface at the superior (Fig. 18, SO) and inferior sutural openings (Fig. 18, IO). In the overlapped area (Fig. 18, C) the fibres are orientated at 45° to the calvarial surface extending from the endocranial surface of the parietal bone margin anteriorly to the ectocranial surface of the frontal bone margin posteriorly. Some variation in fibre direction in this area does occur, but orientation is predominantly in the direction described.

Osteogenesis is active at the sutural area but some interesting differences are apparent from the control. Instead of large clusters of osteogenic cells occurring at the proximal ends of the bone margins these areas are undergoing resorptive remodelling changes as demonstrated igure 19. 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).

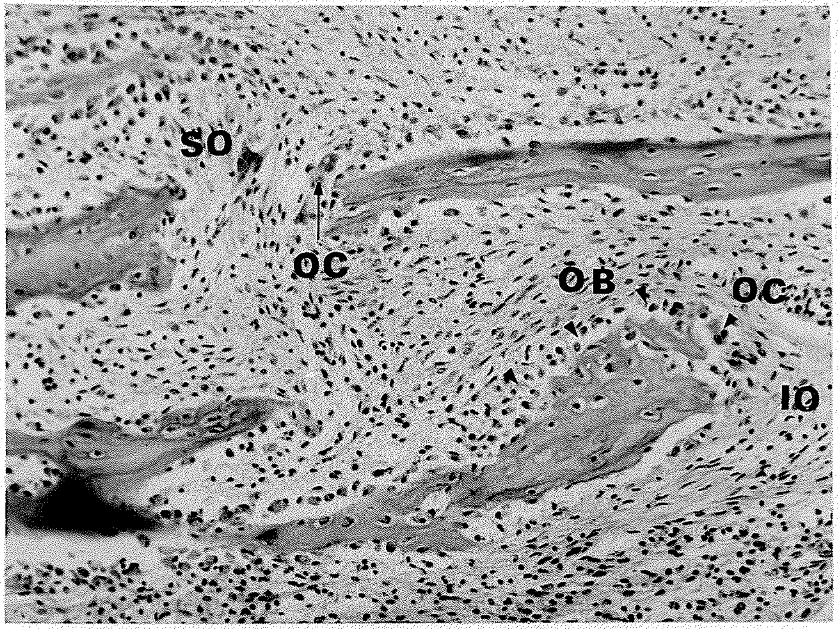
Stained with H and E (X 200).

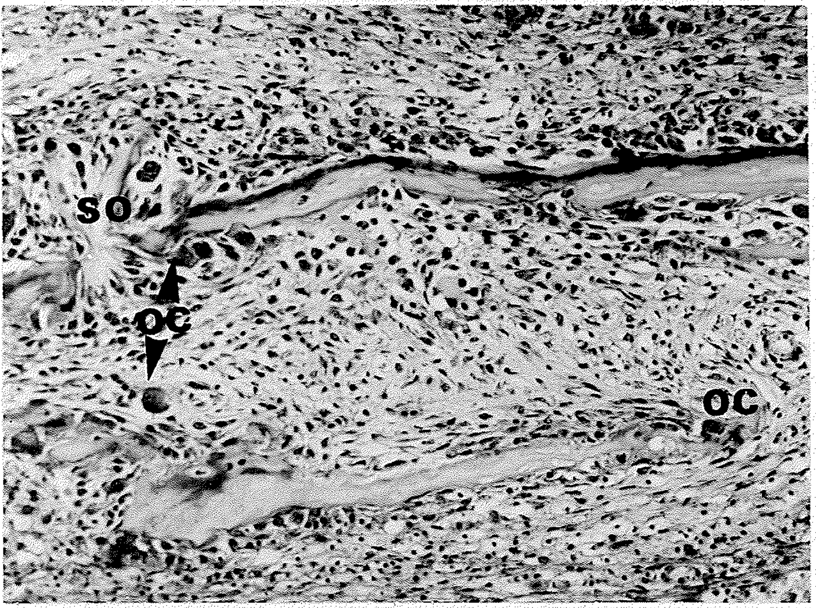
igure 20. Ten day repositioned coronal sutural area.

Note fusion at superior sutural margins (SO) and and resorptive remodelling by osteoclasts (OC).

Stained with Trichrome (X 200).

63b





by the presence of osteoclasts (Fig. 19, OC). Osteoclasts are observed on other surfaces of the bone margins, so that resorptive remodelling changes are not restricted to the bone ends. No osteoclasts were observed in the control sutures examined but the possibility of osteoclasts being present during other time periods or in areas which were not sectioned still exists. Osteogenesis is so active on the ecto- and endocranial surfaces of the opposing bony margins (Fig. 19, OB) that they seem to be increasing more in thickness than in length. This is in contrast with the control, and might explain the fact that the opposing bony margins appear more closely approximated than in the control.

In one portion of the sutural area, bone formation at the superior opening is so extensive that bony fusion occurs between the opposing margins (Fig. 20, SO).

At the cut surfaces of the repositioned segment an extensive inflammatory response (Fig. 21, IR) is present along with an immune response as indicated by the presence of lymphocytes and plasma cells. The presence of osteoclasts (Fig. 22, OC), on the bone ends means that bone resorption is active at these surfaces. Extensive esteogenic activity is occurring on the ectocranial and endocranial surfaces of some bone surfaces adjacent to the cut ends (Fig. 21, OB). This appearance is consistent with the marginal lipping observed on dried skulls adjacent to cut bone edges.

The intact coronal sutural area medial to the repositioned segment but on the left (operated) side (Fig. 23) also demonstrates an increased thickness at the

Figure 21. Inflammatory response in incision area (IR).

Left: Frontal bone (FB).

Right: Repositioned segment (frontal margin) (RS).

Note bone apposition (OB) on superior surface of frontal margin.

Stained with Trichrome (X 80).

Figure 22. Area similar to (RS) in Figure 21.

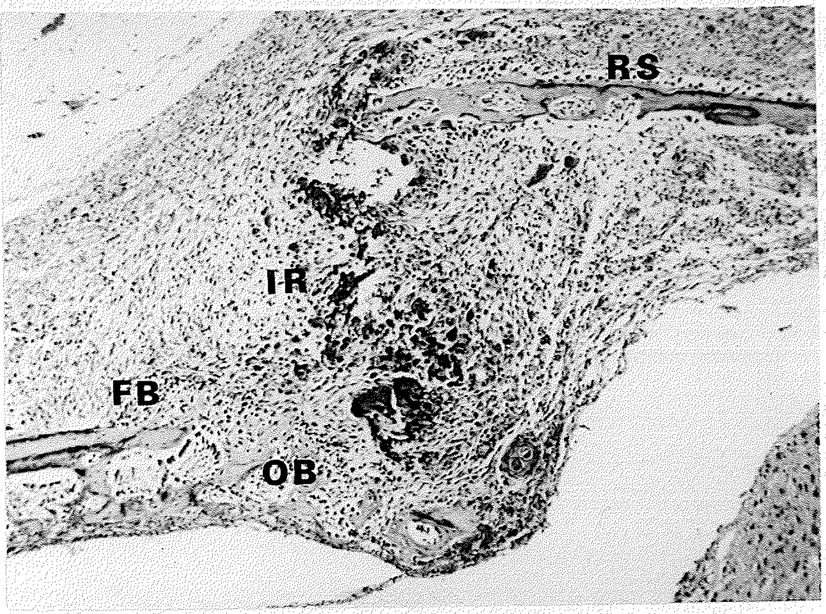
Note osteoclasts near bone ends (OC).

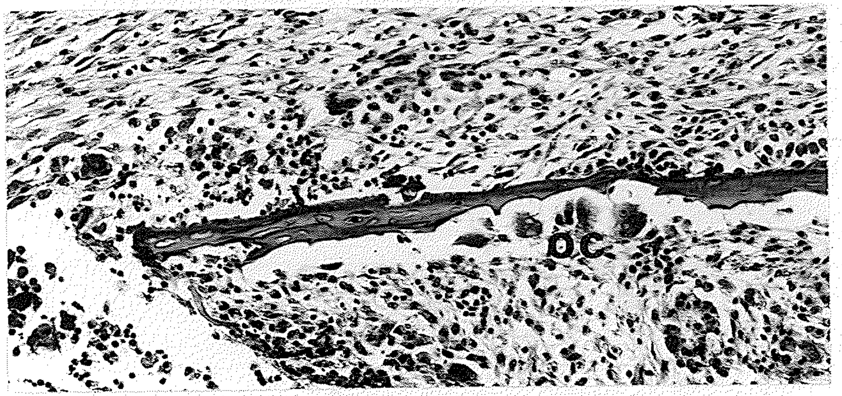
Stained with H and E (X 200).

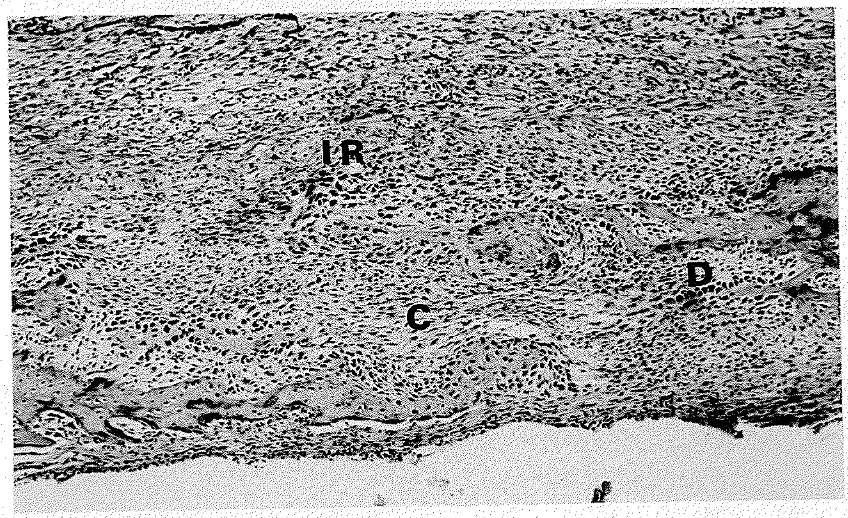
Figure 23. Ten day intact coronal sutural area medial to repositioned area.

Note open diploic spaces (D) and parallel orientation of central layers (C). Inflammatory response (IR) limited to superior cranial surfaces.

Stained with Trichrome (X 80).


RS
IR
FB
OB


OC


IR
D
C

opposing bony margins and a greater number of open diploic spaces. These effects are noticeable for some distance along the frontal and parietal bony margins of the repositioned segment, as well as the bones adjacent to this segment. The intervening cellular and fibrous components of the suture are similar to the control. The most significant finding is that the fibre direction within the suture is parallel to the calvarial surface (C) as in the control. This contrasts markedly with the more perpendicularly orientated fibres of the repositioned sutural area. As before, the inflammatory response (IR) is limited to the superior cranial surfaces of the sutural area.

Twenty-three Day Control

At 23 days the coronal sutural area presents the same general morphological features as the ten day control. There is extensive overlapping of both the lateral (Fig. 24) and the medial (Fig. 25) portions of the sutural area. The lateral portions demonstrate an interdigitating pattern which is not seen medially. The opposing bony margins are thicker than the ten day control and only a few diploic spaces open directly into the suture (Fig. 24, D) while the remainder are completely enclosed by the bony margins.

The suture contains the five intervening layers although the relative proportions of the various layers is changed. The osteogenic layer adjacent to the bony

Figure 24. 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 diploic spaces (D) present to a minor degree.

Stained with H and E (X 80).

Figure 25. Twenty-three day control coronal sutural area (medial aspect).

Only small number of osteogenic cells at margin ends (OB) with a shift towards margin surfaces (small arrows).

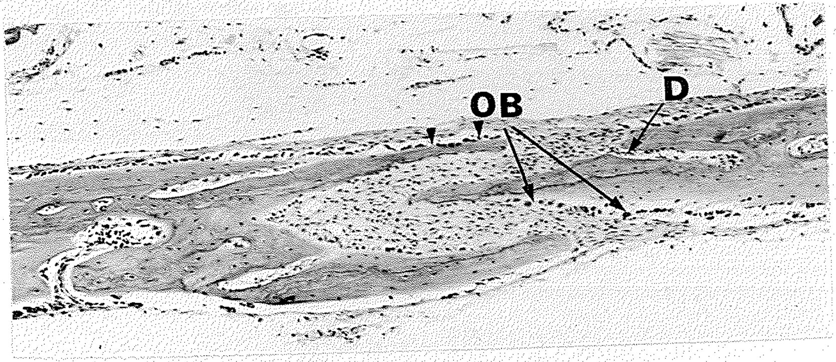
Stained with Trichrome (X 80).

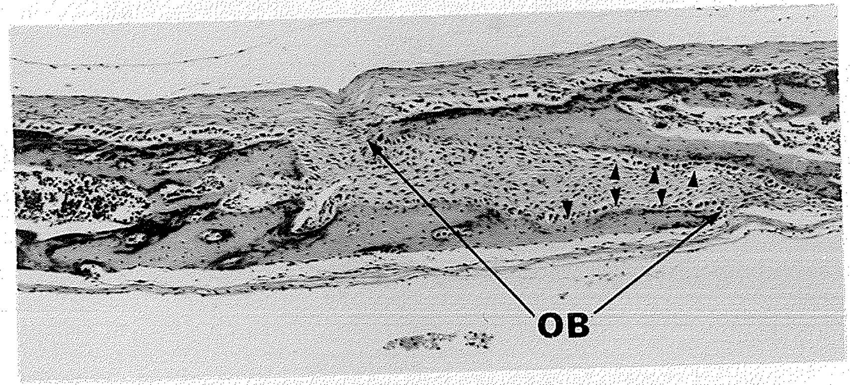
Figure 26. Twenty-three day repositioned coronal sutural area.

Osteogenic layers within suture and on vault surfaces adjacent to sutural area (OB) but reduced at margin ends (large arrows).

Note diploic spaces (D) and perpendicular fibre orientation of central layers.

Stained with H and E (X 80).


OB
D


OB


D
OB
OB

margins is present but the clusters of osteogenic cells are no longer evident at the proximal ends of the opposing bony margins (Fig. 25, OB). Only a few cells are noted in these areas. However, a greater concentration of osteogenic cells, relative to the projecting proximal bone edges, are noted on the superior and inferior surfaces of the opposing bony margins. In Fig. 24, OB this is well illustrated. Here the osteogenic cells are seen on the ectocranial surfaces of the superior frontal bone margin, and the endocranial surface of the projecting parietal margin. This is consistent with the observation that although the degree of overlapping does not appear to change, the bones at the sutural area are thicker than at ten days.

The intervening fibrous layer of the suture is composed mainly of a dense central layer with fibres orientated parallel to the calvarial surface, similar to the ten day control, with a diffuse fibrous network adjacent to the bone surface. In the overlapped area, although fibre orientation is predominantly parallel, some variation does exist.

Twenty-three Day Experimental

The 23 day experimental sutural area is quite different from the control. The shape of the sutural area varies from mild overlapping, a butt-end type (Fig. 26), or in some cases complete sutural fusion occurs (Fig. 27).

The thickness of the opposing bony margins is markedly increased but this effect is less noticeable the farther away from the sutural area one examines. Numerous diploic spaces are present at the sutural area and are lined with osteogenic cells and filled with hemopoietic tissue (Fig. 26, D).

The bony sutural margins are lined with an osteogenic layer indicating that bone formation is active in these areas (Fig. 26, OB). This layer is minimal on the superior and inferior aspects of the bony margins (Fig. 26, large arrows). The relative proportion of osteogenic cells at the sutural area is in favour of the central areas compared to the control. Extensive osteogenic activity is noted on the superior and inferior surfaces of the parietal and frontal bones adjacent to the sutural area (Fig. 26, OB).

The intervening fibrous layers are composed predominantly of dense collagenous fibres which run parallel to the opposing bony margin at a specific area. In the case of a butt-end type of sutural area as in Fig. 26, this central fibrous area runs perpendicular to the calvarial surface. This is in contrast to the control suture where fibre orientation is opposite to the above description.

In some sutural areas of the repositioned segment, complete fusion across the suture occurs. In these areas cartilage is noted and sutural fusion in most cases is due

Figure 27. Twenty-three day repositioned coronal sutural area.

Note cartilagenous fusion (CA), osteogenic areas within suture (OB) and reduced at bone ends (large arrows).

Stained with H and E (X 80).

igure 28. Twenty-three day intact coronal sutural area medial to repositioned segment.

Concentration of cells is shifted from within suture (small arrows) to sutural margins (large arrows).

Note bevelling and interdigitation compared to repositioned area.

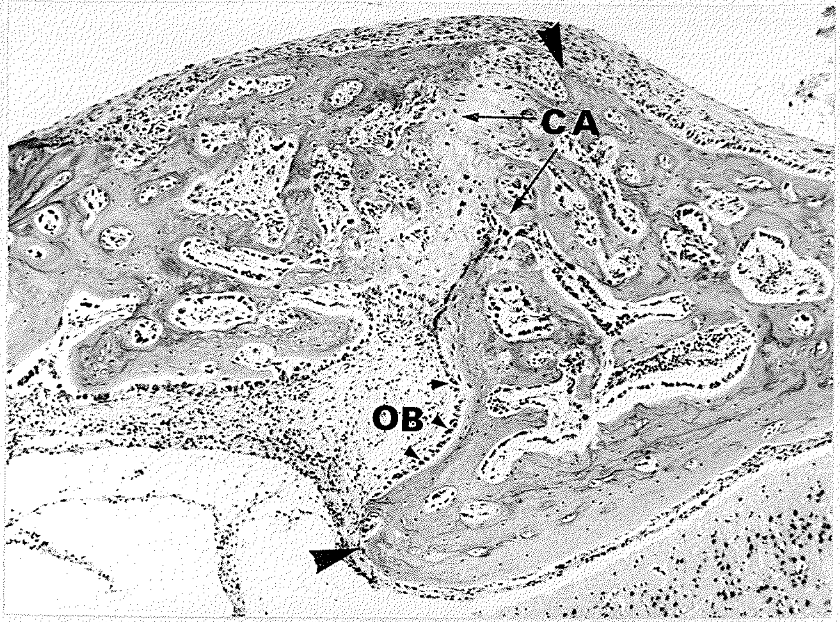
Stained with Trichrome (X 80).

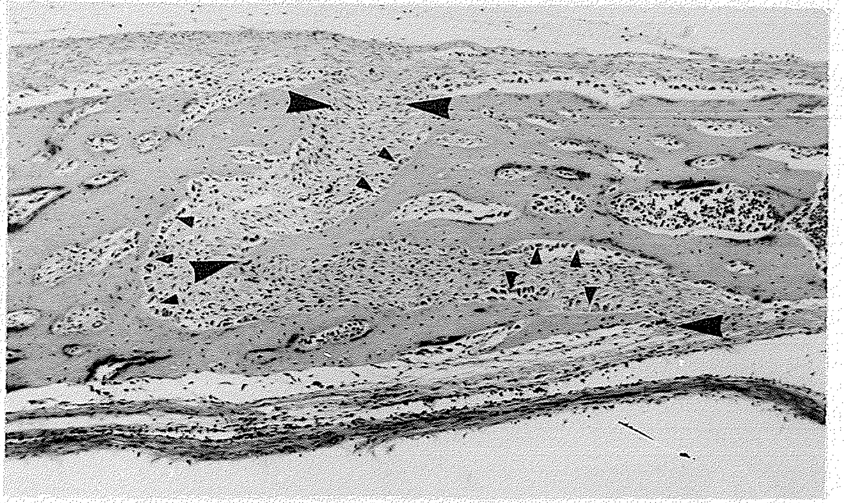
igure 29. Thirty-eight day control coronal sutural area (lateral aspect).

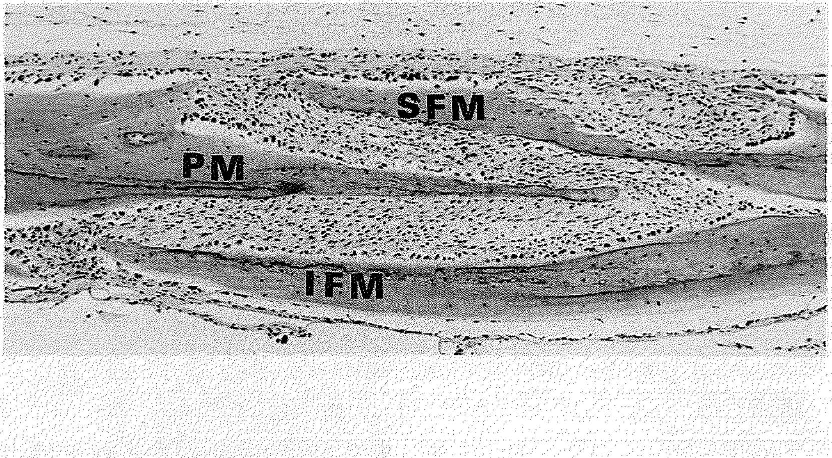
Left: Parietal margin (PM).

Right: Superior frontal margin (SFM).
Inferior frontal margin (IFM).

Stained with H and E (X 80).


CA
OB




SFM
PM
IFM

to cartilagenous union (Fig. 27, CA). Surrounding the cartilage are layers of bone lined with osteogenic cells. At certain places, the cartilage is infiltrated and replaced by bone implying that the fusion involves the process of endochondral bone formation. The cartilage is confined to the more central portions of the sutural area and at no time is it found on the superior or inferior surfaces of the sutural bony margins. Osteogenic activity occurs along the opposing bony margins within the sutural area but is minimal at the bone ends in the region of the superior and inferior sutural openings (Fig. 27, large arrows).

The intact coronal sutural area (Fig. 28) on the left (operated) side but medial to the repositioned segment shows a morphological pattern which is different from both the repositioned and the control sutural area. The overlapping and interdigitation is similar to the control, but the increased thickening and numerous diploic spaces of the opposing bony margins is similar to the repositioned sutural area. The osteogenic areas are concentrated at the projecting bone ends (Fig. 28, large arrows) which is not seen to the same degree in the repositioned suture. There is still a significant proportion of osteogenic cells on other opposing bony surfaces (Fig. 28, small arrows).

The fibre orientation of the intervening central layers is parallel to the calvarial surface. The area of the coronal suture described exhibits the thickening of the opposing bony margins seen in the repositioned segment and the fibre orientation and cellular distribution of the control suture.

Thirty-eight Day Control

The morphological type of sutural area is no different at 38 days than at 23 days. The lateral portions are overlapped and interdigitated (Fig. 29) while the medial portion is overlapped with no interdigitation. In all portions, the amount of interdigitation is more extensive than in the 23 day control. The interdigitation of the opposing margins is so extensive in the lateral portions of the sutural area that the parietal margin (Fig. 29, PM) is almost "swallowed up" by the superior and inferior margins of the frontal bone (Fig. 29, SFM, IFM). Diploic spaces are found in isolated areas of the parietal and frontal bones adjacent to the sutural area but are only rarely found within the bony margins of the sutural area (Fig. 30, D).

The five intervening layers of the suture are similar to the 23 day control. Active osteogenesis is present on all bony sutural margins and fibre direction within the central areas is predominantly parallel to the calvarial surface (Fig. 30, C). The relative proportion of the cellular to fibrous component cannot be distinguished from the 23 day control.

Figure 30. Thirty-eight day control coronal sutural area (lateral aspect).

Note parallel oriented central layers (C) and few diploic spaces (D).

Stained with Trichrome (X 80).

Figure 31. Thirty-eight day repositioned coronal sutural area.

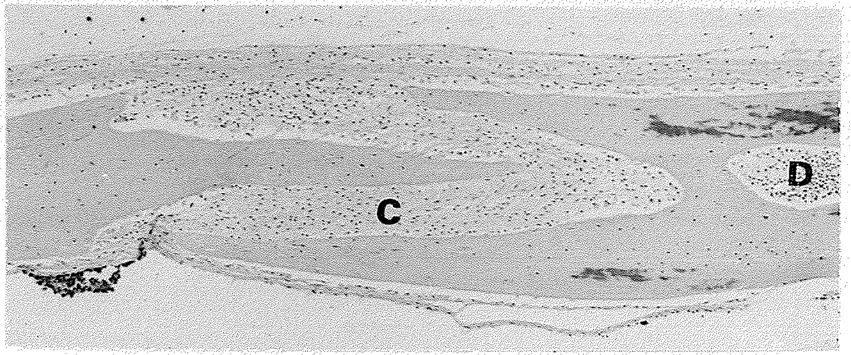
Minimal osteogenesis occurring within suture (small arrows) with increased number of diploic spaces (d) at margins and adjacent areas (D).

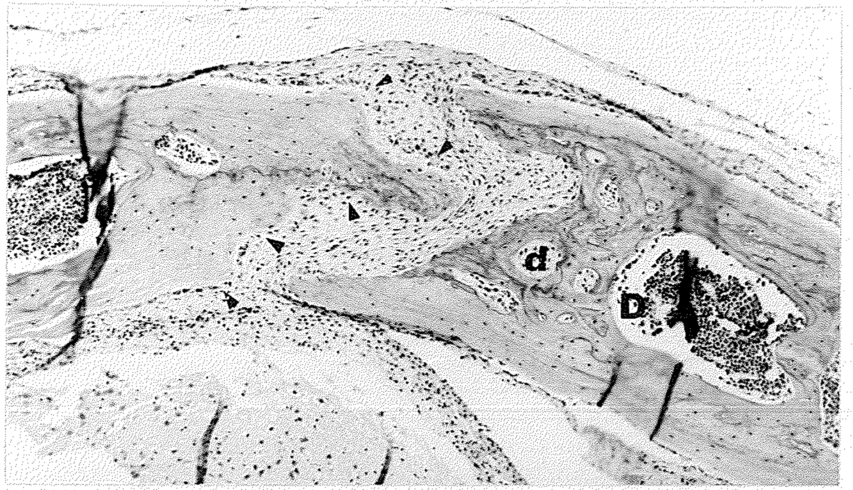
Stained with H and E (X 80).

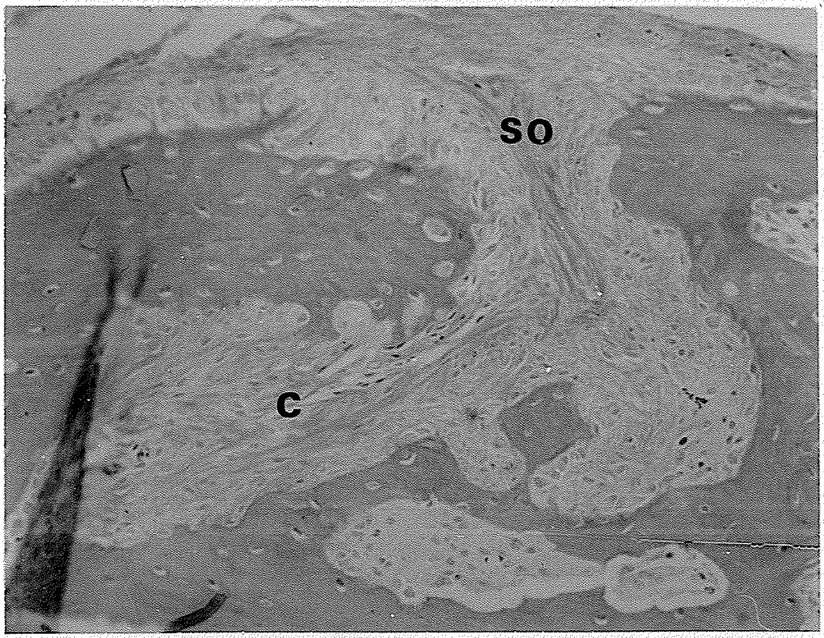
Figure 32. Magnified area similar to Figure 31.

Note perpendicular orientation of fibres at superior sutural opening (SO) and oblique orientation in overlapped areas (C).

Stained with Trichrome (X 200).


C
D


d
D


SO
C

Thirty-eight Day Experimental

At 38 days minimal overlapping and interdigitation occurs but is markedly reduced when compared to the control (Fig. 31). In some portions of the sutural area the opposing margins are flat and result in a butt-end type of sutural area. The thickness of the opposing sutural bony margins is greater than the control but is lesser than the 23 day repositioned bony margins. Large diploic spaces are present within the frontal and parietal portions of the repositioned segment (Fig. 31, D) while smaller spaces are present within the bony sutural margins (Fig. 31, d).

The five intervening sutural layers can be identified. Minimal bone growth is occurring on all sutural bony margins as seen by the single layer of osteogenic cells on these margins. In Fig. 31 (small arrows) this osteogenic activity is illustrated on the parietal bony sutural margin. No areas of resorptive remodelling could be demonstrated at this stage.

The fibre orientation of the central fibrous layer is perpendicular to the calvarial surface at the superior (Fig. 32, SO), and inferior sutural openings. In the overlapped area (Fig. 32, C) the fibre orientation varies but is at a 45° angle to the calvarial surface in most cases. Although fibre direction varies and is often disorganized, there is a general pattern for the repositioned sutural area which is quite different to that seen in the control.

The differences in fibre orientation within the repositioned segment are associated with the differences in morphological pattern of the opposing bony margins. None of the sutural areas examined at 38 days for the repositioned segment demonstrated any cartilage or sutural fusion.

The intact coronal sutural area medial to the repositioned area on the left (operated) side (Fig. 33) shows many of the features seen in a similar area at the 23 day stage. The opposing bony margins are thicker and have more diploic spaces than in the control. There are many more interdigitating bony margins compared to the control. However, the extent of interdigitation of each margin does not appear to be greater, at least in the sections examined. The number of interdigitating margins increases, the thicker the bone at the sutural area becomes.

The intervening layers of this area of the suture are similar in design to the control. An osteogenic layer covers all bony sutural margins (Fig. 34, large arrows) and fibre orientation of the central fibrous layer is generally parallel to the calvarial surface (Fig. 34, C).

igure 33. Thirty-eight day intact coronal sutural area medial to repositioned segment.

Note thickened margins and maintenance of bevelling and interdigitation.

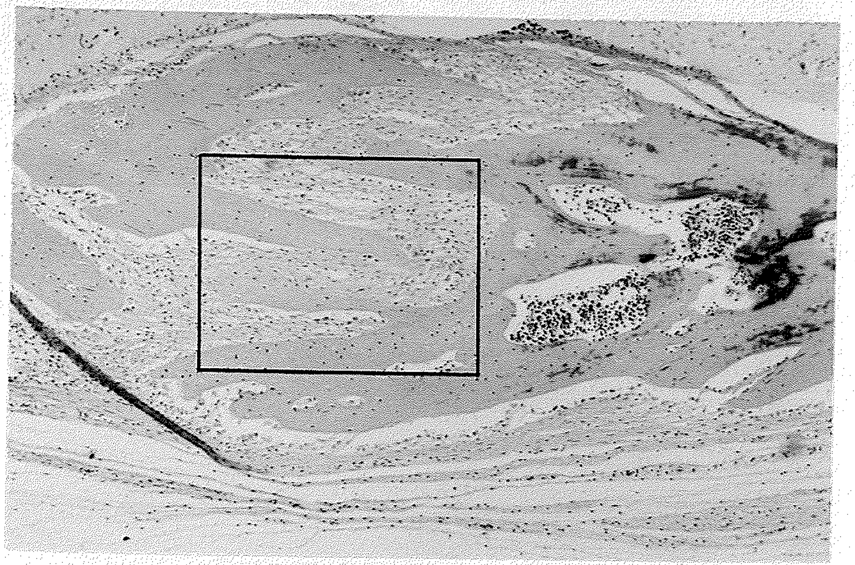
Stained with Trichrome (X 80).

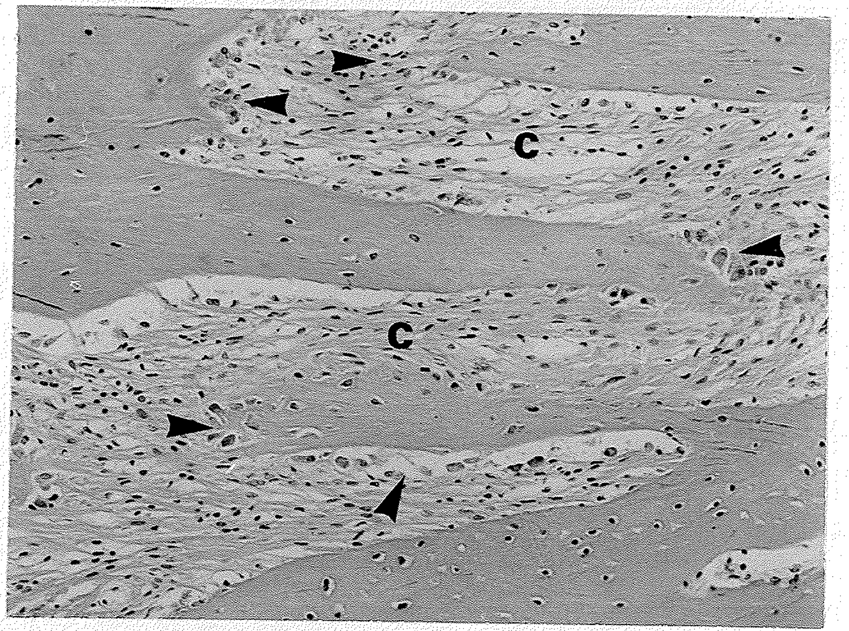
igure 34. Magnification of area in square from Figure 33.

Note increased osteogenic activity at sutural margins compared to repositioned area (arrows) and parallel orientation of central layers (C).

Stained with Trichrome (X 200).

76b





QUANTITATIVE I

LINEAR ANALYSIS OF VITAL STAINING

The linear analysis will be discussed under four main headings corresponding to the following groups:

(1) Control groups.
(2) Incision group.
(3) Extirpation group.
(4) Repositioned group.

Reference will be made in the results to a series of tables which are summaries of the relevant data from the linear analysis. Each series of tables, representing the linear sagittal and coronal analyses will have a key which will explain the method of tabulation and the different abbreviations used.

All time periods referred to in this section will be in "experimental days" corresponding to the injection intervals for the vital-staining. The corresponding postnatal day of age can be obtained by adding an increment of four to the time period mentioned. All figures will refer to the number of days postnatally.

Control Groups

The morphology of the coronal sutural area has been described in detail previously in Section II. It is a bevelled, interdigitating type of sutural area where the parietal bony margin overlaps the frontal bony margin

(Fig. 35). The increments of bone growth at various time intervals on opposing bony margins are listed in Table III. It is evident that the growth increments vary depending on the bone margin and the time interval observed. The two frontal bone margins are similar in growth rates except between the 8th-18th experimental days (interval BC) in which growth at the inferior margin is significantly greater ($P \leqq .05$) than the superior margin. The parietal bone margins differ throughout the experimental period (interval AB not obtained for the inferior margin) with more growth occurring at the superior margin. The total amount of growth on the superior parietal bony margin is greater compared to the inferior frontal margin for this experimental period.

The anterior metopic sutural area (Table IX) is of the butt-end variety. The growth increments at this area are small compared to the coronal sutural area but are relatively constant over the 42 day interval. No growth increment could be obtained for interval A-B due to extremely fast growth rate during this period, which is associated with resorptive remodelling between the superior and inferior aspects of the calvaria at the sutural area. This results in resorption of the first stain line. Bone growth measurements for interval AB are missing in other sutural areas studied for the same reasons. No significant left-right differences in increments of bone growth are noted.

The posterior metopic sutural area is initially a

TABLE III

LINEAR ANALYSIS SHOWING NORMAL GROWTH INCREMENTS IN VARIOUS SUTURAL AREAS

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

Figure 35. Forty-six day control coronal sutural area.

Left: Parietal bone.

Right: Frontal bone.

Non-decalcified section photographed under ultraviolet light (X 80).

Figure 36. Forty-six day coronal sutural area within incision area.

Left: Parietal bone.

Right: Frontal bone.

Note increased overlapping and interdigitation of opposing margins.

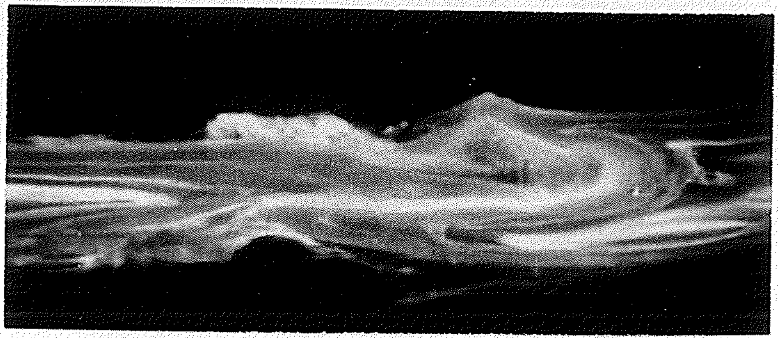
Non-decalcified section photographed under ultraviolet light (X 80).

Figure 37. Forty-six day repositioned coronal sutural area.

Note change toward butt-end type and thickened margins.

Non-decalcified section photographed under ultraviolet light (X 80).

80b



butt-end type, but at 42 days, minimal growth is occurring at the opposing superior sutural margins, while the inferior aspect of the sutural area shows fusion (Fig. 38). The superior margins are growing at the same rate as the anterior metopic sutural margins until the 18th experimental day, but progressively less growth is apparent throughout the experimental period. The pattern of vital-staining reveals that sutural fusion occurs somewhere between the 8th and the 18th experimental days.

The anterior (Fig. 41) and posterior sagittal sutural areas are quite similar in many respects. The morphological pattern is generally the butt-end type but some overlapping (bevelling) does occur. The superior and inferior margins of both sutural areas have similar growth rates and no left-right differences are noted. The posterior metopic sutural area shows less growth at interval CD (18th-28th experimental days) compared to the anterior sagittal but no differences are apparent at the other intervals studied. All four sutural areas studied in the coronal plane show markedly less growth than the coronal sutural area.

Incision Group

The coronal sutural area within the incision area (Fig. 36) is in continuity with the adjacent bony surfaces of the calvarium as the incision areas are healed except in isolated areas of the lateral incisions. The sutural area appears thicker supero-inferiorly than the control although igure 38. Forty-six day control posterior metopic sutural area.

Note fused inferior border.

Non-decalcified section photographed under ultraviolet light (X 80).

igure 39. Forty-six day posterior metopic sutural area adjacent to extirpated area.

Left: Right side of skull.

Right: Left side of skull.

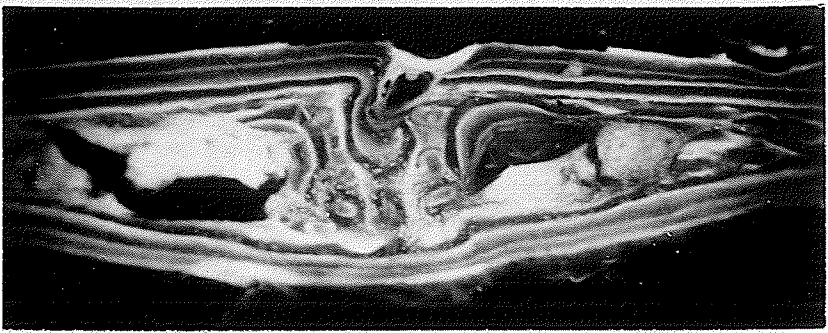
Note lack of fusion of inferior margins and change to the bevelled type.

Non-decalcified section photographed under ultraviolet light (X 80).

igure 40. Lipping of bone margin adjacent to extirpated area.

Non-decalcified section photographed under ultraviolet light (X 80).

82b



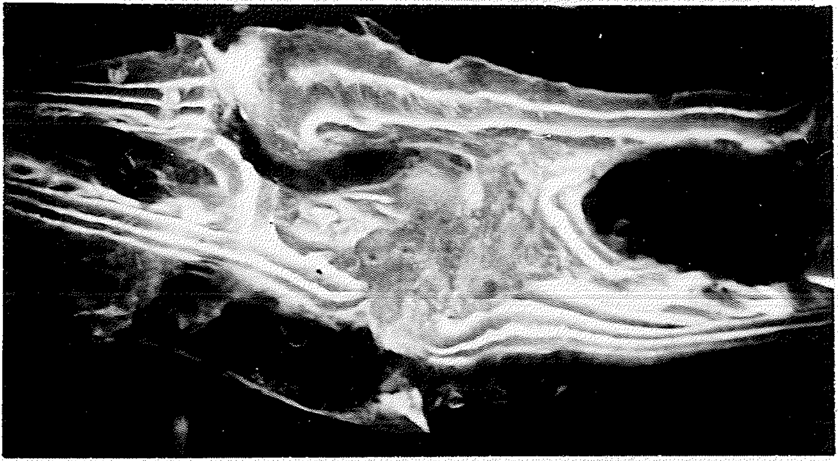


Figure 41. Forty-six day control anterior sagittal sutural area.

Note butt-end type of sutural area.

Non-decalcified section photographed under ultraviolet light (X 80).

Figure 42. Forty-six day anterior sagittal sutural area adjacent to extirpated area.

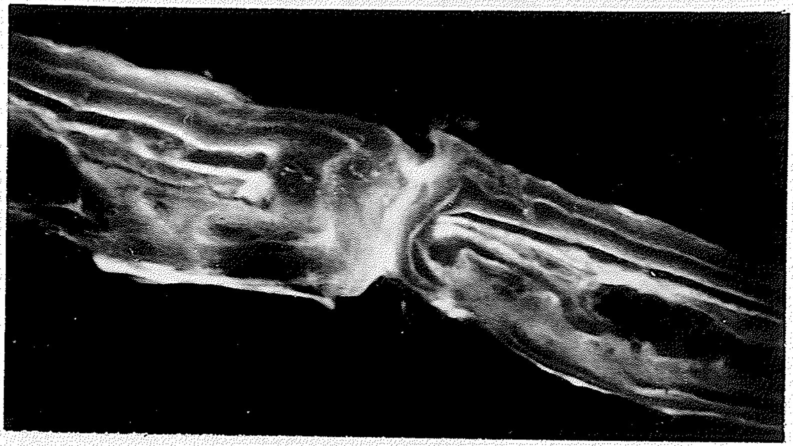
Left: Right side of skull.

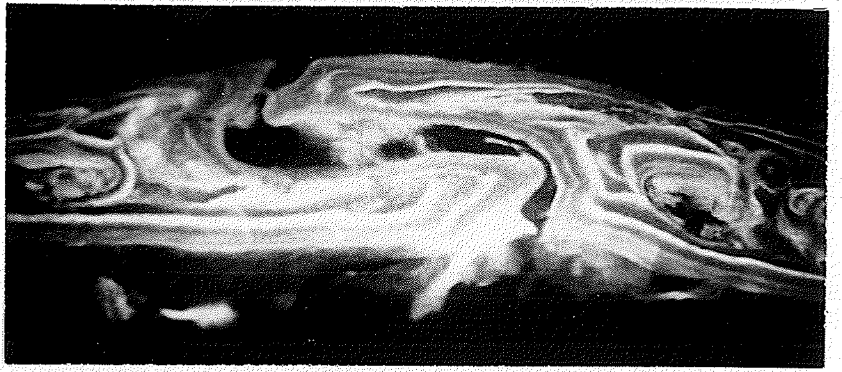
Right: Left side of skull.

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 (X 80).

83b





linear analyses of the bone surfaces adjacent to the sutural area do not show a statistically significant difference in bone growth from the control. The increments of bone growth are greater than the control on the superior frontal bone margins (Table V, Site 9) for interval BC and on the superior parietal margin (Table VI, 11) for interval CD. This alteration of growth rate on the superior bony sutural margins results in a posterior relocation of the superior opening of the suture. No significant difference in growth increments on other bone margins could be demonstrated.

Marked differences in morphological pattern and growth increments at other sutural areas are noted. The anterior metopic sutural area maintained its butt-end type of morphology but the left superior bone surface adjacent to the sutural area demonstrated more growth relative to the right side than in the control animal (here, the right side was greater than the left, Table IX, Site 3) for the increment BC. This results in an increased thickness of this bony margin. Figure 39 (representative of Groups III and V) resembles an overlapping type and no fusion of the inferior bony margins is noted. The sutural area appears thicker than normal supero-inferiorly. Linear analysis (Table X, Site 5) indicates a greater increment of growth at the left superior sutural margin than the right for interval BC. The result is a relocation of the superior sutural opening toward the right side.

The anterior sagittal sutural area (Table XI) is changed morphologically to the overlapping type. Variation in the amount of overlapping occurs but rarely is the butt-ended variety observed. No left-right difference in growth could be demonstrated on the superior bony margins (Site 5) but on the inferior margin (Site 6) the left side shows greater growth than the right for interval CD resulting in a minor relocation of the inferior sutural opening. Although no statistically significant difference is noted on the superior margin, in many cases a difference is present, but due to the variation between various areas of the suture within a particular block, this difference is sometimes camouflaged.

The posterior sagittal sutural area (Table XII) does not show much difference in overlapping and it retains its butt-end type of morphology. However, the fact that minor changes in pattern do occur is suggested by the greater increment of growth on the right inferior margin (Site 6) relative to the left for interval BC. Although overlapping is not clearly demonstrated, an increased thickness on the inferior bone surface adjacent to the sutural area (Site 4) on the left side is noted for intervals BC and CD.

The interparietal sutural area is also affected as seen by the greater increment of growth on the left anterior (parietal) bony margin (Table V, Site 22) compared to the right for interval BC. This results in a greater overall amount of growth (interval AE) for this margin (Table VIII, Site 22).

Growth alterations on bone surfaces in the vicinity of the surgical area are noted. There is an increased amount of growth on the left superior surface of the frontal bone (Table IV, Site 3) anterior to the cut surface for interval BC. The inferior surfaces in this area (Table VII, Site 4) shows an increased amount of growth over the experimental period (interval AE). Alterations in growth of the bone margins adjacent to the lateral incision sites are noted, principally in the posterior metopic and anterior sagittal regions. Sites 1 and 7 (Table X and XI) show greater increments of growth on the left side for interval BC. In most cases the increased growth during interval BC is reflected in a total increase in growth in these areas over the experimental period (interval AE). In general, there appears to be an increased amount of growth during the 8th-18th experimental days of the bone margins adjacent to the incision sites. Both the superior and inferior margins are affected but no preference could be established. The thickening of these margins is referred to as "lipping", and is demonstrated in Figure 40.

Extirpation Group

The coronal sutural area that was extirpated does not re-establish itself and the defect is filled in with a fibrous connective tissue layer. All bony margins surrounding the defect show alterations in bony growth which results in "lipping" of these margins (Fig. 40). The superior surfaces of the anterior and posterior margins (Table V, Sites 3, 17) show increased growth for the interval BC relative to the control side. The posterior margin also demonstrates increased growth during interval DE (Table VII, Site 17). The lateral margins show increased growth for intervals BC in both the posterior metopic region (Table X, Sites 1, 7) and in the anterior sagittal region (Table XI, Sites 1, 8) and for interval DE (Table X, Site 7). The fact that the superior or the inferior bony margin can be affected means that no preference towards either one is clearly shown.

The intact coronal sutural area medial to the extirpated area but on the operated side shows growth alterations similar to the coronal sutural area within the incision area of group V. The superior frontal margin shows increased growth for interval BC (Table V, Site 9). The inferior frontal margin, as well as both parietal margins, show increased growth for increment CD (Table VI, Sites 9, 10, 11). The result is an increased amount of overlapping at the sutural area and a relocation of the superior sutural opening posteriorly relative to the contralateral side. This is consistent with observations made on the dried skulls. Increased growth on the left superior parietal surface (Table VI, Site 13) adjacent to the parietal sutural margin during interval CD is demonstrated.

Other sutural areas also show alterations in morphology and increments of bone growth. Many of these are similar to those changes seen in the incision group. The anterior metopic sutural area is thicker on the left side than the right with an increased growth occurring on the superior surface of the sutural margin during interval BC (Table IX, Site 3). The posterior metopic sutural area shows similar morphological changes as in the incision group (Fig. 28) but no left-right differences in bone growth could be established. However, the increased thickness of the left margin is due, in part, to increased growth on its superior surface (Table X, Site 3) during interval BC. The anterior sagittal sutural area shows an increased thickness and overlapping of its opposing margins. The overlapping occurs in such a way that the left superior surface overlaps the right inferior surface so that there is a relocation of the superior sutural opening to the right side. This increased overlapping is due to increased growth of the left superior margin (Table XI, Site 5) and of the right inferior margin (Table XI, Site 6) during interval BC. The increased growth of the right inferior margin is continued through interval CD as well. The increased thickness of the left margin over the right is verified by the linear analysis which shows an increased growth during interval BC of the inferior bony surface adjacent to the sutural margin (Table XI, Site 4). The posterior sagittal sutural area (Table XII) shows increased growth on the left side at Site 6 during interval BC resulting in some overlapping and increased growth at Site 3 (interval BC) and Site 4 (interval CD) resulting in an increased thickness of the left side compared to the right.

Growth alterations on other bone surfaces on the left side, not in the immediate vicinity of the extirpated area are demonstrated (Table XII, Site 1, BC, and Table XII, Site 7, BC, CD, DE, AE).

Repositioned Group

The coronal sutural area within the repositioned group shows marked changes both morphologically and in incremental growth pattern compared to the control (right) side of the animal. The morphological changes (Fig. 37) have been described in detail histologically in Section II. These changes are mainly a reduction in the amount of overlapping of opposing margins, to minimal overlapping or even a butt-end type of sutural area. Linear analysis reveals a marked reduction in growth rates during the different time intervals. The inferior frontal margin (Site 10) shows less growth than the right side for all intervals studied except for CD where no difference is noted. Both parietal margins (Sites 11, 12) showed a reduction in growth for all intervals except AB where no measurements could be made. The total amount of growth at all these margins is less than the control side although Site 10 (Table VIII) is the only margin showing a significant reduction in growth for interval AE due to the missing values for interval AB at the other margins.

Growth on the bone surfaces adjacent to the sutural area is affected. The superior frontal surface (Site 7) shows more growth during interval BC (Table V) than the control side while the superior parietal surface (Site 13) shows more growth during interval CD (Table VI). For the other time intervals these surfaces, as well as the inferior bone surfaces, do not demonstrate a significant difference from the control side in growth rates. No difference in growth rates could be demonstrated on the cut margins of the repositioned area except for the increase in growth during interval BC (Table V) of the inferior surface of the cut frontal margin (Site 6). In some of the cut margins examined, the pattern of vital-staining reveals that bone resorption took place and this is consistent with the histological findings in Section II.

The cut surfaces of the intact frontal bone shows increased growth during interval BC (Table V, Site 3) and a greater total amount of growth for interval AE (Table VIII). The cut parietal margin (Site 17) shows an increased growth for all time intervals and a total increase in growth for interval AE. These results are consistent with the "lipping" previously described at these margins. Since no linear coronal data was obtained for this group, no mention can be made of any growth alterations in sutural areas in this plane, but results from dried skulls, and the histology would lead one to believe that the changes are similar to groups III and V.

KEY

LINEAR SAGITTAL ANALYSIS

(1) Stain intervals

AB - 8 days - Table IV.
BC - 10 days - Table V.
CD - 10 days - Table VI.
DE - 14 days - Table VII.
AE - 42 days - Table VIII.

(2) Linear Sites 1-22.

(3) Abbreviations

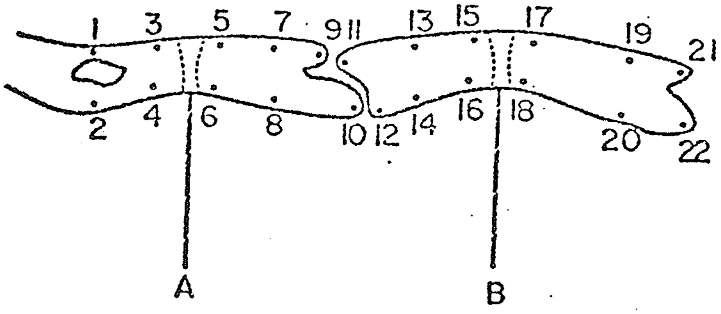
G Groups (I, II, IV, V).
- No significant L-R differences in bone growth. Symbol in upper square.
Two blank spaces in one group - missing data.
▽ L-R difference at $p \leqq .05$.
▽▽ L-R difference at $p \leqq .01$.
BL Blocks on left side.
BR Blocks on right side.
| Inside blocks (Medial sagittal sections) - Symbol in lower square BL and upper square BR.
O Outside blocks (Lateral sagittal sections) - Symbol in upper square BL and lower square BR.

A L-R difference in Groups II, IV or V is noted only if the difference is significantly greater than the L-R difference at the same site for the control group. If $L > R$ symbol is placed in upper square and if $R > L$ symbol is placed in lower square in each group.

TABLE IV

SITES

A B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
G I	–		–	–	–	–				–	–						–	–				–
G III			–																			
G IV			–	–		–											–	–				
										▽												
G V	–		▽		–	–				–	–						–	–				–
B L											O						O	O				
	I		I	I	I	I				I												I
B R	I		I	I	I	I				I	I						I	I				I
																		O				



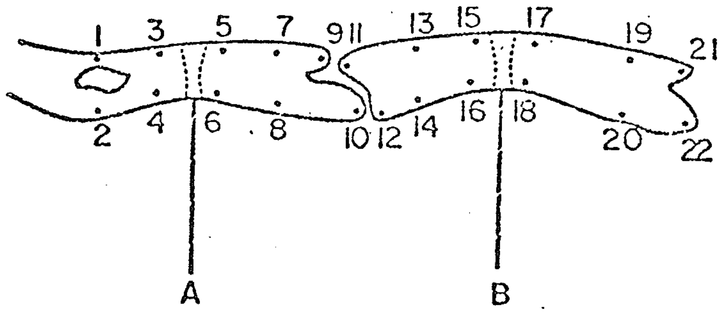
A – B MISSING IN GROUP III

A CUT BONE EDGE IN GROUP V

A and B CUT BONE EDGES IN GROUP IV

TABLE V

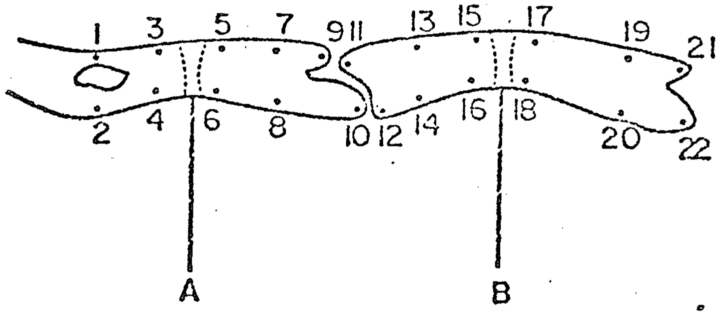
	SITES																					
B C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
G I	–	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	–		–
G III	–	–	▽	–	–	–	–	–	▽	–	–		–				▽	–	–	–	–	–
G IV	–		▽	–	–	▽	▽	–	–				–	–			▽	–	–	–		
										▽	▽	▽										
G V	–		–	–	–	–	–	–	▽	–	–	–	–	–			–	–	–	–	–	▽
B L												O		O				O			O	
	I	I	I	I	I	I	I	I	I	I	I	I	I				I	I	I	I		I
B R	I	I	I	I	I	I	I	I	I	I	I	I	I	I			I	I	I	I		I
							O			O	O		O								O	



A – B MISSING IN GROUP III
A CUT BONE EDGE IN GROUP V
A and B CUT BONE EDGES IN GROUP IV

TABLE VI

	SITES																					
C D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
G I	–	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	–		–
G III	–	–	–	–	–	–	–	–	▽	▽	▽		▽				–	–	–	–	–	–
G IV	–		–	–	–	–	–	–	–	–			▽	–			▽	–	–	–		–
											▽▽	▽										
G V	–		–	–	–	–	–	–	–	–	▽	–	–	–			–	–	–	–	–	–
B L																		O			O	
	I	I	I	I	I	I	I	I	I	I	I	I	I	I			I	I	I	I		I
B R	I	I	I	I	I	I	I	I	I	I	I	I	I	I			I	I	I	I		I
							O		O	O	O	O		O							O	



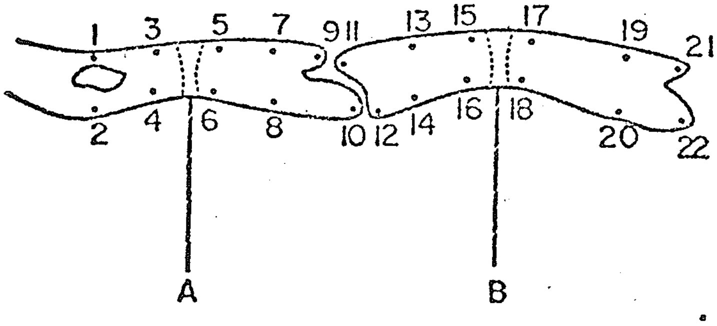
A – B MISSING IN GROUP III

A CUT BONE EDGE IN GROUP V

A and B CUT BONE EDGES IN GROUP IV

TABLE VII

	SITES																					
DE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
G I	–	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	–		–
G III	–	–	–	–	–	–	–	–	–	–	–		–				▽	–	–	–	–	–
G IV	–		–		–	–	–	–	–				–	–			▽	–	–	–		–
				▽						▽	▽	▽										
G V	–		–	▽	–	–	–	–	–	–	–	–	–	–			–	–	–	–	–	–
B L														O				O			O	
	I	I	I	I	I	I	I	I	I	I	I	I	I				I	I	I	I		I
B R	I	I	I	I	I	I	I	I	I	I	I	I	I	I			I	I	I	I		I
									O	O	O	O	O	O							O	



A – B MISSING IN GROUP III

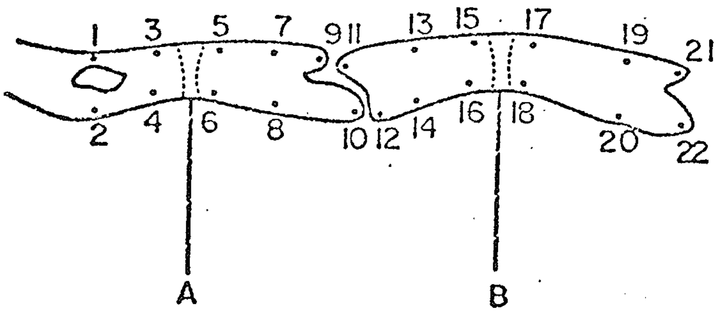
A CUT BONE EDGE IN GROUP V

A and B CUT BONE EDGES IN GROUP IV

TABLE VIII

SITES

AE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
G I	–		–	–	–	–				▽	–			–			–	–				–
G III			▽															–				
G IV			▽	–		–								–			▽	–				
										▽▽												
G V	–		▽		–	–				▽	–						–	–				▽▽
B L											O			O			O					
	I		I	I	I	I				I								I				I
B R	I		I	I	I	I				I	I			I			I	I				I
																	O					



A – B MISSING IN GROUP III

A CUT BONE EDGE IN GROUP V

A and B CUT BONE EDGES IN GROUP IV

KEY

LINEAR CORONAL ANALYSIS

(1) Blocks

Block 1 - Anterior Metopic - Table IX.

Block 2 - Posterior Metopic - Table X.

Block 3 - Anterior Sagittal - Table XI.

Block 4 - Posterior Sagittal - Table XII.

(2) Stain Intervals

AB, BC, CD, DE, AE.

(3) Sites

Block 1; 1-7.

Blocks 2, 3, 4; 1-8.

(4) Abbreviations

G - Groups (II, III, V).

Symbols for significance levels or missing data and their placement in specific spaces are identical to the linear sagittal analysis.

TABLE IX

SITES

AB	1	2	3	4	5	6	7	
G II								
G III								
G V								

SITES

DE	1	2	3	4	5	6	7	
G II	–	–	–	–	–	–	–	
G III	–	–	–	–	–	–	–	
G V	–	–	–	–	–	–	–	

SITES

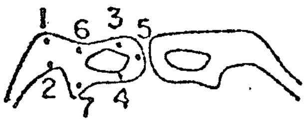
BC	1	2	3	4	5	6	7	
G II	–	–		–	–	–	–	
			▽▽					
G III	–	–	▽▽	–	–	–	–	
G V	–	–	–	–	–	–	–	

SITES

AE	1	2	3	4	5	6	7	
G II								
G III								
G V								

SITES

CD	1	2	3	4	5	6	7	
G II	–	–	–	–	–	–	–	
G III	–	–	–	–	–	–	–	
G V	–	–	–	–	–	–	–	



ANTERIOR METOPIC

BLOCK 1

TABLE X

SITES

AB	1	2	3	4	5	6	7	8
G II			–			–		
G III								
G V			–			–		

SITES

DE	1	2	3	4	5	6	7	8
G II	–	–	–	–	–	–	–	
G III	–	–	–	–	–	–	▽	
G V	–	–	–	–	–	–		
							▽	

SITES

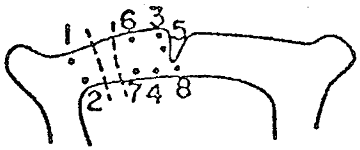
BC	1	2	3	4	5	6	7	8
G II	–	–	–	–	–	–	–	
G III	▽	–	▽	–	–	–	▽▽	
G V	▽▽		–	–	▽▽	▽	–	

SITES

AE	1	2	3	4	5	6	7	8
G II						–	–	
G III								
G V						▽	–	

SITES

CD	1	2	3	4	5	6	7	8
G II	–	–	–	–	–	–	–	
G III	–	–	–	–	–	–	–	
G V	–	–	–	–	–	–	–	



POSTERIOR METOPIC

------ DOTTED AREA IS EXTIRPATED IN GROUP III

BLOCK 2

TABLE XI

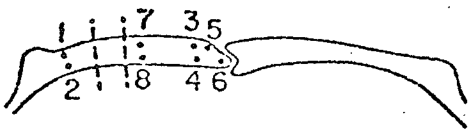
	SITES							
AB	1	2	3	4	5	6	7	8
G II	–	–					–	–
G III								
		▽						
G V	–	▽					▽	–

	SITES							
DE	1	2	3	4	5	6	7	8
G II	–	–	–	–	–	–	–	–
G III		–	–	–	–	–	–	–
	▽							
G V		–	–	–	–	–	–	–
	▽							

	SITES							
BC	1	2	3	4	5	6	7	8
G II	–	–	–	–	–	–	–	–
G III	▽	–	–	▽	▽		–	▽
						▽▽		
G V	▽▽	–	–	–	–	–	▽	–

	SITES							
AE	1	2	3	4	5	6	7	8
G II	–	–					–	–
G III		–						
G V	▽	–				▽	▽▽	–

	SITES							
CD	1	2	3	4	5	6	7	8
G II	–	–	–	–	–		–	–
						▽		
G III	–	–	–	–	–	–	–	–
G V	–	–	–	–	–	▽	–	–



ANTERIOR SAGITTAL

·----· DOTTED AREA IS EXTIRPATED IN GROUP III

BLOCK 3

TABLE XII

SITES

AB	1	2	3	4	5	6	7	8
G II							▬	▬
G III								
G V							▬	▬

SITES

DE	1	2	3	4	5	6	7	8
G II	▬	▬	▬	▬	▬	▬	▬	▬
						▬		▬
G III	▬	▬	▬	▬	▬	▬	▽▽	▬
G V			▬	▬	▬	▬	▬	▬

SITES

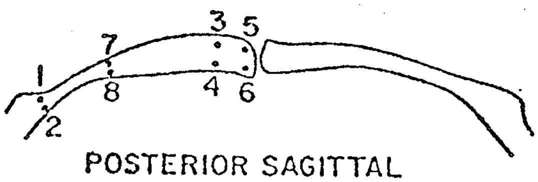
BC	1	2	3	4	5	6	7	8
G II	▬	▬	▬	▬	▬	▬	▬	▬
G III	▽	▬	▽	▬	▬	▽	▽▽	▬
G V			▬	▽	▬		▬	▬
						▽		

SITES

AE	1	2	3	4	5	6	7	8
G II							▬	▬
G III							▽▽	▬
G V							▬	▬

SITES

CD	1	2	3	4	5	6	7	8
G II	▬	▬	▬	▬	▬	▬	▬	▬
G III	▬	▬	▬	▽	▬	▬	▽	▬
G V	▬	▬	▬	▽	▬	▬	▬	▬



POSTERIOR SAGITTAL

BLOCK 4

CHAPTER V

DISCUSSION

The growth of the calvaria is an integrated process of osteogenesis and of proliferation of intervening soft tissues within the originally membranous cranial capsule. At the onset of ossification each calvarial bone is surrounded by a portion of this capsule which separates it from the adjacent bones. Any soft tissue area may be regarded as a suture. Consideration of the functional role of sutural growth on growth of the skull 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. Another problem is what initiates and controls the growth and morphological pattern of the sutural area. Why do certain sutural areas continue to grow while others fuse? What processes control sutural fusion? The last major consideration is what is the relationship between sutural growth and growth of the calvaria as a whole. Many techniques have been used to study some of these problems. Some experiments have looked at normal growth and morphology (Pritchard et al., 1956) while others (Moss, 1954) have looked at sutures under experimental conditions. In using a combination of techniques and in looking at the coronal sutural area under normal and experimental conditions, this study has attempted to study certain aspects of the problems mentioned above.

Normal Sutural Morphology

The morphology and histological detail of the coronal sutural area was established to act as a control for changes observed in the repositioned coronal sutural area. The normal structure of this area at four days consists of two approaching bony margins, each with several projecting bony trabeculae which are "capped" by clusters of osteogenic cells. Prahl (1968) describes the approaching parietal margin as having only one single bone trabeculae. She also states that the overlapping of opposing margins decreases towards bregma. This could not be confirmed in this study due to the variation of the morphological pattern in different regions. The sutural area is in the early growth stage of differentiation with open diploic spaces intermingled with new woven bone at the sutural margins. This is consistent with Moss's (1954) findings. At this stage and more so at the ten day stage the suture can be described as consisting of five intervening layers between the opposing bone margins - two cellular or cambial (Pritchard et al., 1956) layers which are osteogenic and are next to the bone surface; two diffuse layers with thin fibres radiating from the bone into the fifth central fibrous layer. Running from one bone margin to the other on the superior and inferior aspects of the sutural area are the periosteal layers or uniting layers (Pritchard et al.) which are in continuity with the intervening sutural layers. This point - the continuity of the pariosteal and intervening sutural layers has been stressed by both Scott (1956) and Troitsky (1932). The two diffuse fibrous layers described in this study are referred to as Sharpey's fibres by Pritchard because they run from the bone to the middle zone and he considers them part of the cambial layers. Pritchard's description of a loose middle zone and the division of the dense central fibrous layer into two capsular layers could not be supported in this study. The reason may be that the diagram concerning the general structure of a sutural area shown by Pritchard et al. does not fit the coronal sutural area for the period under investigation. The fibre orientation of the central zone is predominantly parallel to the calvarial surface, although some variation, especially in the overlapped areas does exist. This suggests that tension, not pressure, as believed by Prahl (1968) is operative at the sutural area. Prahl's hypothesis was based, however, on a description of orientation within a small variable portion of the suture within the overlapped area. The parallel orientation of collagen fibres in response to tension is demonstrated in the midpalatal suture of monkeys that had received rapid maxillary expansion, where sutural fibres were stretched and oriented in parallel fashion from one sutural bony margin to the other (Murray and Cleall, 1971). As will be pointed out later, other external forces other than tension transmitted to this area during the growth period may influence fibre direction. The coronal sutural area passes through various growth stages where interdigitation and bevelling become more pronounced. Diploic spaces at the sutural area become less numerous and closed off from the suture as opposing margins become thicker. The proportion of fibres to cells becomes greater with age, and by 38 days the central fibrous layer predominates while only a single layer of osteogenic cells is found next to the bone margin. The direction of fibre orientation is the same and the five intervening layers of the suture can be recognized. It is apparent that at least until 23 days of age the suture shows very active growth and by 38 days the growth phase is much reduced and the suture is close to its definitive form which, according to Moss (1954), is observed during the third postnatal week. At this stage the fibrous tissue predominates and the definitive suture consists of a middle cellular layer surrounded by two fibrous layers similar to Weinmann and Sicher's (1955) description. Regardless of the minor difference in histological description, it is clear that the coronal sutural area at this stage is not in a period of very active growth and it is difficult to see how such a suture possesses the histological structure which has been postulated (Weinmann and Sicher, 1955) as necessary for the existence of an expansive force in this tissue.

Incremental Growth at Sutures

In order to understand the mechanisms of cranial growth, the sites of most active cranial growth and the relative contribution of these sites to the total increase in size of the skull (rates of growth in different areas) must first be documented. Massler and Schour (1951) located the sutural areas as the sites of most active growth in the vault and clearly demonstrated that proportional changes in the vault are due to unequal growth at different sutures. Baer (1954) reported that the size of the increment contributed by each of the adjoining bones at a common suture is not always the same and concluded that since differential growth takes place at a common tension line (suture), intracranial pressure as suggested by Massler and Schour (1951) is not the only factor controlling growth of bones in the cranial vault. Baer (1954) maintains that the differential growth at the sutural margins is a means of adjustment to the changing relationship of adjoining bones with growth. Both these studies are cross-sectional in nature using a single stain technique for measurement. A more detailed description of growth increments along all opposing bony margins at various sutural areas is accomplished in this study using a multiple bone marking technique similar to Cleall, Wilson and Garnett (1968) in their documentation of normal craniofacial skeletal growth of the rat. This study lends support to previous reports (Baer, 1954; Moore, 1949; Yen and Shaw, 1963) that the sutures are sites of the most active cranial growth and that differential growth occurs at different sutural areas and at opposite margins within a particular area.

The coronal sutural area under investigation shows differential growth on all four projecting opposing bony margins (Sagittal Sites 9, 10, 11, 12). The finding that the overlapping parietal margin (Site 11) shows a greater total increment of growth than the frontal margin (Site 10) is consistent with Cleall's (1968) findings in rats of an older age group. Problems arise in establishing sutural growth patterns when growth becomes unmeasurable due to limitations in techniques. This was one problem Brash (1934) had when he used older madder fed pigs to study sites of cranial growth, which led him to conclude that the suture contributed little to vault growth.

The linear analysis at various cranial sutural areas indicates that the rates of growth and the morphological pattern (overlapping and interdigitation) are intimately connected. The butt-end type of sutural area seen in the anterior metopic and mid-sagittal sutural areas shows about one-tenth the amount of growth as the coronal sutural area which has extensive bevelling and interdigitation. The posterior metopic sutural area fuses on the inferior aspect somewhere after the 12th day of age, while the superior margin shows diminishing, minimal growth. Maximum vault width is complete by the 20th day while growth in length is essentially completed by the 34th day (Moss, 1954) postnatally. The type of sutural area then is related to the growth and expansion of the neurocranium.

This study supports, in part, Massler and Schour (1951), and Isotupa et al. (1965) who feel that cranial sutural morphology reflects the velocities and direction of growth in different cranial regions. Other factors, however, must be involved. The bevelling and interdigitation is minimal at four days of age at the coronal sutural area, but by ten days it is noticed especially at the lateral aspects of this area. The extent of interdigitation continues to increase throughout this experimental period which is complete at 46 days postnatally. According to Baer (1954) the major expansion of the neurocranium has occurred by the first 20 days of life so that this change in sutural pattern cannot be attributed to expansion of the neurocranium alone. It may be that certain external factors such as the continued upward rotation of the viscerocranium (that part of the skull which is derived from the branchial arches), and the flattening of the vault in the region of the coronal suture (Hoyte, 1971), after the 20th day, may be important. Moss (1961) stresses the influence of such external forces as the muscles and attaches minor importance to neurocranial growth in establishing coronal sutural interdigitations. That these other external factors are important is not surprising when one is aware of the functional continuity of the periosteal layers of the cranium and the intervening sutural layers as described previously.

Sutural Location

An important question regarding the role of the suture in calvarial growth is to what extent is the shape of an individual calvarial bone predetermined by the location of its sutures? Troitsky (1932) felt that calvarial bones would not cross sutural planes and therefore that the shape of individual bones was predetermined. Unfortunately, the bones of his older experimental animals had already passed through their growth phase. Girgis and Pritchard (1958) felt that sutural deviation occurred when osteogenic cells on one side were destroyed and ossification from the undamaged side resulted in overgrowth of that bone margin and a shift of the sutural line. They believed that sutures are plastic both before and after they have differentiated, as long as the skull is still growing. Moss (1954), however, believes that sutures remain adaptable up to the end of the first week after which neurocranial growth is much reduced. Results from this study support Moss and Pritchard in that sutural lines are plastic and subject to experimental alteration. The sutural deviations are opposite to those described by Girgis and Pritchard. In their experiments overgrowth occurred in the direction of the operated side whereas deviations of the mid-sagittal and posterior metopic sutural areas in this study were to the non-operated side. The reason for this is that the osteogenic layers on the operated side was damaged allowing the non-damaged margin to overgrow its normal boundaries. In this experiment sutural deviations occurred in all groups. It appears that the osteogenic layers of the sutural margins adjacent to the operated site were not damaged allowing those margins to respond to the surgical intervention by deviating to the non-operated side. The reason for this deviation is due to increased growth at various sutural margins in such a way as to result in a shifting of the superior sutural openings. Because the morphology of the posterior metopic and the anterior sagittal sutural areas was such that the left superior margin overlapped the right inferior margin, the sutural deviation, as seen from the dorsal aspect of the skull, occurred towards the non-operated (right) side. What caused the increased osteogenesis at the sutural area is debatable. Pritchard (1946), in studying bone of the rat, feels that the increased osteogenesis in his experiments could have been due to mechanical stresses set up by the pressure of the exudate or certain humoral conditions. He felt that it was unlikely that mechanical stresses from the exudate would limit the inflammatory response in his study to the outer surface of the skull; they would almost certainly affect the intracranial osteoblasts as well. In this study, the inflammatory response was also limited mainly to the outer surfaces. Pritchard favours the theory that osteogenesis is initiated by the same stimulus which causes the inflammation, such as the products of damaged tissue, blood clot, or microorganisms - in other words, humoral stimuli. These results would also favour the latter theory.

The sutural deviations in the incision group were to be used as a base line or sham for changes occurring in the other groups as surgical procedures were minimal and bleeding was almost non-existent. However, since all groups showed similar deviations it did not seem to matter whether incision or extirpation of the coronal sutural area was done. The sutural deviations were the same and were assumed to be due to the same stimuli - changes in humoral conditions. Since the majority of bone growth changes at the sutural area occurred between intervals BC and CD which would correspond to the 12th to the 22nd days of age for the rat, these results disagreed with Moss (1954) who felt that osseous overgrowth at sutural areas following extirpation of an adjacent bone did not occur after seven days of age. He felt the sutures had passed into their latter phase of the growth period and that after this their position becomes determined. It is possible that his measurement techniques were not fine enough to observe any changes.

It has been established in this study that sutural patterns were plastic for the period studied (up to 22 days of age) and that changes in these patterns resulted in deviations in sutural location. The location of the suture was determined by the rate of growth on various sutural margins and therefore the form of the individual calvarial bones is also determined by the rate of osteogenesis occurring at its sutural margins. This form is not predetermined by the sutural location as suggested by Troitsky (1932) but can be experimentally altered during the period under investigation.

Controlling Factors in Sutural Morphogenesis

Various theories have been promulgated regarding the factors controlling osteogenesis at the sutural area including genetic or intrinsic factors, mechanical stresses and local humoral conditions. In the previous section it was pointed out that when the sutural area is in functional continuity with the remainder of the skull, certain humoral conditions can alter the growth rates at its margins resulting in deviations. The coronal sutural area within the incision area in group V demonstrated increased growth at its margins during intervals BC and CD (Tables V, VI, Sites 9-11). Since this area was in functional continuity with the rest of the skull (as evidenced by the osseous union of incised areas), the forces normally acting on the sutural area were present, but an additional external influence resulted in an altered morphological pattern (increased bevelling and interdigitation, Fig. 36). The fact that the adjustment at the suture occurred before the

22nd day of age, when the inflammatory response was very active, would indicate that the same humoral conditions were responsible for these changes as in the other deviated sutural areas.

In order to test the relative importance of genetic and mechanical factors, it was felt that if the structural unity of the skull were disturbed and the sutural area was isolated from its normal functional context, the mechanical factors would be minimized and the intrinsic (genetic) factors controlling sutural morphology could be expressed. These results furnish evidence that cranial sutural morphology is not predetermined and can be experimentally altered by removal from normal functional continuity. The repositioned sutural area showed a decreased growth at its opposing bony margins at all time intervals throughout the experimental period. It was felt that since the break in continuity was present throughout the experimental period and that the inflammatory response was an influence before the 22nd day, the changes occurring at the sutural margins were due primarily to its being out of its normal functional context. This point is further substantiated if the intact coronal sutural area immediately adjacent to the repositioned area is examined (Fig. 28 and 33). It has the thickened margins similar to the repositioned area which are associated with the humoral changes, but it has other morphological features which are quite different. The interdigitation and bevelling of its sutural margins, which are only rarely seen to a minimal degree in the repositioned segment, are due to its functional integrity with adjacent skull bones. The linear analysis shows increased increments of growth for the superior bone surfaces (Sites 7, 13) adjacent to the sutural margins of the repositioned segment during intervals BC and CD, respectively (Tables V, VI) and no significant changes for other time intervals compared to the control side. These changes point out that the repositioned area was a viable piece of bone throughout the experimental period.

The results from other implant studies are consistent with this study. Watanabe and Laskin (1957) implanted the zygomatico-maxillary sutural area in the rat abdomen and demonstrated a lack of growth at its margins by a metallic implant technique. Moss (1957) demonstrated a decreased sutural growth and a change in morphology to the butt-end type in coronal sutural area implants in the brain. He feels that the flat sutural area type is intrinsic to all sutural areas and that this is modified secondarily by forces normally imposed at the sutural areas when they are in structural unity with adjacent skull elements. These modifications include bevelling and interdigitation of opposing margins. That some autonomy of growth should be given the sutural area is supported by this study as measurable growth, although much reduced, was recorded at the repositioned area throughout the experiment, and in some areas minor overlapping and interdigitation was seen. It may be that some mechanical stresses were still active at the sutural area preventing a complete return to the butt-end type described by Moss (1957). Moss feels that the external forces at the coronal sutural area causing interdigitations are due to muscular activity and spatial re-orientation of the viscerocranium after the cranial growth period is over. Watanabe and Laskin (1957) and Massler and Schour (1951) feel that tension acting at the sutural area during its growth period are the forces that modify sutural morphology. This study supports both theories. Results indicate that fibre orientation at the suture is tangential to the calvarial surface indicating that tension transmitted to the suture by growth of contiguous structures is an important controlling factor. In the repositioned area fibre orientation was opposite to the intact coronal sutural area medial to it, indicating that tension was not acting at this sutural area. The changes, therefore, in morphology could be due in part to a lack of tension.

How the change in fibre orientation affects sutural patterns is not yet clear. It may be that changes in fibre direction may alter the response of the osteoprogenitor cells so that osteoclasts rather than osteoblasts are formed. Two possible mechanisms could explain the presence of osteoclasts at the ten day repositioned coronal sutural area: (1) a mechanochemical effect (Justus and Luft, 1970) which causes osteoclast production when extracellular calcium levels are raised by load changes in surrounding bones; or (2) changes in the piezo-electric properties of the bony margins (Bassett, 1968). This resorptive remodelling was not seen in any of the intact coronal sutural areas. As pointed out previously tension at the suture associated with expansive neural cranial growth affects sutural morphology. But, other external factors, such as muscle forces and spatial relocation of distant bones, as suggested by Moss (1961) and supported in this study, are important both during and after neurocranial growth is finished.

Sutural Fusion

The sutural fusion noted at ten days and 23 days of age in the repositioned segment is not surprising. Watanabe and Laskin (1957) felt that the gradual decrease in cellularity and ultimate bridging of new bone in their transplanted sutures was comparable to what normally occurs in the cranial sutures when the active growth period has ended. This study lends support to the influence of growth in maintaining sutural patency. When the effects of growth are no longer acting at the sutural area or are minimized a great deal as in the repositioned sutural area, fusion may occur. The growth rates at the sutural margins then determines, in part, whether obliteration occurs and as demonstrated in this study the growth rates of the repositioned sutural margins were reduced throughout the experimental period. When the growth rate at the posterior metopic sutural area was increased experimentally, no fusion of the inferior margin occurs and the morphology changed to the bevelled type of sutural area. However, it must be mentioned that the rate of osteogenesis at the sutural area can be modified by a variety of factors not just growth associated with increased size of the skull. Growth at the metopic sutural area was modified by humoral conditions in this study which probably had no direct relationship with growth of the cranium as a whole. Moss (1958b) states that sutural fusion does not occur when bones are removed from their normal functional matrix. He believes that fusion of the metopic suture in the rat is a response to extrinsic forces and that a mechanical advantage is gained for the skull by this fusion. These extrinsic forces are imposed on this sutural area by the several attachments of the falx cerebri during rotations of various cephalic components with growth. This study would support in part such a mechanistic concept because of the changes in fibre orientation observed at the repositioned coronal sutural area which may have set up stresses not normally present resulting in sutural fusion. Herring (1972) also supports this concept as she demonstrated that sutural fusion in pigs takes place in highly stressed areas but she admits the possibility that sutural growth or adjustment complicates the relationship. Giblin and Alley (1944) feel that a primary condition for sutural fusion appears to be the absence of movement. Since the functional continuity of the coronal sutural area in this study was disturbed so that it could not adjust to movement of adjacent bones to the same degree, the hypothesis of Giblin and Alley might be a valid one.

The presence of secondary cartilage (arising independently of primary cartilage of the chondrocranium) is difficult to explain. Moss (1958b) demonstrated a transitory type of cartilage which is replaced by endochondral bone formation in the metopic suture of the rat during fusion. Moss feels that anoxia occasioned by a poor blood supply during avascular periods in the sutural tissues resulted in cartilage production. Since bone formation was very active at certain sutural surfaces in the repositioned segment, it does not seem probable that anoxic conditions were present. It could be that some mechanical factors, such as stress (Sitsen, 1933), associated with changes in fibre orientation, caused production of chondroblasts rather than osteoblasts. It could also be that certain conditions associated with the inflammatory process resulted in cartilage production.

Some histological features of sutural fusion could be described in the repositioned coronal sutural area. The collagen fibres within the suture lose their tangential orientation to the calvarial surface and become predominantly perpendicularly oriented. In the overlapped area, fibres are directed 45° to the calvarial surface from the inferior surface of the parietal margin posteriorly, to the superior surface of the overlapped frontal margin (Fig. 18). The direction of osteogenesis at the sutural area shifts from the ends of the bone margins to their superior and inferior surfaces which results in an increased thickness and a closer approximation of the margins. The morphological features of overlapping and interdigitation are reduced or lost due to osteoclastic resorption at the ends of the sutural margins. Ultimately, there is a bridging across the sutural area which may be either osseous or cartilagenous. In areas of cartilagenous union, the processes of endochondral bone formation is observed. Cartilage may be an intermediate step in bony sutural fusion although it could only be demonstrated in certain sections.

Normal sutural fusion provides ridigity to the skull against the forces of muscle pull and results when growth at a sutural area is minimal or when displacement of the bones adjacent to the sutural area is absent. It would appear that bevelling and interdigitation provide more resistance to the separation of the bones by external forces but also allow slow gradual angular movements imposed by growth at other points in the skull.

Role of Sutures in Calvarial Growth

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. Also, only minimal changes in bone growth occurred at a distance from the coronal sutural area and these were associated with lipping of cut margins. Since the minor adjustment changes at the sutural areas adjacent to the surgical area in both groups were similar, and since growth changes diminished the farther away from the surgical area one examined, it was felt that growth of the skull as a whole was not affected. If the sutural areas were primary sites of expansive growth as outlined by Weinmann and Sicher (1955), then greater changes in skull form would have been expected in the extirpation group compared to the incision group. As indicated by the fibre orientation of the functionally intact coronal sutural areas of all groups studied, some form of tension must be acting at the suture.

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 they are isolated from their 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 two facts: (1) the influence of the inflammatory response which results in an increased thickness of the sutural margins is acting before the 22nd day postnatally, whereas the changes associated with a lack of functional continuity occur throughout the experimental period; (2) the fibre orientation within the functionally intact coronal sutural area where tension is acting is opposite to the repositioned area adjacent to it.

This study supports Moss's theory of the functional matrix whereby the expanding neural mass is the primary force causing a separation of cranial bones during growth. Linear analysis indicates that the sutural areas are the most active areas where growth occurs in response to the separation of the bones, resulting in an increase in size of the calvarium.

In addition to responding to the internal expansive forces of the brain, the sutural area has to remain united during relative displacements occurring between unequally growing parts and at the same time prevent dislocation or separation by external forces, including the pull of muscles. It does this by altering its morphological pattern by bevelling or interdigitation of its margins, or in areas where growth has ceased and displacement of adjacent bones is minimal, by fusion to provide stability to the skull in that area.

In summary, the neurocranial sutures are sites where active growth takes place secondary to the expanding neural mass. Since both sutures and bones exist in a functional matrix which includes dura, neural tissues, spaces, and muscles, their morphology reflects the varying functional needs of this matrix.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The aim of the present investigation was to experimentally alter the coronal sutural area in the rat in order to study how cranial sutural areas adjust to the altered external environment, and to determine some of the controlling factors. In the light of these findings, the functional role of the suture in calvarium growth has been re-examined.

The sample consisted of 25 control and 31 experimental Long-Evans strain male rats. Two control groups, one sagittal and one coronal, and three experimental groups - an incision, an extirpation, and a repositioned group - were studied. Subgroupings of the control sagittal and repositioned groups were made for histological examination at 4, 10, 23 and 38 days postnatally. Using a multiple bone marking technique beginning at five days postnatally, all other groups received intraperitoneal injections of vital-stains on the 1st, 8th, 18th and 28th experimental days with sacrifice on the 42nd experimental day.

The methods of analysis included examination of dried skulls from standardized photographs, histological examination of the coronal sutural area in the control and repositioned groups, and linear analysis of various cranial regions and related sutural areas in all other groups. Here, incremental measurements of bone growth at specific sites were subjected to statistical appraisal and the left and right side differences were compared between groups.

The qualitative and quantitative evaluation of the results from this study suggest the following new findings:

(1) Stimulation of the growth rate at sutural margins experimentally, results in morphological changes, including an increased amount of bevelling and interdigitation, delays in sutural fusion, and sutural deviations, as observed from the dorsal aspect of the calvarium.

(2) Isolation of the coronal sutural area from its normal functional demands results in a change in shape to minimal overlapping and interdigitation or to the butt-end type. Resorptive remodelling associated with changes in fibre orientation, and alteration in both the rate and direction of growth at the sutural margins are some responsible factors.

(3) Sutural fusion appears to be dependent on the growth rate at the opposing bony margins, the direction of fibre orientation within the suture, and the absence of movement between adjacent bones. The presence of cartilage in the fused area may be an intermediate step in complete osseous union.

(4) Experimental alteration of sutural morphology suggests that external rather than local intrinsic factors are the more important determinants of sutural form.

The following results lend support to findings from other investigations:

(1) The coronal suture consists of five intervening layers between opposing bony margins and two periosteal or uniting layers when studied from the 4th to the 38th day postnatally. The suture is highly cellular at four days and mainly fibrous in structure by 38 days postnatally. In all stages, the fibres are arranged predominantly tangentially to the calvarial surface.

(2) Differential growth was demonstrated at various calvarial sutural areas and between different margins at a specific sutural area.

(3) The sutural areas are the sites of most active bone growth in the calvarium and the direction of fibre orientation suggests that tension is acting at the sutural area. Results from extirpative and repositioning procedures demonstrated the sutures to have little autonomy of growth and were not primarily involved in the expansion of the neurocranium.

(4) The location of calvarial sutures is determined by the relative growth rate of the adjacent bones and can be altered experimentally.

(5) Under normal conditions, sutural area morphology is influenced by a variety of factors, including the velocities and directions of growth in different cranial regions, and the relative displacement or the absence of movement between adjacent bones.

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GLOSSARY

Linear Sites - Sagittal

1. Superior calvarial surface where the cerebral and olfactory portion of the frontal bone meet.
2. Inferior calvarial surface where the cerebral and olfactory portion of the frontal bone meet.
3. Superior calvarial surface midway between point 1 and the middle of the cerebral portion of the frontal bone.
4. Inferior calvarial surface midway between point 1 and the middle of the cerebral portion of the frontal bone.
5. Mid-point of the superior calvarial surface of the cerebral portion of the frontal bone.
6. Mid-point of the inferior calvarial surface of the cerebral portion of the frontal bone.
7. Superior calvarial surface adjacent to the coronal sutural area.
8. Inferior calvarial surface adjacent to the coronal sutural area.
9. Superior frontal bony margin at coronal sutural area.
10. Inferior frontal bony margin at coronal sutural area.
11. Superior parietal bony margin at coronal sutural area.
12. Inferior parietal bony margin at coronal sutural area.
13. Superior parietal surface adjacent to the coronal sutural area.

14. Inferior parietal surface adjacent to the coronal sutural area.
15. Superior surface of cut bone edge of the repositioned parietal segment.
16. Inferior surface of cut bone edge of the repositioned parietal segment.

(Sites 15 and 16 were used only in the repositioned segment and correspond to the same regions as Sites 17 and 18. They were inserted so that both sides of the incision area could be compared in group IV.)

17. Mid-point of the superior parietal surface.
18. Mid-point of the inferior parietal surface.
19. Superior parietal surface adjacent to the anterior lambdoidal sutural area.
20. Inferior parietal surface adjacent to the anterior lambdoidal sutural area.
21. Superior parietal bony margin at the anterior lambdoidal sutural area.
22. Inferior parietal bony margin at the anterior lambdoidal sutural area.

Linear Sites - Coronal

Block 1 (Anterior Metopic Area)

1. Superior surface of the frontal bone medial to the temporal ridge.

2. Inferior surface of the frontal bone medial to the temporal ridge.
3. Superior frontal surface adjacent to the anterior metopic sutural area.
4. Inferior frontal surface adjacent to the anterior metopic sutural area.
5. Mid-point of the anterior metopic sutural margin.
6. Mid-point on the superior surface of the frontal bone.
7. Mid-point on the inferior surface of the frontal bone.

Block 2 (Posterior Metopic Area)

1. Superior surface of the frontal bone medial to the temporal ridge.
2. Inferior surface of the frontal bone medial to the temporal ridge.
3. Superior frontal surface adjacent to the posterior metopic sutural area.
4. Inferior frontal surface adjacent to the posterior metopic sutural area.
5. Mid-point of the superior sutural margin.
6. Mid-point on the superior surface of the frontal bone.
7. Mid-point on the inferior surface of the frontal bone.
8. Inferior surface of the fused frontal bone beneath the superior sutural opening.

Block 3 (Anterior Sagittal Area)

1. Superior surface of the parietal bone medial to the temporal ridge.

2. Inferior surface of the parietal bone medial to the temporal ridge.
3. Superior parietal surface adjacent to the anterior sagittal sutural area.
4. Inferior parietal surface adjacent to the anterior sagittal sutural area.
5. Superior bony margin at mid-sagittal sutural area.
6. Inferior bony margin at mid-sagittal sutural area.
7. Mid-point on superior surface of parietal bone.
8. Mid-point on inferior surface of parietal bone.

Block 4 (Posterior Sagittal Area)

All sites in this area correspond to similar sites in the anterior sagittal area (Block 3) except that they are located more posteriorly on the calvarium.