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EXPERIMENTAL TRANSPLANTATIONS OF CRANIAL AND FACIAL SUTURAL ARTICULATIONS: A HISTOLOGICAL ASSESSMENT.

by

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Submitted in partial fulfillment of the requirements

for the Degree of Master of Science in Dentistry

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May 24, 1988

"Experimental Cross-transplantations of Cranial and Facial Sutural Articulations: A Histological Assessment."

Abstract

by

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The objectives of this experimental study were to evaluate histological responses following cross-transplantations of the contrastive metopic and internasal sutures, and to determine the nature of their integration and metamorphosis in the respective new functional environments. Twenty two Hartly guinea piges were utilized. They were divided into three groups: a non-surgical control group, a surgical control group, and an experimental group. Vital dyes were injected weekly to each animal. Three histological procedures were used: paraffin embedded decalcified sections for observations of fine cellular structures, ground sections for uv visualization of the vital dye labelling and, in conjunction with the vital dyes, microradiographs to determine bone density and new metopic and internasal sutures for measurements of the amounts of expansion taking place at the levels of these sutures. The results demonstrate that expansion and growth activity at these sutural junctions are minimal, that the sutural characteristics relate to the nature of the functional environment, and that, following transplantation to exchanged sites each suture will adopt the characteristics and come to conform with the functional requirements of the new site.

DEDICATED

To my parents, Jacques and Françoise De Coster,

to who I owe everything.

AND

To my brothers, Olivier and Patrick for being

wonderful individuals.

ACKNOWLEDGEMENT

I would like to extend my sincere appreciation and gratitude to all the individuals who by many different ways supported me in this study. Without them this work would not have been made possible, and my life would not be the same. My sincere gratitude and appreciation to:

Donald Enlow, professor and chairman of the orthodontic department, for making me understand and appreciate facial growth and development, for being an enthusiast, knowledgeable and wise advisor and for translating my "Belgian English" into a sophisticated literature.

Robert Cederquist, professor and chairman of the orthodontic department, Columbia University for guiding me and advising me in this field where he has so broad knowledges and understanding.

Ordean Oyen, associate professor of oral biology whose interest, experience and dedication has been so helpful throughout this study, and who also provided me with most of the surgical and photographic instrumentations.

James Simmelink, associate professor of oral biology, and director of research who guided me with many advises and made available to me the sophisticated histological set up that I needed to conduct this study.

Edward Rossi, chairman of the department of oral pathology, who provided me with the facilities and products of the oral pathology department.

Stanley Hirsh, professor of oral pathology, who was always

prompt to help me when I needed it.

Lawrence Scaletta, professor of oral biology for giving me access to the UV light microscope.

Adolf Vozka, whose skills and imagination provided me with custom made instruments.

Charlotte Geleev, Lyda Mumaw, Mary Ann De Carlo, Tom Slabe who taught me and helped me with histological techniques and preparations. Catherine Nichols who, even overloaded always, had time to help me.

Denita Fletcher, for her patience in typing this manuscript. The classmates and friends of the orthodontic department, especially Man-Ching Cheng, Glenn Goodrich, Andrew Slodov, Jarya Thimaporn, Roxana Mussa and Jing-Yi Shiau for their support, sensitive and intelligent friendship that I will never forget.

Marcia Dunner whose friendship and unselfishness has helped me to feel at home away from my country.

The entire faculty of the orthodontic department for teaching me so much and so well.

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INTRODUCTION

Sutural articulations are synarthrosial, immovable joints found only among facial and the calvarial flat bones. Osteogenic potential in the area of a suture allows for the complex differential and interrelated development of the multiple craniofacial components during the active period of growth.

Two main types of sutures exist, the facial type and the cranial type. Their respective morphology, embryonic development as well as the functional environmental influences acting upon them have been shown in previous studies to be significantly different (Pritchard et al., 1956, Koskinen 1982, Kokich 1986).

The objective of this study is to compare and contrast, on the basis of these morphological, developmental and functional differences, the nature of reactions of both types of sutural articulation, facial and cranial, to new functional environments following cross-transplantations.

This experiment will also address the relative intrinsic specificity of the sutural tissue. That is, whether a given suture type is structurally, functionally, and developmentally committed only to its original site and conditions, or whether it is a functionally and structurally adjustable and adaptable tissue with no intrinsic specificity. Resolution of this question might offer a new approach to the treatment of certain craniofacial deformities, such as the Treacher-Collins syndrome or cleft palate anomalies. A calvarial sutural transplant in the area of the defect, where a facial sutural junction previously failed to form, thus, might be

biologically feasible. If the functional soft tissue environment is appropriate or managed appropriately, the transplanted sutural segment could be responsive to this new environment and become histogenetically active in further regional developmental expansion of the collapsed area, improving significantly the prognosis of such malformations.

II. REVIEW OF LITERATURE

Sutural bone growth was once believed to be genetically determined and responsible not only for the active expansion of the calvaria, but also for the forward and downward shift of the ethmomaxillary composite. The growth movements of whole bones around sutural articulations were then presumed to be initiated by bony proliferation from the sutural fibrous connective tissue (Weimann and Sicher, 1955). Based on functional theories of craniofacial growth (Van der Klaauw, 1946; Moss, 1960, 1962; Enlow, 1968; Van Limborg, 1970) as well as numerous investigations (Scott, 1954; Pritchard, et al, 1956; Moss, 1957, 1959, 1960; Watanabe, 1957; Young, 1959; Koski, 1968; Petrovic et al., 1968; Perrson, 1973; Linge, 1973; Smith and McKeown, 1974) the concept that sutures represent primary growth centers has been strongly and repeatedly denied.

Sutural articulations, rather, are now viewed as secondary sites of growth activated by functional stresses in response to triggering effects of soft tissue growth and expansion. Watanabe (1957), by transplanting zygomaxillary sutural tissue with segments of attached bone into the abdomen of guinea pigs, demonstrated that removal of a suture did not affect facial growth, and that the transplanted articulations freed from functional stress ceased growth activity along with fusion between the bone segments. The findings of Young (1959), who experimentally related increased and decreased cranial volume with consequent alterations in the magnitude of calvarial sutural growth, showed that increased or

decreased intracranial pressures have a proportional stimulating or retarding effect on sutural connective tissue proliferation and bone formation.

These studies, as well as many others (Hinrichsen and Storey, 1968; Shapiro, 1976; Jackson and Kokich, 1979; Meikle et al., 1979; Cederquist, 1984) led to and strongly support the view that tensile forces across a suture contribute to the regulation of sutural tissue proliferation and related osteogenesis, and that compressive forces inhibit bone formation at sutural margins (Hinrichsen and Storey, 1968; Tuenge and Elder, 1974; Shapiro, 1976; Cederquist, 1984). These relationships also have been demonstrated clinically in orthodontic procedures involving the use of a palatal expander to correct a skeletal transverse deficiency of the maxilla (Melsen, 1972; Linge 1976), or the use of anterior and posterior pull headgear as a means to orthopedically augment or restrict the forward displacement movement of the maxilla by modifying the stresses on the circummaxillary sutures.

Sutural Configuration

Three types of sutural morphology can be described, a "butt-end" type with the bone margins facing each other as two relatively straight edges, a serrated type with projections interdigitating from one bone to the other, and a "wedge-shaped" squamosal type, with the bone surfaces slightly overlapping each other at their borders.

The degree of serration relates to the amount of growth, the magnitude of functional stress and the duration of activity at a

given suture (Massler and Shour, 1951; Moss, 1957; Smith and McKeown, 1974; Kokich et al., 1976; Markens and Oudhof, 1980).

Moss (1957), by transplanting sutural segments intracerebraly in the growing rat, has shown that the serrated configuration freed from functional influences would change to a butt-end structure. He then concludes that the intrinsic morphology of all calvarial sutural areas freed from normal functional demands is a flat, butt-end articulation, and that both bevelling and interdigitation are responses to extrinsic forces.

In accordance with this concept, Smith and McKeown (1974), in a study where they detach and replace in situ segments of the coronal suture in rats, found 23 days postoperatively that either a butt-ended configuration or a minimal overlapping structure resulted. These authors, with many other investigators (Massler and Shour, 1951; Moss, 1957; Enlow, 1968; Herring, 1972; Smith and McKeown, 1974; Kokich et al., 1976; Herring, 1972; Smith and McKeown, 1974; Kokich et al. 1976; Koskinen, 1976; Foley and Kokich, 1979; Markens and Oudhof, 1980; Oudhof, 1982) supports the view that morphology of sutures reflects the biomechanical forces to which this tissue is exposed.

Sutural interdigitations are the result of a differential growth pattern and osteoblastic distribution along the sutural margins (Massler and Shour, 1951; Smith and McKeown, 1974; Koskinen, 1976; Watzek et al., 1982). Furthermore, this pattern seems to accentuate the sutural digitations with age since it has been shown, as previously mentioned, that the degree of serrations is a factor of the duration of activity at the suture (Massler and Shour, 1951;

Kokich 1976). As described by Massler and Shour (1951) cellular distribution and resultant bone accretion does not have to be symmetrical on both sutural margins within the same area any more than it has to be the same along the whole sutural pathway.

Sutural junctions are also believed to function as limited-motion articulating surfaces of the craniofacial complex. Sutures prevent bone separation under external forces, resist and absorb shocks from the functional environment and allow slight movements between bones of the cranial and facial skeleton (Pritchard, et al. 1956; Moss, 1957, 1960; Koskinen, 1976; Markens and Oudhof, 1980; Van der Linden, 1985).

The serrated, butt-end or wedge shaped configurations are related not only to the functional demands to which the area is subjected, but also to the physical nature of the forces a given suture must adopt in order to maintain regional integrity within the cranio-facial complex as a whole (Moss, 1957; Herring, 1972; Linge, 1973; Koskinen, 1976; Van der Linden, 1985).

Interlocking, three dimensional interdigitations better accommodate shearing forces than the end-on-end type of suture. Furthermore, some investigators (Koskinen, 1976; Van der Linden, 1985) believe that sutural serrations can be visualized as special devices allowing greater mechanical advantage during growth, since the sutural surface area will be greatly increased by the three dimensional, dentate configuration.

Sutural Tissue Morphology

Sutural width (Watanabe, 1957; Linge 1973, Perrson 1973,

Koskinen, 1976), sutural fiber orientation (Watanabe, 1957; Moss, 1960; Linge, 1973, Perrson, 1973; Smith and McKeown, 1974; Koskinen, 1976) and composition (Moss, 1954; Pritchard, 1956; Watanabe, 1957) has been shown to be in close relationship with age and the activity of a suture.

Watanabe (1957), after intra-abdominal implantation of the zygomatico-maxillary suture in guinea pigs, demonstrated a gradual narrowing of the sutural tissue, as well as a reorientation of the sutural fibers parallel to the bone margins. He further observed a relative reduction of the cellular content in sutural tissue composition which Pritchard et al. (1956) observed as a relative increase in fibrous connective tissue composition with the advancing developmental stage of the suture.

Sutural Activity and Ankylosis

Fetal head constraints (Graham 1979) and intra-uterine compression (Koskinen, 1982) have been suggested as contributing or causative factors in premature craniosynostosis, even though compression on cranial and facial sutures following birth has failed to produce bony fusion in controlled experimental studies as well as clinically in orthodontic patients. Moss (1960) in an experimental study in which the periosteum was stripped over several cranial sutures in 84 day old rats to induce sutural fusion, came to the conclusion that there are three possible factors which can explain the differential onset of fusion of some sutures over some others, which are:

- The maturational stage of the sutural area. Only presumptive, immature sutures respond by fusion.

- The relative growth of the brain. Neo-natal fusion was uniform only in sutural areas overlying non-expanding portions of the brain.

- The differential functional demands. Only sutural areas whose morphology indicated relative non-function fused consistently.

Supporting Moss's views, Koskinen (1982) suggested that differences in the maturational stage of the facial and cranial skeleton and of the sutural tissue, as well as the relative degree of growth of a given area, could be factors in the occurrence of cranial synostosis in conjunction with fetal head compression. These factors would also explain why premature synostosis among facial sutures never occurs in these conditions, since:

1. Human facial bones develop a fibrous periosteal capsule around themselves by 17 weeks in utero. In contrast, cranial primordia develop toward each other in a preformed, continuous fibrous membrane (the "ectomeninx"). The layered, differentiated periosteum of the facial suture would have a protective effect against fetal compression.

2. The more prominent shape, size and the faster growth rate of the neurocranium expose its sutures to higher compressive forces in utero than in the facial skeleton.

Premature craniosynostosis has been shown to occur in response to various other causes. Some syndromes, such as Apert's or Crouzon's, are under familial genetic influence. According to Hunter and Rudd (1973, 1974) 8 percent of premature sagittal synostosis patients are of genetic origin. Autosomal dominant and recessive forms have been described with the rate of occurrence

being particular to different sutures (Hunter and Rudd, 1973, 1974; Cohen, 1977). The genetic penetrance varies from very high to complete in some familial instances of sagittal synostosis (Cohen, 1986).

Other factors found to be contributory causes of premature craniosynostosis are metabolic disorders such as hyperthyroidism and rickets, and some drugs with teratogenic effects such as diphenylhydantoin and retinoic acid. Premature synostosis may also result in association with complicating factors involving malformations such as microcephaly, encephalocele or shunted hydrocephaly (Cohen, 1986). Experimentally induced synostosis by means of periosteal implants (Alhopouro, 1972), methyl 2-cyanocrylate adhesive (Persson, 1979; Foley and Kokich, 1980; Babler and Persing, 1982; Nappen and Kokich, 1983), or other methods have been performed in attempts to understand the histogenic mechanisms of sutural synostosis together with compensatory growth at the other sutures, while relating those sutures to natural syndromes such as Apert's or Crouzon's. These studies showed the detrimental effect of a single sutural synostosis on the overall development of the craniofacial complex (Alhopouro, 1972; Babler and Persing, 1982), supporting Virchow's early observation (1851) that cranial deformity in subjects with premature craniosynostosis could be explained on the basis of inhibition of growth perpendicular to the synostosed sutures with some form of compensatory growth at other sutures.

Regenerative Potential of Sutures

Cranial sutures have demonstrated a considerable regenerative

potential. Calveriectomies performed in prematurely synostosed children (Hanson, et al., 1977; Hahn and Jane, 1978) and in controlled studies utilizing rodents (Simpson, 1957; Muke and Meyer-Glauner, 1977; Mabbutt and Kokich, 1979; Mossaz and Kokich, 1981) have shown complete re-skeletization of calveriectomized skulls with newly formed sutures in their proper anatomical locations. Perrson, et al. (1979) observed that craniectomy following immobilization of 30 day old rabbit coronal sutures with methyl 2-cyanocrylate adhesive produces marked vault deformity, but that compensatory angular alterations yielding a normal morphology subsequently occurred by 90 days of age.

Several investigators (Simpson, 1957; Moss, 1959; Muke and Meyer-Glauner, 1977; Hahn and Jane, 1978; Mossaz and Kokich, 1981) demonstrated the importance of the dura mater to the osteogenic potential and the activity of the sutures as well as their ability to regenerate new bone after calveriectomy procedures.

The dural membrane is attached to the basicranium at the crista galli, the lesser wings of the sphenoid and the petrous crests of the temporal bones. These attachments establish tensional stresses from the basicranium to the dura and on to the sutural tissue.

According to several investigators (Moss, 1959; Hahn and Jane, 1978), malformations or alterations of the basicranium will transmit abnormal tensional stresses to the dura mater leading to the premature closure of certain sutural articulations.

In an experimental study in which the falx cerebri of growing rats was sectioned, Moss (1960) demonstrated that normal fusion at the posterior interfrontal suture had failed to occur. Other

studies agreed with this finding. Hahn and Jane (1978), by plicating the dural membrane along certain lines of tension after calveriectomy of 40 severely synostosed children, showed that no recurrence of synostosis had occurred using this technique.

Cranial and Facial Sutures

Pritchard and associates (1956) described facial sutures as comprised of five layers: two cambial, two capsular, and one middle mesenchymal layer. If these authors believe that this five-layered pattern continues from the early developmental stage to adulthood, most other researchers regard it as a transient feature of the immature sutural junction leading to a final three-layered pattern (Enlow, 1968; Kokich 1976).

Facial sutures develop by proximation of facial embryonic bones within an intervening loose mesenchymal tissue. The bones' primordia immediately become covered by a differentiated periosteal membrane. This early formation of a periosteum has been suggested as a protective factor against fetal compression and the premature synostosis of these facial sutures (Moss, 1959; Koskinen, 1982) that would help to explain why facial synostosis is virtually non-existant, in contrast to calvarial sutures.

Cranial sutural development is quite different in comparison to facial sutures. The embryonic calvaria is formed from various ossification centers, each located in specific areas of the calvaria. As the neural mass rapidly expands during fetal life, the bone primordia are kept widely separated with intervening fibrous connective tissue, the ectomeninx. When brain expansion slows, the

bone edges grow closer, and the ectomeninx then become layered into pericranial and dural strata (Pritchard, 1956). These developments induce concomitant initiation of sutural morphogenesis (Kokich, 1986).

In addition to the significant developmental and evironmental differences between the facial and cranial sutural locations, these two contrasting sutural types do not respond to the same kinds of stimuli at the same time or to the same extent. The growth of the calvaria relates directly to the expansion of the brain, with about 90% of its circumference achieved by 2 years of age and 65% already acquired before birth (Scott, 1958). In contrast, facial growth progresses for many more years in conjunction with many other basic circumstances.

Whereas the cranial structures respond essentially to only one primary stimulus, brain expansion, the facial sutures must resist, adapt and respond within a sophisticated, developing functional environment, including complex muscle attachments and functions, masticatory forces, progressive dentition development, enlarging airway, lingual development and function, orbital development, and basicranial associations and constraints. For these facial sutures, more often interdigitated, their morphology reflects their constant, on-going and complex responses.

If a common pathology of the cranial sutures is their predisposition for premature synostosis, facial sutures may sometimes be lacking in presence altogether. Their embryonic primordia can fail to differentiate, as seen in cleft palate children or zygomatico-maxillary sutural junction involving the

Treacher-Collins syndrome.

As stated, the purpose of this study has been to challenge, in a relatively straightforward experimental procedure, the adaptative potentials of these two different kinds of sutural articulations subsequent to cross-transplantations of respective segments. In response to the exchanges of localized environmental influences they then become subjected to, determinations of their respective capacities for adjustive craniofacial growth and the timing involved, thus, is the objective in view of their dissimilar characteristics as outlined above.

The following points are evaluated: Do the sutures remain patent and retain original sutural histology in the three experimental groups studied? Does the surgical cut itself become involved? Does sutural-equivalent growth occur in each of the exchanged areas? Will the cross-transplants be accepted or undergo resorptive removal and replacement? On the basis of differences in the periosteal covering tissues of both types of suture, will regeneration and reconstruction of the transplanted tissues occur? Will the facial suture undergo metaplasia to become, structurally and functionally, a calvarial-type suture, and the converse? Will the exchanged sutures produce new bone of the types in their new or their former locations? And finally, can these two contrasting sutural types potentially be utilized clinically in autotransplants to constrain or to promote subsequent facial or calvarial growth?

III. MATERIALS AND METHODS

a. Description

Twenty-two Hartly guinea pigs were utilized in this study. Ten experimental, 7 sham control, and 5 control animals (Figures 1, 2). The animals were chosen and surgerized without consideration of sex. They were housed in the dental school vivarium at Case Western Reserve University. Food and water was provided ad libidum. The experimental period lasted 28 days. During this time, the animals were weighed each week from the day of surgery to the day of sacrifice (Figure 3).

Contrastive vital dyes were injected intraperitoneally at the same weekly times until the last week before sacrifice. These dyes were, in sequence, xylenol orange (0.3g/kg), Alizarin complexon (0.075 g/kg), fluorexan (0.0484 kg), and alizarine combined with fluorexan as the final marker (Figure 4).

In the non-surgical control group amalgam markers were implanted around the internasal and metopic sutures, and weekly frontal and sagittal x-rays were taken. A special cephalostat was designed to assure the consistency of head posture and craniometric measurements (Figures 5,6). Surgery was performed for each animal between 24 and 26 days of age, just after weaning. An aseptic surgical field and sterilized instruments were used.

The animals were anesthetized intraperitoneally with 30 mg/kg of pentobarbital (nembutal)^{R} and a local injection of xylocaine^{R} 2% at the area of surgery (Figure 7).

After the surgical area has been shaved, a parasagittal soft tissue incisions were made over the internasal and metopic sutures

(Figure 8). The skin and the periosteum were reflected and the surgical bony cuts to be made first outlined with an india ink pen (Figure 9). A flexible negative template 7 mm long and 3 mm wide was used to standardize the bony cuts and to allow an accurate fitting of the segments in their new sites when the transplants were switched (Figure 10).

The bony incisions were started with a round burr in a dental hand piece, with finishing cuts made with a scalpel blade (Figure 11). The transplants were carefully elevated and detached from the underlying dura mater at the calvarial site and from periosteal and nasal septal attachments at the facial site. The experimental group had these bony segments with their contained sutures transplanted from the nasal to the frontal site and vice versa.

The sham control group, after a complete detachments were made, had both transplants replaced into original locations. Before closing the wounds, the transplant locations were identified with 2 india ink marks on the bone surface adjacent to the middle of the transplant. The control non-sham group did not receive anything else but a soft tissue surgical opening, as described previously, except for 4 holes made with a reverse conic burr, one on each side of the internasal and metopic sutures, which were filled with regular dental amalgam to serve as radiographic markers (Figure 12). This was not done for the other groups because of the danger of a fracture of the nasal bones if additional trauma was created in the important transplant area.

Once surgerized, the areas were cleaned and desinfected with an iodine solution. The surgical soft tissue margins were approximated

avoiding undue compression, with 4-0 nylon sutures (Figure 13).

The animals were sacrificed at one month with an overdose of pentobarbital (nembutal)^{R} intraperitoneally and intracardiac. The thoracic cage was then opened for vascular fixation. The vena cava was sectioned for drainage, and 10% buffered formalin fixative then injected into the left ventricule (Figure 14). The heads were skinned, decapitated and placed in fixative.

After 3 to 5 days the experimental areas were removed along with segments of surrounding structures. The specimens were each trimmed into a trapezoidal configuration to facilitate orientation for histologic sectioning and subdivided in 2 to 4 blocks (Figure 15), with the middle cut dividing the transplant transversally into two equal parts. One-half each of the sample groups were decalcified and paraffin embedded, and the other half embedded in bioplastic for ground sectioning and vital dye visualization.

A 0.7% EDTA solution was used for decalcification, with constant agitation maintained by magnetic stirring rods, during the two to three days leading to complete decalcification. The samples were washed in running tap water for 5 hours and processed in a tissue processor (Autotechnicon^{R}) for a cycle of 12 hours. The samples were then soaked in the vacuum infiltrator for one hour and embedded in paraffin blocks.

The sections were sliced with stainless steel disposable blades mounted on a conventional microtome adjusted for 6 microns. The mounted sections were stained with hematoxylin-eosin and examined under ordinary and ultraviolet light.

The ground sections were cut by means of power-driven diamond

waffered blade at 350 microns and then polished to about 100 microns. The sections were covered using a routine mounting medium and examined using both light and ultraviolet inspection. Microradiographs of the ground sections were also made using a Hewlett Packard faxitron x-ray system with Kodak high resolution photo plates. The time of exposure was 90 minutes at a voltage of 20 kvp.

b. Rationale for the Choice of These Specific Materials and Techniques

Sutural growth activities subsequent to the experimental surgical procedures were monitored by means of the amalgam markers around them (non-sham controls) and by injections of vital dyes. These particular sutures that were chosen was based on their contrasting anatomic locations (facial versus cranial) and also because of their striking differences in gross and histologic configuration, nature of associated contiguous tissues, and the contrasting developmental and functional stresses relating to them. The ease of surgical access allowed a simple, clean, non-traumatic procedure. These features would favor a relatively straightforward interpretation of contrastive results. The young age of the animals and the large amount of remaining growth enhance the likelihood that information pertaining to the nature of responsive sutural growth and adaptative potential can be obtained.

The decalcified, paraffin embedded sections were intended to provide information on the fine morphology of the structures involved, the vitality of the implanted bone, types of newly formed bone, patterns of remodeling apposition and resorption, as well as

the characteristics of sutural fiber regeneration and orientation in the surgical areas.

The ground sections marked with fluorescent vital dyes allowed the mapping of new bone formation in the transplant areas and adjacent structures. Microradiographs, as a complement for ground section evaluation, show the new outlines of bony remodeling as they relate to the more dense, older bone.

A four week observation period was utilized so that histogenic trends of subsequent growth and remodeling could be noted. The responsive fates of the original tissues, rather than merely observation of final gross results, thus could be evaluated in relating the results with previous functional and morphologic situations.

The guinea pig is easily handled, has a relatively larger head size compared with rats, and extensive use in previous sutural growth studies make it one of the best known and reliable animals for this kind of experiment.

FIGURE 1: Hartly Guinea Pigs

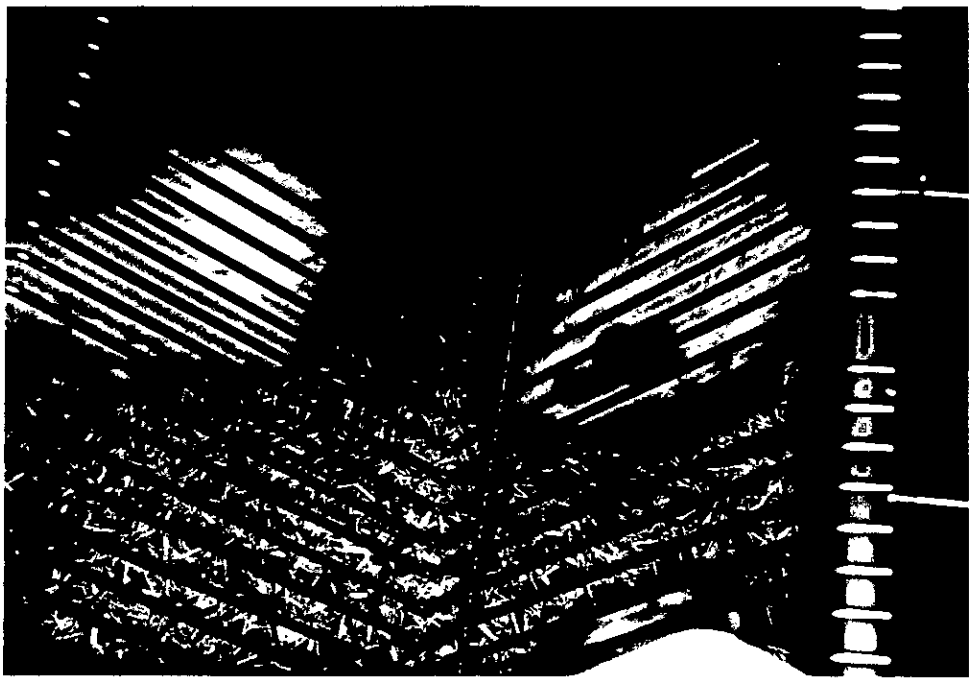


FIGURE 1

FIGURE 2: Groups and Sex Distribution

FIGURE 2

Control, Non-Surgical Group

Animal	Sex	
I	F	3F, 2M
VIII	F	
XIII	M	
XVIII	M	
XXII	F	

Surgical Control Group

Animal	Sex	
II	M	2F, 5M
IV	M	
V	F	
X	F	
XI	M	
XIV	M	
XXI	M	

Surgical, Experimental Group

Animals	Sex	
III	M	4F, 6M
VI	F	
VII	M	
IX	F	
XII	F	
XV	M	
XVI	F	
XVII	M	
XIX	M	
XX	M	

TOTAL: 22 Animals

-9 Females

-13 Males

- 5 Non Surgical Control

- 7 Surgical Control

-10 Experimental

FIGURE 3: Weight increment from 25 days of age to sacrifice, at about 55 days of age.

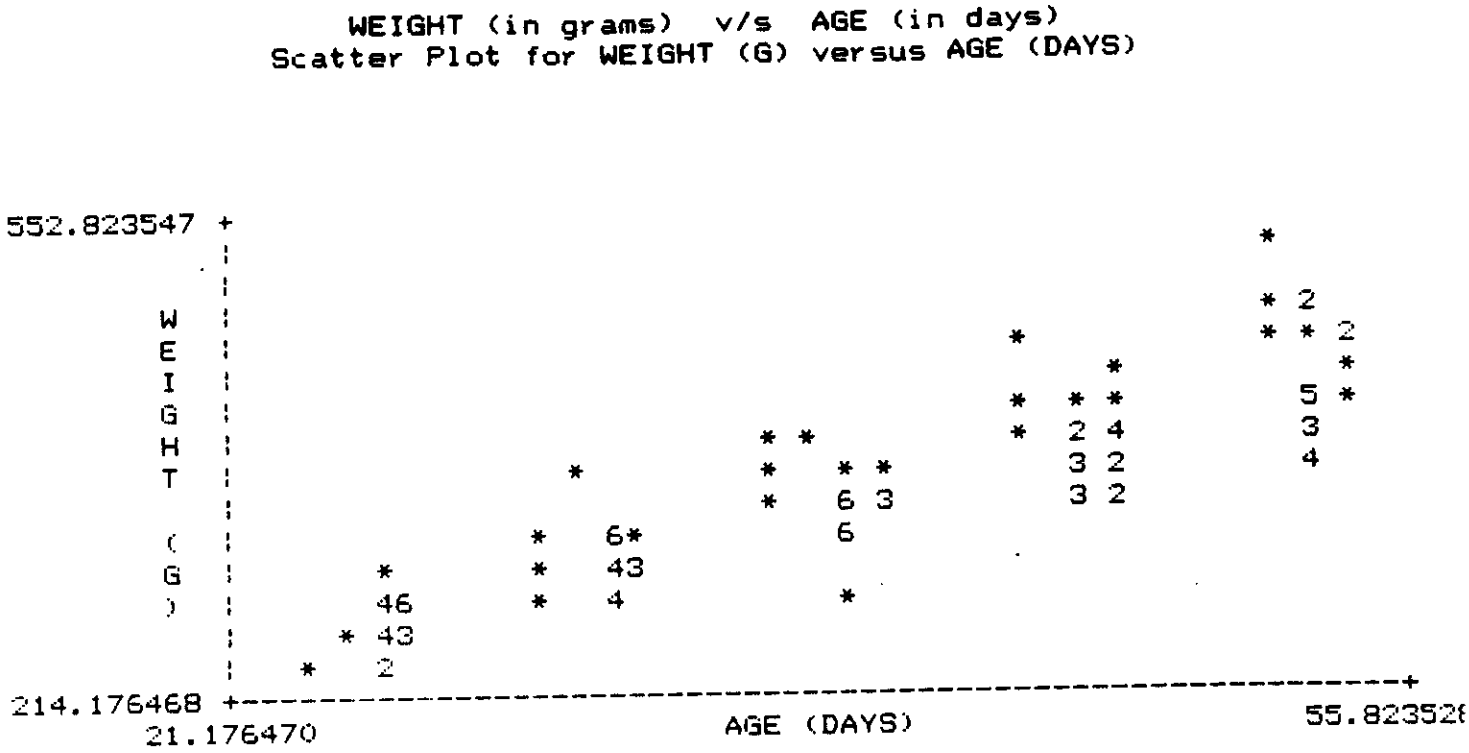

WEIGHT (in grams) v/s AGE (in days)
Scatter Plot for WEIGHT (G) versus AGE (DAYS)
552.823547
214.176468
WEIGHT (G)
21.176470
AGE (DAYS)
55.823528

FIGURE 3

FIGURE 4: Vital Dyes under UV Light 100X
Orange Line: xylenol orange (day of surgery)
Red Line: Alizarine complexone (2nd week)
Green Line: Fluorexan and (3rd week)
Darker Green Line Fluorexan + Alizarine Complexone
(4th week)

FIGURE 5: Frontal Cephalogram
Head Positioned in a Custom Made Cephalostat



FIGURE 4

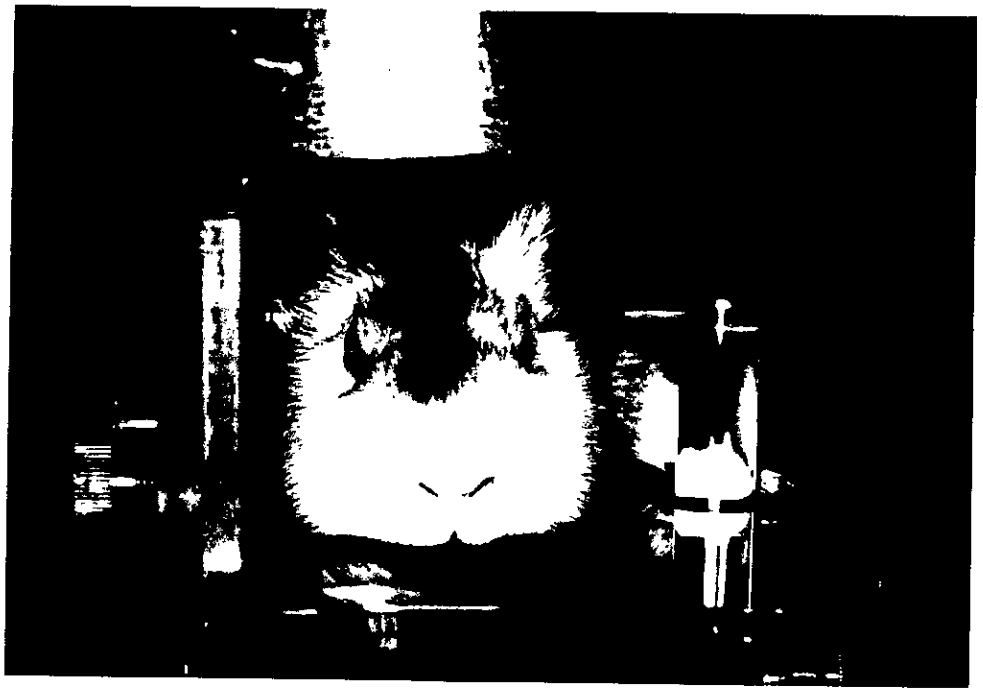


FIGURE 5

FIGURE 6: Sagittal Cephalogram

FIGURE 7: Injection of Pentobarbital Intraperitoneal



FIGURE 6

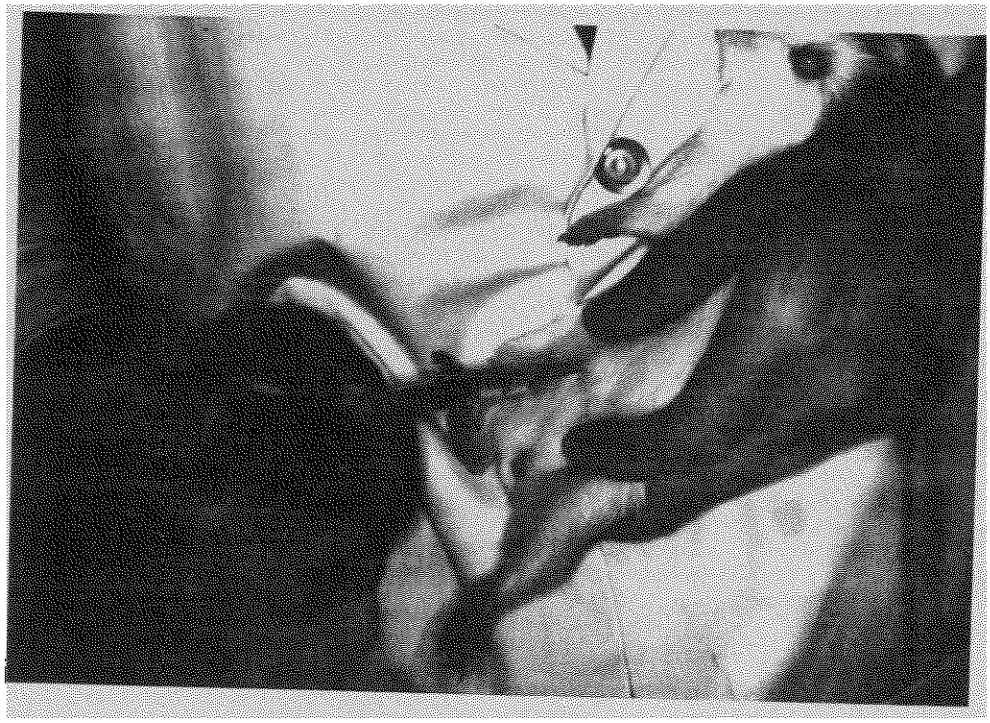


FIGURE 7

FIGURE 8: Parasagittal soft tissue incision over the nasal and frontal area

FIGURE 9: Flexible template for accurate outlining and standardization of bony cuts.

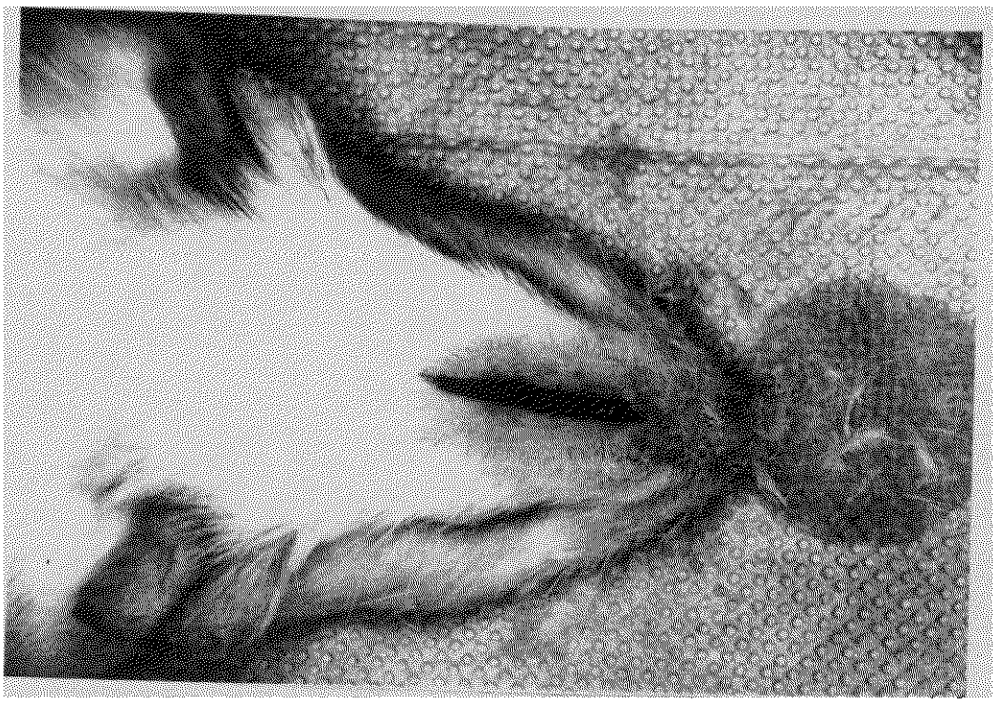


FIGURE 8

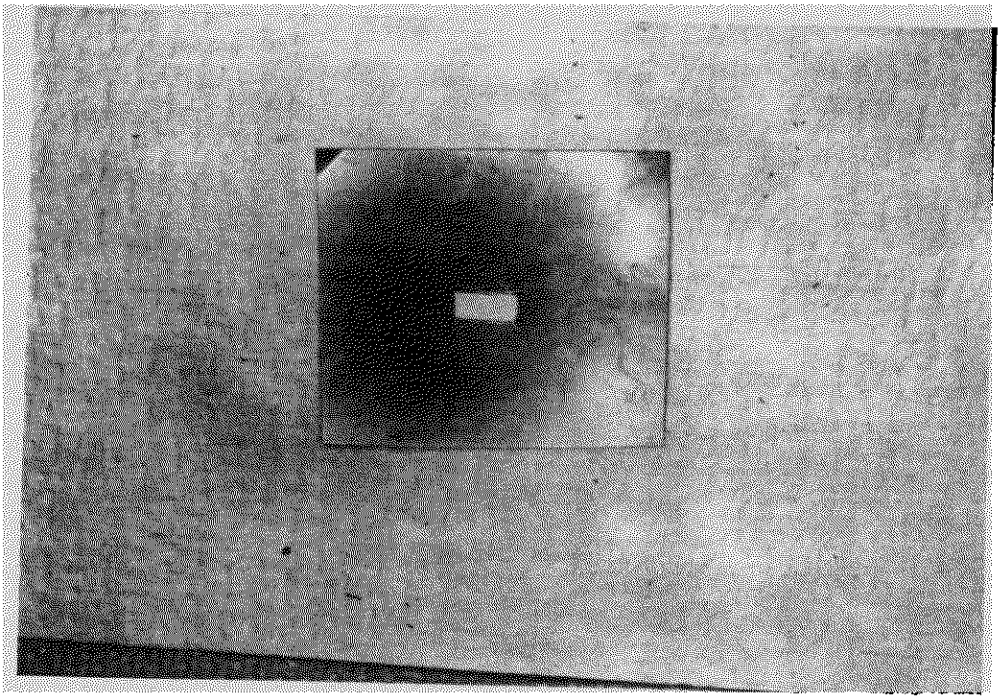


FIGURE 9

FIGURE 10: India Ink outlines of surgical bony cuts

FIGURE 11: Surgical bony incisions



FIGURE 10

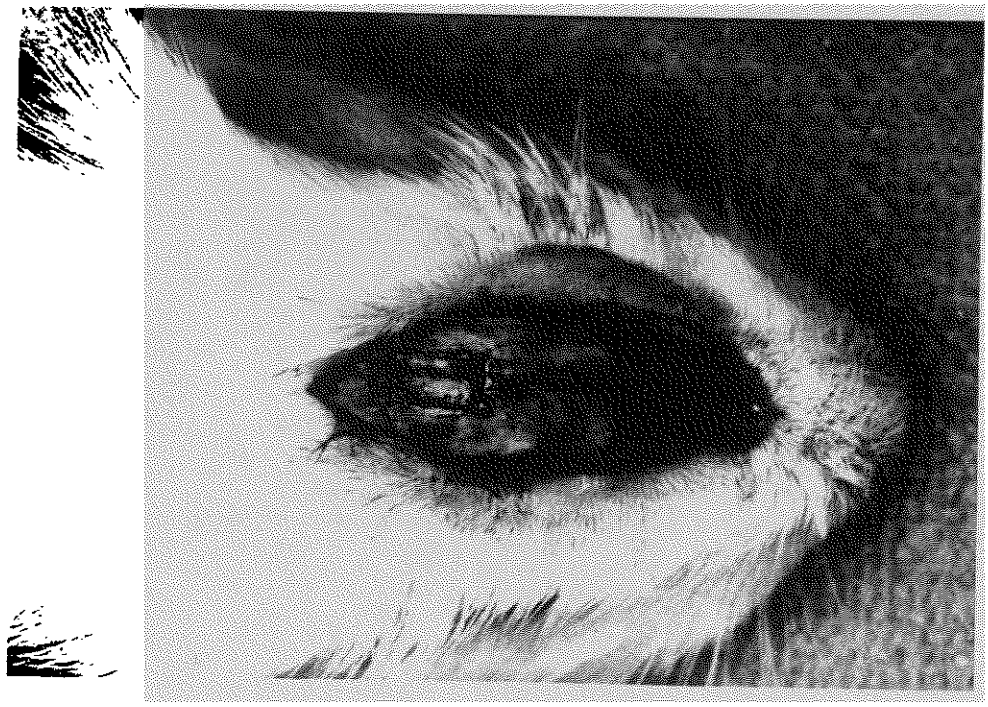


FIGURE 11

FIGURE 12: Amalgam implants around the metopic (above) and internasal sutures.

FIGURE 13: Soft tissue wound closure and desinfection. No undue compression.

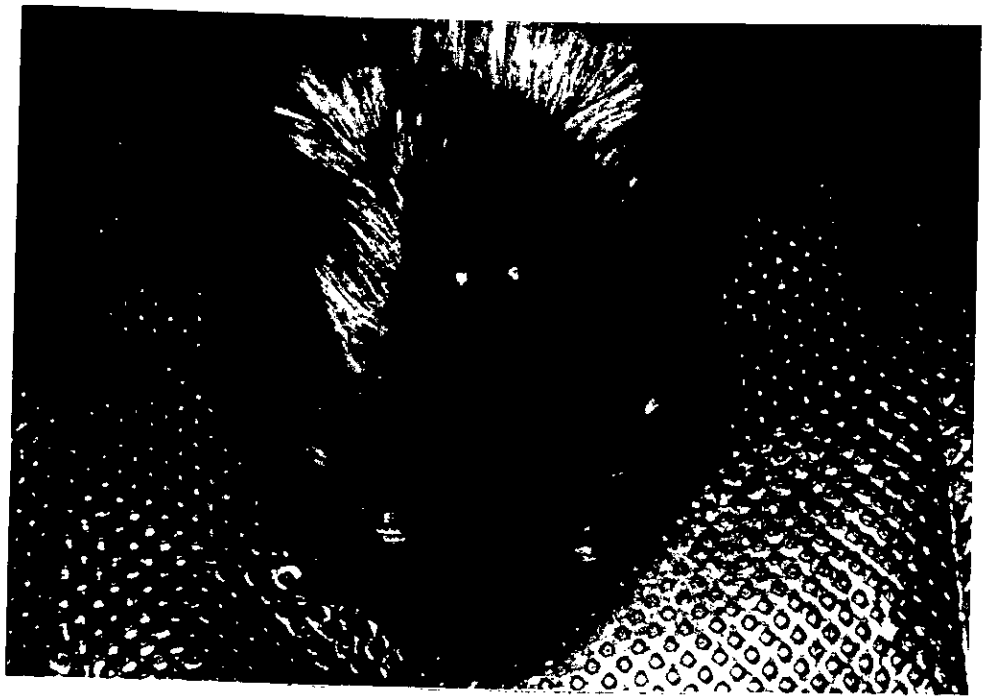


FIGURE 12



FIGURE 13

FIGURE 14: Thoracic cage opened. Vena Cava sectioned. Injection of 10% buffered formalin fixative in the left ventricule for vascular fixation.

FIGURE 15: Trapezoid outlining of the histological samples for easy embedding and orientation.



FIGURE 14



FIGURE 15

IV. RESULTS

I. Cephalometric Observations Control Groups (Figures 16, 17, 18)

Weekly sagittal and frontal headfilms for each animal having implant markers allowed measurements of transverse facial and cranial expansion at the internasal and metopic sutures as well as relationships with anteroposterior development at the fronto-nasal suture. The internasal suture shows a maximum lateral expansion of 0.4 mm, and the maximal expansion at the level of the metopic suture was 0.2 mm. The sagittal cephalograms show an increment at the fronto-nasal suture ranging from 2.1 to 3.2 mm of anteroposterior enlargement.

II. Histological Evaluation (Figure 19)

1. Non surgical Control Group

a. Nasal sites (Figure 20)

The internasal sutural is essentially the serrated type. Its configuration varies slightly depending on location. Anteriorly the suture is almost straight but posteriorly, closer to its fronto-nasal junction, it becomes more serrated and convoluted (Figures 21, 22).

In the middle area of the internasal suture, osteogenic activity at the sutural junction, (as revealed by the vital dyes), is very slight, the sutural pathway is clear and patent, and no trace of sutural closure is observed. The fibers are tensed and show a perpendicular orientation to the bone edges. The nasal bones are of a cancellous type with extensive marrow spaces. When compared with the frontal bone site, it appears thicker, less

lamellar, and more cancellous.

The dyes show a moderate extent of new bone apposition on the mucosal side of the nasal bones, slightly away from the sutural surfaces. A more marked extent of outer periosteal bone deposition had occurred away from the internasal suture, closer to the much more active nasomaxillary sutures.

b. Frontal sites

The interfrontal suture also varies depending on the area. Anteriorly the configuration is more convoluted, becoming more butt-like in its middle part (Figures 19, 20). In this portion (Figure 21), the sutural fibers are oblique or parallel to the bone edges, and the sutural width is narrow compared with the internasal suture. In most cases a bony fusion is seen posteriorly, close to the coronal suture. The limited uptake of vital dyes shows slight osteogenic activity at the sutural margins, and a marked extent of endocranial bone apposition had occurred laterally away from the suture (Figure 2). In some instances, periosteal ectocranial accretion is observed. The frontal bone is mostly of a lamellar type having only few marrow spaces.

2. Surgical Control Group

a. Nasal Sites

In its anterior portion, before the surgical site, the suture resembles the normal nasal control, the fibers are tensed and some bone growth activity had taken place. Nevertheless the sutural configuration is straighter than in the non-sham control group (Figure 22).

In the transplant area, the suture shows some activity. Some

bone apposition had taken place at the edges of the sutures as revealed by the vital dyes. The sutural fibers seem to have adopted their normal, perpendicular orientation with the bony walls. This is a sign of their relative tension and activity.

The sutural configuration mostly appears straighter in the repositioned segment than it was before the experiment or in the area adjacent to the transplanted area. This sutural straightening as revealed by vital dyes occurs by bone apposition in the pits of the digitations, with the tips relatively absent of new bone deposition (Figure 23). The bone in the experimental area is very cellular, no obvious resorption seems to have taken place, and the transplant is vital and formed of normal cancellous bone as it is in the non-sham control samples.

Osteophytes are often seen in the areas of surgical cuts or even in the area of the suture. This callous bone formation can be either on the inner surface (endophytes) or on the ectocranial surface of the nasal bones (Figure 24).

In a few cases a sutural bony bridge had formed. In other instances the suture appears patent, clear and the tissue looks broad and tensed. The vital dyes demonstrate some periosteal deposition laterally away from the transplant. Some inner periosteal deposition can also be observed, slightly apart from the suture and, mainly, at the surgical edges. Thus, a significant bone healing process had occurred, which most of the times led to the complete filling of the surgical cuts (Figure 25).

b. Frontal sites

The metopic suture within the implant area has a strict

butt-end type of configuration. The sutural tissue subsequent to the surgical procedure had become narrowed, atrophic-like in appearance, and its fibers had become oriented parallel or oblique to the bone edges (Figure 26). In most of the samples, a slight to moderate amount of bone apposition had occurred on the sutural margins. In most of the cases, an inner or outer periosteal bony bridge was seen to have developed across the suture.

The development of osteophytes on the endocranial or, more often, ectocranial bone surface had usually occurred in this group. The fine-cancellous non-lamellar deposits were seen mostly around the surgical cuts and at the sutural edges. They contributed to the bone healing process in response to the surgical cuts as well as in sutural closure. This healing following surgical trauma occurred less frequently or, perhaps, just less rapidly than in the facial sites (Figure 27).

Surgical repair of the frontal bone cuts originates essentially from the inner and outer periosteal projections of surrounding bone toward the transplant. This "lipping" of the inner and outer surfaces of the frontal bone does not involve accretion from the middle part of the cut edges (Figure 28). The nature of repositioning of the in-situ replaced cranial segment with its suture seems to have had an influence on the nature of subsequent endocranial and/or ectocranial periosteal deposition. It appears that if the segment has a slightly deeper new position than the old calvarial margin surrounding it, the bony repair projections originate largely from an endocranial source. Otherwise, an outer periosteal bone formative reaction was preferred.

The bony edges as well as the outer periosteal part of the transplant itself did not show any obvious signs of periosteal ectocranial apposition, nor had resorption within the transplant occurred. The inner transplant surface varied from almost no new bone formation at all to a significant extent of endocranial osteogenic activity.

Adjacent to the osteophytes, the new bone deposits on the perimeter surfaces surrounding the surgical site, as well as on the transplant itself, was mostly of a lamellar type with only few small marrow spaces.

3. Experimental Group

a. Nasal Sites, Receiving Metopic Transplants

A cranial frontal bone segment with its suture was transplanted into the facial (internasal) sutural site. The original, butt-end configuration of the cranial segment was essentially maintained in most cases. The sutures were usually patent and active some new bone accretion could generally be observed at their margins. The sutural fibers retained a perpendicular attachment orientation into the bone edges (Figure 29). In some instances a sutural bony bridge had formed.

Even though most cases showed retention of a butt-end sutural morphology, some demonstrated a gradual formation toward a more convoluted configuration, with new digitations projecting from the former straight, butt type articulation edge, as revealed by the vital dies (Figure 30).

In all cases a significant extent of new bone formation had occurred on the inner periosteal side of the transplant, much more

so than the relatively slight deposits seen in the other cross-transplant site or in either of the two control groups (Figure 31). It is comprised of fine-cancelous bone with many marrow spaces.

Little or no outer periosteal apposition on the transplant was observed in this group, and some slight resorptive activity was sometimes seen. Obvious asymmetries of the depository pattern peripheral to the surgical site was seen in some specimens, with one side showing outer periosteal bone deposition but with the contralateral side showing inner periosteal deposition.

The surgical cuts in most cases had healed and closed or had become very close to full closure (Figure 32). The margins of the peripheral nasal bones thus appear to be very active in reparative osteogenesis and very quick to fill the trauma area created by the surgery. This rapid response as well as an intense contribution of the whole nasal bone periphery and the cross-implanted cranial segment is in contrast to the extent of reaction and healing process observed in the cranial region that had received a cross-facial transplant. At the posterior limit of the surgical area, the original butt-end sutural configuration became more convoluted than in the anterior part of the transplant. This is in accordance with the gradually increasing interdigitization of the internasal suture seen in the control more posteriorly. In instances where inner periosteal deposition is marked, a lengthening of the suture that resembles more closely the normal facial suture is seen. The original thin and lamellar segment of the cranial frontal bone had become much thicker by inner-side cancellous bone formation. The

osteophyte type of formation mentioned above occurred less frequently than in the nasal-to-frontal sites.

b. Frontal Sites, Receiving Nasal Transplants

Most of the nasal-to-frontal transplants still show an overall convoluted sutural configuration even though new bone deposition at the sutural edges were tending to straighten the sutural pathway. Apposition was occurring in which the bottom of the sutural digitation inlets were selectively receiving more bone than the tips of these projections.

The degree of serration along the implanted segments seemed to be changing anteroposteriorly, with front part (closer to the anterior surgical borders) being less convoluted. This appears to be a feature imported along with the original suture since the equivalent controls also have this gradual increase in digitization from anterior to posterior. Sutural fibrous connective tissue orientation is parallel or oblique to the bone edges. The absence of bone-to-suture tension in the sutural fibers, as well as the narrow width of the suture itself, thus demonstrates a lack of pattern activity of the sutural tissue that would serve to re-establish the original suture-to-bone relationship (Figure 33). In most samples sutural bony fusion can be observed in the posterior portion of the implant (Figure 34).

The surgical cuts by the conclusion of the experiment, in most instances, had not received bone regeneration or repair. Fibrous connective tissue rather filled the defect. However, abundant osteophytes have formed around the periphery of the transplant segment (not on the segment itself or with the cuts). These are

mainly ectocranial, as also seen in the sham control group, although some endocranial periosteal bone proliferation was also involved in this repair process (Figure 35). The transplant is made of its original nasal cancellous bone, and no evidence of significant endo- or ectocranial apposition can be observed on its surfaces. The surrounding old cranial bone is mostly lamellar, and its thickness has been "leveled" with the transplant by new endocranial apposition. A lesser extent of equivalent endocranial deposition is also seen in both the cranial and sham control groups located in specific areas of the calvaria.

FIGURE 16: Comparison of skulls of a 25 day old and an adult guinea pig. Metopic and internasal sutural segments removed on the 25 day old guinea pig at the metopic area relative constancy of the transverse dimension.

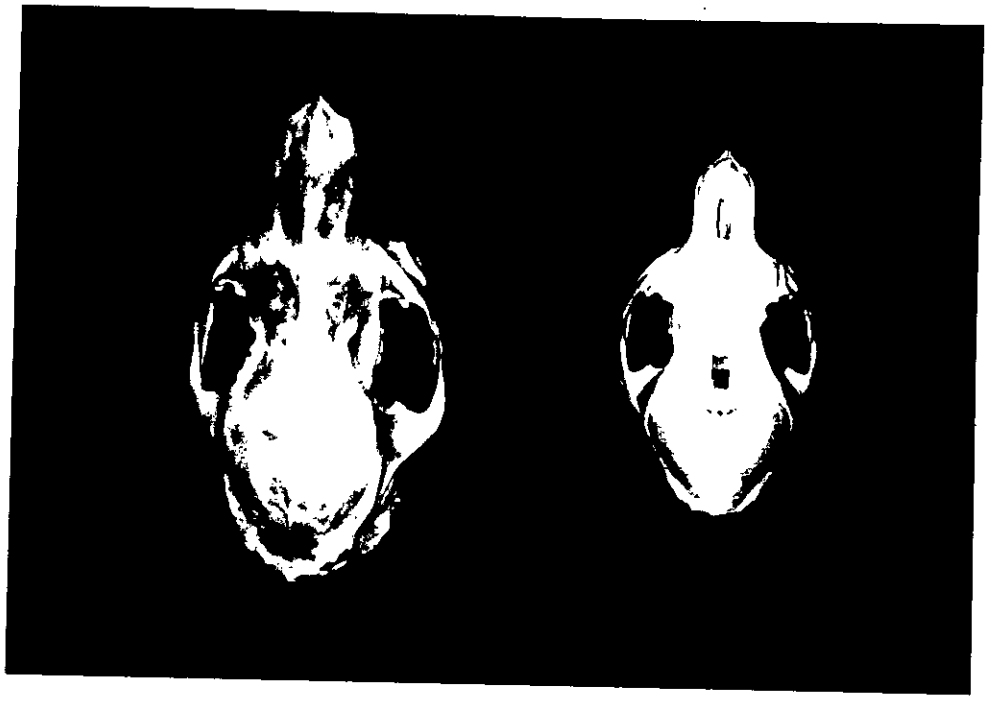


FIGURE 16

FIGURE 17/18: Non surgical control group animals N 1, 8, 13, 18 and 22.
Demonstration of the relatively inactivity of metopic and internasal sutures.
The activity of the fronto-nasal suture is shown to be tremendously high in comparison.

IMPLANTS

Animal #8

	Surgery				Sacrifice	
DATE:	1/24	1/30	2/7	2/13	2/21	Dif.
FACIAL	a= 2	2	2.1	2.1	2.2	0.2
	c= 4.4	4.5	4.6	4.6	4.6	0.2
CRANIAL	a= 3.9	4	4	4.1	4.1	0.2
	c= 7.6	7.6	7.6	7.6	7.6	0.0
SAGITTAL	21.9	22.8	23.7	24	24.6	2.7

Animal #13

	Surgery				Sacrifice	
DATE:	1/30	2/7	2/13	2/20	2/27	Dif.
FACIAL	a= 2.6	2.6	2.7	2.8	2.8	0.2
	c= 5.5	5.5	5.5	5.6	5.6	0.1
CRANIAL	a= 3.2	3.2	3.2	3.2	3.2	0.0
	c= 6.1	6.1	6.2	6.2	6.2	0.1
SAGITTAL	22.2	23.5	23.9	24	24.7	2.5

*a is the inner most distance in millimeters between the implants on the radiographs.

c is the outer most distance in millimeters between the implants on the radiographs.

Sagittal measurements have been averaged.

IMPLANTS

Animal #18

DATE:	2/14/ Surgery	2/21/	2/27/	3/6/	3/12/ Sacrifice	Dif.
FACIAL FRONTAL	a= 1.6	1.7	1.7	1.7	1.8	0.3
	c= 4.7	4.7	4.8	4.9	4.9	0.2
CRANIAL	a= 2.8	2.7	2.7	2.7	2.7	0.1
	c= 5.9	5.9	6	6	6	0.1
SAGITTAL	24.3	24.7	25.4	25.8	26.4	2.1

Animal #22

DATE:	2/22	2/28	3/6	3/13	3/20	Dif.
FACIAL						
	a= 2.2	2.3	2.3	2.4	2.4	0.2
	c= 4.6	4.7	4.0	4.9	5	0.4
CRANIAL						
	a= 3.9	3.9	3.9	3.9	3.9	0.0
	c= 6.6	6.6	6.7	6.7	6.7	0.1
SAGITTAL						
	22.8	24.3	25.3	25.4	26	3.2

FIGURE 19: Table summarizing observations. No numerical data are presented in the text. The observation period being very short, the recording of certain phenomena may be a matter of their presence or absence at a certain point in time.
(A, B, C, D, E, F)

FIGURE 19-A
NASAL SITE: NON-SURGICAL CONTROL GROUP

Location Within the Suture	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within Suture	Bone Deposition Outer and Inner Periosteal Surfaces	Bone Resorption
Anterior Portion:	Relatively straight	Fibers perdendicular to the bone margins	Very wide sutural apposition	Inner side deposition near suture; outer deposition away from suture	None
Middle Portion:	Convoluted	Fibers perpendicular to the bone margins	Less wide than the anterior part	Inner side deposition near suture; outer deposition away from suture	None
Posterior Portion:	Even more convoluted than the middle part	Fibers perpendicular to the bone margins	Slight sutural apposition	Inner side deposition near suture; outer deposition away from suture	None

FIGURE 19-B
FRONTAL SITE NON-SURGICAL CONTROL GROUP

Location Within the Suture	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within Suture	Bone Deposition Outer and Inner Periosteal Surfaces	Bone Resorption
Anterior Portion:	Convoluted	Perpendicular or oblique to bone edges	Moderate to slight	Inner side deposition near suture; outer deposition away from suture	None
Middle Portion:	Butt-end	Circumferential	Slight to none	Inner side deposition near suture; outer deposition away from suture	None
Posterior Portion:	Butt-end	Circumferential	Complete sutural ankylosis	Inner side deposition near suture; outer deposition away from suture	None

FIGURE 19-C
NASAL SITE: SURGICAL CONTROL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within The Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of Implant
2	Straighter than Control	Perpendicular to bone margins	Suture Patent Slight activity	Outer apposition on adjacent nasal bone.	Both Cuts healed with new bone	None
4	Became Straighter	Oblique to Circumferential	Suture patent very slight activity	Slight inner and outer apposition on implant and adjacent nasal bone	Both cuts healed	None
5	Became Straighter	Perpendicular to bone margins	Suture patent almost no bone apposition at the sutural margins	Inner and outer apposition on adjacent bones and transplant	Both cuts healed	None
10	Became Straighter	Perpendicular to bone margin	Bone bridge on the outer surface of the suture	Outer apposition on adjacent bones	Both healed with Epiphytes present	None
11	Became Straighter	Perpendicular to bone margins	Patent and wide. Slight activity	Slight outer apposition on transplant, more on adjacent bones	One completely healed. The other side nearly so	None

FIGURE 19-C (Continued)
NASAL SITE: SURGICAL CONTROL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within The Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of Implant
14	Had become straighter but convolutions beginning to reform	Perpendicular to bone margins	Suture Patent and wide Slight deposition	Outer periosteal apposition on adjacent bones	Both healed with new bone	Slight outer surface transplant
21	Had become straigher but convolutions beginning to reform	Perpendicular to bone margins	Patent and wide moderate deposition	Slight outer apposition on adjacent bones	Both healed	None

FIGURE 19-D
FRONTAL SITE SURGICAL CONTROL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition within the Suture	Bone Deposition on Outer and Inner Periosteal	Surgical Cut	Resorptive Rejection
II		No suture	Complete sutural closure	Outer apposition on the transplant Inner and outer apposition on adjacent bones	Both cuts still patent	None
IV	Straight	Circumferentiated	Outer bony bridge over the suture; remaining sutural pathway reduced	Outer apposition on transplant Inner and outer apposition on on adjacent bones	Osteophytes surgical cut healed	None
V	Straight	Circumferential	Sutural closure from inner periosteal surface; Remaining sutural pathway reduced	Important inner periosteal apposition on transplant slight inner and outer apposition on on adjacent bones	Important osteophytes formation. Both surgical cuts patent.	None
XI	Straight	Perpendicular to oblique sutural fibers orientation	Suture patent and wide; slight activity	Inner apposition on the transplant Inner and outer apposition on the adjacent bones.	Both surgical cuts patent	None

FIGURE 19-D (Continued)
FRONTAL SITE SURGICAL CONTROL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition within the Suture	Bone Deposition on Outer and Inner Periosteal	Surgical Cut	Resorptive Rejection
XIV	Overall slight with slight convolutions	Circumferential	Suture patent thin sutural pathway almost no activity	Inner apposition on the transplant Inner and outer apposition on adjacent bones	One surgical cut healed	None
XXI	Straight	Circumferential	Sutural closure remaining pathway reduced	Inner apposition on the transplant Inner apposition on adjacent bones	One surgical cut healed	None

FIGURE 19-E
NASAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of the Implant
3						Important Resorption
6	Straight but slight formation convolution	Perpendicular orientation of fibers to bone margins	Suture Patent but osteophyte formation at the level of the suture. Moderate activity	Outer periosteal apposition on the adjacent bones	One side healed, the other side nearly so	None
7	Overall straight but formation of convolutions	Perpendicular	Suture patent and wide. Moderate activity	Slight inner and outer apposition on adjacent bones	One side healed	None
9	Straight	Circumferential orientation	Suture patent but narrow. Almost no activity	Inner apposition on transplant	Both surgical cuts patent	None

FIGURE 19-E (Continued)
NASAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of the Implant
12	Straight	Circumferential	Suture patent but narrow. No activity	Outer periosteal apposition on the adjacent bone on one side. Inner apposition on the transplant on one side.	Both surgical cuts healed	None
15	Slightly convoluted	Perpendicular	Suture patent and wide almost no activity	Inner apposition on the transplant outer apposition on adjacent bones	One surgical cut healed	None
16	Convoluted	Perpendicular	Suture patent and wide slight activity	Slight inner apposition on the transplant Important outer apposition on adjacent bones	Endophytes both surgical cuts healed	None
17	Straight	Perpendicular	Suture patent and wide slight activity	Important inner apposition on the transplant	Both surgical cuts healed	None

FIGURE 19-E (Continued)
NASAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fiber Orientation	Bone Deposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of the Implant
19	Straight but formation of slight convolutions	Perpendicular	Suture patent and wide Slight activity	Inner apposition on the transplant Outer apposition on adjacent bones	One surgical cut healed	None
20	Straight but formation of slight convolutions	Perpendicular	Suture patent slight activity	Inner apposition on the transplant Outer on adjacent bone	Endophytes both surgical cuts healed	None

FIGURE 19-F
FRONTAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fibers Orientation	Bone Disposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of Implant
III						Important Resorption
VI	Became straighter but still has slight convolutions	Perpendicular to the bone margins	Suture patent slight activity	Slight inner and outer apposition on the transplant inner and outer apposition on adjacent bones	Osteophytes both surgical cuts patent one almost closed	None
VII	Became straighter	Circumferential	Sutural closure from inner periosteal apposition narrow remaining no activity sutural pathway	Slight outer apposition on the transplant important inner and outer apposition on adjacent bones	Osteophytes both surgical cuts patent	None

FIGURE 19-F
FRONTAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fibers Orientation	Bone Disposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of Implant
IX	Slightly convoluted	Circumferential	Suture closure from outer surface remaining sutural pathway is still wide	Important outer apposition on the transplant Inner and outer apposition on adjacent bones	Outer osteophytes and endophytes one surgical cut healed	None
XII	Almost straight	Perpendicular to bones margins	Suture wide and patent		Both surgical cuts patent	Important resorption
XV	Relatively straighter	Circumferential or oblique	Suture patent but narrow slight activity	Slight inner apposition on the transplant Inner and outer apposition on adjacent bones	Both surgical cuts patent	None
XVI	Slightly convoluted	Perpendicular and oblique	Suture patent but narrow Moderate activity	Slight inner apposition on transplant Inner and outer on adjacent bones	Osteophytes one surgical cut almost closed	None

FIGURE 19-F
FRONTAL SITE EXPERIMENTAL

Animal	Sutural Configuration	Sutural Fibers Orientation	Bone Disposition Within Suture	Bone Deposition on Outer and Inner Periosteal Surfaces	Surgical Cut	Resorptive Rejection of Implant
XVII	Almost straight	Circumferential	Outer bony bridge. The rest of the suture is narrow almost no activity	Slight inner apposition on the transplant. Inner and outer on adjacent bones	Both surgical cuts patent	None
XIX	Straight	Oblique	Osteophyte over the suture pathway suture still wide Moderate activity	Outer apposition on the transplant Inner outer on adjacent bones	Both surgical cuts closed	None
XX	Straight	Oblique or circumferential	Outer bony bridge	Inner and outer apposition on adjacent bones	Osteophytes both surgical cuts patent	None

FIGURE 20: Internasal suture. Non surgical control group. Sharpey's fibers are embedded perpendicular to the nasal bone margins and appears tensed. The suture is wide, patent, and serrated.

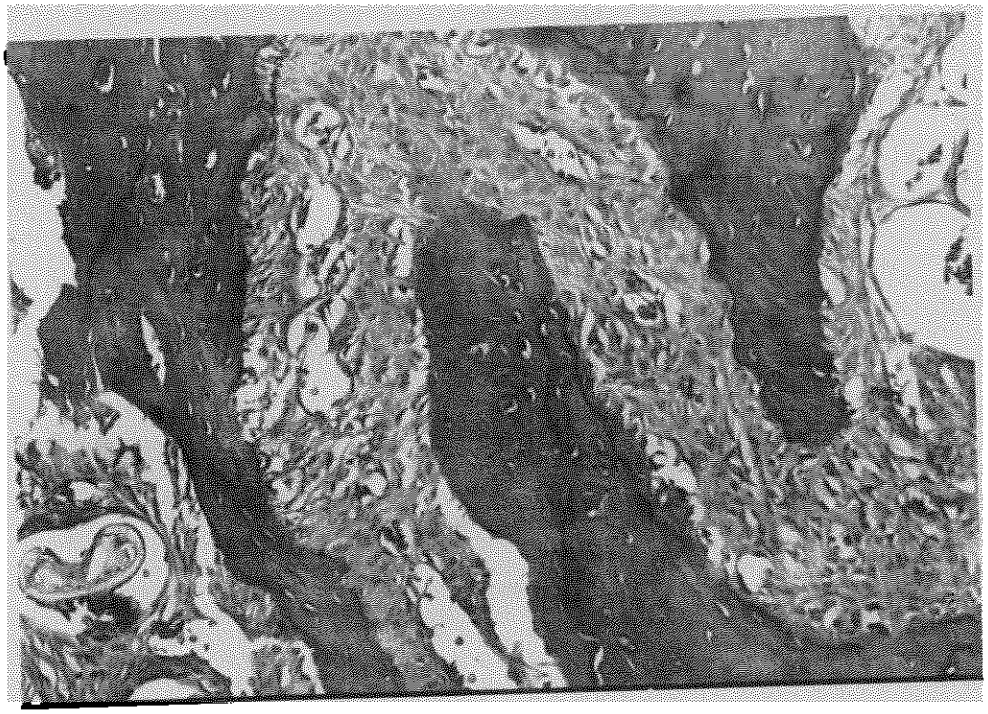


FIGURE 20

FIGURE 21: Internasal suture, anterior portion, non surgical control group. The suture is relatively straight, wide with Sharpey's fibers perpendicular to the bone. The appearances is of an immature sutural junction. The adjacent bone is cancellous and very cellular.

FIGURE 22: Metopic suture, middle portion, non-surgical control group. The sutural tissue is oblique or circumferential to the bone margins, and sutural width is narrow and straight. The adjacent bone is lamellar and less cellular than in a cancellous type.

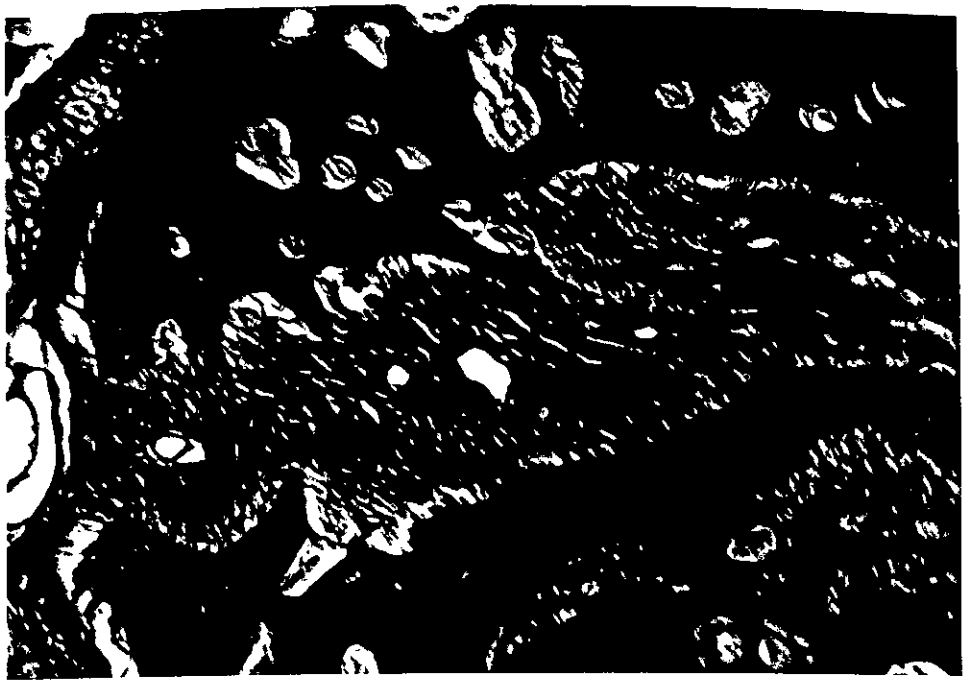


FIGURE 21

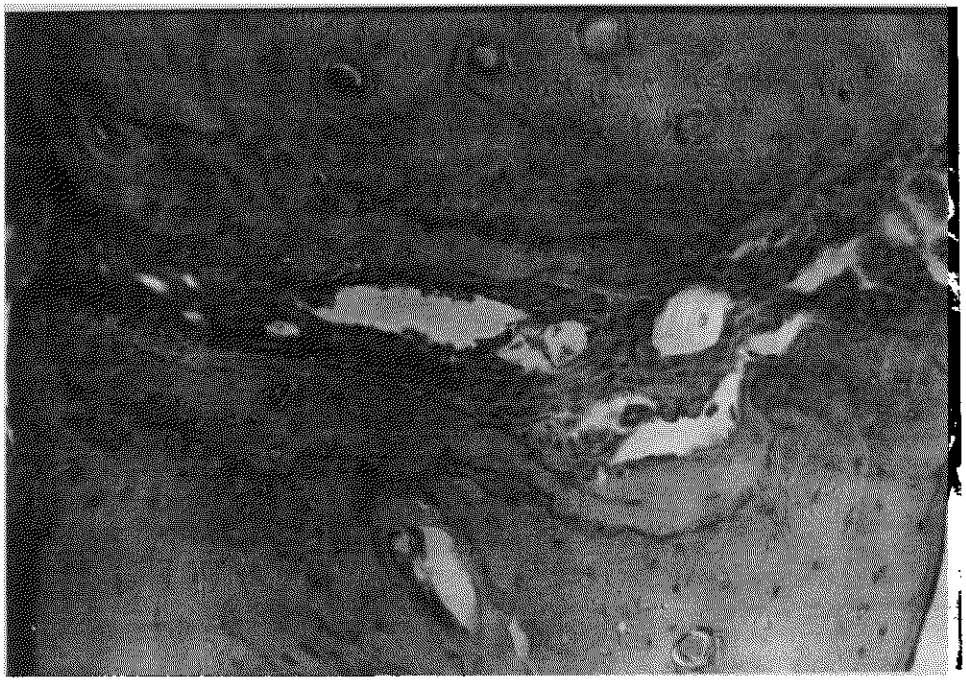


FIGURE 22

FIGURE 23: Metopic suture, anterior portion, non-surgical control group. Fibers are perpendicular to the bone margins, and the suture is wide and slightly convoluted. On the right, some inner periosteal, more cellular bone apposition had taken place.

FIGURE 24: Metopic suture, posterior portion, non-surgical control group. Suture closure had occurred in the middle of the section. A horizontal line of the previous sutural junction is still observable.



FIGURE 23



FIGURE 24

FIGURE 25: Internasal suture, surgical control group. Bone accretion occurred at the edges, as seen by the fluorescent dyes. Straightening of the sutural edges had occurred.

FIGURE 26: Internasal suture surgical control group. The sutural edges are straighter, and fiber orientation is perpendicular to the bone margins.

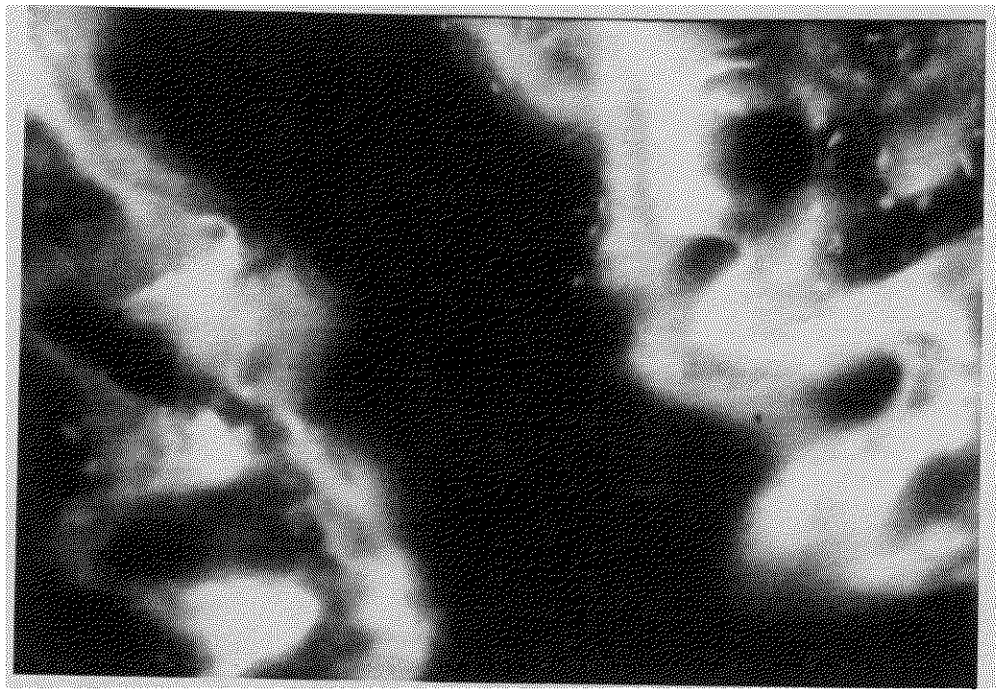


FIGURE 25

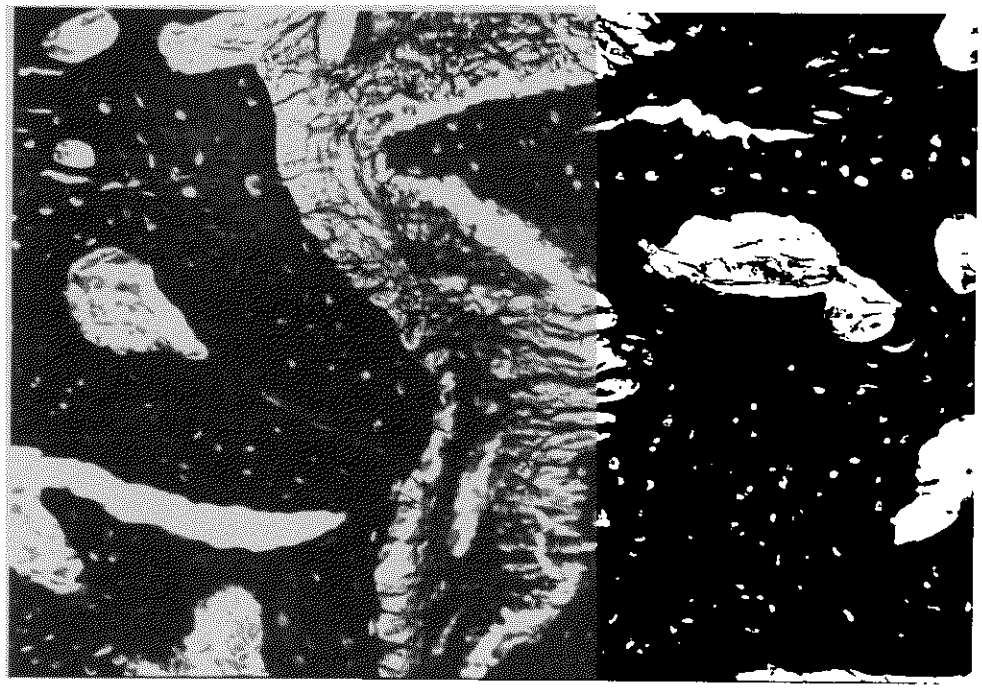


FIGURE 26

FIGURE 27: Nasal Site, surgical control group. The surgical cut had near-complete healing. Osteophytes present.

FIGURE 28: Nasal site, surgical control group. The surgical cut has healed. Above: Dark area: Nasal area surrounding implant. Bottom right: Nasal implant. The surgical cut has healed, as seen by the flourescent marker lines.



FIGURE 27

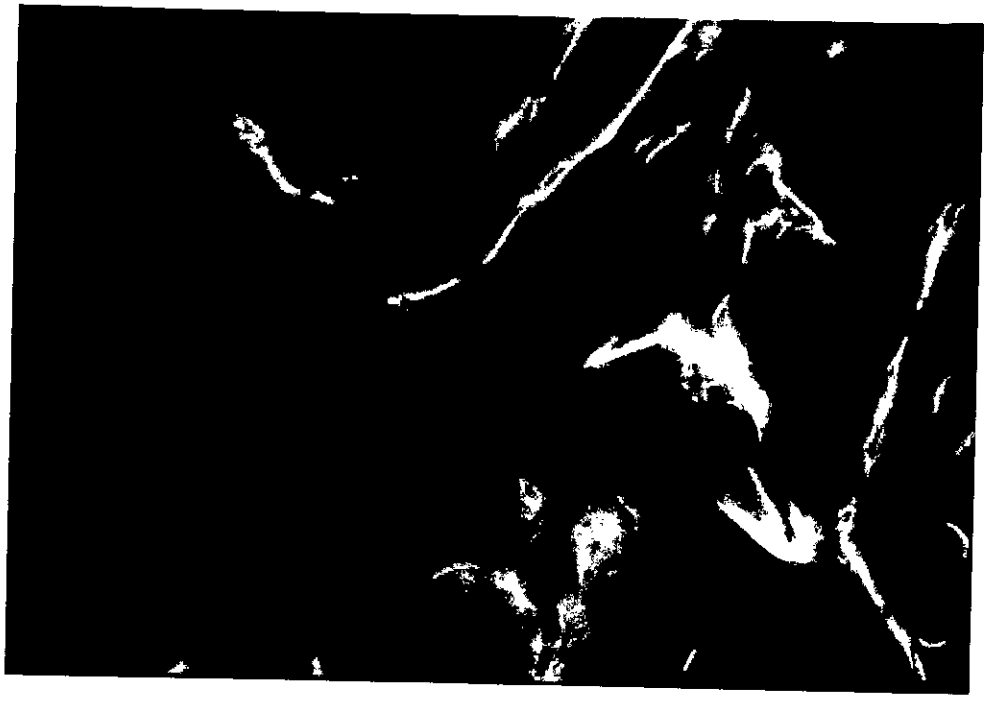


FIGURE 28

FIGURE 29: Metopic suture, surgical control group. Slight accretion had occurred on the surgical edges. Inner periosteal deposition is seen on the right.

FIGURE 30: Metopic suture, surgical control group. On the right: an inner periosteal bony bridge had closed the suture. The rest of the suture shows a circumferential orientation of the sutural fibers. The width of the suture has become very narrow.

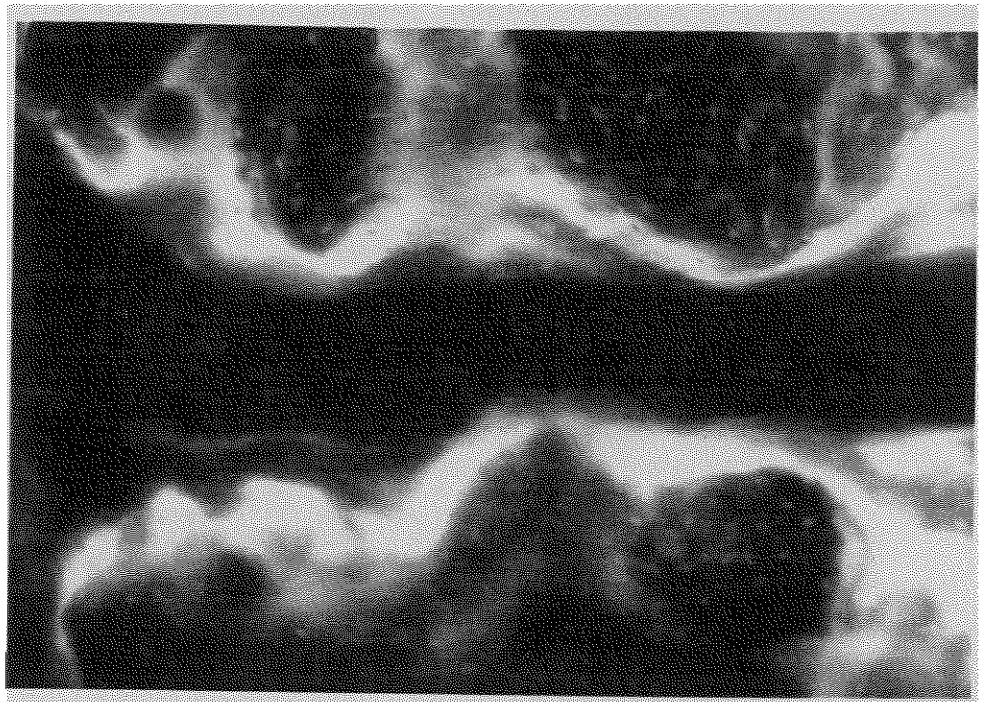


FIGURE 29



FIGURE 30

FIGURE 31: Frontal site, surgical control group. The surgical cut remainds patent. Inner and outer periosteal deposition on the transplant and the adjacent frontal bone is seen. Osteophyte formation (on the right) had occurred.

FIGURE 32: Cranial-to-facial transplant. Bone accretion had formed at the sutural margins (green fluorescence) with formation of serrations.



FIGURE 31

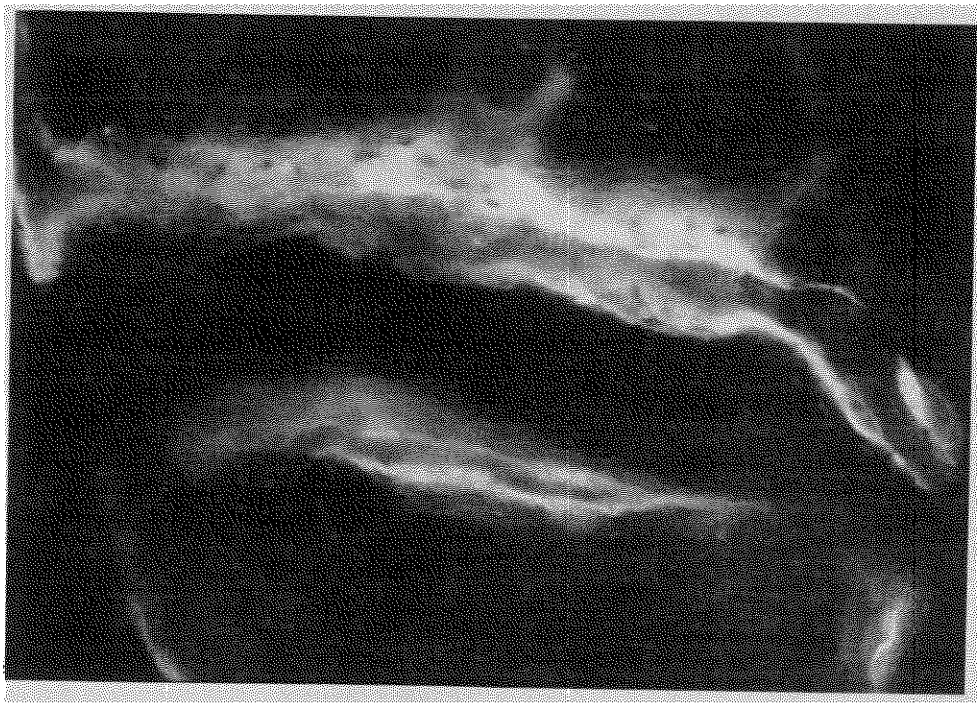


FIGURE 32

FIGURE 33: Cranial-to-facial transplant. Attachments of sutural fibers are perpendicular to the bone margins (on the right). Wide sutural span.

FIGURE 34: Cranial-to-facial transplant. Inner periosteal apposition (on the right) has formed at the level of the suture. Sutural thickening is seen.

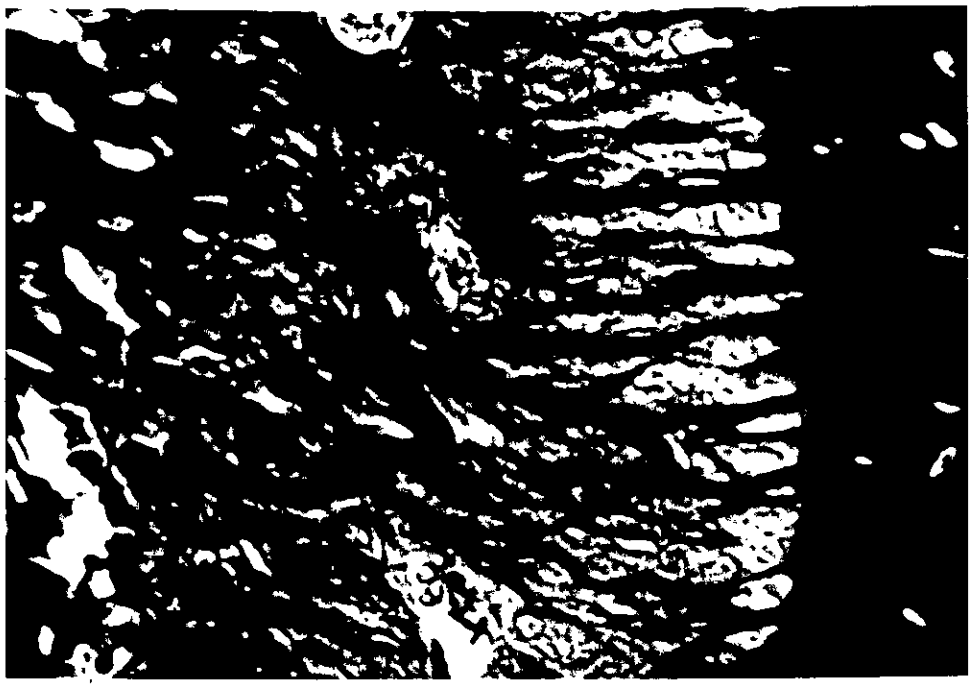


FIGURE 33

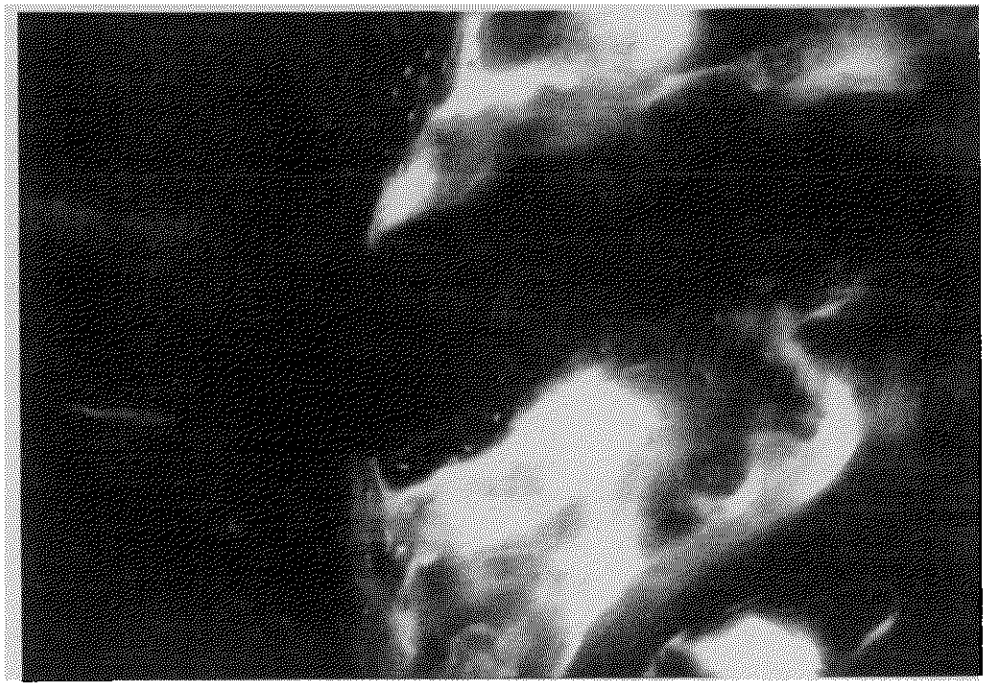


FIGURE 34

FIGURE 35: Cranial-to-facial transplant. The surgical cut has healed (green fluorescence). Above: the dark area is the transplanted frontal bone. Bottom left: the dark area is the adjacent nasal bone.

FIGURE 36: Facial-to-cranial transplant: transplanted internasal suture. On the right is seen inner periosteal deposition. Some bone accretion in the depth of a convolution is seen above.

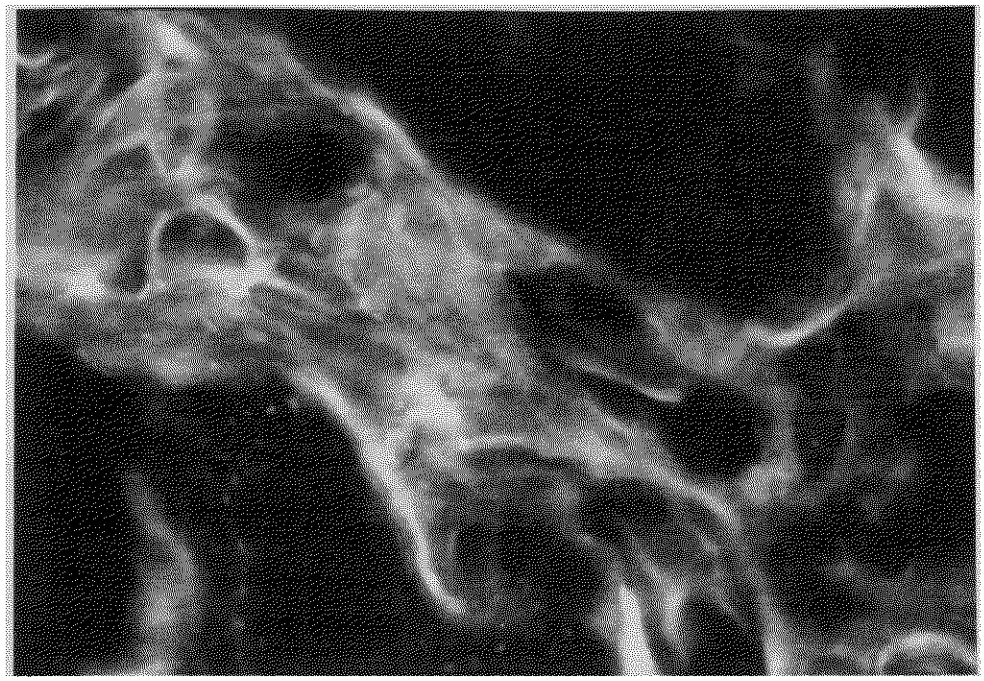


FIGURE 35

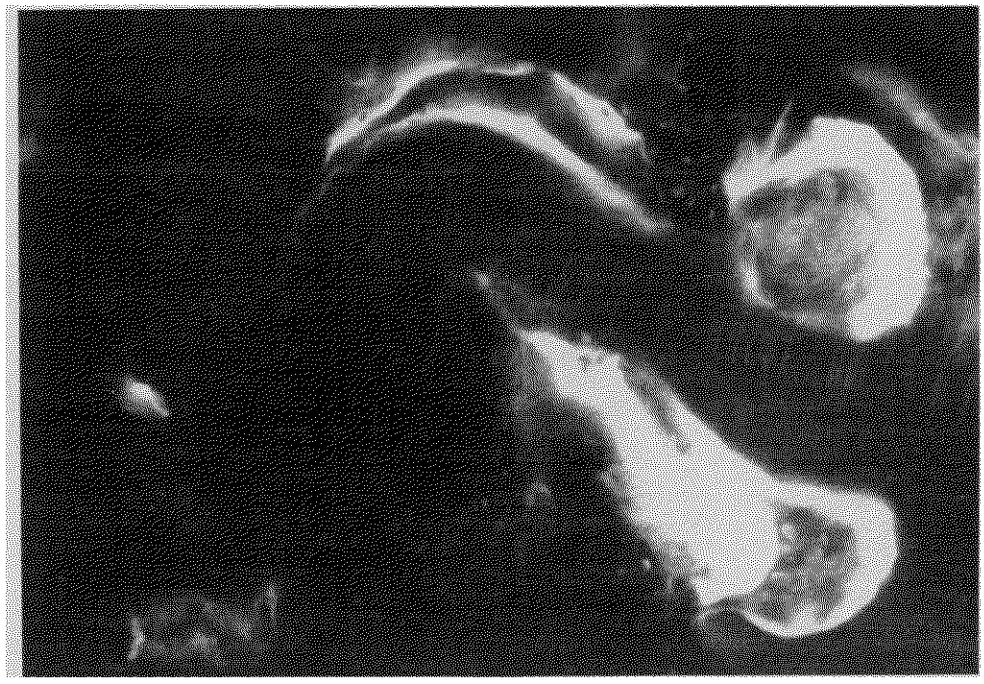


FIGURE 36

FIGURE 37: Facial-to-cranial transplant. Sutural closure from endocranial surface is seen on the right

FIGURE 38: Facial-to-cranial transplant microradiograph. Showing osteophyte formation at the ectocranial surface on a surgical cut (above) surgical cut still patent. Slight bone apposition around the nasal transplanted bone (left) and around the frontal bony margin.



FIGURE 37



FIGURE 38

V. DISCUSSION

Sexual dimorphism has not been considered in this study; the animals were choosen at random by litter and no attempts have been made to select or avoid one sex over the other. Nevertheless, the males grew faster than the females. But, as already observed with rats by Massler and Shour (1951), no other striking characteristics demonstrated significant differences.

Measurements of the distances between amalgam implants on frontal headfilms showed that the internasal and metopic sutures were relatively inactive growth sites during the period surveyed, with growth at the internasal suture slightly exceeding expansion seen in the metopic suture.

The anterior part of the metopic suture is convoluted. Its fibers are perpendicularly oriented to the bone margins, and the suture appears wide and active. Proceeding posteriorly, the suture gradually becomes less serrated, more butt-like, the fibers seem increasingly parallel and the sutural width narrows. In its extreme posterior portion close to the coronal suture, the metopic sutural junction was usually fused with no trace of the above description evident.

The progressively changing characteristics of this metopic suture seem to recapitulate the natural fate of the tissue as described by various investigators (Massler and Shour 1951; Watanabe, 1957; Pritchard, 1956; Moss 1954, 1960; Smith and McKeown, 1974; Linge, 1973; Koskinen, 1976; Kokich, 1976). These authors further believe that sutural configuration, sutural width, fiber

content and orientation are expressions of sutural maturity and activity. Moss (1960), inducing craniosynostosis in growing rats, observed that differential synostosis of calvarial sutures occurs:

- in non-growing area of the brain
- in areas where no functional stresses occur.

In this regard, the metopic suture belongs to the category of sutures which should fuse, at least in its posterior part at an early age. The internasal suture is also not subjected to further expansion, but the tissue characteristics seem to demonstrate a certain amount of functional stress along the suture.

The normal facial suture of the non-sham controls demonstrates an increasing anteroposterior gradient of convolution, such that both sutures, internasal and metopic, have their highest level of serration in the region of their junction with the frontonasal suture, which gradually decreases as the sutures extend away from this junction. Based on the sagittal measurements of the implants, extent of separation that occurred, and the evaluation of the vital dyes, it is clear that very active sutural growth occurred in the frontonasal suture area. This seems to reject the possibility of an intrinsic sutural growth potential, since the gradual changes in both sutural morphology tend to reflect a gradient of functional stresses along both sutures which is not interrupted by the sutural entity. The peak of this gradient would be at the level of the very active frontonasal junction, and would decrease anteriorly, posteriorly, and laterally from this point.

The very high degree of convolution of the frontonasal suture reflects an important growth activity that adds support to the

concept that sutural serration is a measure of functional extrinsic forces, as previously pointed out by Watanabe (1957), Moss (1960), Smith and McKeown (1974), Markens and Oudhof (1980), and Massler and Schour (1951).

In the surgical control group, removal of the convoluted nasal suture segment with replacement in situ, isolated this segment from any extrinsic forces. The original, serrated configuration gradually faded and become a more butt-end type. This is in accordance with Smith and McKeown's (1974) experiment on the coronal suture.

The results obtained in this study are consistent with those of Moss (1957), in which he concluded, after observation of the loss of convolutions in the coronal suture implanted intracerebrally, that the butt type of configuration is intrinsically determined. The serrations thus representing the expressions of extrinsic stimuli.

In the nasal region of the sham control group, the sutural segment anterior to the surgical site became significantly straighter when compared with the non-sham controls. It seems that the breaking down of the sutural continuity had reduced the stresses along this suture and then affected its morphology. These results suggest that sutural patency and configuration are not only the effect of growth activity in a given area, but also reflect normal functional stresses associated with muscular function and attachment. The sham control fiber pattern in the nasal transplant tends to orient more parallel to the bony walls, whereas in the non-sham control animals, the fibers are more perpendicular. This change in orientation may be a response to decreased external

stimuli and the tensile forces across the suture, as has been demonstrated by Watanabe (1957), Linge (1973), Persson (1973), and Smith and McKeown (1974). It seems then that the bony transplant with this segment of suture, isolated from the triggering effect of tensile forces, undergoes gradual metaplasia of the sutural tissue and sometimes ankylosis of the articulation. This further supports the already well known fact that tensile forces across a suture significantly affect its activity and patency (Young, 1959; Moss, 1960; Enlow, 1968).

In the present study, it was observed that transplanted sutural segments, after implantation in their new sites, adopted the structural characteristics of the sutural site they were implanted into.

When a metopic sutural segment was implanted in a nasal site, the features of the implanted suture tended to loose their metopic attributes, and after healing of the surgical cuts and as bone continuity became reestablished in the nasal area, functional activities appeared to reexert their influences on sutural bone growth as well as on cellular content and fiber tension, with slight convolutions beginning to form. Even though the actual expansion of the internasal suture has been shown to be relatively minimal, other functional influences, such as muscular function and attachment, may account for the changes in configuration, sutural width and sutural fiber tension. It appears, then, that under the experimental conditions described, the implanted metopic suture underwent metaplasia leading gradually to the adoption of the internasal sutural characteristics. Furthermore, the inner periosteal

deposition seen on a transplant had a tendency to "level" the thinner lamellar frontal bone and also take on the histologic form of the new bony site.

Conversely, when a internasal sutural segment was implanted into a metopic site, the characteristics of the transplant were gradually lost, their configuration changed to a more butt-type, the fibers became more parallel to the bone margins and the sutural width was significantly decreased. Sutural bony bridges were also observed in some cases. Moreover, the thicker facial bone was "leveled" with the adjacent frontal bones by inner periosteal apposition on the adjacent surfaces. It appears, then, that sutural characteristics relate to the functional environment, and that implanted into another site gradually adopt the characteristics of the former suture originally occupying in this site.

At sacrifice, the surgical cuts in the nasal sites, both sham and experimental, were nearly or already healed, in contrast with the frontal sites where healing seemed to be slower and less complete.

Osteophytes and callous bone repair are observed in all groups. Nevertheless, these frontal site responses appear more often and more consistently in the experimental and sham controls than in the nasal sites. Repair tissue origins can be either from inner periosteal formation (endophytes) or from the outer surface.

Mabbutt and Kokich (1979), in an experiment involving calvariectomy in 14 day old rabbits, demonstrated complete reskeletization by 26 weeks post-operatively. This demonstrates the high degree of regenerative potential in the calvaria. In our

study, the frontal surgical cuts in the experimental and sham groups did not heal as rapidly or as completely in the nasal sites. The timing of the operation, in the relatively fast growing guinea pig, can explain these discrepancies.

The sutural tissue retained its characteristic form, with Sharpey's fiber attachments and layered organization. It is preferred as the growth site articulation over the surgically created gap. A longer- term study may bring a different answer to this question, since, in calveriectomy studies (Simpson 1957; Hanson et al., 1977; Mabbutt and Kokich, 1979) sutures seem to redevelop. Nevertheless, the age of the animals was different in our study, and the ability of the area to regenerate a sutural tissue may be very much reduced.

VI. SUMMARY AND CONCLUSION

In this experimental study cranial and facial sutural articulations demonstrated striking divergences in morphology, reactive development, and formation of enveloping osteogenic tissues in response to the changed functional milieu. The study's objective, by means of cross-transplantations of cranial and facial sutural segments, was to clarify the nature of these differences. That is, are they purely responsive expressions of local functional environments or, conversely, unalterable characteristics that identify a specific sutural type? The sample was divided in three groups: a control group, a sham control group, and an experimental group. The control sample allowed determination of relative contribution of both sutures in a normal, non-surgical situation by means of metallic implant markers. The sham sample allowed distinction between surgical trauma and experimental results, and finally, the experimental sample addressed the specific questions cited above.

The results point to the following:

1. Implants and vital dyes show that the articulations of both the facial and cranial sutural types to be relatively slow-growing, even though the functional stresses along their fibrous attachments are different, as reflected by their sutural morphologies, states of tension, and respective widths. The internasal suture is of a convoluted type, with a perpendicular orientation of its fibers to the bony margins, and presents a relatively wide and uninterrupted sutural pathway. The cranial (metopic) suture has a butt-type of

configuration, with an oblique, almost parallel orientation of its fibers to the bone edges, and a narrow sutural span.

2. In the cranial metopic pathway, sutural configuration and activity was found to be different in different areas. The anterior part is more convoluted but progresses posteriorly to a more butt type, and finally undergoes ankylosis in its extreme posterior segment.

3. A convoluted type of sutural segment, isolated from the main sutural pathway and the extrinsic forces acting upon it, will tend to adopt a butt-type of responsive sutural configuration.

4. When a metopic sutural segment is transplanted to a facial (internasal) site, the metopic segment adapts itself to the new milieu, with the sutural configuration then becoming serrated, its fibers having an arrangement perpendicular to the bone margins, and its sutural span becoming wider.

5. Conversely, when an internasal sutural segment is transplanted into the frontal site, the sutural configuration becomes straighter, its fibers become aligned obliquely to the bone margins, and sutural width decreases.

6. It appears, then, that sutural morphology, fiber orientation, and sutural width are factors that relate to the nature and amount of extrinsic forces acting on them when a suture is transferred to a different milieu. Further, the transplanted suture adapts and assumes the morphology, fiber arrangement and the sutural activity that this new milieu dictates.

7. Surgical wounds tend to heal faster in the facial, internasal site than in the frontal site. This effect may be

related to the more rich vascular network in the nasal area.

8. Osteophytes from inner and outer periosteal origins can be found in all groups. Nevertheless, they are more abundant in the frontal site. We believe that such callous formation in the cranial site is essentially a process of bone repair, in contrast with the facial site where healing is not always associated with callous formation.

9. Frontal surgical cuts did not heal fully in the 25 day old guinea pig. The dramatic regenerative potential observed in previous studies appears to be a measure of the level of maturity of the calvarial osteogenic tissue.

10. Results suggest that the sutural tissues are interchangeable between the different sutural locations, even when the tissues have striking contrastive characteristics. This can be explained by the observation that most of their characteristics relate to functional environment.

11. It is concluded that sutural tissues are appropriate for early manipulation in craniofacial surgery as transplantable sites for subsequent growth.

Nevertheless, a great deal of experimental study will be required to determine possible donor sites and appropriate timings for surgical transplants.

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