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The Short Term Histological Changes in the Sutures of the Facioskeletal Complex Resulting from Rapid Palatal Expansion in the Rhesus Monkey

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THE SHORT TERM HISTOLOGICAL CHANGES IN THE SUTURES

OF THE FACIOSKELETAL COMPLEX RESULTING

FROM RAPID PALATAL EXPANSION IN

THE RHESUS MONKEY

by

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VITA

Craig A. Sinclair was born in Detroit, Michigan on May 21, 1947.

He was graduated from Las Lomas High School in Walnut Creek, California in 1965. He entered San Jose State College and was graduated with a Bachelor of Arts degree in 1970.

In September, 1970, he began his professional training at Loyola University School of Dentistry and was graduated with the degree Doctor of Dental Surgery in 1974.

Upon graduation he began graduate studies in the Department of Oral Biology, Loyola University, Maywood, Illinois, in July, 1974.

DEDICATION

To my wife Norina, for her assistance in construction of this thesis, and for her love and encouragement through my years of professional education.

To my parents for their guidance and encouragement through my educational years.

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CHAPTER I

INTRODUCTION

Facial harmony is dependent upon the coordinated growth of the jaws, facial bones and related structures.

The facioskeletal complex, composed of several bones, undergoes many changes during growth. The amount and direction of bone growth is primarily the result of inherent and genetically controlled cellular function.

External factors, such as muscle attachments and functional requirements probably influences some of the changes. Also the growth pattern and function of one bone may be partially controlled by the growth and function of adjacent skeletal components.

In general, bones react to the forces placed upon them, and if the magnitude or direction of the forces is altered then a change in the shape or relationship with other bones of the complex can be expected.

In the facioskeletal complex, the bones are intimately related either suturally or by proximity of position. Since there is a close interrelationship between the bones, it is possible that alterations in the arrangement of the bones in one region of the complex may result in compensation adjustments in peripheral sutural connections.

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The relative amounts and location of these changes is probably influenced by skeletal morphology, age and sex differences, as they apply to bone elasticity.

It is expected that local alterations would continue until a physiologic and functional equilibrium is achieved.

One must understand fully the changes that can occur when splitting the suture between two bones within the middle of the patients' skull.

CHAPTER II

STATEMENT OF PROBLEM

The purpose of this study was to determine and evaluate the short term histological changes in the sutures of the facioskeletal complex in the Rhesus monkey resulting from rapid palatal expansion.

Many studies have been undertaken to determine the histological changes in the midpalatal sutures resulting from expansion. Other researchers have studied the related sutures, but in most cases the result was a subjective description of the histological findings.

The purpose of this study was to determine the labeling index and cell density, using tritiated thymidine, of the cellular components found in sutural areas related to the midpalatal suture. The sutural areas, (Figure 1), studied were the frontozygomatic, zygomaticotemporal, zygomaticomaxillary and the nasal complex of sutures.

By this study possibly a more objective and quantitative determination of compensating adjustments in intimately related areas to the palatal expansion can be established.

FIGURE 1

DIAGRAM OF LATERAL VIEW OF MONKEY SKULL
SHOWING THE FACIAL SUTURES STUDIED
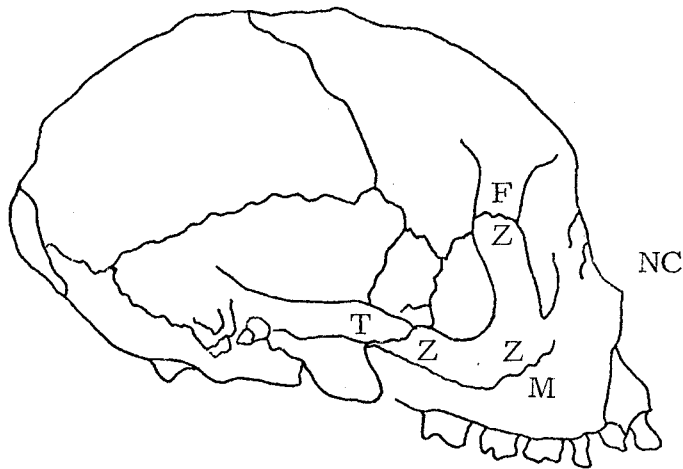
FZ = frontozygomatic suture
ZT = zygomaticotemporal suture
ZM = zygomaticomaxillary suture
NC = nasal complex of sutures

CHAPTER III

REVIEW OF THE LITERATURE

A. Structure of the Facioskeletal Complex:

In order to intelligently understand the affects of rapid palatal expansion on the facioskeletal articulations, the structure of the complex must be reviewed.

One who understood the complexity and structures of the facio-skeletal complex was Sicher (1970). Sicher described the maxilla and the skeleton of the upper face as a biological and mechanical unit which is connected to the base of the skull.

In the study of this area Grant (1962), Arey (1966), Pansky and House (1969), Sicher (1970), and Graber (1972) described the facioskeletal complex as consisting of three vertical pillars on each side which arise in the basal part of the alveolar process and abut with the base of the skull. These three pillars are the canine pillar, zygomatic pillar and the ptery-goid pillar, (Figure 2). These three pillars are curved in order to go around the orbit and nasal cavity.

The canine pillar originates near the maxillary canine. It follows the lateral border of the piriform aperature, then continues as the frontal

FIGURE 2

SUPPORTING PILLARS OF THE
MAXILLARY SKELETON
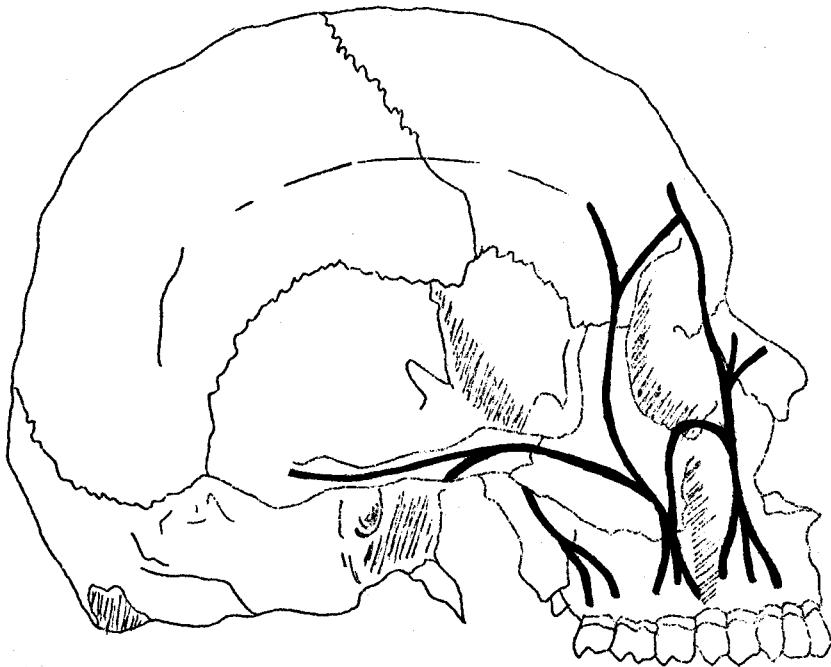
Canine Pillar
Zygomatic Pillar
Pterygoid Pillar

process of the maxilla and finally joins the supraorbital rim. The inferior portion is between the nasal cavity and the maxillary sinus.

The zygomatic pillar originates in the area of the maxillary first molar as the zygomaticoalveolar crest. It continues laterally into the zygomatic process of the maxilla, and then into the zygomatic bone. The inferior part of the zygomatic pillar consists of the lateral wall of the maxillary sinus. The zygomatic pillars are structurally adaptive to lines of stress.

Lines of stress divide in the body of the zygomatic bone (Figure 2). One division is formed by the ascending frontal process of the zygomatic bone abutting against the zygomatic process of the frontal bone which is directed downward at the lateral end of the superorbital rim.

The second division of the zygomatic pillar is the zygomatic arch which is attached at the base of the skull and as the lateral root of the zygomatic arch.

The pterygoid pillar is the pterygoid process of the sphenoid bone. The superior portion is attached to the base of the skull while its inferior end is joined to the posterior end of the maxillary alveolar process by the pyramidal process of the palatine bone.

Due to the curve of these pillars, horizontal connections are present in order to prevent collapse.

The canine and zygomatic pillars are connected above and below the orbit by the supraorbital and infraorbital rims respectfully. The superior connection resists the forces transmitted to it by the anterior and middle maxillary pillars. The horizontal connection between the posterior aspect of the horizontal zygomatic pillar and the superior end of the pterygoid pillar is a strengthening of the bone in front of the oval foramen connecting the articular tuberculum with the root of the pterygoid process.

The system of the pillars on one side are connected to those of the other side by means of the hard palate. The palate forms a vaulted supporting arch between the bases of the right and left alveolar processes.

Considering the structure of the entire facioskeletal complex, there is a direct interrelationship between all parts in form and function and as one component is stressed or altered, there is adaptation by the other components as part of the overall adjustment to attain a physiological equilibrium.

B. Maxillary Growth and Development:

In recent years there has been much research, Brodie (1941), Bjork (1951), Bjork (1955), Scott (1957), Bjork (1967), Sicher (1970), Wragg (1971) concerning maxillary growth and development. In order to

be able to assess the changes brought about by rapid palatal expansion, it is important to understand normal development.

Our present knowledge of maxillary growth and development is best summarized by Enlow and Bang (1965), and Enlow (1968).

Enlow and Bang (1965) state that as the maxilla increases in size, its various parts and regions come to occupy, in sequential order, new positions in the bone. This requires a mechanism of structural adjustment which brings about actual shifts in the location of specific parts in order to maintain constant shape and relative positions.

The postnatal growth of the human maxilla parallels that of the mandible in that forward and downward movement of the growing bone as a whole is a result of growth which takes place in a posterior direction with corresponding repositioning of the entire bone in a forward course. This growth pattern is one of several adaptations to the presence of teeth in the maxilla and mandible, and it makes possible elongation of the dental arch at its free ends. Such growth permits a progressive increase in the number of teeth which can take place only at the posterior ends of the dental arch. It also involves a complex series of corresponding remodeling changes in all of the various parts of both the maxilla and the mandible.

The generalization that the maxilla is thrust downward and forward by growth in posterior and superior parts of the bone is an over-

simplification and, if not qualified, can lead to inaccurate assumptions. Growth does occur in this manner in certain specific areas, but it also proceeds in a complex variety of other directions in different major regions of the maxilla. The overall size of the face increases by a series of specific growth movements in several individual areas which proceed away from each other, thereby drawing out the dimensions of the maxilla in several different directions, (Figure 3).

Bone deposits are added along the posterior margin of the maxillary tuberosity. This functions to lengthen the dental arch and to enlarge the anterior-posterior dimensions of the entire maxillary body. Coordinated with this increase is the progressive movement of the entire zygomatic process in a corresponding posterior direction. This sequence serves to maintain continuously the constant position of the zygomatic process relative to the remainder of the maxilla. The separate zygomatic bone also moves in a posterior course by a combination of resorption from its anterior surfaces and deposition along its posterior side. The face simultaneously enlarges the breadth by proportionate bone apposition on the lateral surface of the zygomatic arch with corresponding resorption from its medial surface.

The floor of the orbit faces superiorly, laterally and slightly anteriorly. Resorption from the lateral surface of the orbital rim functions to make way for the laterally moving orbital surface of the

FIGURE 3

GROWTH DIRECTION OF CRANIAL
BASE AND FACIAL SUTURES
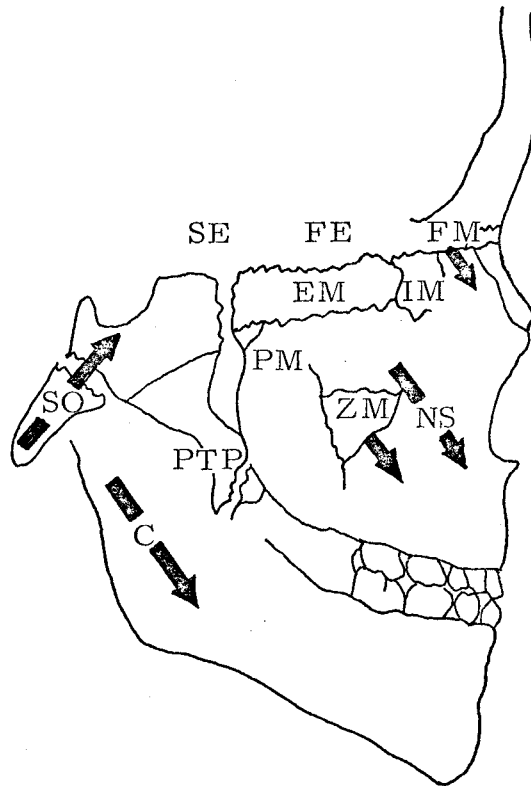
ZM= zygomaticomaxillary suture
FM= frontomaxillary suture
LM= lacrimal-maxillary suture
EM= ethmoidal-maxillary suture
FE = frontoethmoidal suture
SO = Spheno-occipital synchondrosis
 C = reflection of condylar mandibular growth
NS = nasal septum
SE = sphenoethmoid suture
PTP= pterygopalatine suture
PM= palato maxillary suture

maxilla in the floor of the orbital cavity. The nasal area of the maxilla together with its separate nasal bones, also faces in a similar lateral, anterior, and superior direction. Growth proceeds in these same directions by surface bone deposition, thereby increasing the internal size of the nasal cavity by an elongation and expansion of its vertical and horizontal dimensions. The bony cortex lining, the inner surface of the nasal cavity, undergoes periosteal surface removal of bone as its endosteal side receives simultaneous deposits of new bone.

The palatine processes of the maxilla grow in a generally downward direction by a combination of surface deposition on the entire oral side of the palatal cortex with resorptive removal from the opposite nasal side, as well as from periosteal labial surfaces of the anterior maxillary arch.

The premaxillary part of the maxilla grows in a downward direction. The surface orientation of this area is such that downward movement is brought about by resorptive removal from the periosteal surface of the labial cortex which faces away from the direction of growth. The endosteal side of its cortex and periosteal surface of the lingual cortex receive new bone deposits. This growth pattern also produces a slight recession of the incisor area in a posterior direction, a situation also present in the human mandible.

Despite which theory is supported, Enlow (1966), Scott (1957), or Moss (1955) the overall result of maxillary growth is the same.

C. Structure and Growth of a Suture:

Sutures throughout development exhibit five distinct layers of cells and fibers between the edges of adjoining bones; Pritchard, Scott, Girgis (1956); Scott (1957); and Bjork (1966). Also the inner and outer boundaries of the suture are marked by fibrous strata. Therefore, there are five intervening layers and two uniting layers comprising the basic structure of a suture, (Figure 4).

The intervening layers comprise pairs of cambial and capsular layers continuous with the cambial and deeper fibrous strata respec- tively of the periosteum covering the non-sutural surfaces of the bones and a middle loose zone. The uniting layers run directly between the outer fibrous layers of the periosteum on each side.

Sutures arise somewhat differently in the face and cranium. In the face the cambial and capsular layers are already present before the suture is formed. The middle and uniting layers are derived from the mesenchyme between the approaching bone. In the cranium the capsular layers are not formed until the cambial layers have almost met. The uniting and middle zones are derived from the delamination of the fibrous ectomenix between the bones.

FIGURE 4

DIAGRAM OF THE SUTURAL LAYERS
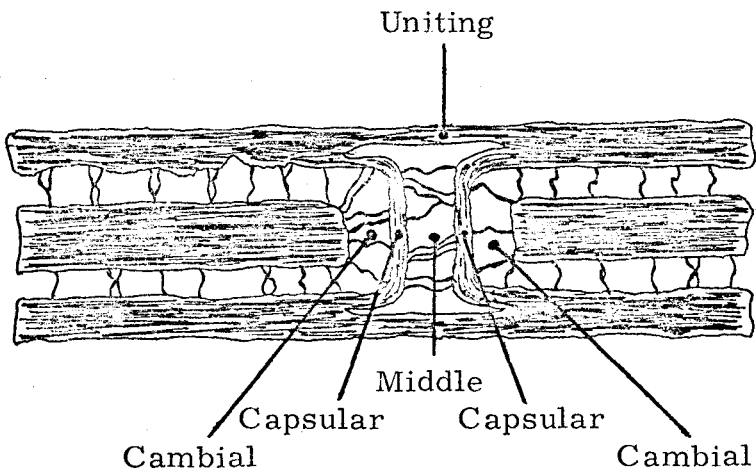
As the suture matures its cambial layers thicken but their predominant direction of the fibers remain parallel to the sutural faces of the bones. The middle layer becomes increasingly vascular. The uniting layers form the strongest bond between the bones. Cartilage, either of the hypertrophic or the hyaline type, is found at the margins of the bones in some sutures during the neonatal period. Cartilage union across a suture is occassionally found.

Areas of partial synostosis are found in some palatal and sagittal sutures both during and after growth of the skull.

Considering their development and histological organization, sutures form a strong bond between adjacent bones while allowing slight movements, and at the same time allow marginal expansion of the bones during growth.

D. Palate Splitting:

1. Rapid Mid-Palatal Expansion:

Rapid mid-palatal expansion was introduced by Angell in 1860 and during that period, controversy arose whether or not the mid-palatal suture was actually separated by the expansion appliance.

Eysell in 1886, a rhinologist, suggested rapid mid-palatal expansion as treatment for nasal insufficiency. Then in the early 1900's, mid-palatal expansion declined in use as more conventional means of widening

the dental arch were advocated. Contrary to the ideas in America, the Europeans continued to use mid-palatal expansion.

The use of rapid mid-palatal expansion was renewed in America due to the work of Brodie et al. (1938). They showed by cephalometric appraisal, that actual bone changes accompaning orthodontic treatment were restricted to the aveolar process.

Harvold (1950) was one of the first to advocate rapid mid-palatal expansion as an adjunct to cleft palate treatment. Using monkeys, his study showed that in addition to movement of the teeth, alveolar processes and basal segments, there was also evidence of movement of adjacent bony structure in the sutural areas. Additional investigators, Gerlach (1956), Thorne (1956, 1960) and Krebs (1958, 1959) reported, from their studies, an opening of the mid-palatal suture lowering of the nasal floor as well as the palatal vault. They also reported an increase in nasal cavity width and an associated straightening of the nasal septum, improved nasal breathing, and increased maxillary arch width and length. Gerlach and Thorne found that a diastema which occurred between the central incisors, at an average of 3 mm., closed spontaneously in a few weeks. The closure of the diastema was thought to be due to readjustment of the transseptal fibers. Thorne also noted a tendency for the bite to open in more than half of his cases. In addition to a widening of the apical base, he found tilting of the posterior teeth which lead him to his

idea of overtreatment to allow for the tendency to relapse. Histolog-ically, Thorne concluded that as the stretched fibrous connective tissue begins to rebuild itself, there was bone deposition at the sutural margins.

Debbane (1958) studied the effects of various forces on the mid-palatal suture of cats. Using expansion forces, Debbane produced a downward and backward tipping of the premaxilla. When using contrac-tion forces, the results were an upward and backward tipping of the pre-maxilla. With the expansion forces, a greater degree of opening of the mid-palatal suture in the area of the premaxilla region was noted than in the maxillary region. There was a lack of opening in the area of the palatine bones although there was opening in the surrounding suture. New bundle bone was deposited on the edges of the palatine sutures, and Debbane attributed the stimulation to the stretching of connective tissue fibers in the suture.

Bjork (1955) was the first investigator to use metallic implants to study the expansion of the mid-palatal suture. Krebs (1959) using vital-lium implants, reported that the increase in the width of the dental arch was twice as much as that of the maxillary buccal segments. The increase in the width of the nasal cavity was less. He also noted that the maxillary segments rotated in the frontal plane. A retainer was used during the retention period in order to maintain the attained dental arch width, however, there was a tendency for relapse.

Haas (1959, 1961, 1965) studied palatal expansion on eight pigs and was able to produce 15 mm. of expansion in ten days. Following this study, Haas used his palate splitting technique on ten patients with maxillary or nasal insufficiency. In one case eight mm. of palatal expansion was reported along with four and one half mm. of widening in the nasal cavity.

Haas concluded from his studies that the expansion by orthopedic forces was indicated in Class III and pseudo-Class III malocclusions, in cases of severe maxillary constriction, and nasal insufficiency.

Isaacson, Wood, and Ingram (1964), Isaacson and Murphy (1964), Isaacson and Ingram (1964) all showed that the resistance of the facial skeleton was as important as the mid-palatal suture in expansion procedures.

West (1964) studied the effects in sutural regions of the maxillary, zygomatic, frontal, vomer, and palatal bones, due to mid-palatal expansion on five monkeys. His results showed that repair in the facial sutures began soon after initial activation and the healing process kept pace with the injury brought on by the expansion.

Cleall et al. (1965) using soft x-rays, occlusal radiographs and histological evaluation, showed that the bony defect in the palatal suture due to expansion, was rapidly filled with new bone. After retention, the sutural area appeared to be normal with no evidence of breakdown. The

outward rotation of the palatal vault, teeth and alveolar processes occurred after separation of the mid-palatal suture and adjustments in the adjacent sutural areas.

Zimring and Iaacson (1965) studied the duration of time the load was stored in an expansion appliance following cessation of active treatment. Their results indicated that load decay occurred through the movement of skeletal structures. Rigid retention was recommended until the articulation reached an equilibrium.

Starnbach et al. (1966) studied the effects of palatal expansion, in Rhesus monkeys, on selected sutures of the facial skeleton. Their study revealed that the fronto-nasal suture showed the greatest histologic response, followed by the zygomaticomaxillary and zygomaticotemporal sutures respectively. Their findings supported the idea of others that changes in the orientation of the bones in one area, may well involve sutural adjustments in remote regions.

Haas (1970) stated that in 100 patients who were expanded 10 to 11.5 mm., exhibited nasal cavity width increased from 3 to 5.5 mm.

Wertz (1968, 1970) found that the gain in nasal cavity width was only 1.9 mm. and that to be insufficient to justify mid-palatal expansion for the sole purpose of increasing nasal efficiency.

Murray and Cleall (1971) showed that the initial separation of the palatal and facial sutures was jointly followed by a repairative process and return of normal sutural morphology.

It is evident from the above literature review that rapid palatal expansion has been substantiated experimentally as well as clinically with the return of normal sutural morphology and permanent widening of the maxillary arch.

Also it is apparent that it is not just the mid-palatine suture that responds to the heavy forces of expansion, but also an adjustment by more remote sutural areas.

2. Indications for Rapid Palatal Expansion:

   a. Class I malocclusions with crossbites due to a functional shift.

   b. Class II malocclusions that are severe mouth breathers associated with a high palatal vault and a narrow nasal aperature literally filled by concha. Often a deviated nasal septum is present. By increasing the internasal capacity, this will facilitate nasal respiration.

   c. Class III malocclusions, treatable without surgical re-positioning, that is due primarily to an under developed maxilla. And the crossbite is due to the inadequacy of the bony base. The profile is flattened especially in the

middle third of the face and there is a crowding of the
maxillary arch.

d. In the cleft palate patient, following closure of the lip and
palate, suffering from collapse of the maxillary fragments
resulting in a poor occlusion and inability to masticate
properly. There is a poor airway due to a constricted
maxilla which results in a mucopurelent discharge and
infection of the sinuses and middle ear. The patient may
be prone to caries and following loss of permanent teeth,
the problem of constructing a prosthesis is difficult due to
scar tissue and a lack of bony support.

Rapid expansion moves the fragments apart into their correct re-
lationship with the mandible. It also improves the facial deformity of the
middle third due to forward movement of the fragments.

Overall, rapid palatal expansion positions the maxilla in a har-
monious relationship to the mandible and corrects the crossbite with an
improvement in facial profile.

3. Contraindications for Rapid Palatal Expansion:

a. Prognathic maxilla, and bimaxillary protrusions.

b. Steep mandibular plane angles.

c. Parental supervision or child cooperation can not be
trusted or depended upon for the periodic activation.

d. If there is a crossbite of one tooth and an easier means of correction is available.

E. Quantitative Autoradiography:

Autoradiography as a qualitative method is one of the best techniques available in determining which cells in an organism or tissue can incorporate a radioactive substance, Rogers (1973).

However, as a quantitative method, autoradiography has some disadvantages, for radiochemical methods are much quicker and the results are more accurately reporducible. The most important advantage of autoradiography is the ability to locate a radioactive product in single cells or in subcellular structures.

Autoradiography can be used to identify types of cells and cells that are synthesizing DNA. Since DNA is stable, once the cells are labeled with radioactive Thymidine, they can be followed for their entire life span.

There are two manners in which autoradiography can be employed as a quantitative method, either as a relative or an absolute measurement of radioactivity, Rogers (1973). Relative measurements of radioactivity are of three types: (1) the fraction of labeled cells in a mixed population of labeled and unlabeled cells; (2) the intensity of the autoradiographic label in single cells or in groups of cells; and (3) the relative amounts of radioactivity in cellular organelles.

An absolute measurement of radioactivity is when the radioactive label intensity is translated into disintegrations per minute per cell.

1. Determining the Fraction of Labeled Cells:

The fraction of labeled cells in a mixed population of labeled and unlabeled cells is of particular importance in the case of tissues labeled with Thymidine$-^3$H.

Three factors must be considered in determining the fraction of labeled cells in a mixed population, Rogers (1973): (1) Is the population of cells to be counted a homogenous population that can be identified on the basis of histological evaluation? (2) What are the criteria for classifying a cell as labeled? (3) What are the limits of accuracy in determining the fraction of labeled cells?

2. Identification of Cell Population to be Counted:

The investigator wants to know the percentage of labeled cells in a homogeneous population. It is necessary to define the cell population under study so that the cells to be counted can be recognized.

It is not always possible, to determine whether or not certain cells belong to a given population, but the number of cells that are difficult to identify is small and therefore the error introduced is minimal.

3. Criteria for Classifying a Cell as Labeled:

A way of classifying a cell as labeled is to set up a minimal number of activated silver grains above a cell as a limit. The number of activated silver grains above a cell is counted, and if the number is above the limit set, the cell is considered labeled and when the number of grains is below the limit, the cell is considered unlabeled.

The limit is usually set by determining the average number of background silver grains for a given area, and then increasing that number by 50 per cent.

4. DNA Synthesis:

The advantage of using thymidine is that it is exclusively incorporated into DNA, Rogers (1973). Before being incorporated into DNA, it is phosphorylated, first to thymidine monophosplate then to thymidine diphoshate, and finally to thymidine triphosplate. The triphosplate is the active form which after the loss of two phosphoric acid groups is polymerized into DNA together with three other deoxynucleotides.

Thymidine is either promptly incorporated into DNA or it is catabolized to non-utilizable products. These non-utilizable products as well as the thymidine and its phosphorlylated derivatives are easily soluble in fixatives whereas the DNA is insoluble.

By fixing the tissue, all radioactivity except that which has been incorporated into DNA is removed. Since DNA is stable, once a cell is

labeled with radioactive thymidine, the cell as well as its descendants remain labeled until their death.

    5. Cell Cycle:

The cell cycle can be defined as the interval between completion of mitosis in the parent cell and completion of the subsequent mitosis in one or both daughter cells (Figure 5).

The cycle consists of:

        (1) S phase - the period during which DNA is synthesized

        (2) G_2 phase - the post-synthetic, pre-mitotic period

        (3) M phase - mitotic period

        (4) G_1 phase - post-mitotic, pre-synthetic period

The S phase and G_2 phase are the time periods required for labeled DNA to show up in metaphase chromosomes, and are the phases in which the labeled cells are studied.

FIGURE 5

DIAGRAM OF THE CELL CYCLE
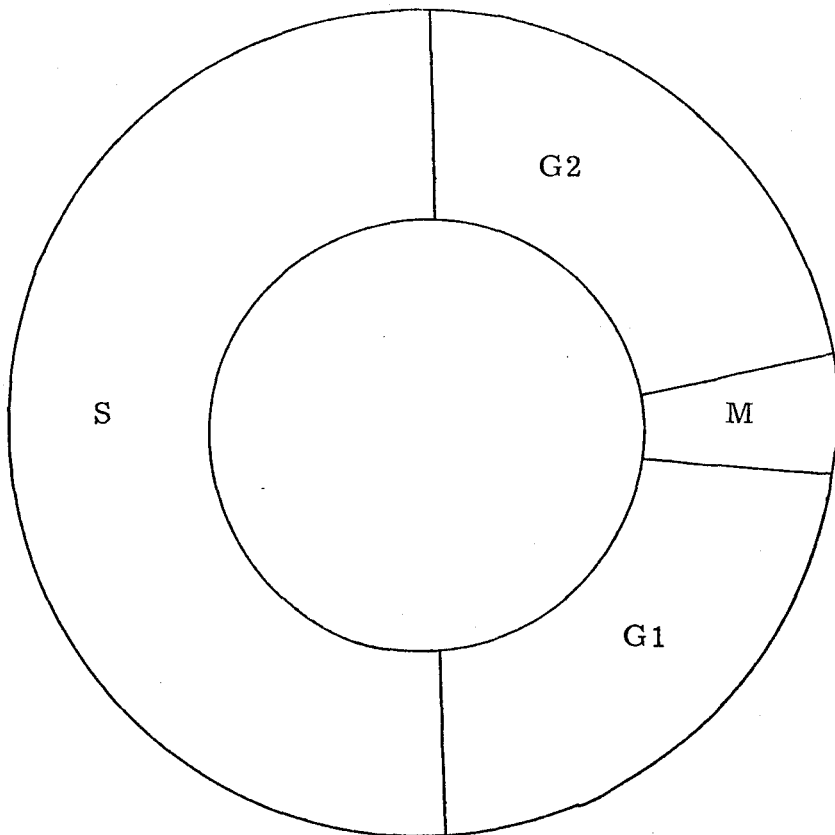
S   = DNA Synthesis period
M = Mitosis period
G2 = pre-mitotic period
G1 = post-mitotic period

CHAPTER IV

METHODS AND MATERIALS

A. Animal Selection:

The object of this investigation was to study the short term histological changes in certain sutures of the facioskeletal complex resulting from rapid palatal expansion in four Rhesus monkeys.

The Rhesus monkey was selected as the experimental animal. The study was conducted using animals in the mixed dentition period, during a time of active maxillary growth. In the human child the best repairatory reaction would be at a time when there is active growth in all areas of the facioskeletal complex.

The Rhesus was chosen since it is felt that the dentition and tissue response are thought to be similar to those of man.

One of the disadvantages to using the Rhesus is that these animals have a normal buccal segment relationship and therefore, the expansion of the maxillary arch tends to move the teeth into an abnormal buccal crossbite arrangement. Also the experimental animals had palates of normal configuration whereas clinically, the rapid palate expansion appliance is usually used for patients with constricted palates.

Another disadvantage is while the Rhesus provides a close approximation to human morphology, structural differences do exist.

The Rhesus have a distinct and separate premaxilla which articulates with the maxillary bones. There is no continuous midline suture running through the premaxilla. The mid-palatal suture joins the two premaxillary-maxillary sutures immediately posterior to the premaxilla, the configuration of the suture outline forming a "Y".

Despite the obvious differences, it was felt that its suitable order characteristics out weighted species differences limiting the direct transference of findings to man.

Though generally it is considered unwise to apply the results of experiments on laboratory animals to reactions in humans, it was considered that the results of the investigation might be applied to certain clinical procedures in the human being.

The experimental design required that the first permanent maxillary molars be present and also either the first permanent premolar or first deciduous molar. The ages of the monkeys had to be in the range of eighteen to twenty-four months which is comparable in tooth and bone development, Baum (1950), to that of a nine year, plus or minus nine months, old child to 11 year, plus or minus nine months, old child.

Six healthy macaca mulatta monkeys were acquired from Primate Imports, Port Washington, New York, according to the experimental standards set.

The monkeys were given permanent tatoos on their chest as a means of identification. Four of the monkeys were female and two were male.

In order to determine which four animals would be experimental subjects, and which two would be the control subjects, a double blind selection was made.

The first control animal had the identification number, 13-C. It was a female and weighed 3.15 kilograms.

The second control animal had the identification number, 413-C. It was a female and weighed 3.60 kilograms.

The first experimental animal was a male and weighed 3.30 kilograms. It's identification number was 213-C.

The second experimental animal was a male, weighed 2.5 kilograms, and was identified as V-084.

The third experimental animal was a female, it's weight was 5.35 kilograms and it's identification was 122-C.

The fourth experimental animal was a female and weighed 4.85 kilograms. It's identification was 800-B.

B. Animal Housing and Care:

The experimental animals were housed in individual squeeze cages at the Animal Research Center, Loyola University Medical Center, Maywood, Illinois. This research facility is directed by Dr. Charles Larson and maintained by Loyola University.

The daily care of the animals, prior to the experimental period, was provided by a full time attendant of the research facility. The feeding and sanitation procedures were standardized.

The animals were fed daily Purina monkey chow biscuits, water and Squibb's Vionate, a multi vitamin for pets, to insure they received a balanced diet. Bananas were also fed daily to facilitate the procedure.

This diet was altered during the colonization period as well as the experimental period.

The hard biscuits were softened with water to the consistency of a soft mash. Added to this were bananas, and ground up vitamins. The animals were fed the new diet twice a day, to ensure that they ate sufficient amounts to maintain their approximate body weights under the stress of the experiment.

The mash was placed in a 50 cc. syringe with one half inch in diameter plastic tubing connected to the end.

The tubing could be gently placed in the animals mouth and then slowly forced out of the syringe.

The new soft diet as well as the manner in which it was delivered was necessary to prevent any damage to the expansion appliances, and to aid in feeding since during the colonization and experimental periods, the restraining collars prevented the animals from using their hands to aid in feeding.

Due to being placed in restraining collars, water bottles were attached to the front of the cages with a long tube entering the cage.

C. Animal Handling:

The animals were quarantined for three weeks upon arrival at the research center. There was an additional colonization period of several weeks prior to the start of any experimental work to acquaint the animals with their surroundings.

The investigators wore surgical scrub suits, face masks, and rubber gloves at all times to protect the animals as well as the investigators from infections during direct contact.

A squeeze cage was used to house and restrain the animals. This is a small, portable cage with a sliding door at one end. The animals were restrained by compressing the movable wall of the cage. The movable wall forced the animal against the front of the cage where the investigator could administer the necessary injections.

One week prior to the experimental period, the animals were placed in plastic restraining collars. There were guide wires from the top to the bottom of the cage which allowed the animal vertical movement but prevented horizontal movement. These collars also kept the animal from damaging the expansion appliances with their hands.

After the collars were placed, the daily examination and activation of the appliances was done under anesthesia. The cage was placed on it's side, and one investigator who wore heavy leather protective gloves, secured the animal by the collar and it's hind limbs. The other investigator injected the anesthesia and atropine. After this the animal could be easily examined, worked on, or the appliance activated.

D. Preparation of the Experimental Animal:

The animal was anesthetized with Sernayle (phencyclidine hydrochloride, 3 mg/kg.) intramuscularly and given Atrosol (atrophine sulfate, 1 ml/20 lbs.) subcutaneously. Following anesthesia, the animal was prepared for the experimental period. These procedures were:

1. The head and neck were measured as well as the cage in order to construct a restraining collar.

2. Preliminary impressions of the upper and lower dental arches were made with base plate wax. From the preliminary models, three sets of custom acrylic trays were constructed.

3. The restraining collars were constructed out of flexible 3/8 inch plastic, which was bolted together in the front and were padded around the opening for the neck. Each collar had holes at each corner for the guide wires.

4. The acrylic impression trays were tied in the mouth of the animal to insure correct size. The trays were then coated on the inner surface with rubber adhesive to grip the impression material that was to be placed in them.

After the adhesive dried, Coe light body rubber impression material was mixed and placed into the tray.

The tray was then placed on the teeth to obtain impressions. This procedure was followed for both dental arches. The beginning study models of the dental arches were made from these impressions by vibrating orthodontic stone into them.

5. The permanent maxillary first molars as well as either the deciduous first molars or permanent first premolars were separated using lightening strips. Preformed ortho-dontic bands were adapted to these teeth. Lower anterior bands were used for the permanent premolars and the deciduous molars while premolar bonds were used for the permanent molars.

After the bands were adapted, an acrylic impression tray, with retention holds, was tied into the mouth. Coe green Stick compound was heated in hot water and adapted to the tray. The filled tray was placed in the mouth over the banded teeth until set. After removal of the impression tray, the adapted bands were gently removed from the teeth and luted into the impression with Kerr's sticky wax. These impressions were poured up in Orthodontic stone and served as the working models on which the expansion appliances would be constructed.

6. Intraoral photographs were taken of the animals.

E. Appliance Design:

Various types of expansion screws are available in several shapes and various sizes depending upon the purpose.

Study models of the maxillary and mandibular arches were made to assess the degree of palatal expansion desired. An expansion screw made by O.I.S. was selected that would allow four millimeters of expansion. The device consists of a threaded center section and two lateral posts.

To avoid as much rotation of the palatal shelves as possible, and thereby realize maximum sutural opening, a fixed and fully rigid appliance design was selected; Haas (1965). A removable expansion screw appliance will primarily tip the teeth laterally. An acrylic body

was preferred rather than an all metal Hyrex type, so that the expanding force is exerted against the alveolar process and basal bone rather than just the teeth, Haas (1965).

F. Appliance Construction:

The appliance was constructed by a direct-indirect method of fabrication; Wertz (1970).

The working models with the bands on the maxillary first deciduous molars or their succedaneous elements and maxillary first permanent molars were used to make the appliances. The lingual aspect of the bands were united with .045 round stainless steel wire, the free ends of which were bent palatally to be embedded in the acrylic position. The lingual bar beside connecting the bands on the same side and adding rigidity, also acts to carry the tooth, in between the bands, buccally rather than leave it in it's original position. An additional .045 round stainless steel wire was attached to the buccal surface of the bands, to unite the bands as well as to aid in stabilization of the bands and prevent displacement.

The expansion screw mechanism was mounted with wax as deeply as possible in the palatal vault with the center of the screw laying directly over the midline and the lateral margins of the screw raised about a millimeter from the palate, (Figure 6).

DIRECT-INDIRECT FABRICATION OF A
MIDPALATAL EXPANSION DEVICE

baseplate wax

expansion screw

solder joint

.045 ss wire

FIGURE 6

.045 ss wire

baseplate wax

expansion screw

acrylic

FIGURE 7

solder joints

bands

.045 ss wire

screw mechanism
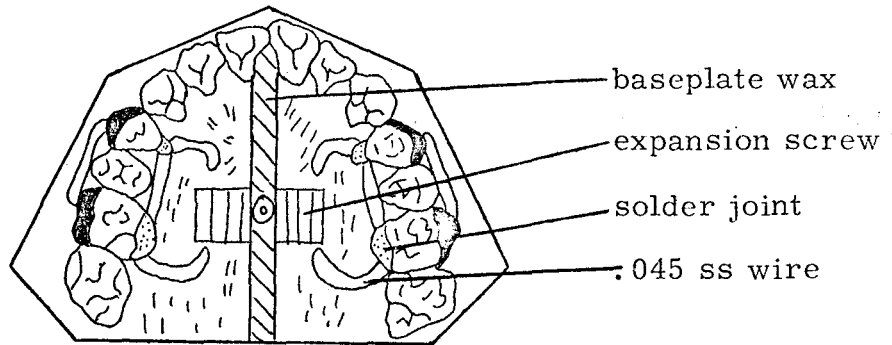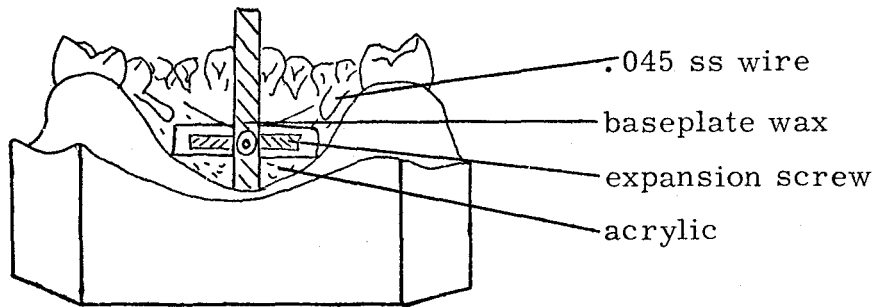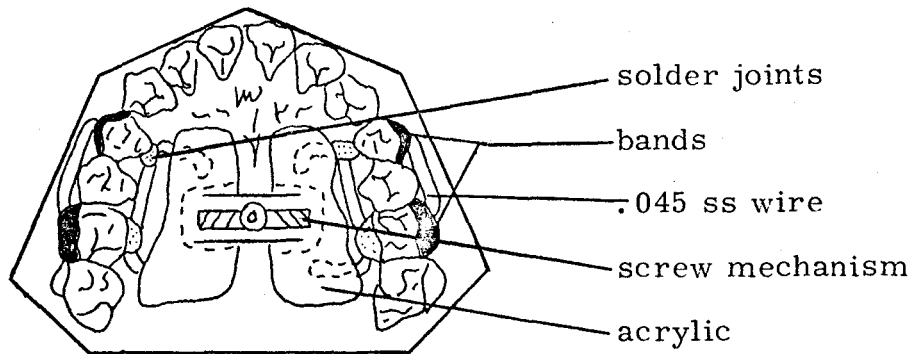
acrylic

FIGURE 8

After two thin applications of separating medium, the acrylic portions were added by the power-drop method. The model and the appliance were placed into a pressure cooker as soon as the acrylic initially set, to increase the strength of the acrylic.

After the acrylic was set, the appliance was removed from the model and the acrylic was cut along the midline until the halves were free. Removal of the wax effects the midline void in which was centered the adjusting portion of the screw mechanism (Figure 7).

The appliance was polished and all edges were rounded off of tissue bearing surfaces, thus minimizing the possibility of tissue irritation. The free gingival margin area was cleared of acrylic and no relief of the palatal surface was done, (Figures 8 and 9).

It was most important that the acrylic which contacts either side of the palatal vault not be trimmed or polished. It was at this point that all the pressure was to be brought to bear so that the mid-palatal suture will open. The abutment teeth act merely to hold the appliance in place.

G. Appliance Cementation and Activation:

The experimental animals were anesthetized with Serneylin (phencyclidine hydrochloride, 3 mg/kg.) intramuscularly and then given Atrosol (atropine sulfate, 1 ml/20 lbs.) subcutaneously. The atropine served to reduce the salivary flow. By reducing the saliva, the intra-
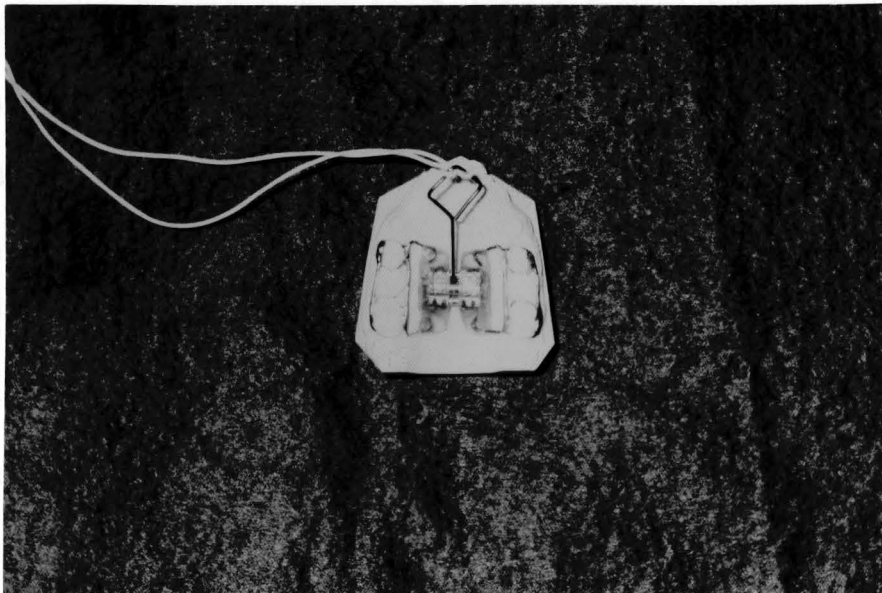
Figure 9. The finished appliance with
activation key.

oral procedures were more easily done and it prevented fluids building up in the lungs while the animal was anesthetized.

Occlusal x-rays were taken using a hand held x-ray machine. The teeth were reseparated with lightening strips and the appliance tried in to readapt the bands before final cementation. After band adaptation and removal of the appliance, the buccal and lingual surfaces of the teeth to have bands, were notched, perpendicular to the long axis, with a bur. The notching provided an additional mechanical lock for the appliance.

The teeth were dried and isolated with cotton rolls, and then cemented in place with Caulk's Grip cement. After the appliance was cemented in place, the initial activation was done. The activation key, attached to a piece of dental floss to prevent accidental inhalation by the animal, was inserted into the screw mechanism at the anterior. The key was pushed posteriorly until it stopped. This amount of activation equaled one quarter of a turn and also equaled one quarter of a millimeter of expansion. The activation procedure was done one more time for a total of one half a millimeter expansion initially.

The appliance was then checked to make sure it was not loosened due to the activation.

On the day that appliances were inserted and activated, the control animals were also anesthetized and given atropine, although only occlusal x-rays were taken.

For the next eight consecutive days, the appliances were acti-
vated two turns each day for one half a millimeter expansion. During
the period of activation following the initial day of appliance insertion,
the animals were anesthetized with Ketaset (ketamine hydrochloride
10 mg/lb.) which is a short acting anesthesia. The animals were also
given atropine. Due to the quickness with which the appliances could
be activated, a short acting drug was used. Each day the appliances
were carefully checked before and after activation to see if they had
loosened.

A total of eighteen, one quarter turns, were accomplished during
the experimental period. Eighteen turns was calculated to yield four
millimeters of expansion. The appliance was designed to be activated
twenty turns before it becomes dissengaged from it's posts and the
halves collapse.

Each day of activation, the control animals were given ketaset
and atropine although nothing else was done.

H. Introduction and Amount of Tritiated Thymidine:

The day after the last activation of the appliances, the animal
was prepared for injection of .7 microcurie (u Ci) of tritiated thymidine
per gram of body weight (specific activity, 1.9 Ci m/M).

All animals were anesthetized and given atropine three hours before sacrafice, and the left arm was shaved to facilitate the thymidine injection.

The animal was restrained on the procedure table in the surgical room. The amount of thymidine, (Table I), was calculated according to body weight and the left arm was scrubbed with soap and water and then rubbed with alcohol. The calculated amount of thymidine was drawn into a 10 cc. syringe and then sterile water was drawn into the syringe until the 6 cc. level was reached. The sterile water was added in order to standardize the amount of fluid injected. Since the water was drawn into the syringe last, during the injection if any fluid was lost do to skin contact, it was most likely water. Finally if any fluid was left in the syringe after the injection, the water served to reduce the concentration of thymidine lost.

A tourniquet was placed superiorly on the left arm, until a vein could be raised. The syringe was inserted and aspirated to insure being in a vein. The tourniquet was removed and the solution was uniformly injected over a one minute period.

Upon completion of the thymidine injection, the animal was placed back in the cage for three hours before sacrafice.

TABLE I

AMOUNTS OF THYMIDINE INJECTED

	Animal	Body Weight	Conc. of Thymidine	Amount of Thymidine
Control	13-C	3.30 kg	.7uCi/g	2.31 ml
	413-C	3.70 kg	.7uCi/g	2.59 ml
Experimental	122-C	5.80 kg	.7uCi/g	4.06 ml
	V-084	2.30 kg	.7uCi/g	1.61 ml
	700-B	5.80 kg	.7uCi/g	4.06 ml
	213-C	4.00 kg	.7uCi/g	2.80 ml

I. Sacrifice of the Animal:

At the time of sacrifice, final x-rays, photographs, and impressions were taken on the anesthetized animal in the preparation room.

In order to sacrifice the animal, an injection of Beuhanasia regular (sodium pentobarbital, 1 ml/5 lbs.) was given intracardially.

Following death, the animal was transferred to the necropsy room where they were decapitated. The head was skinned and the mandible was removed. The top of the skull, from the roof of the orbits to the basioccipital area, was removed with a striker saw. The skull was then placed in 10 per cent Formalin for one hour before it was removed and the rest of the soft tissue removed and the sutures well exposed. The skulls were then placed in fresh 10 per cent Formalin. After several hours in Formalin the sutures were removed with a centimeter of bone on either side. A dried Rhesus skull was used to aid in the location and orientation of the sutures to be removed. Each sutural block removed was placed in an individual specimen bottle containing Formalin, and with a label indicating which animal as well as which suture it was.

The specimen bottles were then given to the technicians for histological preparation.

J. Preparation of Tissues:

After the nasal complex, zygomaticomaxillary, zygomaticofrontal and zygomaticotemporal sutures had been fixed in 10 per cent

Formalin, for three days, the blocks were decalcified for two weeks.
The sections were then reduced in size, embedded and then sliced six
to eight micrometers (uM) in thickness. All sutures were oriented and
sectioned perpendicular to the long axis. After mounting, radioauto-
grams were obtained using Kodak NTB 3 liquid emulsion. The slides
were exposed for four weeks in a light-tight box at 4° C and then
developed, washed, fixed, and stained with nuclear fast red and indigo
carmine. Also hematoxylin and eosin slides were prepared in order to
study sutural morphology.

K.   Method of Measurement:

The labeling index was determined by counting the number of
labeled cells per 1,000 cells counted. A criteria for classifying a cell
as labeled was set. A background count of activated silver grains was
done for each suture and then the number was increased by 50 per cent.
Nuclei with four or more grains were considered labeled. A total of
10,000 cells per suture per animal were counted in order to determine
the labeling index of each.

The cell densities were determined by counting the number of
cells per $110uM^2$ in ten sections. The mean number of cells was then
calculated.

The sections of each suture that were used in determining the
labeling index and cell density were selected for their similarity in

gross sutural morphology. It was the objective to use sections that were from the same approximate location in the suture.

All tissues were studied using a grid with an area of $110uM^2$ at 450 magnifications. The means of the labeling indexes and cell densities were compared using the Student's t Test.

CHAPTER V

EXPERIMENTAL FINDINGS

A. General Observations:

1. During the experimental period there was no separation of the anterior teeth as seen in the patients due to the fact the Rhesus has a separate premaxilla. Due to this difference, there is no continuous mid-palatal suture, and therefore only the palatal shelves separated and not the premaxilla.

2. Following daily activation of the appliances, occasionally blood was seen coming beneath the acrylic. It was determined that the key was being inserted too deeply into the appliance and therefore with the activation, the palatal tissue was being cut by the key.

3. Appliance activation was discontinued when all the experimental animals were approaching a full bilateral crossbite.

4. Upon removal of the appliances after sacrifice, it was noted that there was moderate tissue irritation due to the pressure of the acrylic against the palatal vault. There was also minor tissue irritation due to the bands and the large solder joints near the free gingival margins.

EXPERIMENTAL FINDINGS

B. Physical Findings:

1. Evaluation of the Palatal Expansion Appliance. At the end of the experimental period all the animals were carefully inspected in order to observe and record all physical findings.

It was found that there was an increase in width of the maxillary arches of the experimental animals following expansion when compared to the original maxillary arch models. This increase in maxillary width, measured from the mesial buccal cusp of one molar to the mesial buccal cusp of the other molar, was not noted in the control animals when their original and final maxillary arch models were compared.

An occlusal evaluation of the expansion appliances was made and the amount of opening of the expansion screw was measured to be 4 mm, (Figure 11), when compared to the appliance at time of insertion, (Figure 10).

The buccal occlusion was checked to determine if there was any difference between the control and experimental animals following the experimental period.

The control animals showed normal buccal interdigitation with a normal buccal overjet relationship on the left and right sides, (Figure 12). The vertical dimension was found to be unchanged.

FIGURE 10.. The expansion appliance at time of insertion and
prior to activation.

FIGURE 11. The expansion appliance at time of sacrafice following
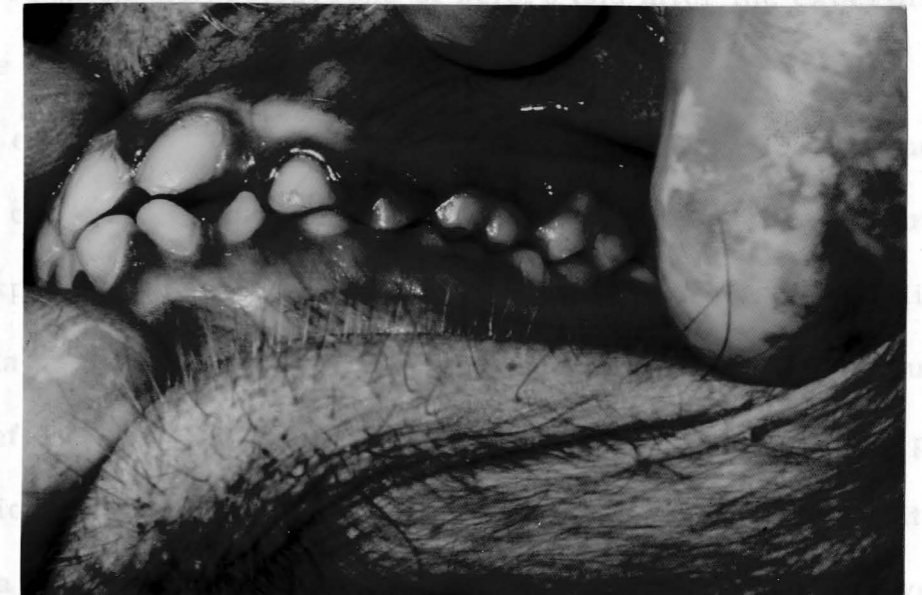complete activation.

FIGURE 10

FIGURE 11

FIGURE 12.

FIGURE 12. The buccal occlusion of a control animal, at the end
of the experimental period, exhibiting normal buccal
interdigitation and overjet.

RIGHT SIDE

LEFT SIDE

The experimental animals showed an abnormal buccal inter-digitation with a resulting increase in vertical dimension and an anterior open bite. The buccal occlusion, (Figure 13), exhibited almost a complete bilateral buccal crossbite. There was also an apparent minimal tipping of the buccal segments in the experimental animals which implied that the heavy expansion forces were mainly transmitted to the mid-palatal suture and not to the anchor abutments. The minimal tipping of the buccal segments was not observed in the control animals.

A roentgenographic analysis of the mid-palatal suture was done on all the animals following the experimental period. The control animals showed no difference in the radiopacity or morphology of the mid-palatal suture when the radiographs of before and after the experimental period were compared.

The experimental animals however did show a difference radiographically of the morphology and radiopacity of the mid-palatal suture following expansion. At the time of insertion of the expansion appliance, the mid-palatal suture appeared to be normal, (Figure 14). The suture showed a definite radiopacity with normal morphology. Following palatal expansion, (Figure 15), the palatal suture was noted to be split. There was a radioluscent area the entire length of the palatal shelves. The separation of the palatal shelves appeared to be parallel and the

FIGURE 13. The buccal occlusion of an experimental animal, at the end of the experimental period, exhibiting abnormal buccal interdigitation, an open bite and a bilateral crossbite.

FIGURE 13

RIGHT SIDE

LEFT SIDE

FIGURE 14. Roentgenogram of the mid-palatal suture before expansion.

FIGURE 15. Roentgenogram of the mid-palatal suture after expansion.

FIGURE 14.   FIGURE 15.

anterior teeth were not spaced which bared out the expected findings since the Rhesus has a separate premaxilla.

C. Histological Findings:

1. The labeling indicies of the individual sutures studied were determined by counting the number of tritiated thymidine labeled cells, (Figures 16 and 17). A criteria was set for classifying a cell as labeled by calculating a minimum number of activated silver grains over a cell. A total of 10,000 cells were counted per suture in order to determine the labeling index. After an index value was obtained, the mean of each group, control and experimental, was determined as well as the standard deviation. To determine the significance of the labeling index values the Student's t Test was used.

a. Nasal Sutural Complex

The labeling indicies for the nasal complex, Table II, in the two control animals were found to be 0.16 and 0.10 with a mean value of 0.13. The standard deviation was $\pm$ 0.4. The experimental animals, Table II, had values of 1.56, 0.15, 1.78, and 1.46 for the labeling index of the nasal sutural complex. The mean value was 1.24 with a $\pm$ 0.73 standard deviation. A comparison of the control and experimental animal labeling indices revealed there was an almost ten fold increase

FIGURE 16. Photomicrograph of an autoradiographic slide of a control animal suture showing the activated silver grains of labeled cells. (X450)

FIGURE 16

FIGURE 17. Photomicrograph of an autoradiographic slide of an experimental animal suture showing the activated silver grains of labeled cells. (X450)

FIGURE 17

TABLE II   60

LABELING INDEX AND CELL DENSITY OF THE
NASAL SUTURAL COMPLEX OF THE
MACACA MULATTA

Animals	Labeling Index / 1,000 cells	Cell Density / 110uM^2
Control		
413-C	.16	123
13-C	.10	115
Mean	.13	119
S.D.	± .04	± 5.65
Experimental		
213-C	1.56	68
122-C	.15	65
V-084	1.78	55
700-B	1.46	61
Mean	1.24	62.25
S.D.	± .73	± 5.62
	$P > .05$	$P < .01$

in cell proliferation in the experimental animals. This large increase

in the experimental animals was determined to be not significant at the

five per cent level.

b. Zygomaticomaxillary Suture

In the zygomaticomaxillary sutures the labeling index values for

the control animals, Table III, were 0.13 and 0.09. The mean value

was 0.11 with a standard deviation of $\pm$ .028. The same suture of the

experimental animals, Table III, had labeling index values of 0.65,

0.05, 0.34 and 0.42. The mean value was 0.36 and the standard devi-

ation was a $\pm$ 0.25. The experimental animals showed a labeling index

of more than three times as large as the control group, however, this

difference was not significant at the five per cent level.

c. Zygomaticofrontal Suture

The values for the labeling indicies of the zygomaticofrontal

suture in the control animals, Table IV, were 0.13 and 0.09 with a

mean value of 0.11. The standard deviation was $\pm$ .028. The exper-

imental animals, Table IV, had labeling indicies of 0.83, 0.10, 0.64,

and 0.80. The mean index value was .592 with a $\pm$ .338 standard devi-

ation. The ratio between the control labeled index and the experimental

was 6:1. The large difference however was determined to be not

significant at the five per cent level.

TABLE III   62

LABELING INDEX AND CELL DENSITY OF THE
ZYGOMATICOMAXILLARY SUTURE OF THE
MACACA MULATTA

Animals	Labeling Index / 1,000 cells	Cell Density / 110uM^2
Control		
413-C	.13	106
13-C	.09	98
Mean	.11	102
S.D.	± .028	± 5.65
Experimental		
213-C	.65	78
122-C	.05	85
V-084	.34	68
700-B	.42	73
Mean	.36	76
S.D.	± .25	± 7.26
	$P > .05$	$P < .01$

TABLE IV 63

LABELING INDEX AND CELL DENSITY OF THE
ZYGOMATICOFRONTAL SUTURE OF THE
MACACA MULATTA

Animals	Labeling Index / 1,000 cells	Cell Density / 110uM^2
Control		
413-C	.13	98
13-C	.09	103
Mean	.11	100.5
S.D.	± .028	± 3.53
Experimental		
213-C	.83	76
122-C	.10	81
V-084	.64	61
700-B	.80	69
Mean	.592	71.75
S.D.	± .338	± 8.69
	P > .05	P < .01

d. Zygomaticotemporal Suture

The labeling indicies of the zygomaticotemporal suture in the control animals, Table V, were 0.15 and 0.13. The mean value was 0.14 and a standard deviation of ± .014. The experimental animals had labeling indicies, Table V, of 0.39, 0.14, 0.50 and 0.37. The mean index value was 0.35 and the standard deviation of ± 0.15.

The mean values between the control and experimental showed a two and one half time increase in the experimental animals, however, the large increase in the labeling index of the experimental animals was not significant at the five per cent level using the Student's t Test.

In all four sutural areas studied it was noted that one of the experimental animals, 122-C, consistently had labeling index values that were well below the values for the rest of the experimental group. The values for this animal very closely resembled the values of the control animals for each sutural index.

The cell density of each facial suture studied was determined by the counting the number of cells per $110uM^2$ in ten sections of each suture. The cell density values as well as the mean value and standard deviations were determined using the Student's t Test.

e. Nasal Sutural Complex

The cell densities of the nasal sutural complex, Table II, for the control animals were 123 and 115 with a mean value of 119. The

TABLE V   65

LABELING INDEX AND CELL DENSITY OF THE ZYGOMATICOTEMPORAL SUTURE OF THE MACACA MULATTA

Animals	Labeling Index / 1,000 cells	Cell Density / 110uM^2
Control		
413-C	.15	133
13-C	.13	124
Mean	.14	128.5
S.D.	± .014	± 6.36
Experimental		
213-C	.39	96
122-C	.14	86
V-084	.50	88
700-B	.37	79
Mean	.35	87.25
S.D.	± .15	± 6.99
	$P > .05$	$P < .01$

standard deviation was a ± 5.65. The experimental animals, Table II,
had cell density values of 68, 65, 55 and 61 with a mean value of 62.25.
The standard deviation was a ± 5.62.

The difference in values between the control and experimental
animals showed almost a 50 per cent decrease in cell densities of the
experimental animals. Using the Student's t Test, this decrease between
the control and experimental animals was found to be significant at the
one per cent level.

   f. Zygomaticomaxillary Suture

The zygomaticomaxillary suture in the control animals, Table
III, had cell density values of 106 and 98 with a mean value of 102.
The standard deviation was ± 5.65. The experimental animals had
cell density values in the zygomaticomaxillary suture, Table III, of
78, 85, 68, and 73. The mean value was 76 and a ± 7.26 standard
deviation.

The difference between the means of the control and experimental
groups showed a decrease in the cell density of the experimental animals
of 30 per cent. This decrease in cell density was significant at the one
per cent level.

   g. Zygomaticofrontal Suture

The zygomaticofrontal suture of the control animals had cell
densities, Table IV, of 98 and 103. The mean was 100.5 and a

standard deviation of a $\pm$ 3.53. The cell densities in the experimental

groups were 76, 81, 61, and 69, with a mean value of 71.75. The

standard deviation was a $\pm$ 8.69.

The difference in the mean cell density values between the con-

trol and experimental animals was a 30 per cent decrease in the experi-

mental group. The 30 per cent decrease between the groups was found

to be significant to the one per cent level.

    h. Zygomaticotemporal Suture

In the control animals the zygomaticotemporal sutures had cell

densities of 133 and 124 with a mean of 128.5. The standard deviation

was a $\pm$ 6.36. In the experimental group the cell density values for the

zygomaticotemporal sutures were 96, 86, 88 and 70. The mean was

87.25 with a $\pm$ 6.99 standard deviation.

The difference between the mean values for the control and

experimental groups was found to be a 25 per cent decrease in cell

density in the experimental group. At the one per cent level it was

determined that the decrease in the experimental group was significant.

Unlike the low labeling index values of one experimental animal,

122-C, the cell density values were all consistent as compared to the

other experimental monkeys.

2.   H. and E. Evaluation of Facial Sutures

The following description was an overall histological evaluation of the facial sutures.

A low power view (X40) of the sutures in the control animals revealed a typical interdigitation of the sutural bony processes separated by fibrous connective tissue. The bony surfaces were found to be smooth with very little appositional and remodeling processes evident.

Under higher power (X450), in the sutures in the control animals it was found that the fibrous connective tissue was uniformly oriented and very dense with a high cellular density (Figure 18). The bony surfaces were relatively smooth with very little evidence of osteoblastic and osteoclastic activity. The different zones of a suture were not evident.

The different sutures, of the experimental animals, varied slightly in their morphology and cellular proliferation depending upon their remoteness to the expansion forces; however, there were great differences in comparison to the sutures of the controls.

In the experimental animals under lower power (X40), it was found that even though there was interdigitation of the bony processes, the connective tissue space between the bone was abnormally wide. The bony surfaces were found to be roughened.

FIGURE 18

FIGURE 19

FIGURE 18. Photomicrograph of an H. and E. slide of the nasal sutural complex in a control animal showing normal cell density, connective tissue orientation, smooth outline of bone surfaces, and normal bone apposition and remodeling. (X450)

FIGURE 19. Photomicrograph of an H. and E. slide of the nasal sutural complex in an experimental animal showing decreased cell density, disorientation of connective tissue, and increased bone apposition and remodeling. (X450)
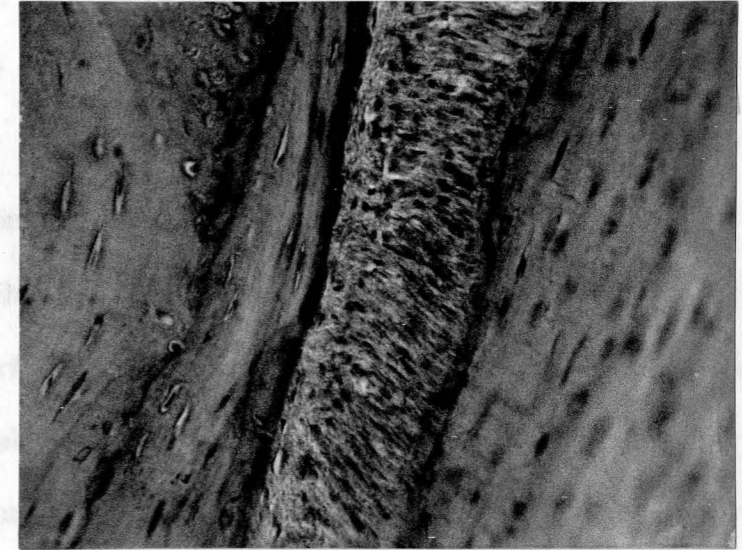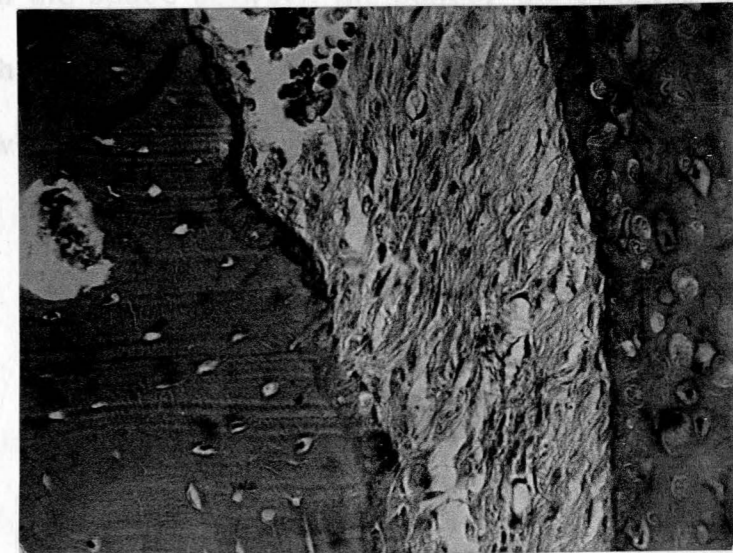
Under higher power (X450), in the sutures of the experimental animals, the connective tissue was found to be less dense with greater intercellular fluid and less cell density (Figure 19). There was greater fiber disorientation in the connective tissue. There were areas of highly stretched tissues as well as areas of heavily compressed tissue. The bony surfaces showed increased apposition and remodeling processes with an abnormally wide area of newly formed bone.

The amount of cell proliferation and differences in sutural morphology depended upon the sutures position in the facioskeletal complex. The farther the suture was from the direct expansion forces, the narrower was the space between the bones, the greater was the cell density and the amount of cell proliferation and changes in sutural morphology were decreased.

CHAPTER VI

DISCUSSION

As previously mentioned it has been determined that heavy expansion forces are generated during rapid palatal expansion and the expansion forces can successfully split the mid-palatal suture. Numerous researchers (Harvold, 1950), (Gerlach, 1956), (Thorne 1956, 1960), and (Krebs, 1958, 1959) stated that they successfully separated the mid-palatal suture. It has also been determined in the studies of Isaacson and Murphy (1964) that a failure to achieve separation of the palatal shelves in adult cleft palate patients may be due to an age dependent increase in the rigidity of the articulations of the facial skeleton. These facial articulations may be more important than the question of ossification of the mid-palatal suture in the application of rapid palatal expansion procedures.

Before determining the effect of the heavy expansion forces on the articulations of the facial skeleton, it is important to evaluate the extent to which the mid-palatal suture was effected in the present study.

The occlusal radiographs of the mid-palatal suture showed a definite separation of the suture in the experimental group. The

separation appeared to be parallel between the palatal shelves. There was also an increase in maxillary arch width with a resulting bilateral buccal crossbite. The abutment teeth showed a minimal change in axial inclination. The moderate tissue damage to the palatal vault indicated that not only were the heavy expansion forces delivered to the abutment teeth but also to the palatal shelves and thereby causing a more effective and parallel split. Haas (1959) in his research on pigs showed that he attained a more parallel split of the palatal suture when using acrylic pads against the palatal vault. In his follow up research, Haas (1961, 1965, 1970) on clinical patients reemphasized the effectiveness and parallelism of the palatal separation when using the appliance. It must be remembered that even though up to ten pounds of force can be generated by the expansion appliance; the amount of force that reaches the rest of the facial articulations is at most a few ounces. Working with expansion appliances and strain gauges, Isaacson and Ingram (1964), Isaacson, Wood and Ingram (1964) showed using clinical patients that large forces were present during expansion and these forces were greatly reduced when reaching the facial skeleton.

The animals in both groups were young and growing specimens; however, one would not expect to find large amounts of growth in the facial skeleton due to the young age of the animals. By doing a gross and serial roentgenographic study by means of metallic implants,

Gans and Sarnat (1951) were able to determine this sutural growth in the facial complex of Rhesus monkeys. The control group revealed normal sutural morphology with minimal apposition and remodeling in all the sutures studied. The amount of cell proliferation in all the sutures was determined to be approximately the same for the controls. Also the cell densities for the two control monkeys were relatively close.

From these findings it can be stated that the nasal sutural complex, zygomaticomaxillary, zygomaticofrontal and zygomaticotemporal exhibited similar amounts of cell proliferation and therefore growth during the experimental period. These findings are similar to those of Starnback, Bayne, Cleall, and Subtelny (1966) who showed normal sutural growth in their Rhesus monkey experiments.

The findings of the experimental group differed significantly from the control group.

The overall morphology of the sutures showed an abnormal widening of the bony processes with a resulting wider connective tissue interval. The connective tissue showed areas of stretching and compression which indicated movement within the sutures. Similar findings were noted in the research of West (1964). This apparent change in the tissue elements of the sutures of the experimental group is consistent

with the findings of Starnback, Bayne, Cleall and Subtelny (1961).

They concluded that the facial sutures showed evidence of great cell-

ular activity.

It was determined that there was a decrease in cell densities

in the sutures of the experimental group. It was found that in these

animals there was an average decrease of 35 per cent in cell density

compared to the controls. This decrease can be accounted for by the

forces applied to the sutural areas. Since the forces caused a

stretching in some areas and a compression in other areas of the su-

ture, there was a resulting cellular reorganization, an increase in

intercellular fluid and vascularization with a decrease in cell densities.

These types of changes in remote sutural areas of Rhesus monkeys

were similarly found in the absorded tetracycline studies of Gardner

and Kronman (1971).

The findings were consistent in all the animals of the experi-

mental group; however, it was determined that the greatest decrease

was found in the nasal sutural complex, followed by the zygomatico-

temporal, zygomaticofrontal and zygomaticomaxillary sutures respec-

tively. The decrease was significant to the one per cent level for all

four sutural articulations.

Due to the decrease in cell density in the experimental group,

one would assume that there would have been an associated decrease

in cellular activity due to fewer numbers of cells. Just the opposite results were found.

The labeling indicies revealed that there was an increase in cellular activity in the sutures. The greatest increase was seen in the nasal sutural complex followed by the zygomaticofrontal, zygomatico-maxillary and zygomaticotemporal sutures. The average increase was found to be 30 per cent. It was determined, using the Student's t Test, that the large increase in cellular activity was not significant at the five per cent level. This apparent inconsistency can be attributed to the low results of one experimental animal, 122-C.

The labeling index for this animal compared to the values of the control group rather than the experimental group. It was felt that there were two possibilities to explain this inconsistency in the results but neither appears to be completely adequate. First there was a possibility that the expansion appliance expanded the abutment teeth through the buccal plates of bone and therefore resulting in a less effective palatal suture split. This possibility was considered due to the fact that a very high labeling index was found in the periodontal ligament of the abutment teeth and a lower labeling index for the palatal suture. The second possibility was that after carefully reviewing the prepared autoradiograms, it was noticed that there were a lot of cells labeled, but the few activated silver grains over the cells was below the

criteria set for the experiment. The apparent low number of visible grains could be due to a processing error.

If the results from the one experimental animal were excluded, the results would be significant to the five per cent level and possibly to the one per cent level due to the large differences in the means between the experimental and control groups.

Though the cell density findings indicated there was a significant decrease in the experimentals, it was evident that the fewer number of cells had a higher rate of DNA synthetic activity as the higher labeling indicies showed. The highest increase was in the nasal sutural complex, which was consistent with the fact that the nasal complex is in the same vertical plane as the palatal suture, where the forces were applied. There is a relative thinness and elasticity in areas of the facial skeleton, particularly the nasal cavity, maxillary sinus and orbital areas (Sicher and DuBrul, 1970) which could have also accounted for the high activity. The next greatest increase in activity was seen in the zygomaticofrontal suture. Displacement of this suture might be accountable due to the relative thinness of the associated orbital area and due to the fact the maxilla is a rigid structural unit and forces applied to it by the appliance would be transmitted to peripheral areas. Similarly Starnbach, Bayne, Cleall and Subtelny (1966) concluded that the rigidity of the area transmitted the expansion.

The zygomaticomaxillary was less affected with the zygomatico-temporal being the farthest from the expansion site and therefore showed the least change.

Since the halves of the maxilla were expanded laterally with associated sutural displacements, and the expansion appliance produced considerable internal stress in the maxillary complex; it might be understandable to find immediate responses. There might have been a signficiant amount of elastic deformation due to the nature of bone resulting in an immediate physiologic response in the form of cellular activity in order to relieve the internal stresses and attain a physiological equilibrium. Isaacson and Ingram (1964) have also stated that with the expansion forces producing internal stresses, there was an immediate response to reduce these forces and attain an equilibrium.

The results attained in this study have for the first time quantitative changes in the craniofacial complex of monkeys undergoing rapid palatal expansion and they therefore reinforce the importance of the craniofacial articulations.

In light of these results, it is suggested that the clinician must carefully consider the total craniofacial skeleton when carrying out an expansion procedure and evaluating the expected results.

CHAPTER VII

SUMMARY AND CONCLUSION

A. Summary:

It was the purpose of this study to determine the short term histological changes of the facioskeletal complex resulting from rapid palatal expansion.

Six Rhesus monkeys, with mixed dentition, were used, two control animals and four experimentals. Four rapid palatal expansion appliances were designed and constructed that would produce four millimeters of expansion. The appliance, with acrylic pads, was selected to allow as much of the expansion force delivered to the palatal suture as possible and also minimal tipping of the palatal shelves. The deciduous first molars or permanent first premolars and the permanent first molars were used as the anchor abutments. The appliance was activated one half millimeter a day until four millimeters were obtained.

Three hours prior to sacrifice, tritiated thymidine was injected into the animals. The histological changes in the sutural areas were determined by histological examination of H. and E. slides, a labeling index per 1,000 cells and cell density count per $110uM^2$.

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The areas studied were the nasal sutural complex, the zygomaticomaxillary, zygomaticofrontal, and zygomaticotemporal sutures.

The histological examination showed the sutures of the control animals to be of normal cell density, connective tissue orientation, smooth outline of bone surfaces, and normal bone apposition and remodeling.

Histological evaluation of the sutural tissues in the experimental animal revealed that there was a decrease in cell density, the sutural connective tissue was abnormally wide and the fibers disorientated. There was an increase in intercellular fluid, vascularization, bone apposition and remodeling.

The labeling index revealed that there was a large numerical increase in cellular proliferation in the sutures of the experimental animals with the nasal sutural complex showing the greatest activity followed by the zygomaticofrontal, zygomaticomaxillary and zygomaticotemporal sutures. Though the experimental results appeared to be significant, the aberrant values of 122-C lead to the conclusions of the Student's t Test that the labeling index values were not significant at the five per cent level.

The cell density counts showed a large decrease in cell densities in the sutures of the experimental animals. The largest decrease was

found in the nasal sutural complex followed by the zygomaticotemporal, zygomaticofrontal and zygomaticomaxillary sutures. These decreases were found to be significant to the one per cent level.

B. Conclusions:

1. An appliance that will split the mid-palatal suture in a parallel manner, with a minimal tipping of the palatal shelves, can be successfully designed and constructed.

2. The rapid expansion appliance produces an increase in the width of the maxillary arch with a resulting bilateral crossbite.

3. The use of a rapid palatal expansion appliance produces changes in remote sutural areas of the facioskeletal complex.

4. The nasal sutural complex showed the greatest labeling index and therefore the greatest proliferative activity followed by the zygomaticofrontal, zygomaticomaxillary and the zygomaticotemporal sutures.

5. In all sutures studied, the experimental animals showed a significant decrease in cell density.

6. Histologically, the sutures of the experimental animals showed a wider zone and greater amount of disorientation of the connective tissue with an increase in intercellular fluid, vascularization, and bone remodeling.

7. The aberrant labeling index found in 122-C undoubtedly accounted for the Student's t Test finding the values insignificant since the difference between experimental and control groups was quite large.

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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_____
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